Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: B

Pharma, Drug Discovery, Toxicology & Medicine

Highlights

- Method of Mathematical Planning
- Development of an Optimal Technology
- Water Contamination by Nitrates
- Treatment used in Ophthalmological Practice

Discovering Thoughts, Inventing Future

VOLUME 22 ISSUE 2 VERSION 1.0

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine

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Volume 22 Issue 2 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

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Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
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Offset Typesetting

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Szmodits László Pharmacist (Hungary. Budapest) Hungarian Pharmacist József Dorner, Pre-Eminent Botanist (1808-1873)

By Szmodits László

Introduction- The author describes the kife and scientific work of the hungaryan pharmacist, Józaef Dorner (1808-1873). Dorner worked for 16 years a general pharmacist. But then turned to a special discipline of his profession and became an excellent botanist. He performed at first florristic research, but later concentrated his attention to plant anatomy and physiology, by systematic application of the microscope, be inaugurated a in new aspect in botanica. Very soon, already in 1853, was Dorner discussing bioenergetics. He was selected to Corresponding Member of the Hungarian Academy of Sciences and was a distinguished botanical expert of the Roval Association of Natural Sciences. He has had been estimated as an excellent teacher as well.

GJMR-B Classification: DDC Code: 581.96805 LCC Code: QK396

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Szmodits László Pharmacist (Hungary. Budapest) Hungarian Pharmacist József Dorner, Pre-Eminent Botanist (1808-1873)

Szmodits László

I. Introduction

The author describes the kife and scientific work of the hungaryan pharmacist, Józaef Dorner (1808-1873). Dorner worked for 16 years a general pharmacist. But then turned to a special discipline of his profession and became an excellent botanist. He performed at first florristic research, but later concentrated his attention to plant anatomy and physiology, by systematic application of the microscope, be inaugurated a in new aspect in botanica. Very soon, already in 1853, was Dorner discussing bioenergetics. He was selected to Corresponding Member of the Hungarian Academy of Sciences and was a distinguished botanical expert of the Roval Association of Natural Sciences. He has had been estimated as an excellent teacher as well.

Life path until 1849

József Dorner was of German nationality. Its original name was József Thurner junior wohl 1834. The time of the name change. Was confirmed by a letter from the Lutheran parish of Győr-Hungary, Was born in Győr on November 2, 1808 (1). His father József Thurner senior merchant in Győr. The mother Susanna Schmidt. He completed primary scholl at his birthplace and then an at Lutheran grammar school in Sopron. Here he liked botany, he studied the flora around Sopron with great interest (2, 3).

At that time was enough to complete 6 high school classes to enter the profession of pharmacist. In 1824, they practiced a Hungarian Crown Pharmacy in Sopron. Here lenarned the Latin names of Medicines and took part in the formulations Technologist also performed an opüeration (4). After 3 three, he passed the internship successfully. Between 1827 and 1831, he was an assistant in Pest and Bratislava (today Slovakia,) the old Hungarian name: Pozsony, German: Pressburg). After his time an assistant, he enrolled at the University Vienna, where he obtained a degree in pharmacy in 1832. In Vienna, he met the chief physician István Endlicher (1804.-1849), a botanist, who had a significant influence on the Work of of József Dorner.

In addition to German, he also spoke French. He was in contact not only with Hungarian botanists, but also with many foreing scientitif. He became friendly with the famous botanist of those times: József Sadler (1792-1849) professors of botany at Pest university and János Heuffel phiysicians botanist and Antal Rochel (1770-1847) warden of the botanical garden ib Pest and Ferenc Adoll Láng (1795-1863). pharmacist botanist. He kept up a correspondence with foreign scientists, including the botanists Eduard Fenzl (1808-1870) of Vienna and H. G. Ludwig Reichenbach (1793-1873) in Leipzig, His father bought the Golden Crown Pharnacy in Bartislava, in 1836. Here owned 4 four, In 1840 he sold the Pharmacy

Mediated by Antal Rochel in 1835, he visited of southern Hungary, Bánát. He met János Heuffel, with whorn they sesarched the local flora. He euhred his Herbárium with the plants collected were. His book on his expencienses was published in 1839, Pressburg. *„;Das Banat in toographisch-naturhistorischer Beziehung, mit besordener Berücksichtigung der Herculesbäder nächst Mehadia und ihrer Umgebungen."* It was such a succens that it aroused the interest of contemporary scientists. This has already been very much notices in the couuntry. In 1840, he sold bis chemist's shop and took a post in the health department of the Governor's Council in Buda. (3,5).

He published two books on chemical technology: 1. on the theory and practice of vinegar production. 1841.- 2. the process of brandy with mashing, making malt and years, Pesth, 1843. (1).

In 1842 the Hungarian Academy of Sciences announced a tender for the change of the Climate. of Hungary about, the flora and fauna- Dorner observed weather data every 2v hours, a day for 5 yerars wird directions. He also often recorded the temperates of the water in a well in Buda, Tabán. He continued this work later until 1850. Was valuable because it dealt extensively with 100 gold honors by Academy in 1847 (5, 6, 7).

In 1846 he decided to compile and publish a manul on the flora of Hungary. Heuffel and Sadler welcomed Dorner's plan and offered to collaborate with him, but due to Sadler's death in 1848 and Heuffel's illness, the plan was never realized. Dorner could not undertake the enormous work alone (1, 4).

Author: e-mail: szmodits.laszlo@t-online.hu

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On March 13 the 1847, was elected a member of the Royal Hungarian Society of Natural Sciences. As one of the notabilities of the botanical committee. He was invited to take over the book collections of the Society. He organized the enlargement of the collections trough exhange (8).

In 1848 during the was of indepedence the wars invited me as a ministerial secretary to the Ministery of Religion and Public Education. Howewer, after the capitulation of 1849. He retook privacy, At that time he devted all his to botany. (3, 9).

His life and work from 1851 until his death

In 1851-53 he gave several lectures in the Hungarian Society of Natural Sciences: parasitic plants, fertilization of plants and plant cells (10.11). In 1853 a book was pulished on grape diseases.

In 1853 he delivered a lecture at the Royal Hungarian Society of Natural Scienses with the title „;The plant kindom and nan". In a paper published in the journal „;Új Magyar Múzeum" (New Hungarian Museum) hr discussed the Darwinist theory of evolution:

„;*The times when botany was confined to the identification, of genera and species... have passed. The new science has assumed a higher standing. It no longer satisfies itself with the classification of forms..., but seareches for their inner relationships."*

In the paper „;The plant cell.". He wrote: *„;The study of cells in the most remarkable part of the science of botany, which with the introduction of the microscope has become a most interesting subject. There exists in the cell a remarkable moving force, so far physically unidentified, whereby the cell content spreads in different ways unnocited in the body of the plant.*"

József Dorner realized that the tissue structure of plants would only become phenomennon of plants, which is based on motion and metabolism. At that time it was still novel in botany.

On December 15, 1858, he was elected a corresőonding member of the Hungarian Academy of Sciences. The title of his inaugural lecture was: „;*Online of the history and application of the microscope.*"

In the 1853, He taught natural science (biological subjects) es in the Lutheran grammar school in Szarvas, Békés country. The from 1860 in a similar school in Pest. Both shools taught in Hungarian, but he also taught Chemistry, Physics, German and French.

Textbooks

1. Elelements of Botany, 1864. Pest,
2. Elememnts of Mineralogy: 1858, 1865, Pest,
3. Elements of Zoology:

– Uninhabited: 1864, Pesth,
– Reptiles, fish, birds. 1863. Pesth,
– Mammals: 1863. 1874, Pesth (2).

Dorner knows first-hand scientific a acievements of the page. He adcvocated the need for permonence knowledge.

„;*We don't want to train botanists and zoologists. We educate young people enriched with touring back ground, who are capable of self-education*" (9).

In 1855 published an article on the history of the fertilization of seed plants. He highligted te role seeds Here (3).

In 1860 he a very interesting arcticle entiiled „;The Hungarian Great Plain, especially the area aeound Szarvas." Whit a historical introduction, Hungary a country of peculiarities and extremes. At the time this settlement was nig village.

Discussed in detail the natural village conditions (bedrock, climate, agricultural production) and the situation pessanty. Worked out the population date of the area. He also pointed and the benefits of urbanization. Perceived differences in social classes.

„;He described the conditions of the poor and wastelful lives of the nobles (9).

In one of his last works, he compared the flora of Pest country that of Lower Austria. This study was completed with a detailed list of plants (1862).

In his paper „;Oak-trees of Budapest", published in 1862, he gave a summary of the Quercus species ocurring in the environs of Budapest on the based of his observations (3).

In 1863 at the IXth Congress of Hungarian Physicians and Natural Scientists, held in Pest, Dorner delivered a lecture on the *„;Cuscuta varieties of the Hungarian meadow.*"

He pointed out that owing, to their parasitic nature, the dodder, species differ from dicotyledons in their tissue structure. He presented details of the germination and development of the dodder, and mentioned the prevention of dodder infestation. During the lecture, he showed mature specimens of Cuscuta. This lecture was a great success and aroused the interest of many of foreign botanist as well (12).

Dorner taught the Hungarian botanical terminology to Paul Ascherson (1843-1913,) a botanist from Berlin, who then translades „;Cuscuta varieties of Hungarian meadow" into German (5).

In 1868, in one of his last academic lectures, he came up with som rare plants from Hungary. Anthemis neilreichii was also detected in Hungary, met was discovered in Lower Austria a few years ago. He also founded the species Cuscuta obstugiflora Hunp et Bonl, which had already disccovered by Paul Ascherson and Viktor Janka in the Lower Tisza (13).

He was constantly enrchting shholl has. The grammar shas Budapest kept is herbarium of 48 fascicules, 15 thousand flat. Today it is in the Budapest Galery of the Natural History-Museum. He also wrote book rewiews.

József Dorner's human characteristics, disease, and death

He was a highly educated, and a very well-preperaded humble man. He also acknowledged supertiosus customs. Trough hís scholarly figure he can easly recoggnize superstars habits as well. He was always a success with the skill of an excellent performer. He was characterized by beautiful Hungarian speech. He justified what he had to say, He calced humor to hís performentes. He wo speak to hin listened intetly to hin.

He married in 1845, but had no children from marriage. His wife died early, so so he lived alone. He also taught it has long illness. He had an incurable heart problem.So died on 9 th October, 1873. Fiume Road in Budapest, his heas still rest in public cemetery todav. The memory Hungarian Pharmacist Pantheon is preserved.

II. Summary

József Dorner was one of the first Hungarian researches in plant morphology snd physiology with a degree in pharmacy. An thet time, he was not yet able to teoch anthe university as sn academy. But with a pen and word, he made great. He was an excellent instructor. I wrote in English so that, readers could get to know the work of Hungarian pharmacists.

„;Respect for the greats and noble tradition of the past, the main guarantee our ascension."

(Vilmos Milkó Hungarian doctor-professor, 1878-1956)

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Pharmaco-Economic Evaluation of the Treatment used in Ophthalmological Practice

By Shoyusuf F. Shodmanov & Shakhnoza Z. Umarova

Abstract- The most important among the clinical forms of glaucoma is primary open-angle glaucoma (POAG), which, according to various authors, occurs in 70%-92% of cases. Due to the high prevalence of POAG, late detection and serious prognosis for visual functions, this disease occupies a special place in clinical ophthalmology. The aim of the study is to conduct a pharmacoeconomic evaluation of Tafluprostvs Travoprost in patients with POAG. Materials and methods of research is pharmacoeconomic methods of analysis, in particular the calculation of the relative risk, the calculation of the probability of events, cost-effectiveness analysis. According to the calculated results of pharmacoeconomic analyzes, Tafluprost was relatively less expensive and more clinically effective than Travoprost in patients with primary open-angle glaucoma. Alternative treatment with Tafluprost contributes to savings in the overall treatment procedure. Therefore, we recommend adding Tafluprost to the list of essential medicines.

Keywords: pharmacoeconomic evaluation, ophthalmology, medicines, relative risk, probabilities of events, cost-effectiveness analysis.

GJMR-B Classification: DDC Code: 617.7 LCC Code: RE1

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Pharmaco-Economic Evaluation of the Treatment used in Ophthalmological Practice

Shoyusuf F. Shodmanov [α] & Shakhnoza Z. Umarova [σ]

Abstract- The most important among the clinical forms of glaucoma is primary open-angle glaucoma (POAG), which, according to various authors, occurs in 70%-92% of cases. Due to the high prevalence of POAG, late detection and serious prognosis for visual functions, this disease occupies a special place in clinical ophthalmology. The aim of the study is to conduct a pharmacoeconomic evaluation of Tafluprostvs Travoprost in patients with POAG. Materials and methods of research is pharmacoeconomic methods of analysis, in particular the calculation of the relative risk, the calculation of the probability of events, cost-effectiveness analysis. According to the calculated results of pharmacoeconomic analyzes, Tafluprost was relatively less expensive and more clinically effective than Travoprost in patients with primary open-angle glaucoma. Alternative treatment with Tafluprost contributes to savings in the overall treatment procedure. Therefore, we recommend adding Tafluprost to the list of essential medicines.

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I. Introduction

The most important among the clinical forms of glaucoma is primary open-angle glaucoma (POAG), which, according to various authors, occurs in 70%-92% of cases. Due to the high prevalence of POAG, late detection and serious prognosis for visual functions, this disease occupies a special place in clinical ophthalmology [1].

The proportion of the disease caused by this type of pathology, requiring surgical treatment, is generally small. However, the absolute number of patients with this pathology is quite large: if, in general, in Uzbekistan, the absolute number of patients (2019) with various forms of eye diseases was more than 600 thousand people, then with glaucoma the number of patients was 9,000 [2, 3].

Despite significant advances in the medical treatment of this disease, the percentage of blindness and low vision as a result of glaucoma remains stable and does not tend to decrease. But with the timely detection of the disease, with the help of drug treatment, you can reduce the level of intraocular pressure (IOP). The issue of introducing a more effective, but less expensive drug remains relevant [4].

The aim of the study was to conduct a pharmacoeconomic evaluation of Tafluprostvs Travoprost in patients with POAG.

II. Materials and Methods

In this research pharmaco-economic methods of analysis, in particular relative risk calculation, probability of events calculation, cost-effectiveness analysis was performed in order to achieve the purpose of the investigation.

III. Results and Discussions

To evaluate the cost-effectiveness of Tafluprost compared with Travoprost in patients with POAG, a Markov analytical model was implemented. The structure of the model was taken from the NICE Guide (2017). Patients were initially classified into glaucoma conditions based on visual field characteristics (Hoddap–Parrish–Anderson criteria) [5].

To monitor patients for more than 3 years, a 1-month cycle was used. During a Markov cycle, members of a cohort may stay at their stage, die, or progress. Because glaucoma can only be prevented from worsening further, none of the cohort members can regress along the clinical path. Based on the HPU (hectopascal unit of pressure) classification system, patients were divided using the mean deviation (MD) value into early (MD less than -6dB), moderate (MD less than -12dB) and severe (MD greater than -12dB). It was believed that the main impact of each strategy was to increase or decrease the risk of developing POAG. However, according to clinical data, the most detailed risk factor for treatment outcomes is adjusting for changes in IOP. To find the relative risk of developing glaucoma of each of the interventions, a systematic search was carried out, moreover, the probabilities of transition between stages were found using another search in the literature. Since the model assumed that an increase in IOP is associated with a further increase in the likelihood of developing glaucoma, this study showed that costs decrease when the progression of the disease is inhibited [6, 7].

Author α: Researcher, Chief specialist, Department of Registration of the State Unitary Enterprise "State Center for Expertise and Standardization of Medicines, Medical Devices and Medical Equipment", Tashkent Pharmaceutical Institute, Oybek Street 45, Tashkent, Uzbekistan.

Author σ: Pharmaceutical Institute of Education and Research, Ph.D. professor, vice-rector.

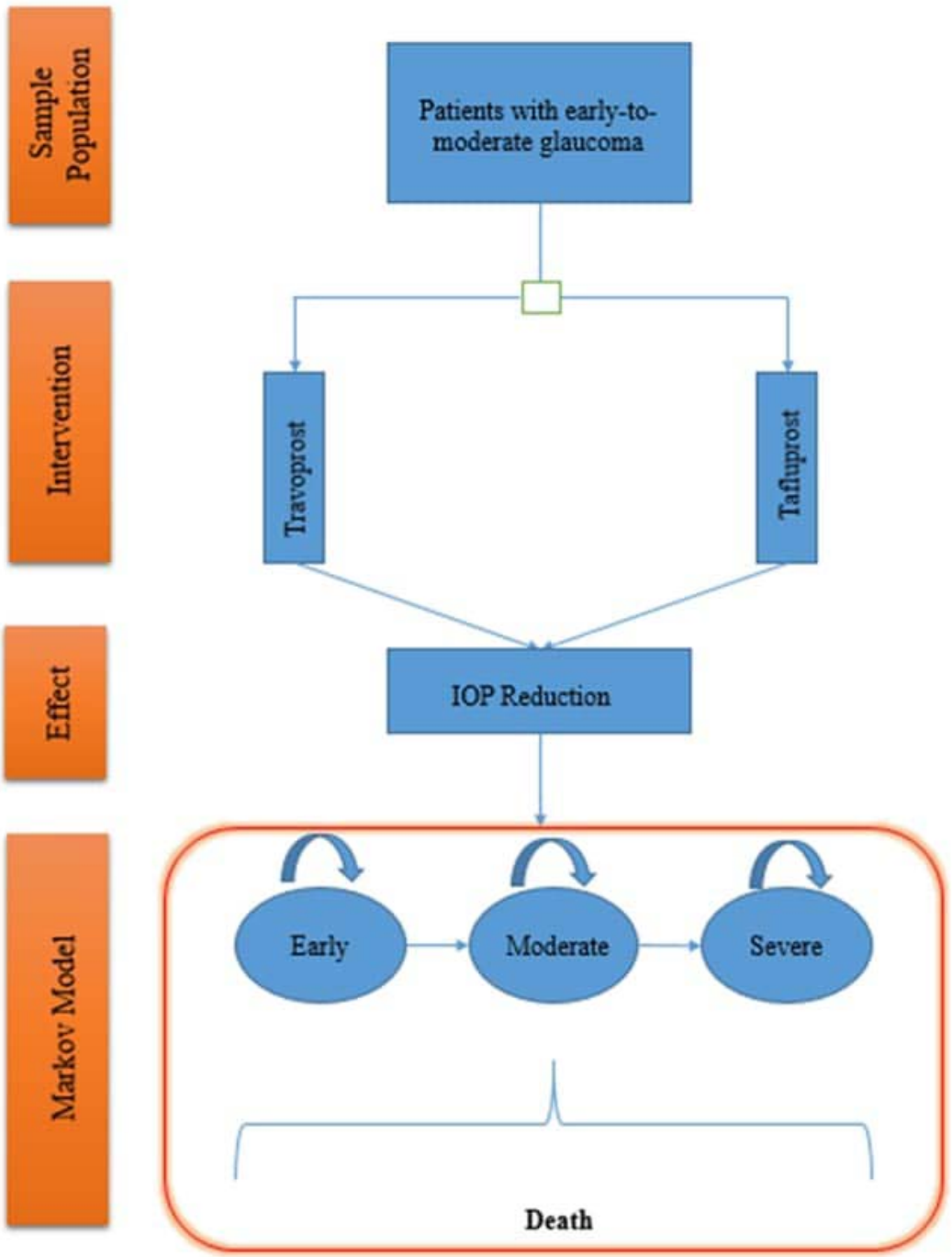

Fig. 1: Initial decision tree integrated with Markov model

Several input parameters were used to populate the model. These baseline data were based on clinical evidence derived from effective evidence review, attempting to broadly allocate resources to guideline updates and supplementary databases. Model inputs have been reviewed and validated by clinical experts. More details on sources - clinical outcomes, utilities and costs are explained below.

Initially, the search was conducted to determine the likelihood of a treatment-related transition during the follow-up period. Since all the data obtained reflected a period of time that exceeded the duration of the cycle in our model, we converted the information into monthly probabilities (Table 1.). The incremental cost-effectiveness study presents the annual risk of progression to routine glaucoma with moderate to severe clinical treatments. Input parameters were based on 601 patients followed up for 5 years in the Primary Glaucoma Treatment Collaborative Study (PGTCS). Calculation of transition probabilities from early to severe glaucoma was based on various combinations of methods, including natural decline, from the study of early overt glaucoma to the NICE methodology [11]. The study reported transition probabilities by estimating the

number of months it takes a normal patient to change from one state of well-being, then to the next, which was found to decrease in efficiency-adjusted MD effectiveness each month. IOP and age were identified as significant risk factors. The total mortality of people over 65 years of age was obtained from WHO sources to calculate the Markov model.

Table 1: Probabilities of transition between health states of patients with glaucoma

Glaucoma Stages	Average monthly probabilities	Min	Max	Author, Year
P (early to moderate)	0.003779977	0.003023982	0.004535973	Rein et al., 2009 Lichter et al., 2001
P (moderate to severe)	0.003779977	0.003023982	0.004535973	Rein et al., 2009 Lichter et al., 2001
Mortality				
Total mortality of the population over 65 years of age	0.000483333	0.000386667	0.00058	World Health Organization, 2016

Since glaucoma is a chronic disease, it has a great impact on many stages of a patient's life. Although glaucoma eventually leads to permanent blindness, the performance of daily activities and the quality of life recognized by individuals seriously affect health in the early stages of the disease. There are many potential causes of the impact of glaucoma on the patient's quality of life, such as loss of visual field, stress and anxiety due to tests in clinics, impairment and cost of medical care [13]. Many authors have investigated the impact of illness at health stages on the quality of life of patients. Utility values for early, moderate, and severe health conditions in glaucoma were derived primarily from two studies. The first study included a cross-sectional study of 434 patients with 5 common eye conditions, including glaucoma. Computerized preference scores were used to rate standard utilities from 0 (death) to 1 (excellent health). The second study analyzed the impact of the applied therapy on the utility of patients with glaucoma. The sample population consisted of 225 patients in the same age group as in our evaluation. To the best of our knowledge, a noteworthy finding from this study is that no interference was found between treatment and utility of glaucoma. When reviewing both studies, no rapid discrepancies were found between beneficial outcomes at different stages of glaucoma. Table 2 shows the detailed utility values used in the model.

Table 2: Utility values used in the economic model

Health status	Utility	Min.	Max.	Author, Year
Early glaucoma	0.92	0.736	1.104	Lee et al. (2008) and Palette Guedes et al. (2015)
Moderate glaucoma	0.89	0.712	1.068	Lee et al. (2008) and Palette Guedes et al. (2015)
Severe glaucoma	0.86	0.688	1.032	Lee et al. (2008) and Palette Guedes et al. (2015)
Death	0.00			

Cost and resource utilization parameters were obtained from a clinical specialist who works at the Specialized Research Center for Eye Microsurgery in Tashkent.

To calculate the monthly intervention costs, we first derived the annual cost of POAG by multiplying the average unit cost by the expected resource use. Each stage of the disease includes the necessary diagnostic costs, the salaries of medical staff and the weighted cost of medicines. Average prices for prostaglandin analogues and eye drop with beta-blockers were obtained from local pharmacies in Tashkent. After the early stage of glaucoma, monotherapy with Travoprost (Travatan 2.5 ml) or Tafluprost (Teflotan 2.5 ml) was prescribed. However, patients with moderate progression are treated with combined beta-blockers and prostaglandin analogues. The operation is applied on both eyes in a severe condition of glaucoma. In accordance with the recommendations of experts, the frequency of medical diagnoses was set to 4 times a year, that is, once every three months. In addition, it is estimated that 15 vials of Teflotan 2.5 ml and Travatan 2.5 ml are consumed annually per patient. Meanwhile, the patient is annually prescribed 7 vials of timolol maleate, 5 ml each, and Oftan® Timolol, 5 ml each.

Table 3: The cost of treatment used in the model

The cost of treatment in one case	Medium Cost (UZS)	Minimum Cost (UZS)	Maximum Cost (UZS)
Diagnostics and laboratory tests			
Visometry Test	21,300	14.910	27.690
Simple optical correction	23,000	16,100	29,900
Biomicroscopy	40,000	28,000	52,000
Simple perimetry	23,000	16,100	29,900
The cost of treatment in one case	Medium Cost (UZS)	Minimum Cost (UZS)	Maximum Cost (UZS)
Tonometry	25,000	17,500	32,500
Gonioscopy	30,000	21,000	39,000
Direct ophthalmoscopy	30,000	21,000	39,000
Reverse ophthalmoscopy	40,000	28,000	52,000
Consultation	27,000	18,900	35.100
Total diagnostic and lab costs per visit	**1,037,200**	**726.040**	**1,348,360**
Care costper visit (20 min)	2.536	1.775	3.296
Doctor cost per visit (20 min)	4,620	3.234	6.006
Total payroll costsfee	**7.156**	**5.009**	**9.302**

Table 4: Cost of eye drops used in the model

The cost of treatment in one case	Medium Cost (UZS)	Minimum Cost (UZS)	Maximum Cost (UZS)
Eye drops			
Taflatan 2.5ml	90,000	63,000	117,000
Travatan 2.5ml	140,000	98,000	182,000
Timolol maleate5ml	30,000	21,000	39,000
*Oftan® Timolol*5ml	25,000	17,500	32,500
Annual cost of intervention per patient	**early glaucoma (UZS)**	**Moderate glaucoma (UZS)**	**Severe glaucoma (UZS)**
Tafluprost	2,415,824	2,800,824	5,815,824
Monthly intervention cost per patient	**201.319**	**233.402**	**484.652**
Travoprost	3,165,824	3,550,824	6,565,824
Comparator monthly cost per patient	**263.819**	**295.902**	**547.152**

Cost-effectiveness study used actual numbers or averages as model parameters. This strategy gives the best estimate of the cost-effectiveness of the Tafluprost intervention, but does not take into account the uncertainty about model inputs or the likelihood of a different sequence of events. A widely used cost-effectiveness measure is to apply the incremental cost-effectiveness ratio (ICER) when comparing Tafluprost and Travoprost eye drops.

During the cost-effectiveness analysis, which gives the best estimate of the cost-effectiveness of Tafluprost, a sensitivity analysis was performed to assess the vulnerability of the model and clinical incidents. To assess the effect of changing one parameter or the parameter that had the greatest impact on the model results, we performed a one-sided sensitivity analysis. When performing a one-sided sensitivity analysis, the valid ranges of the model input data were used. This made it possible to evaluate the individual impact of model inputs on the results. To conduct the Monte Carlo simulation, the model was run on a cohort of 1000 patients and the selected inputs were randomly selected based on the assigned distribution. The results were presented in Table 5, then looking at the cost-effectiveness threshold at the country level, the prospects for the appropriateness of the proposed intervention were assessed.

Table 5: Input range for one-sided sensitivity analysis

Variable	Range	
	High	Low
Overall mortality in patients over 65 years of age	0.00058	0.00039
Cost discount rate	0.003	0.002
Results discount rate	0.003	0.002
The cost of a severe stage per month Tafluprost	$630,047.60	$339,256.40
The cost of a severe stage per month Travoprost	$711,297.60	$383,006.40
Likelihood of switching from moderate to severe glaucoma with Tafluprost	0.004535973	0.003023982
Probability of going from early to moderate glaucoma with Tafluprost	0.004535973	0.003023982
Relative risk of Travoprost	1.235337423	0.823558282
Health Benefits of Early Glaucoma	1.104	0.736
Moderate stage cost per month Tafluprost	$303,422.60	$163,381.40
Health Benefits in Severe Glaucoma	1.032	0.688
Early-stage cost per month Tafluprost	$261,714.27	$140,923.07
Cost of the moderate stage per month Travoprost	$384,672.60	$207,131.40
Early-stage cost per month Travoprost	$342,964.27	$184,673.07
Benefits of Moderate Health in Glaucoma	1.068	0.712

The results of a one-way sensitivity analysis suggested that the variable that strongly influenced the economic model was the utility of the moderate stage of glaucoma. In absolute terms, when the QALY health utility of moderate glaucoma declined, ICER increased nearly 6-fold. In addition, when the risk of spending on Travoprost in early to moderate glaucoma was modified, their ICER showed balanced volatility for both parties per QALY. The results of other models are relatively less sensitive than the above parameters (Fig. 1).

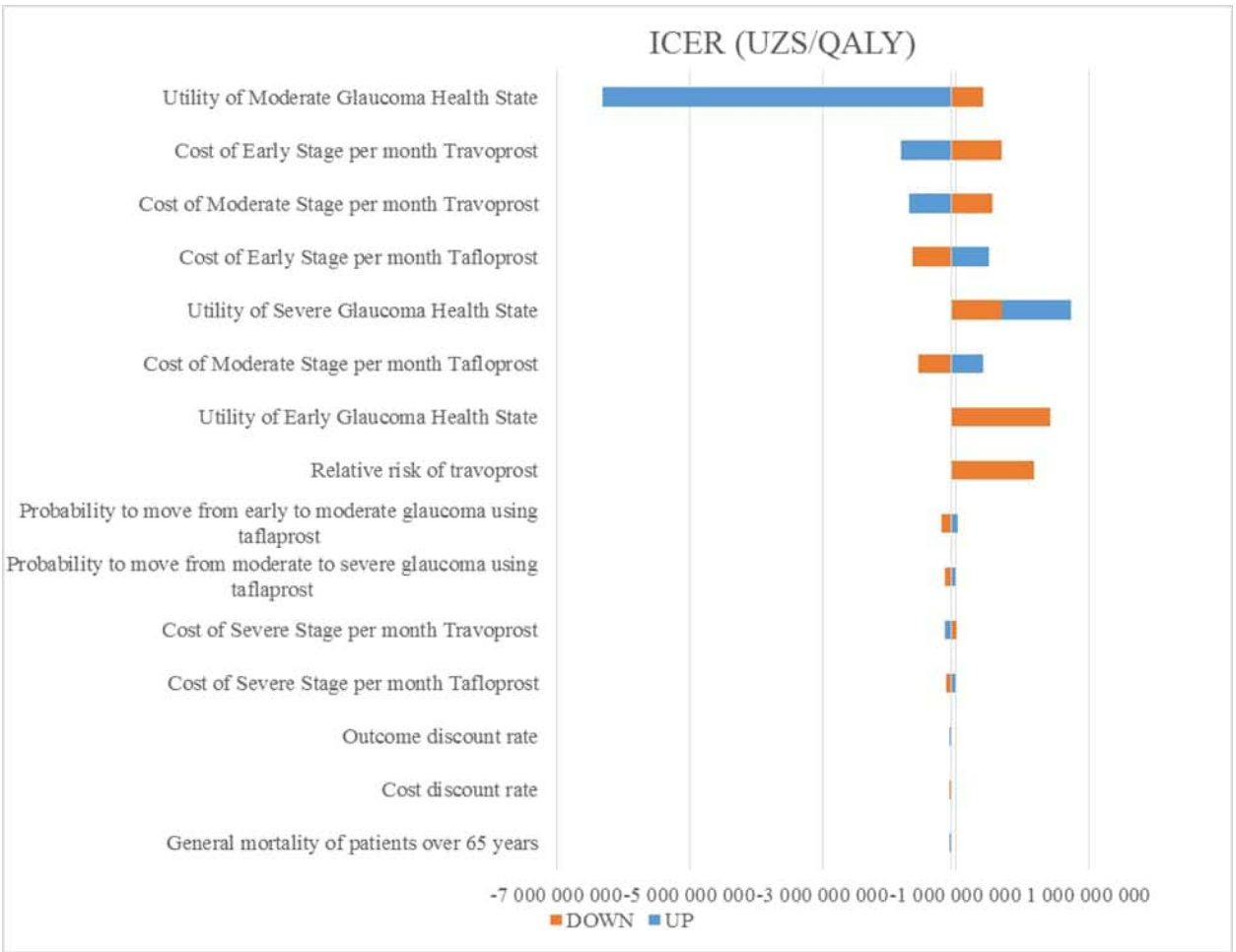

Fig. 1: Results of One-Way Sensitivity Analysis

The results from the cost-effectiveness analysis for the reference case are presented in Table 6. On average, the new intervention dominated by Tafluprost is less costly and clinically more effective than the comparator drug (Travoprost). We calculated that a savings phenomenon could be observed in the treatment of patients with POAG (compared to the Brown study).etal.), so ICER was negative.

Table 6: Results of cost-benefit analysis

Strategy	Average total cost	Average overall effect, QALYs	ICER UZS/QALY
Travoprost	9,721,341	30.878	-1 069 362
Tafluprost	7,582,616	30.880	

ICER = (7 582 616 -9 721 341) / (30.880-30.878) = -1 069 362 UZS/QALY

Next, we performed a budget impact analysis. According to the results of the analysis, it can be seen that in the treatment of glaucoma, when using Tafluprost, you can save 2.138.725 UZS (Table 7).

Table 7: The results of the "influence on the budget"

Transition	Payment	the effect influence on budget, sum	A comment
in treatment glaucoma on Tafluprost with Travoprost	9,721,341 - 7,582,616	= 2,138,725	Saving funds

The cost-effectiveness analysis based on the model showed that the treatment of patients with POAG with Tafluprost has a dominant advantage over the reference drug Travoprost. Despite marginal improvement in quality of life, the Tafluprost intervention resulted in cost savings due to less resource use. However, it is interesting to note that our results are not consistent with a recent US study comparing several prostaglandin analogs in the treatment of patients with glaucoma. Brown Research et al. (2019) showed that Tafluprost is more costly and effective than Travoprost. The additional allowance for the target group was about US$214,828. However, since the economic study took into account cost parameters related to a developed country such as the United States, it is inappropriate to compare with our findings from the perspective of Uzbekistan. Our analysis shows that the exclusive use of Tafluprost rather than Travoprost in the treatment of patients with glaucoma prevents additional economic burden. Under these model assumptions, it has been calculated that delaying progression in early states of glaucoma may prevent patients from taking additional glaucoma medications and even eye surgery in advanced stages of the disease. Based on this, we can assume that Tafluprost would be the most practical option in the reference center environment for the treatment of glaucoma, which would save money for the healthcare system in Uzbekistan.

The study has many strengths. A short analytical decision tree and a Markov model were used to collect epidemiological, clinical, resource utilization, and outcome estimates. The model included the likelihood of glaucoma progressing to advanced stages. In addition, these stages reflect both the clinical and economic consequences of glaucoma. The literature used to derive the specific parameters in our model is based on the sufficient size of the observation period and the target population. We used specific cost data for Uzbekistan, which was unprecedented in this area for individual interventions in the treatment of patients with POAG.

IV. Conclusions

According to the calculated results of pharmacoeconomic analyzes, Tafluprost was relatively less expensive and more clinically effective than Travoprost in patients with primary open-angle glaucoma. Alternative treatment with Tafluprost contributes to savings in the overall treatment procedure. Therefore, we recommend adding Tafluprost to the list of essential medicines.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Ophtalmologic Cystinosis

By Fiqhi Aissam

Introduction- Cystinosis is a very rare lysosomal, autosomal recessive disease (1/200,000 births) caused by a mutation in the CTNS gene (Chz 17) encoding a protein called cystinosine. The intralysosomal accumulation of cystine induces the formation of insoluble crystals responsible for progressive multiple organ failure. Cystinous nephropathy is manifested by failure to thrive, Fanconi syndrome, damage to the renal glomerulus and manifestations affecting other organs appearing as early as 6 to 12 months of life. The specific treatment for cystinosis is cysteamine. The management is multidisciplinary.

We report the case of a boy, aged 6 years, followed for cystinosis diagnosed at the age of 18 months with polyuropolydipsic syndrome. The patient has been treated with oral cysteamine (Cystagon) since the age of 2 years.

GJMR-B Classification: DDC Code: 724 LCC Code: NA500

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We report the case of a boy, aged 6 years, followed for cystinosis diagnosed at the age of 18 months with polyuropolydipsic syndrome. The patient has been treated with oral cysteamine (Cystagon) since the age of 2 years.

On the ophthalmological level,his corrected visual acuity is evaluated at 10/10 P2 ODG, and the intraocular pressure is at 12 mmHg in both eyes. The main complaint of this patient is photophobia. he is treated with cysteamine eye drops (Cystadrops®) at a dosage of 4 drops per day and additional treatment with artificial tears. With the slit lamp, we find a corneal cystinosis of grade 1.50 according to the classification of Gahl (figure 1): deposits of birefringent spindle-shaped crystals accumulating in the corneal stroma, and progressing from the anterior stroma to the endothelium. The appearance is sparkling and multicolored on biomicroscopic examination.

The severity of the corneal involvement can be assessed by corneal OCT.

The fundus does not show retinopathy. Cystinosis retinopathy is very inconstant. Crystal deposits are sometimes found all over the retina, associated with depigmentation and which can progress to retinal atrophy. It can be explored by retinal angiography. The crystals can also deposit on the conjunctiva, iris, ciliary body, anterior lens capsule, choroid or optic nerve and cause various rarer manifestations, such as glaucoma by closing the angle, papillary edema or visual field changes.

Ophthalmologic monitoring for cystinosis should be annual, with visual acuity assessment, Gahl score estimate supplemented by OCT imaging of the cornea, measurement of intraocular pressure and fundus examination.

This case illustrates a well-followed patient who is coping well with his illness.

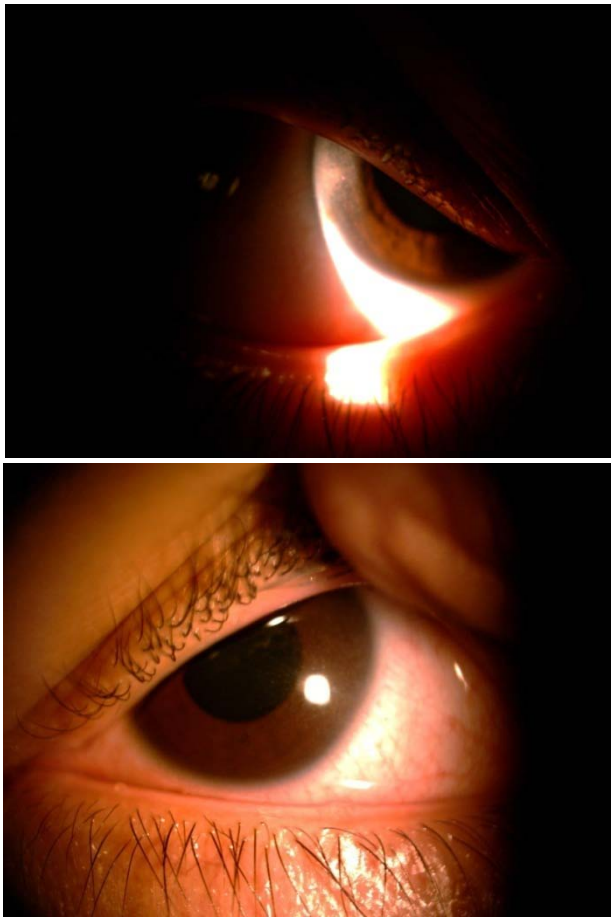

Figure 1: Corneal crystal deposits.

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Author: Hospital Militaire Rabat Maroc. e-mail: fiqhiaissam@gmail.com

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Using the Method of Mathematical Planning of the Experiment in the Development of an Optimal Technology for Obtaining Dry Extract from the Chological Collection “Triflos”

By Nargiza Abdumajidovna Abdurakhmanova & Yokut Saidkarimovna Karieva
Tashkent Pharmaceutical Institute

Abstract- Research is carried out on the development of technology for the dry extract of the choleretic collection "Triflos" by the method of mathematical planning of the experiment. In this case, the method of a four-factor experimental plan based on a 5x5 Greek-Latin square is used. The use of short-term ultrasonic exposure to intensify the extraction of target groups of biologically active substances is scientifically substantiated. The proposed technology is tested in industrial conditions. The dry extract yield was 21%.

Keywords: choleretic collection "Triflos", dry extract, the degree of grinding of raw materials, extractant, temperature, hydromodule, circulating extraction, ultrasound.

GJMR-B Classification: DDC Code: 641.4 LCC Code: TX603

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Using the Method of Mathematical Planning of the Experiment in the Development of an Optimal Technology for Obtaining Dry Extract from the Chological Collection “Triflos”

Nargiza Abdumajidovna Abdurakhmanova [α] & Yokut Saidkarimovna Karieva [σ]

Abstract- **Research is carried out on the development of technology for the dry extract of the choleretic collection "Triflos" by the method of mathematical planning of the experiment. In this case, the method of a four-factor experimental plan based on a 5x5 Greek-Latin square is used. The use of short-term ultrasonic exposure to intensify the extraction of target groups of biologically active substances is scientifically substantiated. The proposed technology is tested in industrial conditions. The dry extract yield was 21%.**

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I. Introduction

The Decree of the President of the Republic of Uzbekistan Shavkat Mirziyoyev "On measures to further improve the provision of the population with medicines and medical products" dated October 31, 2016 is an important factor in expanding the scope of work in the pharmaceutical industry. This document outlines important tasks for increasing the localization of pharmaceutical production by expanding the use of local raw materials, in particular, medicinal plants, and improving the delivery of quality medicines to the population at affordable prices.

One of the tasks provided for in the Decree of the President of the Republic of Uzbekistan dated April 10, 2020 No. 4668 “On additional measures for the development of traditional medicine in the Republic of Uzbekistan” and No. PP-4670 “On measures for the protection, cultural cultivation, processing of wild medicinal plants and rational use of available resources” is the organization of cultivation and harvesting of plants and raw materials of non-plant species used in traditional medicine, conducting laboratory and scientific research in this direction, increasing the export potential of the industry, as well as integrating education, science and production processes. In this regard, the Tashkent Pharmaceutical Institute is carrying out consistent work to ensure the implementation of the priority tasks outlined in the resolutions.

Author: Tashkent Pharmaceutical Institute.
e-mail: nargiza_24.10.1975@mail.ru

One of the promising directions in the field of drug development is the creation of phytopreparations for the treatment and prevention of these diseases. More active usage of phytopreparations, both alone and in combination with synthetic drugs, depending on the severity and nature of diseases, will contribute to the use of drug-saving technologies in clinical practice. Natural biologically active substances of plants are evolutionarily closer to the human body than synthetic ones, they are easily included in metabolic processes and have practically no side effects, and many of them are precursors of physiologically active substances (hormones, mediators). Recent studies have shown that the healing properties of medicinal plants depend on the harmonious interaction of all active substances, which together have a broader effect than individually.

In this regard, studies on the conversion of collections and individual plants used in the form of infusions and decoctions into total preparations-extracts are very promising, since the possibility of their wider use is limited by the imperfection of the dosage form [1,2].

Summarizing the above, we can conclude that the creation of medicines and dietary supplements based on medicinal plant materials using a resource-saving extraction method (due to the maximum depletion of raw materials) is timely and relevant and corresponds to the social order of clinical medicine.

Given the above, the Tashkent Pharmaceutical Institute has developed a choleretic collection “Triflos”, consisting of the following types of herbal medicinal raw materials: flowers of tansy false yarrow, flowers of chamomile, yarrow herb. To date, studies are carried out on its standardization, the establishment of quality standards, as well as the study of specific activity and safety. Cholagogue collection when tested on laboratory rats showed more than 50% increase in bile secretion [3]. The pharmacotherapeutic effects of this collection, which mainly consist in the regulation of phospholipid metabolism in the liver and the normalization of bile flow due to the antispasmodic effect on the sphincter of the gallbladder, according to the opinion prevailing in the literature, is determined by the presence of a number of

phenolcarboxylic acids and flavonoid compounds. So, in the composition of the collection components, along with essential oils, terpenoids, bitter and tannins, resins, organic acids and mineral salts, the presence of chlorogenic, rosmarinic, caffeic, ferulic acids and their corresponding glycosidic derivatives, as well as flavonoids: quercetin, luteolin, acacetin, apigenin and their glycosides. These literature data were confirmed by us experimentally [4].

The purpose of our research is to develop an optimal technology for obtaining a dry extract from this collection using the method of mathematical planning of the experiment.

II. Experiments and its Results

Currently, there are a large number of various extraction schemes designed to increase the yield of active substances, ensure maximum depletion of raw materials and enrichment of the extract with target biologically active substances (BAS). These extraction schemes also include factors that affect the completeness of extraction of biologically active substances, such as the nature and concentration of the extractant, the ratio of raw materials and extractant, temperature, type of extraction, etc. [5-18].

In order to facilitate the laborious process of finding the optimal conditions for extraction, we decided to apply the method of mathematical planning. Based on the characteristics of the technology for obtaining a dry extract, we chose a four-factor experimental plan based on a 5x5 Greek-Latin square. At the same time, the influence of the degree of grinding of raw materials (factor A), the concentration of ethyl alcohol (factor B), the hydromodulus (factor C) and the temperature regime (factor D) on the completeness of the yield of the sum of flavonoids in terms of quercetin in extraction is studied [19].

Factors and their levels are given in Table 1.

Table 1: Factors and their levels used in the experiment

Factor and its levels	Factor value	Factor and its levels	Factor value
The degree of grinding of raw materials (factor A)		*Ethyl alcohol concentration (factor B)*	
a_1	2-4 mm	b_1	50%
a_2	5-7 mm	b_2	60%
a_3	8-10 mm	b_3	70%
a_4	11-13 mm	b_4	80%
a_5	14-16 mm	b_5	90%
Hydromodulfactor (factor C)		*Temperature factor (D)*	
c_1	1:5	d_1	40^0C
c_2	1:10	d_2	50^0C
c_3	1:15	d_3	60^0C
c_4	1:20	d_4	70^0C
c_5	1:25	d_5	80^0C

The 5x5-experiment plan and the results of determining the quantitative content of biologically active substances (the sum of flavonoids in terms of quercetin) are presented in Table 2.

Table 2: The yield of the sum of flavonoids in terms of quercetin in the extract in a four-factor plan 5x5 with three repeated experiments, %

Factor A	Factor B					Totala_i
	b_1	b_2	b_3	b_4	b_5	
a_1	C_1d_1	c_2d_2	c_3d_3	c_4d_4	c_5d_5	
	1,22	2,15	2,14	1,36	1,04	
	1,28	2,09	2,17	1,41	1,09	
	1,21	2,19	2,11	1,33	0,99	
	3,71	6,43	6,42	4,1	3,12	23,78
a_2	c_2d_3	c_3d_4	c_4d_5	c_5d_1	c_1d_2	
	2,15	1,89	2,02	1,56	1,62	
	2,11	1,93	2,06	1,52	1,59	
	2,17	1,84	1,97	1,6	1,67	
	6,43	5,66	6,05	4,68	4,88	27,7
a_3	c_3d_5	c_4d_1	c_5d_2	c_1d_3	c_2d_4	
	1,3	1,55	1,76	1,67	1,62	
	1,34	1,61	1,71	1,74	1,67	
	1,26	1,52	1,8	1,66	1,59	
	3,9	4,68	5,27	5,07	4,88	23,8
a_4	c_4d_2	c_5d_3	c_1d_4	c_2d_5	c_3d_1	
	1,3	1,69	1,56	1,5	1,24	

		1,34	1,65	1,61	1,52	1,2	
		1,26	1,73	1,51	1,47	1,27	
		3,9	5,07	4,68	4,49	3,71	21,85
a_5		c_5d_4	c_1d_5	c_2d_1	c_3d_2	c_4d_3	
		1,01	1,24	1,76	1,37	1,57	
		1,09	1,29	1,73	1,41	1,59	
		1,02	1,18	1,77	1,32	1,52	
		3,12	3,71	5,26	4,1	4,68	20,87
Total	B_i	21,06	25,55	27,68	22,44	21,27	118
	C_k	22,05	27,49	23,79	23,41	21,26	
	D_l	22,04	24,58	27,67	22,44	21,27	

Before carrying out the analysis of variance, the homogeneity of the variance was checked using the Cochran test. The tabular value of the Cochran test for f1=2 and f=25 is 0.22, i.e., uexp, equal to 0.0709, is less than the tabular one, which confirms the equal accuracy of the experiments. Analysis of variance of the obtained results is presented in Table 3.

Table 3: Dispersion analysis of experimental data to determine the yield of the total flavonoids in the obtained extracts

Source of dispersion	Number of degrees of freedom (f)	Sum of squares (SS)	Mean squares (MS)	F_{expert}	$F_{0,05}$	Hypothesis
Factor A	4	2,245	0,56125	328,0885	2,56	a≠0
Factor B	4	1,82652	0,45663	266,931	2,56	b≠0
Factor C	4	1,538827	0,384707	224,887	2,56	c≠0
Factor D	4	1,782227	0,445557	260,4579	2,56	d≠0
Remainder	8	0,15216	0,01902	11,11847	2,13	res≠0
Errorinsidecell	50	0,085533	0,001711			
Totalamount	74	7,630267				

The obtained values Fexp>Ftabl, which indicates the statistical significance of all four studied factors. The value of Fres.in.cell indicates the presence of an interaction between the factors.

Using Duncan's multiple rank test, the differences in the average values of the data on the yield of the total flavonoids in the obtained extracts are studied. It is established that according to the influence of the degree of dispersion of plant raw materials on the response, they can be arranged in the following row: a1 = a2 = a3> a4> a5, i.e. the optimal degree of grinding of raw materials, providing the maximum yield of the sum of flavonoids, are 2-4 mm, 5-7 mm and 8-10 mm. However, taking into account the fact that the excessive dispersity of plant raw materials will ensure the release of not only biologically active, but also ballast substances, which, accordingly, will lead to a contaminated extract, we decided to use raw materials in further studies, the dimensions of which are in the range of 5-7 mm [20, 21].

The influence of the next studied factor - the concentration of ethanol used as an extractant, can be arranged in the following row: b2 = b3>b4>b5>b1, Thus, ethanol at a concentration of 60% and 70% is the optimal extractant. For the purpose of economic feasibility, our choice was stopped at 60% ethyl alcohol.

It is known that the ratio of vegetable raw materials and extractant has a significant impact on the yield of biologically active substances in the obtained extracts. This was confirmed by the results of our mathematical planning. It was found that almost the same output with a slight difference was obtained with a hydromodulus of 1:10, 1:15, 1:20. In order to save the extractant, we recommend the use of a hydromodule equal to 1:10.

The application of the Duncan rank criterion also helped to reveal the influence of the temperature factor on the yield of the sum of flavonoids, this series can be represented as follows: d2 = d3>d4>d1>d5, thus, the temperature of 500C was chosen as the optimal one.

An analysis of domestic and foreign literary sources indicates that the short-term use of ultrasound in the extraction of plant materials stimulates the release of biologically active substances [22, 23]. Given the above, we carried out extraction from the composition of medicinal raw materials, culminating in 10 minutes of ultrasonic exposure. At the same time, an increase in the yield of the total flavonoids in terms of quercetin is observed from 2.34% to 2.6% (1.11 times).

Thus, the proposed technology for obtaining a dry extract from the Triflos choleretic collection is tested under industrial conditions at BALZAM LLC.

The industrial extractor, on which the proposed technology was tested, consists of:

1) Main tank. This is the extraction tank, which is the main element of this system. It mainly serves to extract biologically active substances from medicinal plant materials;
2) A condenser, which serves to cool and return the condensed liquid to the extraction tank;
3) A vacuum condenser, which serves to concentrate and collect the resulting extract;
4) A tank for collecting the extractant (used as a collection) (Fig. 1).

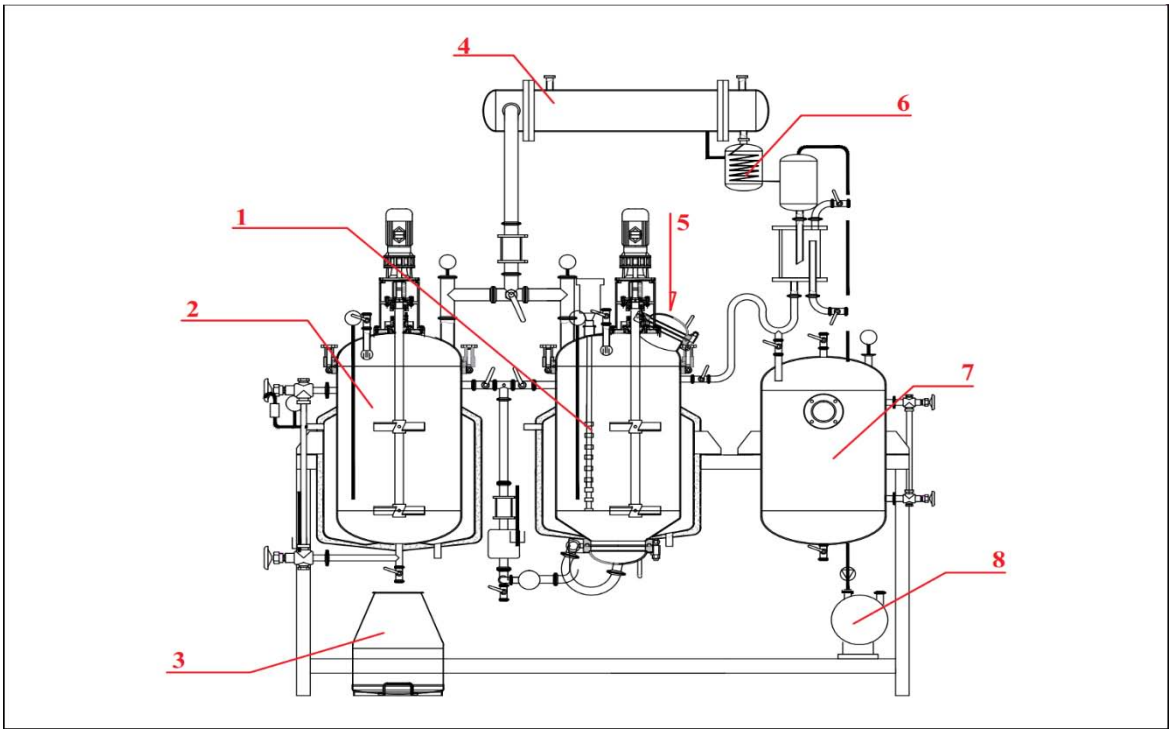

Fig.1: Scheme of the extractor "RUIAN XUANLI MASHINERY TANK". 1-extractor with built-in ultrasonic device; 2-vacuum concentrator; 3-collection; 4- capacitor; 5- loading hopper; 6- refrigerator; 7- tank for alcohol recovery; 8-vacuum pump

This equipment can carry out several operations simultaneously; control and regulation of the temperature regime of the extraction process, use ultrasound, create a vacuum, condense the extractant, recover alcohol, etc.

To obtain a dry extract, medicinal plant raw materials, crushed to a size of 5-7 mm, are weighed and mixed in the ratio: tansy flowers - 15 parts, chamomile flowers - 10 parts, yarrow herb - 10 parts. The mixture of vegetable raw materials was loaded into a special container and soaked with half the amount of 60% ethyl alcohol until a "mirror surface" is formed, left for 24 hours. After soaking, the soaked raw material is transferred to the extractor and 60% ethanol was added, bringing the raw material-extractant ratio to 1:10, the mixture was heated to 500C, and circulation extraction was carried out in the RuianXuanli Machinery Tank extractor. Next, ultrasonic extraction is performed for 10 minutes. After the vacuum extraction process is completed, the liquid extract is pumped through the filter into the reactor and left for 24 hours to settle. Purification of the distillation residue of the water-alcohol extract is carried out by filtration.

Then, the extractant was distilled off in the reactor using vacuum for 3 hours. The extract remaining after distillation of the extractant was spray dried in a high-speed spray dryer "LPG-15 Spray Dryer" (manufactured by RuianXuanli Machinery Co., LTD). (Fig. 2).

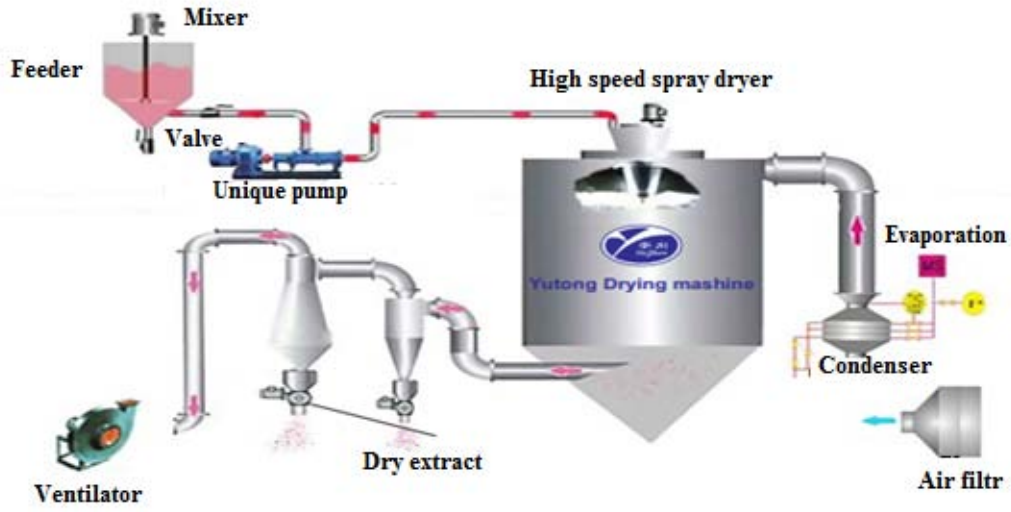

Fig. 2: Spray dryer "LPG-15 HIGH SPEED SPRAY DRYER"

As a result of the research, a light brown dry extract with a weak specific odor and a bitter taste is obtained. The dry extract yield is 21±1.53%.

III. Conclusions

1. Using the method of a four-factor experimental plan based on a 5x5 Greek-Latin square, the choice of the degree of grinding of raw materials, the concentration of ethanol, the ratio of raw materials and extractant, the temperature regime in the development of the technology of dry extract of the choleretic collection "Triflos" is carried out. The results of dispersion analysis shows that the most complete yield of the sum of flavonoids is observed with the following indicators of the above factors: dispersion of raw materials -5-7 mm, 60% ethyl alcohol, hydromodulus 1:10, temperature 500C.
2. The expediency of using ultrasonic treatment for a more complete recovery of target biologically active substances is scientifically substantiated.
3. This technology of circulating extraction using ultrasound is tested in industrial conditions. A dry extract is obtained, the yield of which was 21%.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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By María Fernanda Modarelli, Rodrigo Miguel Bilbao & Osvaldo Juan Ponzo

University of Buenos Aires

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Objectives: To assess the thyroid disrupting action of groundwater contaminated with nitrates and arsenic, by means of a bioassay of chronic toxicity in Xenopus laevis larvae.

Methods: Three experimental groups immersed in water: Control group (C) (n=13) filtered drinking water, Exposed group (E) groundwater (n=18) and Positive Control group (PC) (n=18) filtered drinking water added with 0,007 mg/l of potassium perchlorate. A water physicochemical analysis was performed. The duration of metamorphosis stages, total body length, mortality per group, weight and height were morphologically evaluated. The colloid volume, degrees of hyperplasia, and height of the follicular epithelium of the thyroid gland were histologically evaluated. At molecular level, NIS thyroid symporter protein expression was measured.

Keywords: *endocrine disruptors - xenopus - groundwater - nitrates – thyroid.*

GJMR-B Classification: *DDC Code: 174.95 LCC Code: Q175.35*

Strictly as per the compliance and regulations of:

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Results: The groundwater physicochemical analysis showed the presence of nitrates (values between 24 and 83 mg/l) and arsenic (0.05 mg/l). Prometamorphosis was longer in group E Vs C ($p<0.0001$). In PC only three animals completed this stage ($p<0.0001$). Weight was in increasing order E<PC<C and height E<PC<C ($p<0.05$). Mortality recorded per group was: 10% in group E exclusively ($p<0.0001$). Changes could be noticed in the thyroid glandular histoarchitecture at stage 58NF: hyperplasia grade 1 in C, grade 2 in E and PC ($p<0.0001$). The colloid area and the height of the follicular epithelium were in increasing order PC<C<E ($p<0.0001$). The level of expression in the larval thyroid tissue of NIS symporter was in increasing order C<PC<E ($p<0.0001$).

Discussion: Changes observed in the thyroid gland, as well as the morphological alterations, of *Xenopus laevis* larval development at stage 58NF, could be related to the presence of nitrates and arsenic in the groundwater which cause a synergic disruptive action on the thyroid.

Keywords: endocrine disruptors - xenopus - groundwater - nitrates – thyroid.

Author α σ: Endocrinology Laboratory, Institute of Physiology, Academic Unit II, School of Medicine, University of Buenos Aires. Buenos Aires. Argentina.

Corresponding Author ρ: Endocrinology Laboratory, Institute of Physiology, Academic Unit II, School of Medicine, University of Buenos Aires. Buenos Aires. Argentina. e-mail: oponzo@fmed.uba.ar

I. Introduction

Endocrine disruptors (EDs) affect the normal function of the endocrine system, by interfering with the synthesis, storage, transport, circulating levels, peripheral action and catabolism of hormones. Thyroid disruptors (TDs) are a group of chemical substances that affect the hypothalamus-pituitary-thyroid (HPT) axis in different ways, for example through their capacity to decrease the circulating levels of thyroid hormones (Brucker-Davis F. 1998), or acting directly on their receptors as well as on the enzyme or plasmatic carriers which play a significant role in the mediation of its action (Howdeshell KL. 2022) in humans as well as in animals (Colborn T. et al. 1993).

Contaminated groundwater could vehicle different EDs as nitrates, perchlorates and thiocynates, among others (Zewdie T. et al 2010). Endemic areas of hypothyroidism and goiter with no iodine deficit have been described, being a probable cause the presence of EDs in the water drank by the population living in those areas (Andrada I. et al. 2009).

The correct thyroid function involves a proper activity of the sodium/iodide symporter (NIS) at thyroid follicular cells. In mammals and amphibians, thyrotropin (TSH) stimulates NIS expression being involved in this transcription factors as PAX-8, TFF-1 and TTF-2 (Dohan O. et al. 2003). The NIS symporter inhibition interferes with iodine uptake, decreasing the synthesis of triiodothyronine (T_3) and thyroxine (T_4) which results in a TSH increase. Consequently, a higher stimulation in an attempt to compensate the hormone synthesis, leads to the development of goiter (Crofton K. et al. 2005).

During the spontaneous amphibian metamorphosis, the NIS mRNA expression is low in pre-metamorphous tadpoles and it increases throughout prometamorphosis, as the same time as the increase of mRNA expression of TSH beta subunit (TSHb) at tadpole hypophysis, thus suggesting a TSH regulation in the NIS expression (Opitz R. et al. 2006). Moreover, thyroid hormones play a fundamental role in amphibian and on human fetus development (Zoeller RT. et al. 2004).

Amphibian larvae are used as bioassays for being highly sensitive to the action of different substances present in water, even in LOAEL (Lowest Observed Adverse Effect Level) and NOAEL (No Observed Adverse Effect) concentrations (FETAX.

2000). The metamorphosis of these animals is a process that depends on thyroid hormones, being this influence higher during the prometamorphosis and climax stages (Tietge JE. et al. 2005). In a short period of time larvae suffer structural, physiological, biochemical and behavioral transformations due to T_4 action and to the conversion of T_4 into T_3 in the target organs. These alterations are accompanied by changes in thyroid gland volume, height of follicular epithelium, colloid reabsorption level and iodine uptake. These processes are regulated by T_4 which secretion increases at prometamorphosis onset and continues rising up to the end of metamorphosis (Miranda LA. 1995), to achieve tail resorption. In the case of *Xenopus laevis* the thyroid gland becomes operational at prometamorphosis onset (Nieuwkoop PD et al. 1967), similar to what happens with other anurans (Saxen L. et al. 1957).

Disorders which involve iodine transport and lead to a change in the thyroid hormone synthesis, may cause changes in the growth and metabolism during amphibian metamorphosis, as well as in humans (Shi YB. et al. 1996). Because of this, *Xenopus laevis* larvae can be used as a biologic model to study *"in vivo"* the biological effect of endocrine disruptors (EDs), by evaluating the morphological and functional changes normally induced by thyroid hormones (THs).

This study proposes that the immersion of *Xenopus laevis* larvae "*in vivo*" in groundwater could cause morphological, histological and biomolecular changes which are the result of the presence of endocrine disruptors (EDs) in such water under study (Modarelli MF. and Ponzo OJ. 2018).

II. Methods

We experimented with *Xenopus laevis* larvae from the Endocrinology Laboratory of the Institute of Physiology, School of Medicine, University of Buenos Aires. Larvae used for this experiment were obtained after amplexus (of adult specimens), of only one spawn. Samples from healthy specimens with no malformations and with a homogenous size were selected in accordance with ANFICOR guidelines (Herkovits J. et al. 1999). Selected specimens were placed in transparent containers; one larva by each 500 cm^3 of water, held in stable conditions on a 12 h light: 12 h darkness photoperiod, temperature: 22° C ± 2° C and pH: 7.2 to 8, in filtered drinking water with extraction of chlorine by carbon filter, and were fed ad libitum with balanced feeds (Sera Micron). To reduce specimen stress, the same person changed the water and controlled larvae each 48 h. The protocol was approved by institutional animal care and use committee (CICUAL/UBA: 0003598/2013. Res. 700).

a) Experimental Design

Larvae were divided into 3 treatment groups: a) Control group (C) (n=13) immersed in filtered drinking water; b) Exposed group (E): immersed in 30-meter-depth well groundwater from the southern suburbs of Buenos Aires, Argentina (n=18); Positive Control group (PC): immersed in filtered drinking water added with 0,007 mg/l potassium perchlorate (KClO4) as NIS-inhibiting thyroid disruptor (n=18). All animals underwent 70-day-period treatment. Partial study cuts were made at premetamorphosis, prometamorphosis and climax stages, using Niewkoop and Faber (NF) criteria (Nieuwkoop PD et al. 1967) to determine those different stages. Mortality per group was recorded and morphological changes in larvae such as: total time of metamorphosis, time of premetamorphosis, prometamorphosis, climax, weight and height (Organization for economic Co-Operation and Development. (OECD). 2004) were analyzed. Moreover, thyroid gland histological changes were evaluated like colloid area, height of follicular cell, number of follicles per field, hyperplasia and hypertrophy (Grim C. 2007). Finally, NIS symporter protein expression in the thyroid tissue was studied using Western Blot technique.

b) Histological Technique

Larvae were sacrificed by immersion in MS222 (200mg/l) solution for later histological and biomolecular evaluation. For histological analysis after specimen sacrifice, tissues were fixed in Bouin solution during 24 h and then subjected to a dehydration process with successive passages of 15 min each in increasing alcohol concentrations (70%, 96%, 90%, 100% and Xilol) to be finally embedded in paraffin blocks for staining. The histological slices were 5-micron-thick and dewaxed with Xilol for 15 min, to be later rehydrated by successive passages of 10 min each in decreasing alcohol concentrations (100%, 96%, 90%, 70%). For staining it was used the Hematoxylin and eosin technique.

c) Western Blot

After thyroid extraction by removing lower jaw and a small part of the hyoid bone, samples were homogenized by sonication under refrigeration in lysis buffer (Tris 1,514 g, SDS 6 g and 2 beta mercaptoethanol 5 ml for 200 ml, pH 6.8) and Protease Inhibitor Cocktail (Pierce Biotechnology Inc., Massachusetts, USA) in a ratio of 10 µL per 1 ml of tissue. The supernatant solution was placed at 100°C (boiled in water) for 5 min and then centrifuged at 1600 rpm. The sample protein quantity was measured with Bradford method. Then it was carried out an SDS-PAGE in 12% polyacrylamide gel under denaturing conditions, in an electrophoresis cell (BioRad Mini Protean 3 Cell) for 90 min at 120 volts with transfer buffer (Tris 25mM; glycine 0.2 M; SDS 0.1% and pH 8.3). The loading volume per each sample was 10 to 20 µl. The volume

was decided in relation with the protein quantity present in each sample. Each sample was diluted in loading buffer in a ratio of 1:2 (Tris-HCl 0.065 M, SDS 3%, bromophenol blue 0.1%, β-mercaptoethanol 5% and 10% glycerol, pH 6.8). Beta actin was used as loading control. Afterwards humid electro transference and immunoblotting were performed. For the electroblotting (electrotransfer) a Polyvinylidene difluoride (PVDF) membrane (Amersham, UK) and a cell with transfer buffer were used (25 mM Tris, 192 mM glycine and 20% methanol) for 1 h at 100 mv. Subsequently, three washes were carried out of 5 min each with TBS 1X (TrisHCl 20 mM, NaCl 150 mM pH 7.8) and it was blocked with a TBS solution with Tween-20 0.2% (TBS-T) and 5% p/v of skim milk (Svelty) for 1 h at room temperature (shaking). The membrane was incubated with rabbit polyclonal primary Anti COOH-terminus NIS antibody (Millipore Corp. USA, CAT #ABC1453) at a 1:500 dilution overnight at 4°C and subsequent three washes with TBS-T and one with TBS of 5 min each. After blocking the membrane during 30 to 40 min and subsequent washes with TBS-T of 5 min each, it was incubated for 1 h at room temperature (shaking) with secondary antibody conjugated with peroxidase 1:1.500 of mouse anti-rabbit polyclonal. Finally, in order to perform detection by chemiluminescence three washes with TBS-T and one with TBS of 7 min each were carried out. After the last wash the membrane was incubated for 5 min with the reagent for enhanced chemiluminescence (ECL) (Biorad, cat #170-5060 USA) and it was exposed to X-ray plate (Kodak and GE) during 1 to 5 min and further plate development in darkroom. The developed signal was quantified with software for image analysis Scion Image Version beta 4.0.2.

d) Water analysis

A groundwater sampling from wells of different depth (30 to 60 meter-depth) located in the studied area (Pampeano aquifer) was carried out. The different types of analyzed water (groundwater and filtered drinking water) used in the experiments were storage and transported, sealed and refrigerated to the place where the experiments were performed in new plastic bottles of mineral water emptied and later rinsed with the collected water. Then, the bottles were filled up to the total capacity, no air gap between the lid and the content, and transported refrigerated to the place of processing. All water samples underwent a microbiologic and physiochemical analysis at the National Institute of Industrial Technology (INTI-Parque Tecnológico Miguelete, Argentina).

To determine the presence of nitrates it was used ion chromatography technique by Metrohm´s 881 Compact, column Metrosep A 150/4mm, with carbonate /bicarbonate eluent, chemical suppression with conductivity detection, and calibration by peak area. For other analyzed parameter APHA-methods 2340 were used according to the standard analysis of water for human consumption (Standard Methods for de Examination of Water and Washwater. 1995). Reference values for water for human consumption are those of the Argentine Food Regulations. The term Undetectable was used for concentrations below the detection limit (DL) of the method of analysis.

e) Statistical analysis

Parametric one-way ANOVA tests were performed for the statistical analysis regarding morphology, histology and biomolecular parameters. The normal distribution was verified by means of Kolmogorov-Smirnov and Bartlett tests, and Tukey and Bonferroni post tests were carried out for the analysis of differences. A non-parametric ANOVA with Kruskal-Wallis and Dunn tests were performed for those small and asymmetric samples. For the qualitative variable analysis reflected in the contingency tables as a percentage, it was used as statistical test the Exact Fisher Test and the Katz´s numerical approximation to evaluate the relative risk. In all cases it was considered as significant $p<0.05$ with 95% confidence intervals (CI), and in each case it was determined the interval, the average, the standard deviation; and for the percentage analysis it was used the relative risk. For that purpose, statistical GraphPad Software (Inc. San Diego, California USA, www.graphpad.com) and Infostat (statistical program, digital version 2015. www.infostat.com.) were used.

III. Results

a) Physicochemical analysis results of studied area water

Regarding the nitrate analysis, Sample N°1 (from 60-meter-depth wells) showed a value of 24 mg/l, exceeding EPA regulations as safety limit for water for human consumption (EPA, 2016) and Sample N°2 (from 30-meter-depth wells) showed a concentration of 83 mg/l exceeding the maximum limit of safety for water for human consumption (CAA, 2021) established in the Argentine Food Regulations (Table 1). These results should be analyzed in connection with the rest of the determinations, as nitrates may be acting *per se* or in synergy with other components, generating the thyroid disruptive effect observed in the population of those areas.

The samples from well groundwater (1 and 2) showed a higher conductivity (Table 1) which indicates an increase in salinity, probably generated by the presence of septic tanks in the vicinity (between 1 and 3 meters) of sampling areas and for the possible existence of industries near the studied area which dump liquids such as cleaning water. On the other hand, the total hardness of Sample N°3, being over 330 mg/l, is highly superior to the others, so those may be considered hard waters. This fact could favor the

reactivity of substances with possible disruptive action (De Groef et al. 2006).

High levels of arsenic, above safety level for human health by the Argentine Food Regulations (up to 0.01 mg/l), were observed in the samples of groundwater as well as in purified bottled water drunk by the population living in the area of the study (Table 2).

b) Morphological changes

i. *Total time of metamorphosis. Weight and Height*

Regarding the number of animals which completed metamorphosis in all before mentioned three groups, a very significant difference was observed among the groups. Larvae immerse in filtered drinking water (C) completed metamorphosis in a 100% but just a 38% for those animals in the group exposed to groundwater (E), and none in the positive control group (PC) (filtered drinking water with $KLClO_4$) ($p<0.001$). Larvae growth delay was particularly observed in the transition from stage 58NF to 60NF ($p<0.01$). This delay was too evident and progressive in group PC from stage 54NF ($p<0.0001$), with a 95% confidence interval (CI) (0.6368 - 0.7923) and a relative risk that tends to infinity for the relation C Vs E. And for E vs PC the CI was 95% (0.2077 - 0.3632).

Total time of metamorphosis in group C was 56 ± 1.95 days, and 67 ± 2.01 days ($p<0.01$) in group E. In PC this time could not be determined due to the fact that larvae reached metamorphosis stage 62NF but none of them reached stage 66NF, time when the metamorphosis process is completed. This happened due to the addition of a constant dose of potassium perchlorate (0,007 mg/ml), which caused a total stop of metamorphosis at stage 62NF (Fig. 1). Mortality per group was 10% in group E larvae exclusively ($p<0.001$).

The stage affected was prometamorphosis which is controlled by T_4. It was observed a slower larval growth in group E represented by a delay in the transition from one stage to the following during the prometamorphosis process. This difference was significant in groups E and PC *vs* group C during transition from stage 58NF to 60NF and it was noticed a significant larval growth delay in group E *Vs* C during the transition from stage 54NF to 60NF ($p<0.01$) with 0.69 relative risk (RR). The delay was even more pronounced in PC group larvae vs C, ($p<0.002$), with a 0.46 RR. The weight at stage 58NF was significantly lower in group E Vs C and PC ($p<0.05$), with a 95% confidence interval (CI) (54.157 - 364.69) (Fig. 2). It was noticed a significant difference in height, but this difference was smaller in group E and PC *vs* C ($p<0.05$), with a 95% confidence interval (CI) (8.667 - 5.448: E *vs* C, -6.750 - 4.619: PC *vs* C). No significant difference was observed between groups C and PC (Fig. 2).

From the above analyzed we conclude that the delay occurs in the transition from stage 54NF to 60NF in group E and PC *vs* C, with a larval growth delay in groups E and PC. In group E this delay is evidenced by a decrease in the final size of animals, which achieved less weight and height than the ones in the other two groups (C and PC) (Picture 1).

c) Histological changes

i. *Follicular colloidal area*

During 58NF stage metamorphosis it was observed an increase in the colloid area size in group E Vs C and PC ($p<0.05$) with a 95% CI (E vs C: -9184.7 - 179.62 and E *vs* PC: 2252.2 - 9253) (Fig. 3) ($p<0.001$). On the other hand, the colloidal area was smaller in group C *vs* PC ($p>0.001$) with a 95% CI (-2336.2 - 4.477) (Pic.2).

ii. *Glandular hyperplasia degree*

Thyroid gland hyperplasia during stage 58NF was degree 1 in group C, degree 2 in groups E and PC. The differences observed in group C *Vs* E were highly significant ($p<0.0001$), with a 95% CI (0.3680 - 0.6860) and 0.33 RR. There were also significant differences between groups C and PC ($p<0.002$) with 95% CI (0.2708 - 0.4799). No significant differences were observed between groups E and PC (Fig. 4).

iii. *Number of filled follicles*

The number of filled and empty follicles per gland was also significantly different being the number of filled follicles higher in group E *Vs* C and PC ($p<0.02$) with 95% CI (0.4906 - 0.8303) and 0.66 RR. Nevertheless, there were no significant differences between C and PC groups (Fig. 5).

iv. *Height of follicular epithelium*

The height of the follicular epithelium showed significant differences in groups C *vs* E ($p<0.0001$) with a 95% IC (-181.22 - 96.084) and in groups E *Vs* PC ($p<0.0001$) with a 95% CI (130.56-202.21). In increasing order, the height was less in group PC than in C and in group C than in E (Pic. 3). The average height of the follicular epithelium for each group was: C: 166.23 ± 43.23; E: 284.02 ± 68.12; PC: 128.64 ± 35.69 um (Fig. 6).

d) Molecular changes

i. *NIS expression analysis*

When data logarithmic correction was made it showed that NIS protein expression increases in groups E and PC being this a significant difference ($p<0.05$) (Fig. 7). The average in increasing order was E > PC > C. Registered values measured in optical density were: C: 680.7 ± 196.92, E: 1251.02 ± 702.94, PC: 1059 ± 592.85 (Fig. 6).

IV. Discussion

Endemic regions of hypothyroidism and goiter without iodine deficiency have been described, suggesting the consumption of EDs by the population living in these areas as a predisposing factor. (Blount

BC. et al. 2006). Our study, carried out in the southern suburbs of Buenos Aires, found levels of nitrates and arsenic in the groundwater above the safety limit for human consumption. Furthermore, in studies made in nearby rivers, other pollutants with endocrine disruptive action were detected, among them: lead, chrome, hydrocarbon and polychlorinated biphenyls (PCBs) (Janiot L. 2000). These elements may contaminate the groundwater layers, specially the superficial ones by runoff from water tables in low-gradient streams. In the area of study, the water to drink or to irrigate is obtained from these water layers, being the Pampeano and Puelche aquifers the sources from where most of the population gets their water. The superficial Pampeano aquifer is free and often contaminates the deep Puelche aquifer which is semi confined. The last one represents one of the drinking water reservoirs most important of Argentina and Latin America (Adema MP. 2017 and Ingeniería Geotécnica y Ambiental. 2005).

The action of thyroid endocrine disruptors (TEDs) may alter the synthesis, storage, transport and catabolism of hormone homeostasis (Colborn T. et al 1993) and may decrease the production of thyroid hormones (Kleiman DL. et al. 1989) by acting on membrane transporters such as NIS. In the case of nitrates, the inhibition of the sodium-iodide symporter (NIS) interferes with iodine uptake at thyrocyte level, first step in thyroid hormone synthesis (De Groef B. et al 2006). This leads to hypothyroxinemia with the following increase of TSH (Manzon RG. et al. 2004), which induces cell proliferation as an adaptive response. Thus, generating an increase in gland size and changes in gland histoarchitecture, which in humans may cause goiter development (Brauer VF. et al. 2006).

The decrease in thyroid hormone levels affects human development, as well as it does in larvae metamorphosis. In this study we have shown a delay in the development, and changes in the body morphology during larvae metamorphosis of *Xenopus laevis* exposed to contaminated water, causing caused by thyroid disruption. We have demonstrated a longer period of prometamorphosis and smaller weight and height of specimens at stage 58NF. These differences could be explained by the need to reach a metabolic threshold, which allows them to complete the morphological changes of this stage. This could be determined by the acquisition of an adequate level of thyroid hormones. In case that this does not happen, it can cause a stop in larval development. This fact has been observed in other amphibians and urodele, which develops a state called *neoteny* (Galton VA. 1992), determined by a complete brake on the metamorphic process in adverse environmental situations. This fact is similar to the one observed in our experimental Positive Control (PC) group, in which larvae were exposed to a constant dose (0.007 mg/l) of potassium perchlorate (known as a NIS inhibitor), suffering a complete stop of its metamorphic development; therefore, no larvae completed the metamorphosis process.

The observed differences could be explained by the negative feed-back made by the T_3 and T_4 at tadpole hypophysis level which is operational at prometamorphosis onset. Thyroid hormones may negatively regulate the mRNA expression for the TSH synthesis during metamorphosis. The mRNA expression for the thyroid hormone receptor increases during the larval development throughout prometamorphosis and peaks at climax (Opitz R. et al. 2006). The presence in water of NIS inhibitors, such as nitrates, could be interfering with the proper production of thyroid hormones in larvae.

We have known for years that *Xenopus laevis* are extremely sensitive to water soluble substances as nitrates and perchlorates, even in low concentration, due to their aquatic life. For this reason, *Xenopus laevis* was chosen as experimental model in this work (Kloas W. 2002).

Histologically, the follicles constitute the anatomical functional unit of the thyroid gland in amphibians and in humans. Its follicular epithelium and the colloid constituted by thyroglobulin change their histological appearance depending on the secretory phase. These events may be altered by thyroid disruptors, being the histologic changes a sensitive parameter to determine the level of action of this disruptor (Wolff J. 1998). Our analysis showed a change in gland histoarchitecture like hyperplasia and hypertrophy of the follicular epithelium and an increase of the colloid volume in the thyroid gland follicles in prometamorphic larvae.

The thyrocyte uptakes iodine against gradient by the sodium-iodide symporter (NIS) located at the basement membrane, with energy expenditure. This transporter is inhibited by nitrates and other disruptors. The NIS expression is stimulated by the TSH, which involves the regulation of transcription factor as TTF1, TTF2 and PAX8 (Rivolta CM. et al. 2005).

The inhibitory action of thyroid disruptors on the NIS co-transporter and the changes on the metamorphosis (Furlow JD. et al. 2006 and Degitz S. et al 2006), as well as histological and biomolecular thyroid changes, have been assessed (Hood A. et al. 1999; Below H. et al 2008 and Mukhopadhyay S. et al. 2005). The increase in the NIS protein expression level noticed during larvae prometamorphosis exposed to nitrate contaminated water, could be the result of an adaptive mechanism trying to compensate its functional state.

Differences observed in our study in larval morphology as well as in glandular histoarchitecture during the different stages of Xenopus laevis metamorphosis between E and C groups may be explained by the presence of one or more substances with a thyroid disruptive action in groundwater of the studied area. These substances could be interacting in

a synergetic way on more than one level on the thyroid gland. This could explain what happens with arsenic. The arsenic, as the nitrates, was detected in concentrations considered as unfit for human consumption by the Argentine Food Regulations. The chronic exposure to an excess of arsenic in drinking water has been strongly linked to higher risk in humans. Arsenic has been shown to be a powerful endocrine disruptor in low levels, changing the genic regulation mediated by thyroid receptors (Davey JC. 2008). The synergistic action of nitrates and arsenic could explain the mortality observed exclusively in this group.

V. Conclusion

The nitrates present in groundwater, as well as other possible endocrine disruptors such as arsenic, produce morphological alteration in the *Xenopus laevis* tadpoles, as well as histological and molecular thyroid changes when exposed to this type of water during their metamorphosis. These events are related to an increase of the NIS expression levels during prometamorphosis stage. Despite this adaptive change, it is not possible to compensate for the thyroid alteration generated by nitrates, thus not achieving the morphological changes necessary to adequately complete this stage. New studies must be carried out to better understand the mechanisms that lead to these alterations.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by a Grant of the University of Buenos Aires (UBACYT 20020130 100439BA).

We are grateful to: Dr. J. J. Lopez and his team (Department of Histology, School of Medicine, University of Buenos Aires) for his contribution in the histological analysis.

We thank Angela Ciocca Ortúzar for the manuscript revision.

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Table 1: Physicochemical analysis of groundwater (Pampean Aquifer, Buenos Aires, Argentina) sampling

SAMPLE 1 Well depth: 60 meters	COLOUR	Greenish [(a)]
	ODOR	Odorless
	SEDIMENT	Null
	pH	7.5
	RESIDUAL ACTIVE CHLORINE	0,00 ppm
	CONDUCTIVITY	729 micros/cm [(a)]
	TOTAL DISSOLVED SOLIDS (TDS)	525 mg/l
	TOTAL ALKALINITY (CO_3Ca)	312 mg/l
	TOTAL HARDNESS (CO_3CA)	114 mg/l
	CHLORINES (CL)	36 mg/l
	SULPHATES (SO_4^{2})	20 mg/l
	AMMONIA (NH_4^{+})	Undetectable
	NITRITES (NO_2):	0.01 mg/l
	NITRATES (NO_3):	24 mg/l*
	CHROMIUM (Cr^{+6}):	Undetectable

DRINKING WATER (Extraction of chlorine by filter)	COLOUR	Colorless
	ODOR	Odorless
	SEDIMENT	Plentiful
	pH	7.6
	RESIDUAL ACTIVE CHLORINE	0,00 ppm
	CONDUCTIVITY	685 micros/cm
	TOTAL DISSOLVED SOLIDS	493 mg/l
	TOTAL ALKALINITY (CO_3Ca)	351 mg/l
	TOTAL HARDNESS (CO_3CA)	80 mg/l
	SEDIMENT	18 mg/l
	SULPHATES (SO_4^{2})	20 mg/l
	AMMONIUM(NH_4+)	Undetectable
	NITRITES (NO_2):	Undetectable
	NITRATES (NO_3):	< 5 mg/l
	CHROMIUM (Cr^{+6}):	Undetectable

SAMPLE 2 Well depth: 30 meters	COLOUR	Colorless
	ODOR	Odorless
	SEDIMENT	Barely detectable
	pH	7.3
	RESIDUAL ACTIVE CHLORINE	0,00 ppm
	CONDUCTIVITY	982 micros/cm [(a)]
	TOTAL DISSOLVED SOLIDS	707 mg/l
	TOTAL ALKALINITY (CO_3Ca)	409 mg/l
	TOTAL HARDNESS (CO_3CA)	336 mg/l [(a)]
	CHLORINES (CL)	87 mg/l
	SULPHATES (SO_4^{2})	25 mg/l
	AMMONIA (NH_4^{+})	Undetectable
	NITRITES (NO_2):	0.01 mg/l
	NITRATES (NO_3):	83 mg/l *
	CHROMIUM (Cr^{+6}):	Undetectable
SAMPLE 3 Drinking water (can)	COLOUR	Colorless
	ODOR	Odorless
	SEDIMENT	Plentiful
	pH	7.6
	RESIDUAL ACTIVE CHLORINE	0,00 ppm
	CONDUCTIVITY	395 micros/cm [(a)]
	TOTAL DISSOLVED SOLIDS	284 mg/l
	TOTAL ALKALINITY (CO_3Ca)	175 mg/l
	TOTAL HARDNESS (CO_3CA)	112 mg/l [(a)]
	CHLORINES (CL)	29 mg/l
	SULPHATES (SO_4^{2})	20 mg/l
	AMMONIA (NH_4^{+})	Undetectable
	NITRITES (NO_2):	Undetectable
	NITRATES (NO_3):	< 5 mg/l
	CHROMIUM (Cr^{+6}):	Undetectable

**On or exceeding the limits for safety values.[(a)] Differences in values between drinking water and purified bottled water*

Table 2: Levels of arsenic and others elements.

DETERMINATION TYPE	PURIFIED BOTTLED WATER	GROUNDWATER
TOTAL HARDNESS (CO_3Ca)	57 ± 2.0 %	64 ± 20 %
CALCIUM	12.5 ± 2.5 %	13.5 ± 2.5 %
MAGNESIUM	6.4 ± 3.0 %	7.2 ± 3.0 %
ARSENIC	**0.02-0.07 mg/l**	**0.05 mg/l**
MAGNESIUM	< 0,05	< 0,05
SODIUM+POTASSIUM	132	183

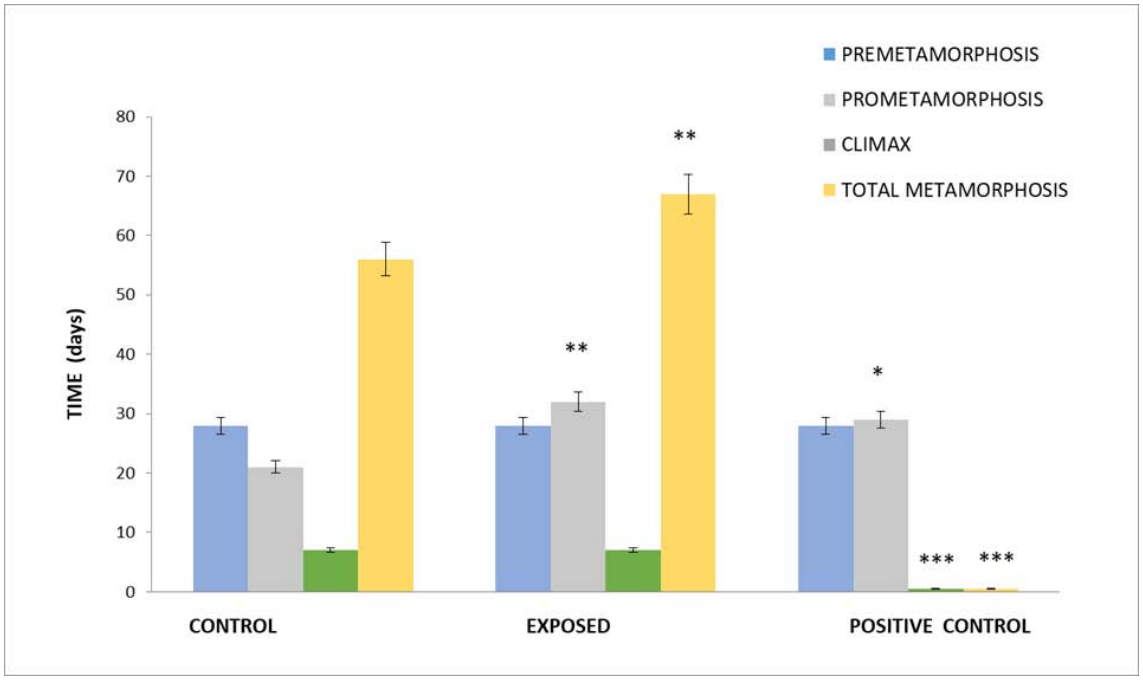


Figure 1: Metamorphosis periods (premetamorphosis, prometamorphosis and climax) per group and water type of *X. laevis* larvae under Control treatment: C, Exposed: E, and Positive Control: PC. *p<0.05, **p<0.001, ***p<0.0001 vs. Control

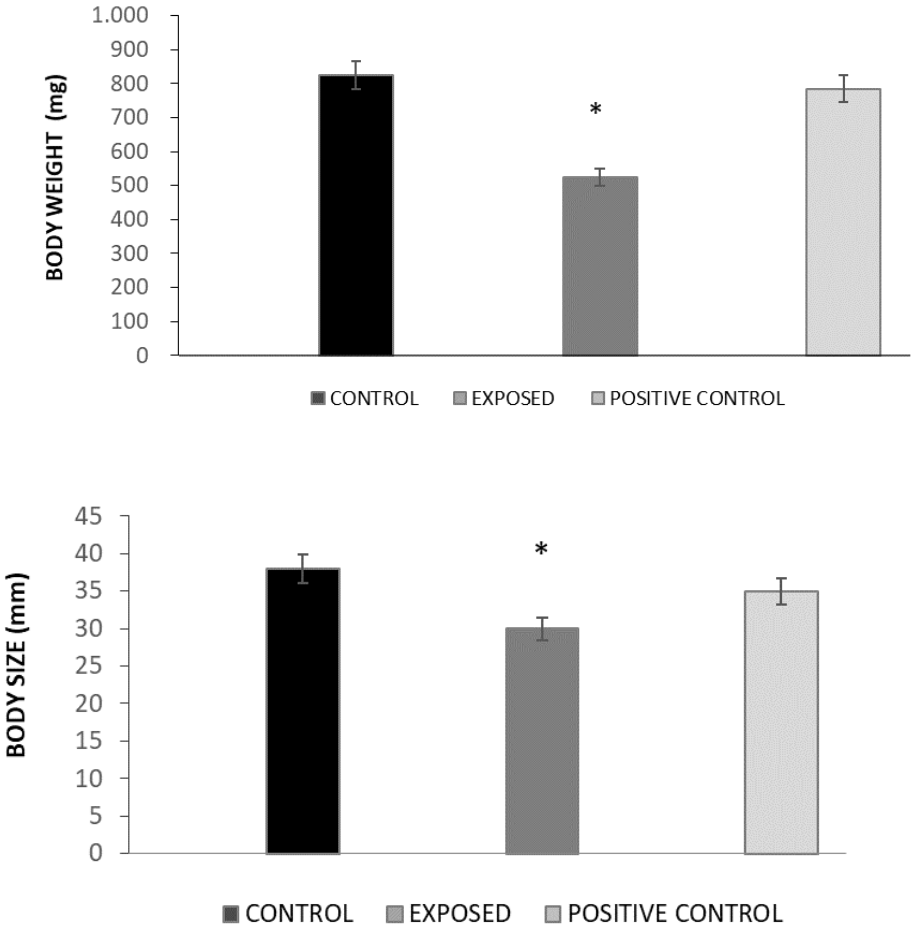
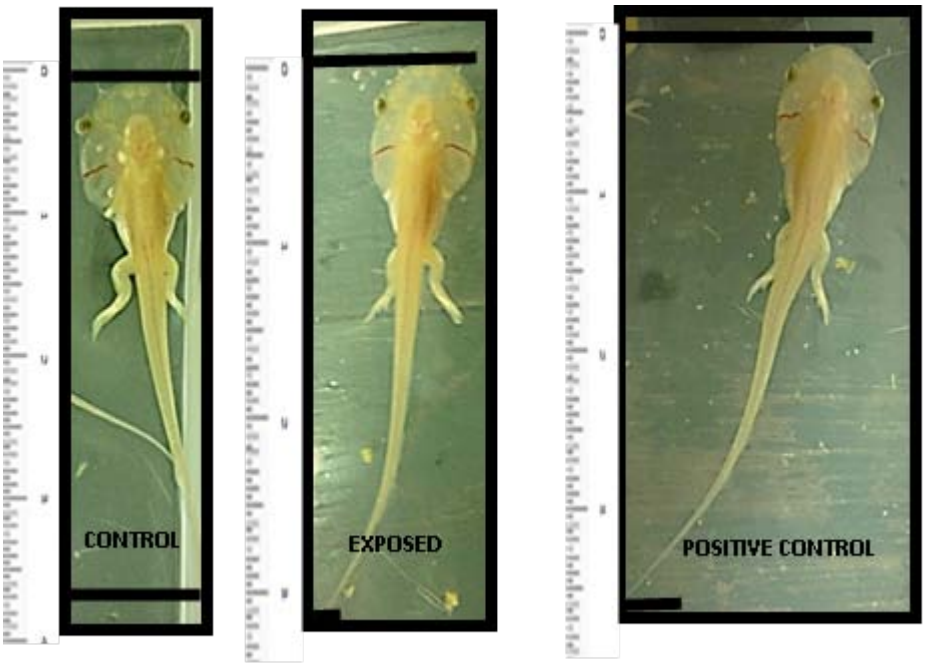


Figure 2: Xenopus laevis larvae body weight and size at stage 58NF. *p<0.05 vs Control.



Picture 1: Larvae morphological development change at stage 58NF (prometamorphosis), showing a delay in the Exposed groups and Positive Control Vs Control.

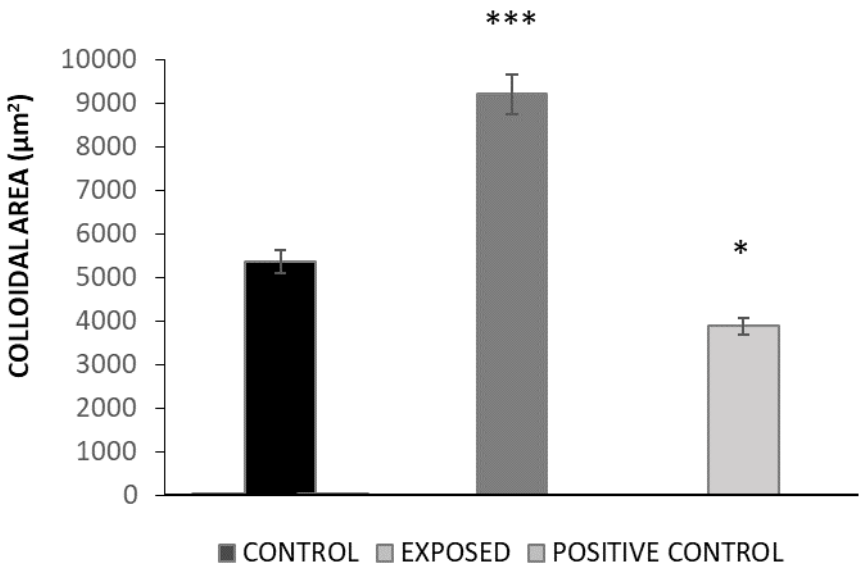
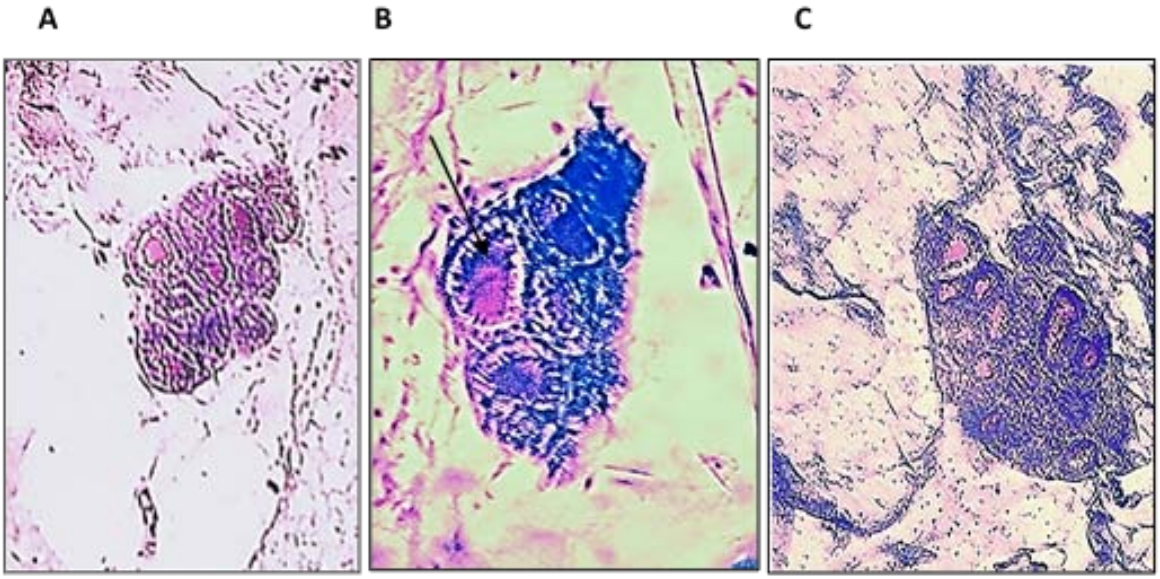


Figure 3: Thyroid gland colloidal area at stage **58NF**. *p<0.05, ***p<0.0001 vs. Control.



Picture 2: Optical microscopy (10x) of the follicular colloid area in Xenopus laevis thyroid glands at stage **58NF**: A) Control, B) Exposed: where a bigger size gland and an increase in the colloid area can be observed, C) Positive Control.

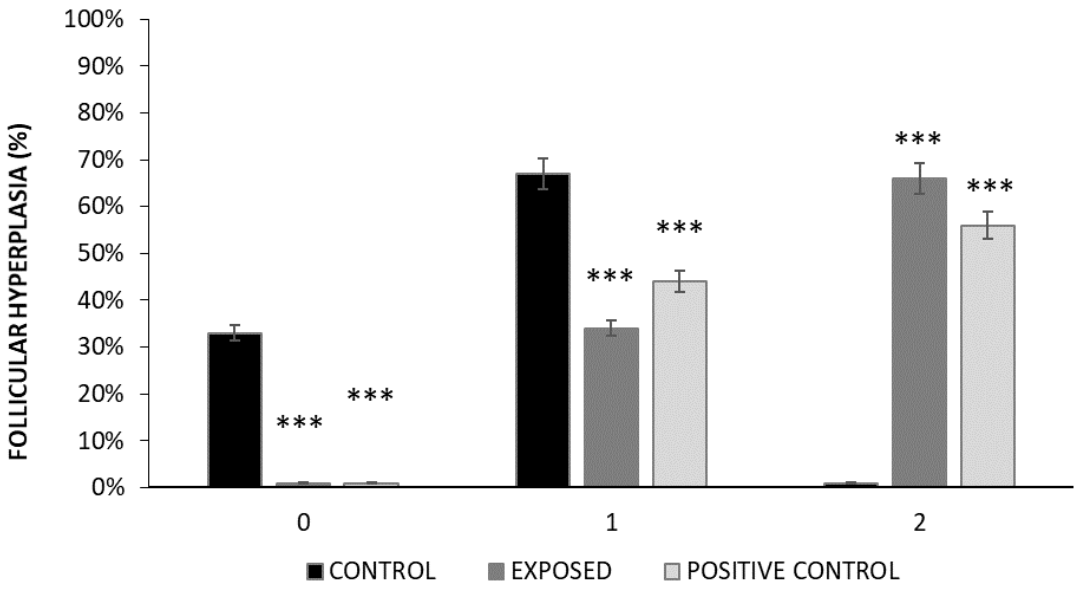


Figure 4: Percentage of follicular hyperplasia degrees (0, 1, 2) in each experimental group during stage **58NF**. ***p<0.0001 vs Control.

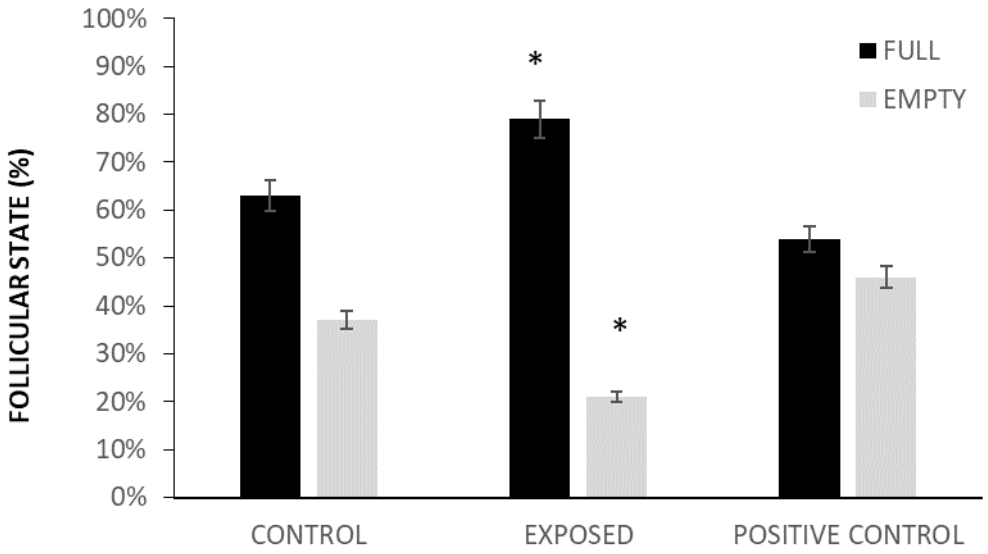
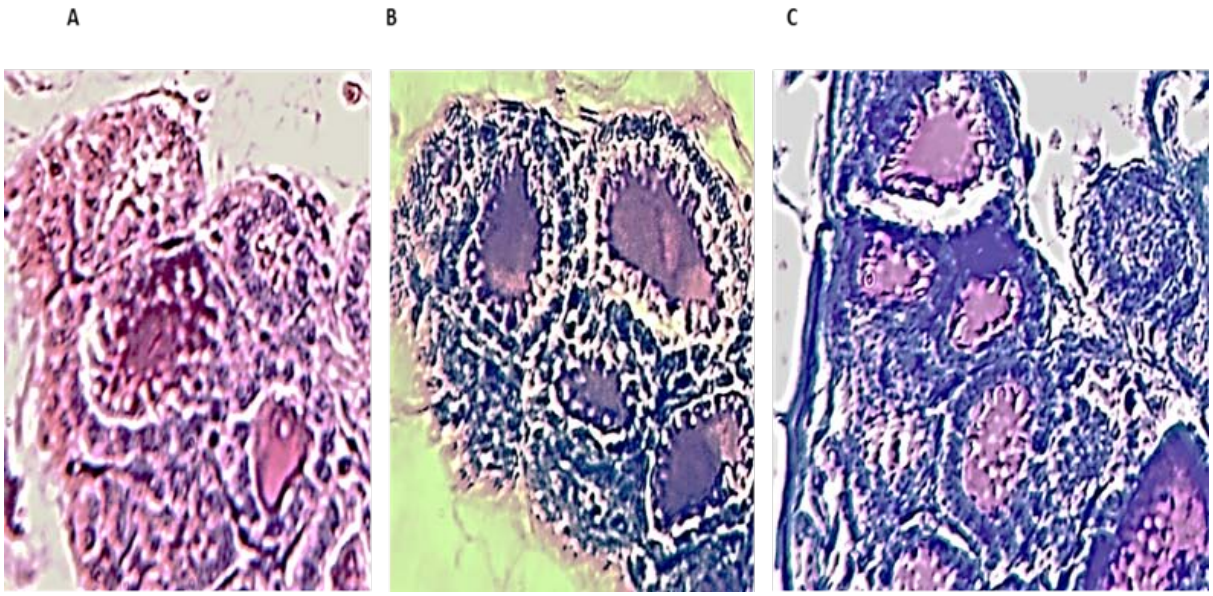


Figure 5: Percentage of filled and empty follicles during stage **58NF**. *p<0.01 vs Control.



Picture 3: Optical microscopy (40x) of the thyroid gland in Xenopus laevis at stage **58NF** showing colloid area, size and degree of follicular hyperplasia: A) Control, B) Exposed, C) Positive Control.

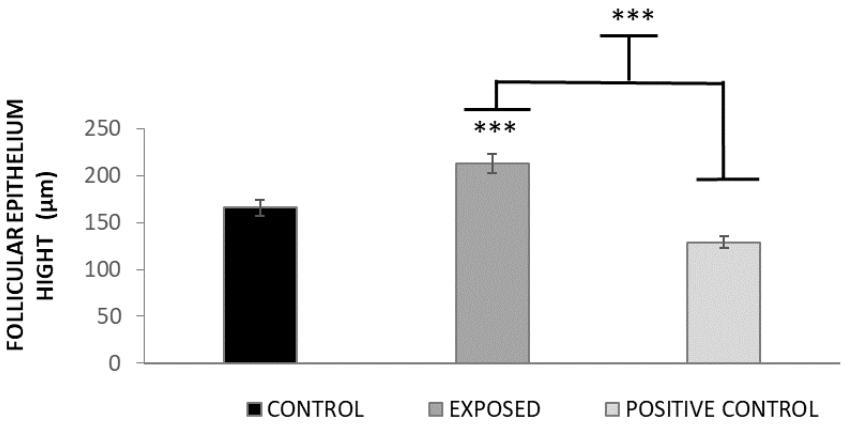


Figure 6: Follicular epithelium height at stage **58NF**. ***p<0.0001 vs Control and vs Positive Control.

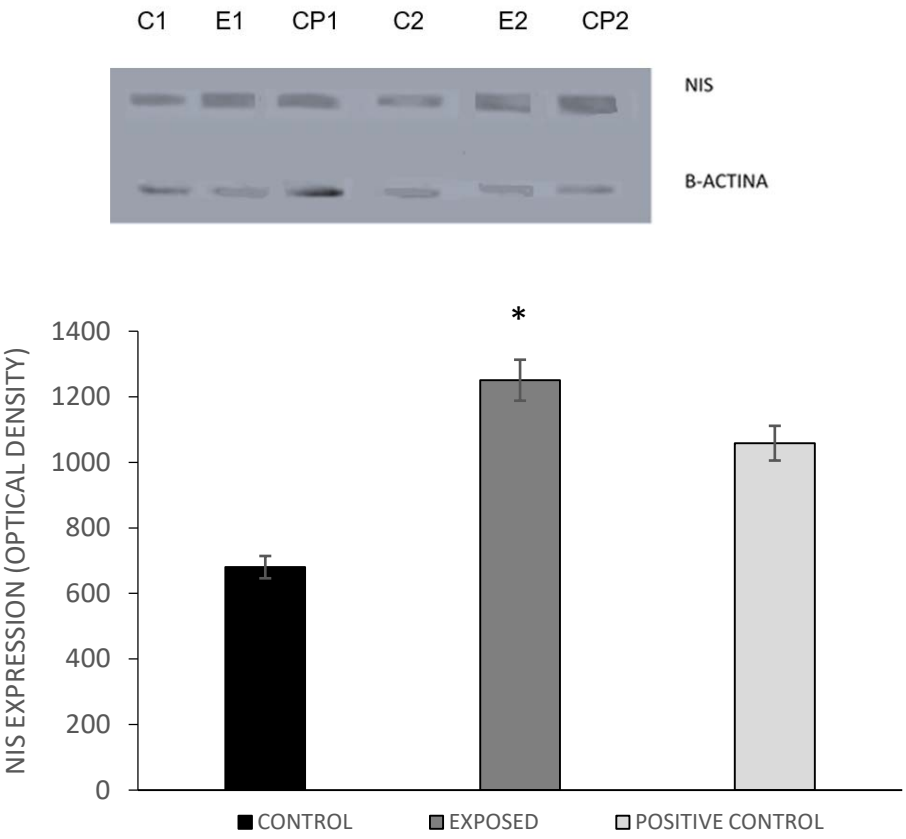


Figure 7: NIS protein expression at stage **58NF** was higher in groups Exposed (E 1, 2) and Positive Control (PC 1, 2) vs Control group (C 1, 2) *p<0.05.

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Development of Dietary Supplement Capsules "Nigelit" using Mathematical Methods

By Kh. K. Bekchanov, S. F. Iskandarova & N. A. Djabbarov

Tashkent Pharmaceutical Institute

Abstract- The research featured the development of formulae and technology of obtaining biologically active additives for functional foods with potential anti-inflammatory activity using a substance containing zinc (microelement) and plant raw material as source of natural biologically active substances. The relevance of the study is comes from the need for more effective use of natural biologically active compounds, the need to expand the range of domestic dietary supplements, in particular, those recommended for the prevention of disorders of the prostate activity and erectile dysfunction, with natural ingredients and import substitution. On the basis of scientific data and the results of the conducted research, substance containing zinc and a promising plant raw material containing biologically active substances were selected; their safety and potential properties were assessed. The optimal ratio and dosage of active ingredients in the composition has been scientifically substantiated.

Keywords: biologically active additives, dietary supplement, raw material, mathematical method, experimental design, factor, optimization parameters, capsules, "nigelit", excipients.

GJMR-B Classification: LCC Code: KF49

Strictly as per the compliance and regulations of:

Development of Dietary Supplement Capsules "Nigelit" using Mathematical Methods

Kh. K. Bekchanov [α], S. F. Iskandarova [σ] & N. A. Djabbarov [ρ]

Abstract- The research featured the development of formulae and technology of obtaining biologically active additives for functional foods with potential anti-inflammatory activity using a substance containing zinc (microelement) and plant raw material as source of natural biologically active substances. The relevance of the study is comes from the need for more effective use of natural biologically active compounds, the need to expand the range of domestic dietary supplements, in particular, those recommended for the prevention of disorders of the prostate activity and erectile dysfunction, with natural ingredients and import substitution. On the basis of scientific data and the results of the conducted research, substance containing zinc and a promising plant raw material containing biologically active substances were selected; their safety and potential properties were assessed. The optimal ratio and dosage of active ingredients in the composition has been scientifically substantiated. The technological properties of the mixtures of active ingredients that determine the choice of technology and the quality of the finished product were studied. Researches were carried out to select the optimal composition for the capsules "Nigelit" using a mathematical method of experimental design, the 4 × 4 Latin square. The experimental results were processed using mathematical methods of statistical and variance analysis, involving the Fisher statistic - Fstatistic (also called the F-ratio) and the generalized desirability function. As a result, the composition and technology of dietary supplement - capsules "Nigelit" were developed.

Keywords: biologically active additives, dietary supplement, raw material, mathematical method, experimental design, factor, optimization parameters, capsules, "nigelit", excipients.

I. Introduction

Erectile dysfunction or the inability to achieve or maintain an erection sufficient for satisfying sexual activity, often becomes a real problem for men. According to the WHO information every 10th managed older 21 has the erectile disorder and after the age of 60 years every third man is not able to perform sexual intercourse. About 35% of men aged 40 to 70 years suffering from partial or total inability to achieve erection [1].

To date, effective approaches of pharmacological correction of disorders of sexual function in men have been developed. Depending on the indications for use, these drugs should have separately or in combination neurotropic, vasodilating, anti-inflammatory, antimicrobial, antihypoxic, immunotropic effect [1]. The basis of drug therapy for erectile dysfunction, as a rule, is made up of phosphodiesterase inhibitors: vardenafil, sildenafil, tadalafil [1]. However, all of these drugs are available with a doctor's prescription. At the same time, significant advances in the prevention and treatment of male genital pathology can be achieved with the use of over-the-counter herbal remedies. Compared to synthetic ones, natural components have a milder and more versatile effect due to the variety of components actively affecting the body and, as a rule, rarely cause side effects [1]. Most of the herbal remedies for correcting erectile dysfunction are presented in the form of biologically active additives.

Today the market of dietary supplements for the correction of erectile dysfunction mainly offers complex drugs of a certain functional orientation, most often combining herbal ingredients with a pronounced stimulating effect on sexual function and a general tonic effect [1].

When reviewing the literature, it was found that one of the types of plant raw materials that have a beneficial effect on the function of the prostate is the seeds of Nigella sativa [2], and of the microelement – zinc [3, 4].

Based on the analysis of the pharmaceutical market of the manufactured dosage forms, we have selected hard gelatin capsules as the rational dosage form for the medicinal preparation under development. Capsules represent a prospective solid dosage form with a number of advantages. For instance, they are attractive in appearance, easy to swallow, contain accurate dose, protect encapsulated medications against light, air and moisture since capsule shells provide a high level of airtightness, can quickly swell up, dissolve and get absorbed in the gastrointestinal tract, have high bioavailability [5].

The aim of the research is to develop the composition and technology for obtaining dietary

Author α: Associate professor at the Department of pharmacy organizing and pharmaceutical technology, PhD in Pharmaceutical Sciences, Tashkent Pharmaceutical Institute.
e-mail: bekchanov.xamdam@mail.ru
Author σ: DSc in Pharmaceutical Sciences, professor, Head of the Department of Pharmacy organizing and pharmaceutical technology, Tashkent Pharmaceutical Institute.
e-mail: iskandarova.shakhista@mail.ru
Author ρ: Associate professor, PhD in Pharmaceutical Sciences, Department of Industrial Technology of Drugs, Tashkent Pharmaceutical Institute. e-mail: nuri_jna@mail.ru

supplements with specified characteristics using plant raw materials - seeds of Nigella sativa and a microelement – zinc.

II. Materials and Methods

Seeds of Nigella sativa, zinc sulphate, their mixture, their mixtures with different excipients and capsules on their base were selected as the materials of research. The technological properties of the materials that determine the composition, technology and the quality of the finished product and the capsule disintegration were studied using conventional methods, described in [6, 7, 8]. In order to select the optimal composition for the capsules "Nigelit" a mathematical method of experimental design, the 4 × 4 Latin square was used. The experimental results were processed using mathematical methods of statistical and variance analysis, involving the Fisher statistic (F statistic) and the generalized desirability function [9, 10, 11, 12]. In order to create granules moisture activated dry granulation (MADG) was used [13, 14, 15].

III. Results and Discussion

For the development of the dietary supplement, firstly, a suitable dose of the microelement – zinc and plant raw material containing biologically active substances were calculated, based on the study of their safety and potential properties. Then the technological properties of the mixture of active components were studied. It was established that the mixture of active components has unsatisfactory technological properties in particular flow ability. This makes obtaining capsules of an acceptable quality from such a material impossible. In order to eliminate these disadvantages, and to produce good-quality capsules, it is necessary to incorporate excipients, and to use granulation, which prevents the segregation (stratification) of the mixture [16].

In order to choose the most rational composition and the encapsulation technology for the capsules "Nigelit", we took advantage of a mathematical method of experimental design, the method of the 4 × 4 Latin square and performed analysis of variance. The use of this method makes it possible to significantly reduce the experimental error and to quantify the effect of various factors on the optimization parameters [9, 10, 11, 12]. In this case, flowability, bulk density, angle of repose of the granular materials and disintegration time of capsules were selected as the optimization parameters (see table 1); while fillers, antiaderents (anti-sticking agents), disintegrants and glidants were selected as factors affecting optimization parameters (see table 2). Technological properties of the granular materials and the capsule disintegration were studied using conventional methods [6, 7, 8]. In order to create granules moisture activated dry granulation (MADG) was used [13, 14]. This method allows for simultaneous mixing and of ingredients, as well as forming of a product with homogeneous dispersion [13]; minimization grinding of such issues with wet granulation as the need for a significant amount of granulating liquid, the duration and energy intensity of the process of mixing the wet mass, sensitivity of individual components of the mixture to high moisture levels, the necessity of using granule-forming *apparatus* in most cases, the long-lasting stage of drying the granules, accompanied by the unfavorable effect of temperature on active pharmaceutical ingredients, as well as the cumbersome equipment required for the air preparation and air purification processes [15]. The main advantage of this granulation method is that the resulting granules do not need to be dried - this speeds up the technological process, reduces labor and energy costs. Moreover, the resulting granules do not need additional grinding due to the characteristic homogeneous particle-size distribution [13]. Purified water was used as the moisturizing liquid for granulation.

Table 1: Optimization parameters for the capsules "Nigelit"

Optimization parameters (Y)			
Y_1	Y_2	Y_3	Y_4
Flowability (10^{-3} kg/s)	Bulk density (kg/m^3)	Disintegration (min.)	Angle of repose (°)

Table 2: Factors affecting the optimization parameters of capsules "Nigelit"

Name of the capsules	Factors			
	A - fillers	B - antiadherents	C - disintegrants	D - glidants
	a_1 - starch	b_1 - magnesium stearate	c_1 - CMC	d_1 - starch

"Nigelit"	a_2 - Prismalac 60	b_2 – calcium stearate	c_2 - MCC Arbocel A 300	d_2 – aerosil
	a_3 - MCC Arbocel A 300	b_3 - stearic acid	c_3 - UAP	d_3 – talc
	a_4 – dextrin	b_4 – kaolin	c_4-Na-CMC	d_4 – PEG-400

In order to select the optimal composition for capsules "Nigelit" various mixtures of active components and excipients were designed and prepared according to the formulations presented in table 3. Each mixture prepared according to the formulations was granulated using moisture activated dry granulation method. Then the technological properties of thesemixtures and the disintegration of the capsules prepared on their base were studied (see table 3).

Table 3: Experiment design matrix and research results on the effect of the excipients on the optimization parameters for capsules "Nigelit"

(Symmetric fractional factorial experiment, 16 out of 256, 1/16 fraction)

Experiment number	Factors				Optimization parameters				*D*
	A	B	C	D	Y_1, 10^{-3} kg/s	Y_2, kg/m^3	Y_3, min.	Y_4, °	
1	a_1	b_1	c_1	d_1	4,5	387	30	40	0,47
2	a_1	b_2	c_2	d_2	4,3	386	32	43	0,44
3	a_1	b_3	c_3	d_3	5,0	400	33	42	0,48
4	a_1	b_4	c_4	d_4	4,1	385	38	46	0,33
5	a_2	b_1	c_1	d_2	4,1	380	32	41	0,42
6	a_2	b_2	c_2	d_1	4,3	381	28	41	0,47
7	a_2	b_3	c_3	d_3	4,8	392	35	42	0,43
8	a_2	b_4	c_4	d_4	3,9	380	40	46	0,29
9	a_3	b_1	c_2	d_3	5,5	390	20	38	0,69
10	a_3	b_2	c_1	d_2	4,8	377	25	43	0,54
11	a_3	b_3	c_3	d_1	5,1	380	28	44	0,52
12	a_3	b_4	c_4	d_4	4,7	378	30	46	0,46
13	a_4	b_1	c_1	d_4	4,4	388	27	43	0,51
14	a_4	b_2	c_2	d_3	5,2	400	25	40	0,61
15	a_4	b_3	c_3	d_2	4,5	386	30	46	0,45
16	a_4	b_4	c_4	d_1	4,8	390	33	43	0,46

Then the results of studying the optimization parameters (table 3) were subjected to analysis of variance which allows us to test the null hypothesis (all means are equal) against the alternative hypothesis (at least one mean is different) with a specified value of alpha (in our case the value of alpha (α) or the level of significance is equal to0.05 or 5%) and probability (in our case the value of probability (P) is equal to0.95 or 95%). In other words analysis of variance allows us to determine the significance of the studied factors A, B, C, D for the optimized parameters Y_1, Y_2, Y_3, Y_4(table 4) at a given level of significance[11, 17].

It should be mentioned that if the F test statistic (observed) is greater than the F critical value, i.e. $F_{statistic}$>

$F_{critical}$, then the hypothesis of similarity or the null hypothesis H_0 is rejected, and the hypothesis of the difference or the alternative hypothesis H_1 is accepted with a level of significance $\alpha = 0.05$ or 5%. This means that the factor significantly affects the change in the output data - the values of the optimization parameter and the data depend on the factor with a probability of P = 0.95 (P = 1 - α) or P = 95% (P = 100 - α). If the F test statistic (observed) is less than the F critical value, i.e. $F_{statistic} < F_{critical}$, then the hypothesis of similarity or null hypothesis H_0 is accepted, and the hypothesis of difference or alternative hypothesis H_1 is rejected with a level of significance $\alpha = 0.05$ or 5%. This means that the factor does not significantly affect the output data - the values of the optimization parameter with a probability of P = 0.95 (P = 1 - α) or P = 95% (P = 100 - α).

Table 4: Analysis of variance of the experimental data from the study of the indicators of the capsules "Nigelit"*

(Four-factor analysis of variance without replication)

Optimization parameters	Source of variance	Degrees of freedom (df)	Sum of squares (SS)	Mean square (MS)	$F_{statistic}$	$F_{critical}$
Y_1–Flowability, 10^{-3} kg/s	Factor A	3	1.26	0.42	3.02	3.49
	Factor B	3	0.45	0.15	0.74	3.49
	Factor C	3	0.73	0.24	1.34	3.49
	Factor D	3	1.66	0.55	5.23	3.49
Y_2-Bulk density, kg/m^3	Factor A	3	268.5	89,5	2.26	3.49
	Factor B	3	78.5	26.17	0.47	3.49
	Factor C	3	156.5	52.17	1.07	3.49
	Factor D	3	467.5	155.83	6.79	3.49
Y_3 – Disintegration, min.	Factor A	3	174.75	58.25	3.41	3.49
	Factor B	3	172.25	57.42	3.32	3.49
	Factor C	3	182.25	60.75	3.69	3.49
	Factor D	3	66.75	22.25	0.85	3.49
Y_4 - Angle of repose,°	Factor A	3	0.5	0.17	0.02	3.49
	Factor B	3	51.5	17.17	5.49	3.49
	Factor C	3	51.5	17.17	5.49	3.49
	Factor D	3	48.5	16.17	4.79	3.49

**Analysis of variance was conducted on the experimental data from table 3, and the statistical indicators in table 4 were calculated, using ANOVA module of the statistics software MiniTab.*

The results of the analysis of variance (table 4) allow us to state the following:

- The selected types of fillers (Factor A) does not have a significant effect on the flowability (Y_1), bulk density (Y_2), angle of repose (Y_4) of the granular materials as well as the disintegration of capsules (Y_3);
- The selected types of antiadherents (Factor B) has a significant effect on the angle of repose (Y_4) of the granular materials and does not have a significant effect on the flow ability (Y_1), bulk density (Y_2) of the granular materials as well as the disintegration of capsules (Y_3);
- The selected types of disintegrants (Factor C) has a significant effect on the disintegration of capsules (Y_3) and the angle of repose (Y_4) of the granular materials and does not have a significant effect on the flow ability (Y_1), bulk density (Y_2);
- The selected types of glidants (Factor D) has a significant effect on the flow ability (Y_1), bulk density (Y_2), angle of repose (Y_4) of the granular materials and does not have a significant effect on the disintegration of capsules (Y_3).

The overall (generalized) evaluation of the optimization parameters - the disintegration of capsules and the technological properties of the granular materials (model mixtures)- was carried out using a desirability function [11, 18]. In order to generalize the values of the optimization parameters that have different units of measurement, we used the well-known and widely accepted Harrington's desirability function, first introduced by him in solving quality control problems of mass production. The Harrington's scale establishes a correspondence between linguistic evaluations of desirability of the values of the indicator *x* and the numerical intervals *d(x)* (table 5) [11, 19].

Table 5: Numerical intervals of the Harrington's scale

Linguistic evaluation	Intervals of the desirability function values *d(x)*
Very good	1.00 - 0.80
Good	0.80 - 0.63
Satisfactory	0.63 - 0.37
Bad	0.37 - 0.20
Very bad	0.20 - 0.00

In order to construct the desirability function scale of the optimization parameters for the capsules "Nigelit"(Fig. 1), the method of quantitative analysis was used with the range of desirability values between 0 and 1 (Table 5). The value $d = 1$ corresponds to the best value of the optimization parameters, while $d = 0$ - to their worst value of ones. The intermediate values of the desirability function reflect specific levels of the product quality: very bad (0.00 - 0.20), bad (0.20 - 0.37), satisfactory (0.37 - 0.63), good (0.63 - 0.80) and very good (0.80 - 1.00). Conversion of the natural values (Y) into individual desirability values (*d*) with a one-sided limit $Y \leq Y_{max}$ or $Y \geq Y_{min}$ was performed using the following equation:

$$d = \exp\left[-\exp(Y')\right] \quad (1)$$

where $Y' = b_0 + b_1$. The coefficients b_0 and b_1 were calculated by assigning the corresponding desirability values d for two of the property values, preferably selected within the range $0.2 < d < 0.8$. The desirability curve (Fig. 1) were plotted in the (Y', d) coordinates based on the equation of the desirability function. At the same time, Y_{max} or Y_{min} on the dimensional scales corresponded to 0 (zero) on the dimensionless scale Y'. The desirability scale (Fig. 1) was used to convert the response values (Y_1, Y_2, Y_3, Y_4) into the dimensionless desirability function (d_1, d_2, d_3, d_4), i.e. to find individual desirability values for the measured values of the optimization parameters Y_i.

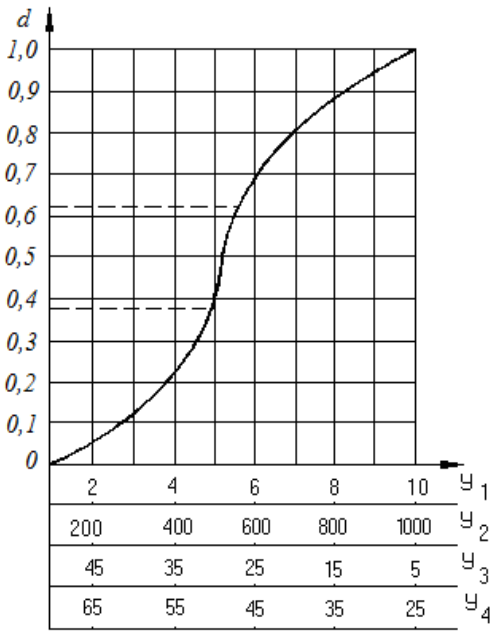


Fig. 1: The Desirability Function Scale of the Optimization Parameters for the Capsules "Nigelit"

Then the overall (generalized) desirability function values were calculated using the formula (2)as the geometric mean of individual desirability values found. It should be noted, this is a more successful approach towards optimization of the parameters for finished products (in our case capsules):

$$D = \sqrt[4]{d_1 d_2 d_3 d_4} \quad (2)$$

where *D* is overall (generalized) desirability function value; d_1, d_2, d_3, d_4 - individual desirability function values.

The values of the overall (generalized) desirability function (*D*) for the capsules "Nigelit" are presented in table 3.

Based on the generalized evaluation of the optimization parameters - the disintegration of capsules and the technological properties of the granular materials (model mixtures), carried out using a desirability function, the excipients can be arranged in the order of preference as follows:

- The type of fillers (Factor A) – $a_3 > a_4 > a_1 > a_2$;
- The type of antiadherents (Factor B) – $b_1 > b_2 > b_3 > b_4$;
- The type of disintegrants (Factor C) – $c_2 > c_1 > c_3 > c_4$;
- The type of glidants (Factor D) – $d_3 > d_1 > d_2 > d_4$.

The optimal composition for capsules "Nigelit" was formulated based on the results of the mathematical method of experimental design and using a desirability function.

The most optimal combination of levels of factors - composition of the excipients that ensure the required indicators for the capsules "Nigelit" (table 3, composition No. 9) was selected based on the values of the overall (generalized) desirability function (*D*) of the optimization parameters. The excipients included in this composition are listed in table 6.

Table 6: The Most Optimal Composition of the Excipients that Ensure the Required Indicators for the Capsules "Nigelit"

Name of the capsules	No. of the optimal composition	Excipients included in the optimal composition
"Nigelit"	composition No. 9 in table 3	MCC Arbocel A 300 (filler – a_3) Magnesium stearate(antiadherent– b_1) MCC Arbocel A 300 (disintegrant– c_2) talc(glidant – d_3)

Based on the results of the mathematical method of experimental design, we recommend the following formulation and technology:

Formulation:

Nigella sativa seeds ground	-350mg
Zinc sulphate	-20 (equal to 7.3 mgof zinc)
MCC Arbocel A 300	-22mg
Talc	-4mg
Magnesium stearate	-4 mg
Average net weight of capsule	-00 mg

Technological process: A moisturizing liquid (water) is sprayed into the dry mixture during the mixing process of seeds ground of Nigella sativa and zinc sulphate with "granule-forming" excipient- MCC Arbocel A 300(filler)in order to form agglomerates - granules. The "drying" of granules is accomplished by adding a "drying" excipient –the rest amount of MCC Arbocel A 300 (disintegrant) into the mixer, during the continuous mixing process. Since the final moisture content of the product obtained by this granulation method usually does not exceed the final moisture content of the granules obtained by traditional wet granulation, we did not perform additional thermal drying of the granules. In the final stage, talc (glidant) and magnesium stearate (antiadherent) are added to the granules. The resulted compact granules have good technological properties which are presented in table 7.

Table 7: Results of the study of the technological properties of the granules for the capsules "NIGELIT"

No.	Studied indicators	Unit of measurement	Obtained results
1	Appearance		Dark brown granules with a strong, agreeable aromatic odor and a spicy, pungent taste
2	Particle-size distribution: +2500 -2500+1000 -1000+ 500 - 500+ 250 - 250	μm, %	 1.5 23.0 47.5 20.8 7.2
3	Bulk density untapped	kg/m^3	390
4	Bulk density tapped	kg/m^3	455
5	Flowability	10^{-3} kg/s	5.5
6	Angle of repose	°	38
7	Residual moisture	%, 70 °C	4.2

As evidenced by the data in Table 7, in contrast to the mixture of active components, the granular material prepared according to the selected composition and technology has satisfactory technological properties.

Taking into account the amount of granular material to be encapsulated, it's density, empty capsule volume capacity and the requirements for uniformity of the capsule contents the capsule size 00E was chosen to encapsulate the calculated dosage. [17, 20]. The process of filling the capsules with the granular material was performed using the capsule-filling machine MF 30.

IV. Conclusion

Thus, based on the results of study of technological properties of the the mixture of active componentsand the granular material prepared according to the selected composition and technology and using the mathematical method of the experimental design, the 4 × 4 Latin square, an optimal composition was formulated and the rational encapsulation technology for the capsules "Nigelit" with an average net weight of capsule (weight of core material) 400 mg was developed.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Quality Assessment of Active Substances that Demonstrate Sedative Effect of "Flegmen" Syrup

By Zaynab E. Sidametova & Nemat K.Olimov

Tashkent Pharmaceutical Institute

Abstract- As a result of the research, the amino acid and elemental composition of "Flegmen" syrup was determined, and it was found that the syrup contains 18 amino acids and 57 macro and micro elements. The accumulation in "Flegmen" syrup of a significant number of amino acids and useful minerals, along with active biologically active groups (flavonoids, saponins), allows us to objectively consider it as a valuable source of an effective sedative.

Keywords: flegmen; amino acids; regel's gooseberry; turkestan motherwort; peppermint; high performance liquid chromatography.

GJMR-B Classification: DDC Code: 363.739460973 LCC Code: TD223

Strictly as per the compliance and regulations of:

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I. Introduction

Amino acids are organic compounds which are considered material unity of all tissues of human body. They are responsible for metabolic processes and energy metabolism, ensuring the functioning of the body. Amino acids directly affect the state of the nervous system, regulating mental activity, mood and sleep [2,5]. A deficiency of even one building block can worsen a person's health and lead to serious biochemical and physiological disorders in the body. Therefore, amino acid supplements are best taken in all cases when you load yourself - physically or mentally, but cannot fill the increased need in a timely manner solely from food. There are three groups of amino acids that are replaceable, irreplaceable and conditionally essential [11, 12].

The deficiency of essential amino acids is compensated by the human body with plant foods, and in the case of medical indications, in the form of medicinal preparations containing these compounds. Many amino acids are not only of great physiological importance, but are highly effective pharmacological substances. Replaceable can be independently synthesized in the body. Essential ones are not synthesized on their own and enter the body with food. Conditionally essential amino acids can be synthesized independently in the presence of essential amino acids. There are several optimal periods for taking amino acids. During training, amino acids will help improve athletic performance, muscle growth and strength, as well as speed up recovery processes after exercise. During diets and drying, amino acids will help maintain muscles and health during forced starvation, as well as speed up the process of burning fat. IN periods of mental load of amino acids will increase intellectual productivity and relieve excessive psycho-emotional stress [9,10].

According to WHO, a significant part of the world's population suffers neuropsychiatric disorders during their lives. For the treatment of these types of disorders, sedative herbal preparations are often used, which is due to their wide range of action due to the presence of a complex of active substances in them, ease of use, ease of dosage, minimum contraindications and side effects.

With this in mind, from the plants most commonly used in the formulation of sedatives and having industrial stocks in the republic, namely, Regel's gooseberry, Turkestan motherwort, peppermint and licorice, we previously developed a collection and based on it a sedative syrup was obtained "Flegmen"[3,13].

Amino acids are an integral part of proteins, they perform one of the most important roles in the body. Almost all tissues are formed from them: skin, hair, ligaments, tendons. There are three types: replaceable, conditionally replaceable and irreplaceable. Non-essential amino acids are supplied to the body with food and can be synthesized in it. Essential amino acids must be supplied to the body from outside. Conditionally essential amino acids can be synthesized by the body from essential amino acids if necessary. There are twenty compounds in nature that form proteins. Non-essential amino acids include: glutamic acid, glycine, aspartic acid, serine, cysteine, tyrosine, alanine, proline. Essential amino acids are those amino acids that our body cannot produce on its own, they must be supplied with protein foods. Essential amino acids include: valine, isoleucine, leucine, threonine, methionine, lysine, phenylalanine, tryptophan, histidine. Conditionally essential amino acids include: arginine, tyrosine, cysteine. Each of them is responsible for a specific function [1,9,10].

As for the sources of obtaining biologically active substances, including amino acids, one of them is vegetable raw materials. In turn, it should be noted

Author α: Doctor of Philosophical Sciences, Associate Professor, Tashkent Pharmaceutical Institute, Oybek Street 45, Tashkent, Uzbekistan. e-mail: info@pharma.uz
Author σ: Doctor of Pharmaceutical Sciences, Professor, Tashkent Pharmaceutical Institute, Oybek Street 45, Tashkent, Uzbekistan.

that biologically active substances of plant origin differ from synthetic compounds in that they are in plants in complexes easily absorbed by the body and biologically available concentrations [11,14]. Based on the above, this work, we decided to devote the study of one of the groups of such biologically active substances of the "Flegmen" syrup - amino acids in order to identify their possible participation in the manifestation of the sedative effect of the drug.

The purpose of the study was the study of the amino acid and mineral composition of the sedative syrup "Flegmen".

II. Experimental Part and Methods

To this end, we decided to review the methods for the analysis of amino acids in various objects (primarily in drugs) described in the literature. At the same time, it was established that there are currently many methods for determining amino acids in various objects. Among them, the most common are methods for the determination of amino acids by reverse-phase and cation-exchange High Performance Liquid Chromatography (HPLC), as well as electrophoretic methods. Considering the high prevalence and a number of advantages over other methods, we preferred HPLC in the choice of the amino acid analysis method. In particular, the improvement of HPLC technology and its wide practical application makes it possible to solve the problems of separation and quantitative determination of very small amounts (10 mg/kg and below) of analyzed components in complex objects. However, the absence of chromophore groups in most amino acid molecules requires a derivatization step when using this method. At the same time, various reagents have been proposed for pre- and post-column derivatization.

Thus, in the works of a number of researchers on a C18 column in the gradient elution mode with a methanol-phosphate buffer mixture with fluorimetric detection, glutathione, glutamylcysteine, and 16 amino acids were simultaneously determined using o-phthalaldehyde as a derivatizing agent. And in the works of a number of authors, naphthalenedialdehyde was used in the determination of desmosine, isodesmosine, and 17 other amino acids. 11-dansyl derivatives of amino acid isomers were separated (UV detection) on a β-cyclodextrin column using a mobile phase methanol–phosphate buffer (pH=6.5) [9, 10].

As you know, for each new drug, a specific analysis method should be developed for it. When developing an HPLC technique, in order to obtain reliable results, it is most important to find the optimal conditions for the analysis, the main of which is the choice of the mobile phase (the phase with the highest selectivity with respect to active substances), the column size, the type and size of the sorbent particles, and the elution mode (gradient or isocratic mode), detection method (conditions), standard, etc. All this, of course, requires appropriate research using various reagents and solvents, as well as a waste of time. However, when reviewing the literature, we came across the HPLC technique [6] used to determine similar substances in a similar object. This circumstance led us to the idea of testing this technique for our case, in order to establish its suitability for determining amino acids in "Flegmen" syrup and, if necessary, modifying it.

The object of the study was the "Flegmen"sedative syrup obtained on the basis of a plant liquid extract. The syrup obtained in this way is a thick solution of a light brown color, with a characteristic odor and a sweet, slightly icy taste [3,13].

The suitability of the chromatographic system was checked by the efficiency of the chromatographic column, the degree of separation of the peaks and the relative standard deviation.

Chromatography of the amino acids of "Flegmen" syrup and a standard mixture of amino acids with a known concentration was carried out sequentially under similar conditions. The results of the chromatographic analysis of the amino acids of the "Flegmen" syrup are shown in fig. one.

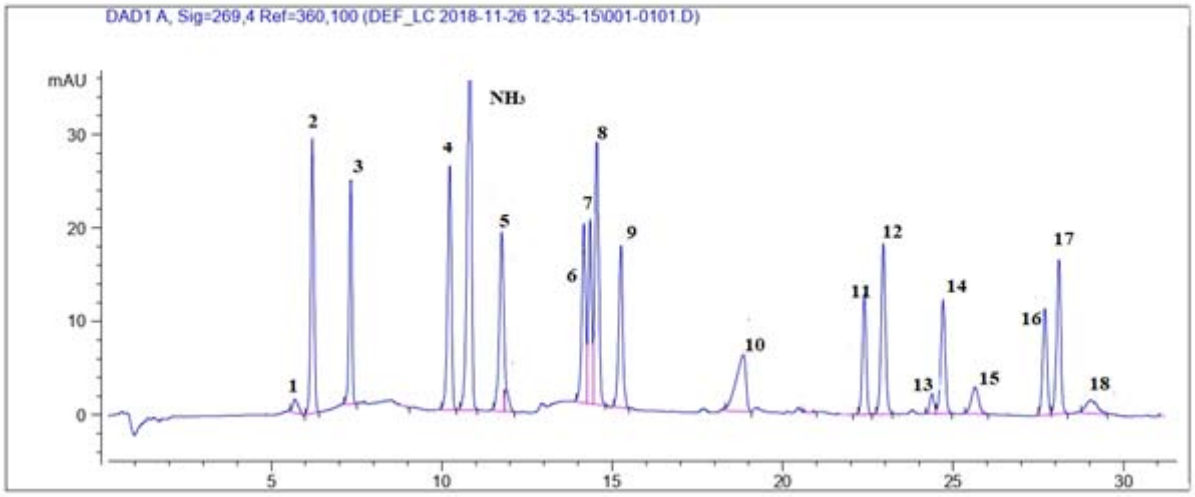

Fig. 1: Chromatogram of free amino acids of "Flegmen"syrup

1 - aspartic acid, 2 - glutamic acid, 3 - asparagine. 4 - serine, 5 - glutamine, 6 - histidine, 7 - threonine, 8 - glycine, 9 - arginine, 10 - alanine, 11 - tryptophan, 12 - methionine, 13 - tyrosine, 14 - valine, 15 - phenylalanine, 16 - isoleucine, 17-leucine, 18-lysine.

Identification and quantification of the amino acids contained in the study preparation was carried out by comparing the retention times and peak areas on the standard amino acid chromatogram with those on the amino acid chromatogram of the study preparation. Analysis of the obtained amino acid chromatograms of the test sample and the standard mixture showed the presence of several amino acids in the "Flegmen" syrup. The results of the analysis with names, chemical formula and amino acid content are presented in Table 1.

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Table 1

No.	Amino acid name	Chemical formula	Content μg/ml
one	Aspartic acid	$C_4H_7O_4N$	0.0240
2	Glutamine acid	$C_5H_9O_2N$	0.0840
3	Asparagine	$C_4H_8N_2O_3$	0.0044
five	Glutamine	$C_3H_7O_3N$	0.0124
6	Histidine	$C_4H_9O_2N$	0.0038
7	Threonine *	$C_6H_9O_2N_3$	0.0292
8	Glycine	$C_2H_5NO_2$	0.0543
nine	argenin	$C_2H_5O_2N$	0.0668
10	Alanine	$C_6H_{14}O_2N_4$	0.0418
eleven	tryptophan	$C_{11}H_{12}N_2O_2$	0.0218
12	Methionine *	$C_5H_{11}NO_2S$	0.0421
13	Tyrosine *	$C_9H_{11}NO_3$.	0.0112
fourteen	Valine *	$C_2H_5O_2N$	0.0177
15	Phenylalanine *	$C_6H_9O_2N_3$	0.0177
16	Isoleucine *	$C_6H_{13}O_2N$	0.0092
17	Leucine *	$C_6H_{13}NO_2$	0.0092
eighteen	Lysine HCl *	$C_6H_{14}N_2O_2$	0.0713
			0.5209

***** *Essential amino acids*

III. Research Results

According to the research data, "Flegmen" syrup contains 18 amino acids, which indicates a high biological value of the sum of amino acids of the studied liquid extract. Among the amino acids of the collection, glutamic acid predominates, which is used in the treatment of diseases of the central nervous system. From this it follows that the amino acids of the studied syrup can participate in the manifestation of the pharmacological activity of the syrup.

Further research was aimed at studying the mineral composition of "Flegmen" syrup.

Trace elements have been known for a long time, but only very recently they have been recognized

as substances necessary for Life. Trace elements are "food mainly for the endocrine glands", more precisely, for enzyme enzymes, since they are catalysts for vital processes. In the impact on the body, all microelements are interconnected and interdependent. Human needs for these "metals of life" are very individual. Minerals make up only 4% of the mass of the human body. Half of this amount is part of the solid parts of the body: bones, teeth, nails, hair, soft tissues. The rest is in the blood, in the intercellular and intracellular fluid.

70-80% of our body mass is water and gases soluble in it - carbon, hydrogen, nitrogen and oxygen, and most of all in our body oxygen is about 60% of body weight, carbon - about 17%, hydrogen - about 10%, nitrogen – only 3% [3].

Micro and macro elements control metabolic processes, maintain the physical and chemical integrity of cells and tissues by maintaining characteristic bioelectric potentials. It is microelements that play the main role in the activity of enzymatic processes necessary for life. That is why their deficiency, as well as excess, will immediately affect human health. It should be noted that the intercellular space contains mainly sodium and calcium, and inside the cells - potassium and magnesium. If the balance between them is disturbed, a person develops various diseases, accompanied by swelling. In this case, the balance should be maintained both between sodium and calcium, and between potassium and magnesium [2,12].

It should also be noted that the minerals contained in plants are divided into two groups: the first, called macroelements, includes potassium, sodium, calcium, magnesium, manganese, silicon, chlorine, phosphorus; plant ash contains at least hundredths of a percent of these elements; the second, called trace elements, include: iron, copper, zinc, iodine, barium, etc. Their content in the ash is thousandths of a percent. Accumulation of trace elements in plants is often selective: different types of plants grow in the same soil conditions, and only some of them are able to concentrate certain microelements [4,7,14].

The determination of the elemental composition was carried out using a highly sensitive multi-element analysis method - mass spectral with inductively coupled plasma (ICP - MS) [15]. As a result of the analysis, the presence of 57 mineral elements was established in the syrup. According to the results obtained, 2 elements (Br and K) were contained in concentrations from 100 to 1000 mg/kg, 2 elements (Na and Mg) ranged from 10 to 100 mg/kg, 2 elements (P and Fe) ranged from 1 to 10 mg/kg), and below 1 mg/kg 51 elements (Fe, Zn, B, Cr, Al, I, Sr, Mn, Cu, Ca, Sc, Ba, Mo, Ni, Li, V, Se, Sb, Zr, Sn , Nb, As, Co, Ga, Ag, Cd, Ta, Cs, Te,W, Tl, Bi, Re, Nd, Ce, Pb, Hf, Y, Gd, In, Sm, La, Er, Eu, Dy , Pr, Lu, Ho, Tm, Au, Hg, Pt)[102; pp.-206-213].resultsand study of the elemental composition of "Flegmen" syrup are presented in table 2.

Table 2: Elemental composition of "Flegmen" syrup

Element	Soda in μg/ml	Element	Sod-e in mcg/ml	Element	Soda - e in mcg/ml
Br	4015	Li	0.016	Nd	0.00018
K	2755	V	0.01	Ce	0.00018
Na	51.9	Se	0.011	Pb	0.00015
mg	16.5	Sb	0.005	hf	0.00013
P	1.96	Zr	0.003	Y	0.0001
Fe	1.41	sn	0.002	Gd	0.00007
Zn	0.22	Nb	0.0016	In	0.00007
B	0.16	As	0.0012	sm	0.00006
Cr	0.14	co	0.0011	La	0.00006
Al	0.12	Ga	0.001	Er	0.00006
I	0.125	Ag	0.0008	Eu	0.00005
Sr	0.11	CD	0.0007	Dy	0.0005
Mn	0.08	Ta	0.0007	Pr	0.00004
Cu	0.08	Cs	0.00055	Lu	0.00003
Ca	0.07	Te	0.0004	Ho	0.000019
sc	0.03	W	0.00046	Tm	0.00001
Ba	0.042	Tl	0.00035	Au	0.0000039
Mo	0.033	Bi	0.00033	hg	0.0000023
Ni	0.01	Re	0.00026	Pt	0.0000023

As the data in table 2 show, "Flegmen" syrup contains 57 elements. Of these elements, calcium, magnesium, potassium, sodium and chlorine, which are part of the cell in the form of ions, are vital. The listed elements are included in the group of macronutrients. Macronutrients in the syrup, the largest quantities are: bromine, potassium, sodium, magnesium, phosphorus, iron, zinc, boron, chromium. Elements found in syruphaving a positive effect on the vital activity of the organism, to a certain extent, contribute to an increase

in the pharmacological value of this medicinal vegetable syrup due to the combination with its main biologically active substances [10].

The detected elements according to the degree of decrease in their content can be represented as the following series: Br> K> Na > Mg >P >Fe >Zn >B >Cr > Al> I> Sr > Mn= Cu> Ca >Sc >Ba>Mo>>Ni >Li >V >Se >Sb >Zr >Sn > Nb > As > Co > Ga >Ag>Cd= Ta> Cs> Te > W > Tl > Bi, >Re >Nd= Ce > Pb > Hf > Y > Gd=In >Sm> La > Er >Eu > Dy > Pr >Lu > Ho >Tm > Au > Hg= Pt

When determining the elemental composition of the syrup, special attention is paid to the content of toxic heavy metals - lead, cadmium and mercury, which the FAO and WHO Joint Commission on the Food Code (Codex Alimentaris) refers to the number of components subject to priority control in international food trade [8,9]. It is shown that the content of toxic heavy metals in the syrup is within the limits allowed by SanPin 0193-06[4]. Comparison of the concentrations of these metals in the studied preparation with their clarks showed that their content practically corresponds to uncontaminated territories, which indicates the environmental safety of raw materials.

Thus, for the first time by the ICP - MS method, the mineral composition of "Flegmen" syrup was determined, in which the content of 57 elements was found. Elements such as bromine, potassium, sodium, magnesium, phosphorus, zinc have been found, which have a pronounced sedative effect and have a beneficial effect on nervous tissue, restoring performance after emotional and physical stress. The data obtained allow us to conclude that the elemental composition of the syrup is very diverse and, accordingly, can have a complex effect. It has been established that the content of toxic heavy metals lead, cadmium and mercury does not exceed the permissible values, which indicates the environmental friendliness and safe use of the syrup in medical practice.

Also, from table 2 it can be seen that in the "Flegmen" syrup such elements were found that are involved in sedative activity.

Magnesium deficiency, even if not too great, can be the cause of heart disease. A serious lack of this mineral leads to disastrous consequences - as a rule, to heart attacks. Lack of magnesium leads to anxiety, fear, confusion, depression. Also, there is hyperactivity, nervousness, stepping from foot to foot, jumping gait, sharpness of movements. Loss of balance, dizziness, fainting, weakness in the arms and legs, blood pressure disorders, cold extremities. The trace element magnesium promotes the absorption of calcium. Bromine is involved in the regulation of the activity of the thyroid gland, as it is a competitive inhibitor of iodine.

The lack of bromine in food leads to insomnia, growth retardation and a decrease in the number of erythrocytes in the blood [2,11]. Phosphorus and bromine have a pronounced sedative effect and have a beneficial effect on the nervous tissue, restoring performance after emotional and physical stress [2,11,12].

Lack of iodine contributes to the development of Graves' disease (goiter). Children and adolescents require more iodine than adults. Iodine is used in atherosclerosis, treatment of syphilis in the tertiary period, inflammatory processes of the respiratory tract, chronic mercury and lead poisoning, to prevent and treat goiter [2,11]. Potassium iodide is prescribed for mastopathy of the mammary gland and other neoplasms in the endocrine glands. Iodine has a sedative (calming) effect on a person, increases mental abilities. Iodine is necessary for the synthesis of the thyroid hormone - thyroxine, as well as for the creation of phagocytes - patrol cells in the blood, which must destroy debris and foreign bodies. Phagocytes capture and digest microorganisms, defective cells.

Lithium prevents the development of neuropsychiatric diseases and has a positive effect on the treatment of schizophrenia [2,11,12]. Zinc deficiency is of exceptional importance, as it not only leads to underdevelopment of the nervous and reproductive systems, but is also deeply linked to immunodeficiency problems. T-lymphocytes in conditions of zinc deficiency are inactive.

Potassium is a very common mineral found in many foods. The best sources of potassium are plant products, especially dried fruits and berries, nuts, seeds, Jerusalem artichoke, potatoes, radishes, cabbage, green vegetables, oatmeal, beets, bananas, bread, currants, tomatoes. Symptoms of potassium deficiency are muscle weakness, heart problems and mental disorders. Low potassium intake can lead to sodium retention and high blood pressure. A potassium-rich diet has been linked to beneficial effects on cardiovascular health. The macroelement potassium is needed, first of all, for the transmission of nerve impulses, to maintain the acid-base balance of the blood, for normal carbohydrate metabolism, to ensure muscle contraction. Its need increases primarily with vomiting and prolonged diarrhea, with profuse sweating, with diuretics, with increased excretion of potassium in the urine, which can be caused, as well as excessive amounts of sodium, coffee, sugar and/or alcohol consumed, or low blood sugar levels. blood.

The macronutrient sodium is primarily needed for normal water exchange between blood cells and tissues, to maintain the acid-base balance in the body, to transmit nerve impulses, to ensure muscle contraction.

A profound lack of sodium can lead to coma and death. Excessive consumption of this macronutrient burdens the kidneys, causes edema (normal water exchange between blood cells and tissues is disturbed), can cause an increase in blood pressure, and leads to

excessive excretion of water and potassium in the urine (which, however, does not relieve edema). Dietary sodium deficiency usually does not occur. Acute deficiency can occur with profuse sweating in combination with the consumption of large amounts of non-sodium fluids, or as a result of vomiting and diarrhea. Symptoms are muscle cramps, lack of appetite, malabsorption of nutrients.

IV. Conclusion

Thus, as a result of the research, the amino acid and elemental composition of the "Flegmen" syrup was determined, while it was found that the syrup contains 18 amino acids and 57 macro and micro elements. Among the amino acids of the collection, glutamic acid predominates, which is used in the treatment of diseases of the central nervous system. Among the macronutrients, such elements as lithium, phosphorus, iodine, magnesium, bromine were found, which have a pronounced sedative effect and have a beneficial effect on the nervous tissue, restoring performance after emotional and physical stress. The data obtained allow us to conclude that the amino acid and elemental composition of the syrup is very diverse and, accordingly, can have a complex effect.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Review on Emulsomes as Carriers for Drug Delivery

By Bandaru Hemanth Kumar, Shaik Farooq Ahmed & Prasanthi D

Abstract- This review determines the introduction to emulsomes, need to the invention, advantages, disadvantages, formulation of the emulsomes, methods of preparation and application of emulsomes. In the recent years attention has been focused on development of vesicular drug delivery system. These emulsomes provide the drug release in a controlled and sustained manner up to 24 hours, whereas the liposomes have shown release up to the mark of 6 hours. Emulsomes comes under the category of the vesicular drug delivery system and these are mainly developed for the purpose to overcome poor bioavailability, protection from harsh gastric environment and from gastric enzymes, which mainly degrade the drug molecules. The success of the emulsomes is for the delivery of drugs to fight against viral infections, fungal infections, dermal therapy, cancer, auto immunity. Mainly the drug is enclosed in the emulsomes and provide existence of drug in systemic circulation. Emulsomal based formulations of genetic drugs, antisense oligonucleotides and plasmids for gene therapy having proper and clear potential for systemic utility are increasingly available.

Keywords: emulsomes, liposomes, emulsions, preparation methods of emulsomes, applications of emulsomes.

GJMR-B Classification: DDC Code: 610.3 LCC Code: R121

REVIEWONEMULSOMESASCARRIERSFORDRUGDELIVERY

Strictly as per the compliance and regulations of:

Review on Emulsomes as Carriers for Drug Delivery

Bandaru Hemanth Kumar [α], Shaik Farooq Ahmed [σ] & Prasanthi D [ρ]

Abstract- This review determines the introduction to emulsomes, need to the invention, advantages, disadvantages, formulation of the emulsomes, methods of preparation and application of emulsomes. In the recent years attention has been focused on development of vesicular drug delivery system. These emulsomes provide the drug release in a controlled and sustained manner up to 24 hours, whereas the liposomes have shown release up to the mark of 6 hours. Emulsomes comes under the category of the vesicular drug delivery system and these are mainly developed for the purpose to overcome poor bioavailability, protection from harsh gastric environment and from gastric enzymes, which mainly degrade the drug molecules. The success of the emulsomes is for the delivery of drugs to fight against viral infections, fungal infections, dermal therapy, cancer, auto immunity. Mainly the drug is enclosed in the emulsomes and provide existence of drug in systemic circulation. Emulsomal based formulations of genetic drugs, antisense oligonucleotides and plasmids for gene therapy having proper and clear potential for systemic utility are increasingly available. The worldwide market for drug delivery systems is growing at an ever-increasing rate and is being fueled by several significant needs, the commercial necessity of extending product life and product portfolios, the clinical need to enhance drug safety and patient compliance and the technological challenge of delivering new therapeutics. Exploitation of new advances in drug delivery technology will give pharmaceutical companies a significant competitive advantage in an increasingly demanding marketplace.

Keywords: emulsomes, liposomes, emulsions, preparation methods of emulsomes, applications of emulsomes.

I. Introduction

a) Emulsomes

Emulsomes are nanosize in range compared with the other vesicular drug delivery system such as niosomes, pharmacosomes, ethosomes. Due to their reduced size, they can be used to increase the bioavailability of drug and as the best carrier for the intravenous delivery as well as the oral drug delivery[1]. Oral route is the best route to reduce the number of adverse effects. Emulsomes are novel oral drug delivery systems which carries lipophilic drugs within it. Emulsomes are lipoidal vesicles which contains solid fat core surrounded by phospholipid bilayer. They are the liposomes with extra single inner phospholipid layer which contains solid fat. The drug release pattern by emulsomes is sustained and slow release and it is also soluble in aqueous phases and can be easily circulated through blood[2]. The Emulsome nanocarrier technology is a lipid-based drug delivery system designed to act as a vehicle for drugs with poor water solubility. Emulsome particles consist of a microscopic lipid assembly with an internal fat core, which dissolve the water-insoluble drugs in the absence of any surfactant or solvent.

Author α σ: Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, Telangana.
e-mails: Bandaruhemanthkumar9493@gmail.com, gpfarooqahmed@gmail.com
Corresponding Author ρ: Associate Professor, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, Telangana, India.
e-mail: prasanthidhanu@gmail.com

b) Need for the invention

Undesirable side-effects are often produced when water-insoluble vehicles are used for the parenteral administration for example thrombophlebitis, hemolysis, or blood coagulation. The potential carriers for fat soluble materials are liposomes and o/w emulsions which minimize such undesirable side effects. However, there are many problems with stability and drug loading capacity which have been reported using either of these delivery systems[3].

c) Liposomes

It consists of one or more concentric phospholipid bilayers, separated by water or aqueous compartments, range from 20nm to 10 μm.

They are

1. SUV (20-100nm)
2. LUV (> 100nm)
3. REV (0.5μm)
4. MLV (2-10μm)

Effective for localized sustained release of drugs in tissues [4].

Drawbacks of liposomes

1. Unilamellar Vesicles- low content of lipid molecules so
   - Low drug loading capacity for lipophilic compounds
   - More suitable for entrapment of water –soluble materials
2. Amount of drug that can be contained therein is limited.
3. MLV liposomes- not appropriate for I.V due to large size.
4. Difficulties in preparation of acceptable liposomal formulations with long-term stability and high drug loading [2].

d) Emulsions

Emulsions are defined as "heterogeneous systems of one liquid dispersed in another liquid in the form of droplets usually exceeding 1 μm in diameter". The two liquids are immiscible and are chemically not reactive or slowly reactive. An emulsion is a thermodynamically unstable dispersed system. This instability causes reduction in its free energy by separating the dispersed droplets into two liquid phases.

Evidenced of emulsion instability during storage:

– Creaming
– Flocculation
– Coalescence [5]

Drawbacks

1. Micro-droplets of size less than 1μm should be achieved to prevent the formation of emboli in blood vessels.
2. Emulsifiers must be coated to lower the free energy at the interface and decrease the tendency of droplets to coalesce. These emulsifiers produce harmful side effects upon injection into the body.
3. It has detergent characteristics because most of them are hemolytic agents which act as membrane solubilizers.
4. Limited formulation options due to restricted emulsifiers for safe parenteral injection.
5. The water insoluble drugs such as phenytoin, amphotericin B, cyclosporin, miconazole, diazepam, etoposide, etc makes the formulation difficult for intravenous use.
6. These drugs are marketed in co-solvent systems such as polyethylene glycol or propylene glycol-ethanol-benzyl alcohol mixtures, which have shown toxicity problems, such as thrombophlebitis on injection.
7. Alternatives to cosolvent systems are micellar solutions or emulsions, but the presence of toxic surfactants in these systems makes them undesirable for intravenous administration[5].

Summary of the invention

To provide pharmaceutical compositions comprising nano-emulsions of particles comprising of lipid core composed of lipid which is in a solid or liquid crystalline phase atleast 25° C, stabilized by at least one phospholipid envelope, for parenteral, oral, rectal, intranasal or topical delivery of both fat-soluble and water-soluble drugs.

This is solid fat nanoemulsion or "EMULSOMES".

Emulsomes, having the characteristics of both liposomes and emulsions [1]

II. Formulation

1. Composition of lipid core

It exhibits solid (or) liquid crystal or mixed solid and liquid crystal phases at room temperature (25°C) when measured in bulk. Lipid compositions suitable for use as the core component of emulsomes may be characterized as being in the solid or liquid crystalline phase at least about 25°C., when measured in bulk form without incorporation into emulsomes. Some lipid compounds present in a mixture optionally may be fluids at 25°C. When pure provided that the lipid mixture as a whole is solid or liquid crystalline in bulk at 25°C. In preferred compositions, at least 90% of the individual lipid compounds present in the core are solids or liquid crystals at 25°C when measured in pure bulk form.

Phase determination is performed on the bulk lipid, incorporation into the emulsome core. The macroscopic phase determination on a bulk sample may be made on a melting apparatus or by spectroscopic means, such as IR, NMR, or fluorescence intensity or anisotropy. Bulk phase determination of an existing emulsome preparation may be performed by first extracting the core lipids, then measuring.

It consists of - triglycerides,

– Monoesters,
– Cholesterylesters & cholesterol,
– Antioxidants,
– Protein components [6]

a) Triglycerides

Available as synthetic triglycerides or mixture of several triglycerides. Fats isolated from natural sources usually are available only as mixtures of triglycerides. Such natural mixtures are suitable for preparation of emulsomes, provided that the melting characteristics of the mixture are such that they exhibit a solid or liquid crystal phase at 25°C.

Triglycerides, which are solid at 25°C have fully saturated fatty acid chains which are incapable of undergoing peroxidation reactions.

Examples of solid fats suitable for the preparation of emulsomes are:

Triglycerides composed of natural, even-numbered and unbranched fatty acids with chain lengths in the C10-C18 range, or microcrystalline glycerol triesters of saturated, even-numbered and unbranched fatty acids of natural origin such as tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin.

Partially hydrogenated vegetable oils may be used to prepare emulsomes which are free of cholesterol or cholesteryl esters [7].

In some the lipid of the hydrophobic core may have a solid to fluid phase transition (melting) temperature between 25°C and physiological temperature (37°C) when measured in bulk. For example, tricaprin melts at 35°C-37°C., and is wholly or

predominantly in the fluid phase at physiological temperature. Tricaprin may be used to form an excellent lipid core for nanoemulsions. Lipid core may be composed of lipid, which is in solid phase at 37 °C.

Ex: higher saturated triglycerides-tripalmitin and tristearin

b) Monoesters

The lipid core may contain monoesters of fatty acids such as waxes

Ex: Esters from beeswax and spermaceti-cetyl palmitate

Preferred waxes are made from saturated or monounsaturated fatty acids and saturated or unsaturated fatty alcohols.

Ex: Arachidyl oleate

Other monoesters include solid monoglycerides such as glyceryl monostearate, and fatty acid esters of short chain alcohols such as ethyl stearate [8].

c) Cholesterylesters & cholesterol

These can be incorporated into the lipid core or the surrounding phospholipid envelope. cholesterol has a polar alcohol group, it tends to incorporate into the envelope monolayers or bilayers rather than into the lipid core itself, and should be considered a component of the phospholipid envelope rather than of the core [9].

Preferred cholesteryl esters are those of saturated or monounsaturated long chain fatty acids, such as palmitoyl or oleoyl, respectively.

Cholesteryl esters may be present in levels up to 50 mol % relative to the triglyceride or other solid lipid core component [10].

d) Antioxidants

Lipid core may contain one or more antioxidants.

The need for antioxidants may be lessened by preparing the lipid core from saturated fatty acids.

Ex: Alpha tocopherol & its derivative
Butylated hydroxytoluene

e) Protein components

Lipid particles of the invention preferably do not contain serum apolipoproteins such as apo B, apo AI, apo AII, or apo E.

The apo B protein has the effect of targeting intravenously administered lipid particles to certain cellular receptors, such as the LDL receptor on hepatocytes and certain other cells.

Other proteins and peptides optionally may be present in emulsomes.

Examples of such peptides and proteins may be cyclosporin, luteinizing hormone releasing hormone (LHRH) and its analogs, calcitonin, insulin, and other synthetic or recombinant peptides.

An example of natural protein is collagen, which may be used to prepare emulsomes with controlled or sustained release properties.

2. Phospholipids: Constitute the surrounding envelope of emulsomes

Ex: 1. Natural phospholipids - soybean lecithin, egg lecithin, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, diphosphatidylglycerol, Cardiolipin, phosphatidylserine, phosphatidylcholine, sphingomyelin.

2. Synthetic phospholipids– dimyristoyl & distearoyl phosphatidylglycerol, dimyristoyl & dipalmitoyl phosphatidylcholine, Hydrogenated lecithin & phospholipids.

The phospholipid component may be either saturated or unsaturated, and may have a gel to fluid phase transition temperature either above or below 25°C.

Ex: egg or soy PC – below room temp
dimyristoyl PC – slightly below room temp
distearoyl & dipalmitoyl PC –above room temperature
emulsomes prepared with phospholipids which are in the gel phase at 37°C are expected to have more rigid bilayer envelopes and longer circulation time in plasma.

Emulsomes may be prepared with molar ratios of phospholipid to total lipid in the range of 0.1 to 0.75 (10 to 75 mol %), more usually 0.1 to 0.5 (10 to 50 mol %). The molar ratio of phospholipid to core lipid typically may be in the range of 0.1:1 to 2:1, usually 0.1:1 to 1:1, often 0.2:1 to 0.9:1, frequently 0.2:1 to 0.8:1, and commonly 0.25:1 to 0.6:1.

On a weight basis, the ratio of phospholipid to core lipid usually falls in the range 0.5:1 to 1.5:1, and frequently 0.6:1 to 1.2:1. [1]

3. Non-natural surfactants: optionally may be incorporated into emulsomes in small amount as less than 0.1% to less than 10% (mol/mol) of total surfactants.

The increasing concentrations of synthetic surfactants progressively decrease the particle size, and higher concentrations than those used are expected to result in formation of micelles (1-10 nm diameter).

4. Negatively charged lipids: These are added to the lipid phase of emulsomes to increase the zeta potential of the composition, thus stabilizing the particles.

Incorporation of these negatively charged lipid compounds in emulsomes results in the formation of phospholipid bilayers with opposing charges, thus increasing the loading of water-soluble molecules in the aqueous compartments formed by the phospholipid bilayers surrounding the lipid core.

Inclusion of negatively charged lipid molecules in emulsomes is to reduce the likelihood of particle aggregation, which minimizes destabilizing processes such as coalescence, flocculation, or fusion. Aggregation is prevented by the repulsive forces between the approaching particles.

Ex: negatively charged lipid molecule-oleic acid negatively charged phospholipid- phosphatidylglycerol, phosphatidic acid, phosphatidylserine, phosphate-dylinositol

Range is 0 to 30-mol % relative to total phospholipid & charged lipid.

5. Incorporation of drugs

Water insoluble compounds – incorporated by dissolving drug in suitable organic solvent along with other ingredients.

Water-soluble drugs- by dissolving in aqeous medium.

Categories of drugs incorporated

Antifungal, antiepileptic & anticonvulsant drugs, beta-adrenergic blockers, aids drugs, anti-anxiety agents, anti-depressants, corticosteroids, anabolic steroids, estrogens & progesterones. [11]

III. Pharmaceutical Preparations of Emulsomes

a) Method

1) Lipid film formation (Handshaking method)

Surfactants/lipids are casted as layers of film on their organic solution using flask rotary evaporator under reduce pressure (or) by hand shaking. The casted films are dispersed in aqueous. Hydration is done with constant hand shaking. The lipids will swell and get peeled off from the walls of round bottom flask at slightly above the phase transition temperature of surfactants used for specific period of time. Swelling of lipid and dispersion of casted lipid film is done by manual hand shaking or by exposing the film to a steam of water saturated nitrogen for 15 minutes, followed by swelling in the aqueous medium without shaking. Hand shaking method produce multi lamellar vesicles (MLV) and non-shaking method produced large unilamellar vesicles (LUVs).

2) Reserve phase evaporation

This technique is comprised of two steps. First prepare a water-in-oil emulsion of phospholipids and buffer in excess organic phase. Second remove organic phase under reduced pressure. The two phases of phospholipids and water are usually emulsified by mechanical methods. Remove the organic solvent under vacuum, it causes the phospholipid coated water droplets to combine to form a gel-like matrix. Further continual removal of organic solvent under reduced pressure causes the gel like matrix to form into a paste of smooth consistency, which is a suspension of LUV. Drug entrapment efficiency is achieved up to 60-65%. This method is used to encapsulate both small and large molecules. Avoid the exposure of drug to be encapsulated to organic solvents and to mechanical agitation as less as possible. Phospholipids are dissolved in organic solvents such as chloroform, isopropylether, or mix two organic solvents to adjust the density to unity that is closer to the density of aqueous phase. Biologically active molecules such as enzymes, protein pharmaceuticals and RNA type molecules may undergo conformational changes, protein denaturation, or breakage of DNA strands due to the harsh conditions of organic solvent exposure and mechanical agitation[7].

3) Ethanol injection method

It is the alternative method used for the preparation of small unilammellar vesicles (SUVs). An ethanol solution of surfactant is injected rapidly through a fine needle into excess of saline or other aqueous medium. Vaporize the ethanol for the formation of vesicles. Narrow distribution of small liposomes (under 100 nm) can be obtained by simply injecting an ethanolic lipid solution in water, i.e. in one step, without extrusion or sonication. This method is a suitable technique to obtain the spontaneous formation of emulsomes with small average radius. Alternatively, the lipid or lipid mixture is dissolved in alcoholic solvent and an aliquot of 200, 500, or 600 ml fast injected at room temperature, 1 ml syringe into the dispersant solution, which contains water or saline solution, of 9.8 ml further diluted to 1:50, 9.5 ml diluted to 1:20 or 9.8 ml diluted to 1:17, respectively. The solution was then vigorously hand-shaken for 20-30 seconds. After that the ethanol solution is fast-injected in a 5% glucose solution. The vesicles had shown average diameter of about 60 nm and may be stable for at least one week[6].

4) Cast film method

Mix the phospholipids and triglycerides in a weight ratio of 0.5:1.0 where triglycerides have a solid to liquid phase transition temperature of greater than 25°C. Suspend the mixture in an aqueous solution at a temperature below the solid to liquid transition temperature in order to reduce the suspension to yield emulsomes. These emulsomes comprise a nano-emulsion of liquid particles having a mean particle diameter between 10-250 nm usually within the range 20 to 180 nm usually and frequency within range 50-150 nm. The size range is determined on a weight percentage basis rather than a particle number basis. Usually, the lipid component may be volatile and chemically un-reactive volatile organic solvent such as dichloromethane or diethylether. Remove the solvent under reduced pressure in a rotary evaporator or under stream of inert gas. The resulting lipid film is then hydrated and dispersed by covering and shaking with an aqueous solution. If the drug component were not included in the organic solution, they may be added to aqueous hydration solution. Size the lipid suspension or dispersion at 800 pressure bars by high shear homogenizer.

5) Detergent removal technique

Phospholipids and a detergent are mixed together to form micellar mixtures. The detergent is

removed from the preparation while the micelles progressively become richer in phospholipid content and the lipids come together to form single bilayer vesicles. Methods such as column chromatography, dialysis or adsorption onto bio beads used to remove the detergent from the preparation. The dialysis technique was first reported for reconstituting biological membranes solubilized with detergents. This method is also applicable for the preparation of emulsomes. Commonly used detergents here are those with high critical micelle concentration. Ex: sodium cholate, sodium deoxycholate, and octylglycoside. In this technique detergent is removed by a flow through dialysis cell from phospholipid detergent mixture. Reports were found that this technique yielded homogeneous population of single layered emulsomes with mean diameters of 50-100nm[4].

Applications of Emulsomes

1. Entrapment of water insoluble drugs
   - Neuroprotectant drug HU-211
2. Encapsulation of water –soluble drugs
   - Adaprolol- Maleate
3. For controlled release
4. As blood substitutes or oxygen carriers
   - Stable blood–substitute perfluorodecaline formulation
   - Perfluorotributylamine formulation
5. Can be lyophilized
6. In anti-viral therapy (anti HIV)
   - AZT- CDS in emulsomes
   - Brain enhanced delivery of AZT-Q by AZT-CDS-emulsomes
7. For ophthalmic use
   - 1% Indomethacin
8. For topical use as creams
   - 1% Indomethacin
   - Diclofenac & ketoprofen
9. In anti-fungal therapy
   - Miconazole
   - Amphotericin-B
10. Antiepileptic & anticonvulsant
    - Diazepam
    - Phenytoin
11. For sustained & targeted delivery
    - Zidovudine – to liver
12. For immunization
    - HIV-1 neutralising antibodies in genital & respiratory tracts of mice intranasally immunized with oligomeric gp160 formulated in emulsome[3]

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Usage of Nivolumab- Platinum Containing STAT1 Molecule for Suppression PD-1/PD-L1 Genes in PD-1/ PD-L1 Expressing Cancer Cells

By Waleed O. Atta
Assiut University

Abstract- Blockage of PD-1 proteins by immune checkpoint inhibitors showed an accepted therapeutic effect in cancer. But; tumor microenvironment exerts its antitumor influence by various mechanisms. Malignant cells have the ability of PD-1/PD-L1 protein over synthesis, which can be a defense action against immune checkpoint inhibitors and immunotherapy. Binding nivolumab with platinum-containing STAT1 will be used to reduce PD-1 genetic level. Nivolumab has the option of endocytosis, while STAT1 is the transcription factor that binds to the DNA, specifically PD-1 gene. STAT1 is the activated protein in response to multiple cytokines stimulation of cancer cells, which are the same for increasing PD-1/ PD-L1 upregulation. The used STAT1 in our therapeutic strategy is the activated form and loaded with platinum particles for damaging DNA bases in PD-1 promoter regions upon translocation to the nucleus. STAT1-platinum molecule is connected to nivolumab Fc region by solamargine polymer for selective cancer cell targeting.

Keywords: A5 complex, IFN-γ endocytosis, cytokines, solamargine, phosphorylation.

GJMR-B Classification: DDC Code: 784.2 LCC Code: M3.1

Strictly as per the compliance and regulations of:

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I. Background

a) PD-1/ PD-L1/PD-L2 Levels and Functions

PD-1 is a surface glycoprotein and is presented on CD4+ and CD8+ T cells, natural killer [NK] cells, natural killer T [NKT] cells, B cells, macrophages, and dendritic cells [DC] subsets. Also; PD-1 is expressed on tumor cells and TAMs [tumor associated macrophages] of many cancer types such as melanoma, non-small cell lung cancer, and head and neck squamous cell cancer [Baumeister et al. 2016]. PD-1 surface receptors should be suitable because it controls the immune balance towards self-antigens. Its deficiency or increased level causes altered immune response, lethal immune response and autoimmune disorder. PD-1 deficiency in murine models by genetic knockdown or blocking its signaling pathway results in serious immunopathology during acute infection via elevated levels of cytokines that result in tissue damage [Barber et al. 2006, Frebel et al. 2012]. Other harmful possibilities can occur, such as autoimmune dilated cardiomyopathy and autoimmune encephalomyelitis. [Sage et al. 2018] PD-1 has an inhibitory function on binding with PD-L1 and PD-L2 expressing cells [Latchman et al. 2001]. PD-L1 and PD-L2 receptors are expressed on hematopoietic cells such as CD8+, CD4 + T cells, B cells, dendritic cells, macrophages and non-hematopoietic cells like hepatocytes, vascular endothelial cells, epithelial cells, myocytes, pancreatic islet cells, placenta and eye cells. Also, PD-L1 and PD-L2 are expressed on tumor cells and stromal tumor cells [Sun et al. 2018]. PD-1 inhibitory signals play a critical immune modulatory response by induction regulatory [Treg] and natural [T reg]. As result, immune modulatory molecules, such as anti-inflammatory cytokines transforming growth factor-b [TGF-b] and interleukin-10 [IL-10], are secreted [Attanasio et al. 2016]. Activated Treg cells show high PD-1 levels, and their blockage will inhibit Treg cells` essential function.

PD-1 is overexpressed on M1 and M2 macrophages within the tumor tissue that represent tumor-associated macrophages [TAMs]. M1 macrophages have an early tumorigenic effect, while M2 macrophages stimulate metastasis [Tamura et al. 2018, Pollari et al. 2018] PD-1 receptors within the tumor different cells inactivate T cells, B cells, Natural killer cells and dendritic cells, by that way it inhibits the phagocytic action of T cells and other cellular immune response against tumor cells [Gordon et al. 2017]. The cytoplasmic tail of PD-1 entails two structural motifs: ITIM and ITSM. Once binding to PD-L1/PD-L2, the tyrosine residues are phosphorylated, which permits the efficacy of cytoplasmic tyrosine phosphatases such as SHP2. These phosphatases attenuate the signal of the TCR and CD28 [Berraondo, 2019]. PD-1 expressing tumor cells by that mechanism can convert CD8+cytotoxic cells into exhausted cells. PD-1 can stimulate and induce T regulatory cells to consider tumor antigens as self-antigens and escape from phagocytosis within the tumor microenvironment [Jiang et al. 2015]. Also, PD-1 disturbs T cell metabolism by glycolysis suppression and lipolysis stimulation [Patsoukis et al. 2015].

b) PD-L1/ PD-1 Overexpression and Association with Stat1 Level

Tumor cells can induce stromal cells and TAMs to express PD-L1 directly by cell to cell contact or indirectly through secretion specific mediators such as IL-4, IL-6, IL-10, IL-13, CXCL8, SPP1 and IFN- γ [Lu et al.

Author: Faculty of medicine, Assiut University, Sohag, Egypt.
e-mail: dangersurgery@gmail.com

2019]. In that way, tumor cells recruits surrounding cells for macrophage resistance by PD-L1 increased transcription. STAT1 is activated by the same activators of PD-L1 such as IL-4, IL-13 [Wang et al. 2004], CXCL8 [Chen et al. 2019] α and TNF-[Wang et al. 2000].

The number of PD-1 membrane receptors is increased by IFN- γ that activates Janus Kinases [JANs] that phosphorylate STAT1; in turn, activated STAT1 is transferred to the nucleus and acts as a transcriptional factor to enhance interferon-stimulated genes replication [ISGs] [Walter MR 2020]. Also, Anti-PD-1 antibody activates STAT1 through IL-12 activation [Lu et al. 2109].

STAT1-Pt molecule selection to be included in a therapeutic approach after linking to nivolumab can be an effective therapy. As illustrated above, PD-1/PD-L1 transcriptional cascade reactions by cancer cells share the same activators of STAT1. So, PD-L1 and PD-1 enhancement is resulted by malignant cells` mediators against nivolumab, helping STAT1-Pt transported to the nucleus [fig 1]. So endocytosis of the nivolumab-activated STAT1-Pt complex into the cancer cell will permit deposition of platinum loaded on STAT1 onto the Nucleus [in response to malignant cell defense], specifically PD-1 and PD-L1 gene promoter regions. Also that can be upregulated by the advantage of nivolumab that can be endocytosed more than other anti-PD1 antibodies [Ben Saad et al. 2020].

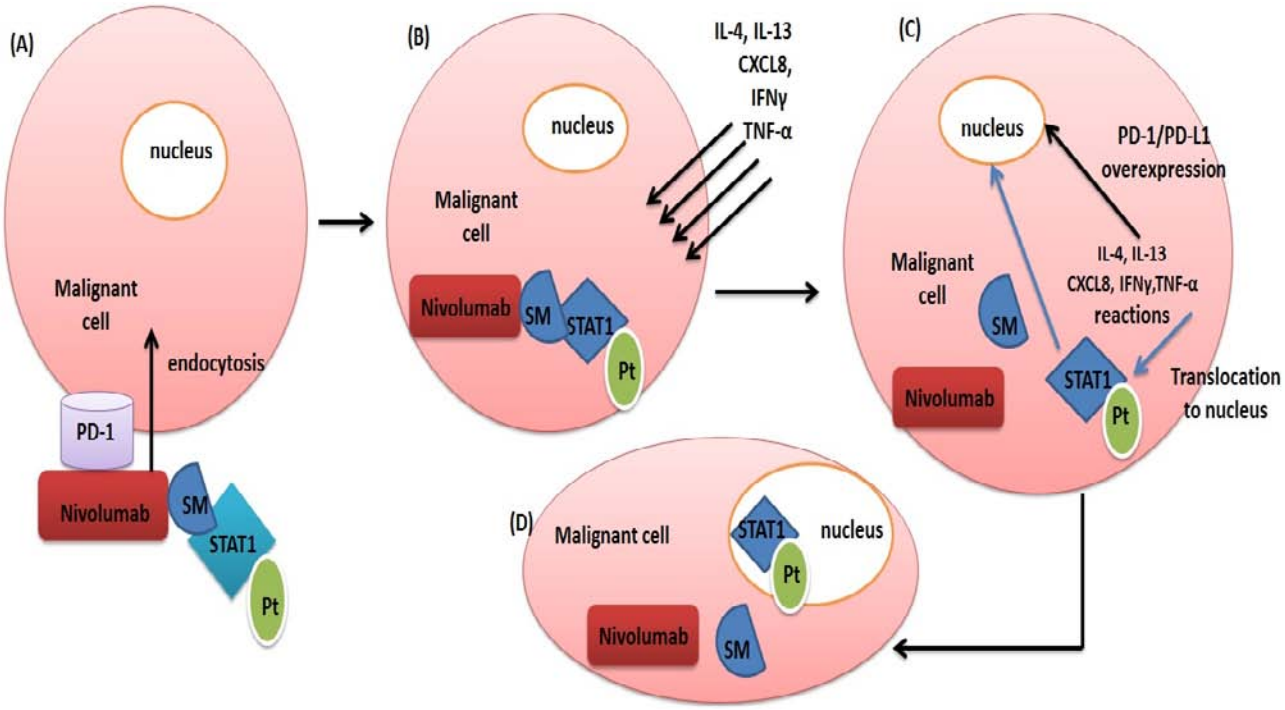


Figure [1]: Stages of nivolumab-STAT Pt endocytosis and functioning.[A] Endocytosis of the molecule into the malignant cell after binding to PD-1 receptor.[B] Nivolumab is dissociated from STAT1-Pt by the action of malignant cell glycosidase enzymes and releasing solamargine polymer [SM] free within the malignant cell cytoplasm. Malignant cells secrete IL-4, IL-13, CXCL8, TNF-α and IFNγ for PD-1/PD-L1 genetic stimulation by various intracellular signals. [C] IL-4, IL-13, CXCL8, TNF-α and IFNγ stimulate signaling pathways for elevation PD-1 surface proteins and simultaneously STAT-1-Pt translocation to the malignant cell nucleus.[D] STAT1-Pt attaches to the malignant cell nucleus [PD-1 promoter region] the platinum molecules loaded on STAT1 making adducts with PD-1 promoter region adenine and guanine bases, so damaging the base of PD-1 gene.

c) Solamargine Specific Anti-Tumor Properties

Solamargine`s selective anti-cancer efficacy makes it a candidate for directing nivolumab specifically to malignant cells. Solamargine used in treatment of cancer cell lines of Ehrlich Carcinoma, Leukemia [K562], Colon Cancer [HT-29, HCT-15], Liver Cancer [HepG2, PLC/PRF/5, SMMC-7721], Lung Cancer [A549], Gastric Carcinoma [AGS], Pancreatic Carcinoma [MIA, PaCa-2], Renal Adenocarcinoma [786-0], Uterine Adenocarcinoma [HeLa 229], Ovarian Carcinoma [JAM], Mesothelioma [NO36], Glioblastoma, Astrocytoma [U87-MG], Prostate Carcinoma [DV-145, LNCap, PC-3], Melanoma [A2058], Breast Cancer [T47D, MDA-MB-231], Osteosarcoma [U20S] and Squamous Cell Carcinoma [A431, SCC4, SCC9, SCC25][Bill, 2013].

Solamargine has multiple anti-cancer mechanisms such as stimulation the intrinsic and extrinsic pathways of apoptosis, increased function of external death receptors [TNFR-1, Fas receptor, TNFR-

1-associated death domain [TRADD], Fas-associated death domain [FADD], elevation of the intrinsic ratio of Bax to Bcl-2 and oncosis [Sun et al 2010].

Solamargine polymer will be the bridging molecule between nivolumab [after glycosylation its Fc portion] and STAT1-Pt complex. [Chemical and biochemical reactions will be discussed later] Nivolumab, like other immune checkpoint inhibitors, are related to exaggerate immune-related Side effects, such as colitis, hepatitis and skin disorders because of cross-reaction with healthy PD-1 presenting hematopoietic cells [Dyck et al. 2017]. Those autoimmune adverse reactions can be avoided by solamargine, in other words, it will restrict nivolumab binding with normal PD-1 presenting cells and will facilitate selectivity towards PD-1 expressing malignant cells and TAMs only.

Solamargine molecular formula is C45H73NO15 with a mass of 868.04 Da. Its systematic name is [22R, 25R]- spiro-5-ene-3ßL-α -L-rhamnopyranosyl-[1 2glu]-0-α--yl-rhamnopyranosyl-[14glu]-ß-D-glucopyranose. [fig.2]

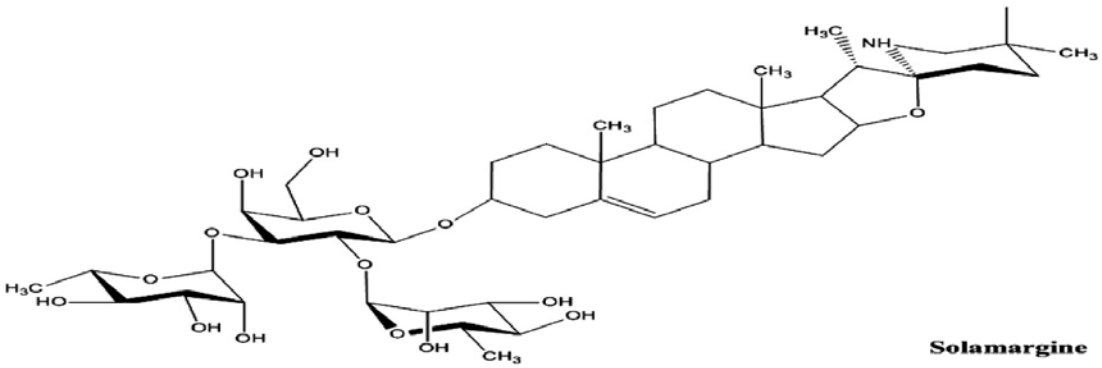


Figure [2]: Molecular formula of solamargine.

d) Structure of Nivolumab-Stat1- Platinum Molecule

The innovated molecule consists of nivolumab [anti PD-1 MAB] glycosylated with glucopyranose of solamargine. Solamargine β-solamargine polymer through its Fc region with is bound to glycosylated cisplatin molecules loaded on seven lysine residues of biochemically activated synthetized STAT1.[Fig 3]

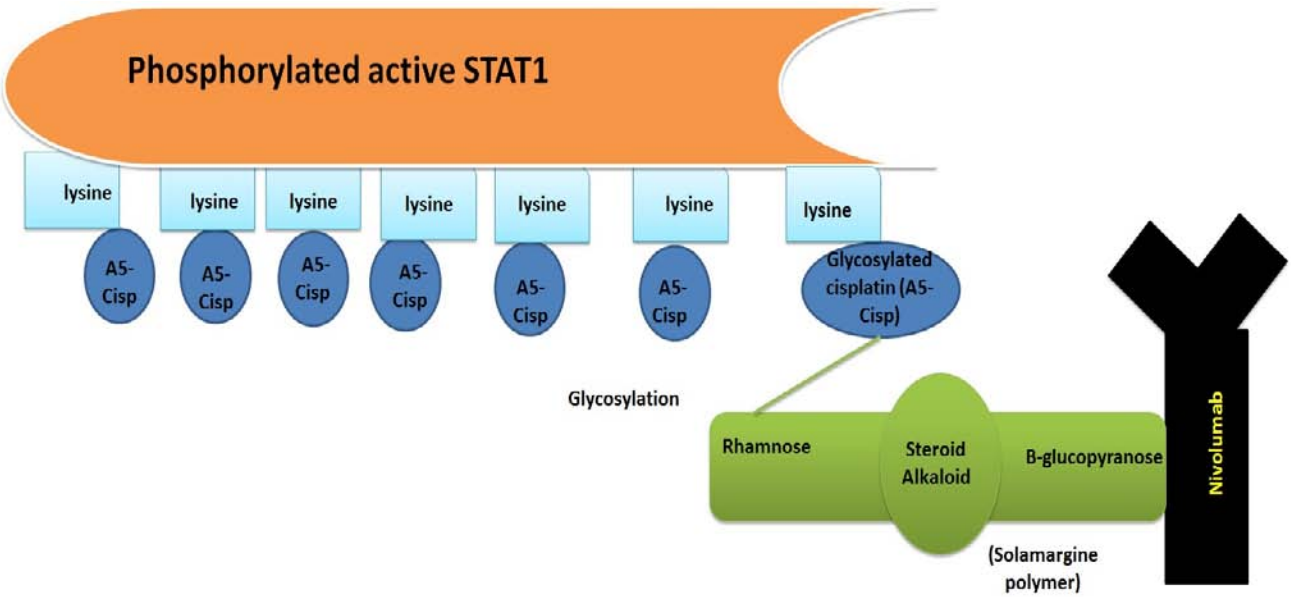


Figure [3]: Structure of nivolumab–STAT1 Pt molecule. Nivolumab Fc portion is glycosylated glucopyranose residue]. Glycosylated cisplatin molecules are β-with solamargine polymer [loaded on STAT1 lysine residues [reaction discussed later]. Glycosylated cisplatin is attached to solamargine polymer [rhamonse moiety] by rhamnosyl transferase.

e) Nivolumab-Stat1 Pt Therapeutic Mechanisms

On Nivolumab-STAT1 Pt administration, it runs within the body circulation towards PD-1 presenting malignant cells due to the presence of multiple targeting elements. The first is nivolumab`s nature a monoclonal antibody [Anti-PD1] however, it can be directed towards PD-1 expressing hematopoietic cells such as CD4+ and CD8+ T cells, natural killer [NK] cells, natural killer T [NKT] cells, B cells, macrophages, and dendritic cells resulting in immunosuppression. Also, it can bind to hepatocytes, vascular endothelial cells, epithelial cells, myocytes, pancreatic islet cells, placenta and eye, initiating autoimmune adverse reactions. Here the role of solamargine polymer comes. Solamargine glycoside is considered as an attracting factor for cancer cell requirements for proliferation and spread. Also, it is characterized by selective tumor cell binding. The third is the activated STAT1 which is the needed transcriptional factor for malignant cells to overexpress PD-1 as a defending pathway against nivolumab. So cancer cells and TAMs will uptake the activated STAT1 because it is their rescue to escape from the immune system, After all, it is responsible for increasing PD-1 expression on their surface membranes.

Also, nivolumab-STAT1 Pt molecule is a concentrated anti-cancer therapeutic molecule. Nivolumab is a human immunoglobulin G4 PD-1 immune checkpoint inhibitor antibody that attenuates PD-1 interaction with PD-L1/PD-L2 receptors and stimulates anticancer immunity. It showed good therapeutic parameters [prolonged PFS and increased response rate] in the treatment of non-small-cell lung cancer [NSCLC], melanoma, renal cell carcinoma [RCC] and other cancers. [Guo et al. 2016]. Nivolumab [IgG4] Fc region consists of double heavy-chain Cγ2 and Cγ3 constant domains that are bound to two Fabs, comprising VH and Cγ1 [heavy chain] and VL and Cj/k [light chain] domains, through a hinge. The Fc region has the dominant role for functioning. A biantennary oligosaccharide moiety, covalently attached to Asn297 in the Cc2 domain, contains two N-acetylglucosamine residues, and a branching mannose residue to which α[1–3] and α[1–6] 'arms' of mannose and N-acetylglucosamine residues are attached. The oligosaccharide moiety can additionally contain a fucose residue, attached to the first N-acetylglucosamine residue, and galactose and sialic acid residues attached to the α[1–3] and α[1–6] arms [Davies and Sutton 2015.].

Activated STAT1–Pt molecule will be a trap for malignant cells. Malignant cells use activated STAT1 for tumor spread and immunity resistance [Messi et al. 2017]. This is observed in the reduction of NK cellular activity in multiple myeloma, acute myeloid leukemia [AML], and acute lymphoblastic leukemia [ALL] [Bellucci et al. 2015] after IFNγ stimulation. While in head and cancer, wild type of EGFR induces JAK2/STAT1 activation that promotes the antitumor effect by PD-L1 over-transcription [Concha-Benavente et al. 2016] Interferon regulatory factor 1 [IRF1] , which is a downstream activator of STAT1 just after IFNγ stimulation, has an enhancing effect of PD-L1 genetic activity [Lee et al. 2006].

Activated STAT1 contains 2-acetyl serine which is essential for protein integrity [Bienvenut et al. 2012]. Lysine residues 114, 175, 29, 366, 525, 637 and 665 are methylated. Methylation gives the advantage of the antiviral function. Methylation is done by methyltransferase SETD2 [Chen et al. 2015]. Lysine residues without methylation are the target ones to be conjugated with glycosylated cisplatin molecules. That conjugation will not affect STAT1 function in malignancy. Glutamic acid residues 657, 705 are ADP-ribosylated by PARP 14. Glutamic acid ADP ribosylation suppresses STAT1 phosphorylation [Iwata et al 2016]. During synthesis and purification of activated STAT1-Pt molecule, ADP ribosylation of glutamic acid will be avoided. Tyrosine 701 residue is phosphorylated in response to Janus protein-tyrosine kinase and epidermal growth factor receptor stimulation after IFNγ induction [Quelle et al. 1995, Iwata et al 2016]. Tyrosine 701 phosphorylation also is one by KIT-Asp [816] mutants in neoplastic mast cell lines [Chaix et al. 2011]. Serine residues 708, 745 phosphorylation occurs through IFN-α/β induction by IKKε, so serine residues 708,745 phosphorylation is essential for STAT1 activation [Perwitasari et al. 2011]. Serine 727 phosphorylation and tyrosine 701 phosphorylation is really necessary for STAT1 activation. Serine 727 phosphorylation occurs by the action of etoposide and PKCdelta. [Brodie and Blumberg 2003, Wen et al. 1995]

Glycosylated cisplatin are combined with purified active STAT1 lysine residues. Glycosylated cisplatin [platinum IV] are prodrugs that undergo activation to platinum II by malignant cell reductants such as ascorbic acid and glutathione. Being a prodrug and activation inside malignant cells only minimize possible side effects to a significant extent. Glycosylation helps attachment to lysine residues and at the same time, glycosylation to rhamnose residues of solamargine [fig.2]. Also, platinum IV drugs are favored other than platinum II ones because they are more stable and have longer half-life than platinum II drugs. Glycosylation adjusts steric hindrance and length to enable cisplatin for a reduction potential and positive shift to the cancer cells. It is not forgettable that platinum IV drugs have lipophilicity more than platinum II. Lipophilicity permits more access of platinum IV drugs for tumoral cellular uptake and DNA adenine-guanine platination. The used platinum IV drug in STAT1-Pt is A5 complex of cisplatin [fig. 4]. It is known that A5 has more efficacy towards HeLa, A549, MCF-7 and PC3 cancer cell lines other than cisplatin and oxaliplatin. [Jing, et al., 2016].

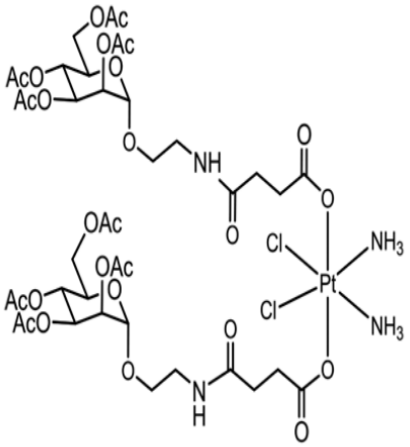


Figure [4]: Structure of A5 cisplatin complexes.

f) Chemical and Biochemical Steps for Nivolumab-Stat1-Platinum Molecule

1. *Purification of activated STAT1:* HeLa cells have a major role in STAT1 cultivation. HeLa cells will be incorporated with Lysine 6- dehydrogenase gene delivered by exosome pDNA [plasmid DNA] containing the enzyme gene [Munagala et al. 2021], then keeping PH 10.1 and temperature 70 o C [Heydari et al. 2004]. 70°C will not affect STAT1 protein integrity because its denaturation temperature is 95°C [Sisler et al. 2015]. The resulted STAT1 within HeLa cells will be unphosphorylated and deaminated lysine residues. Then IFN stimulation of HeLa cells for tyrosine phosphorylation. [Kim and Maniatis 1996]. However, it is inactivated in the nucleus by unknown tyrosine phosphatase PTP and purified as Stat1-PTP from HeLa nuclear extract. [ten Hoeve et al. 2002] Then E6-E6AP complex [one of the Human Papilloma Virus E6 oncoproteins] can be used to combine with Stat1-PTP and degrade it in vitro to yield purified phosphorylated STAT1 [Jing et al. 2007]. The purified STAT1 is phosphorylated at its tyrosine 701 and serine 727 because of IFN stimulation besides deaminated lysine residues 114, 175, 296, 366, 525, 637 and 665, and that is the wanted form to be used in our molecule [fig.5].
2. A5 complex molecules of cisplatin will be reacted with deaminated Lysine residues of purified STAT1 [not methylated] by lanthionine biosynthetic enzyme B [LanB] proteins in the presence of glutamate, ATP and Mg2+. [Garg et al. 2103]. This in vitro dehydration reaction between the glycosidic component of 7 molecules of A5 complex cisplatin and 7deaminated lysine residues of phosphorylated STAT1 to result in STAT1-A5 cisplatin molecule. [fig.6]
3. Production of glycosylated Nivolumab with solamargine polymer: Transgenic immunization for human immunoglobulin loci with genetically recombinant Chinese hamster ovarian cells expressing human PD-1/PD-L1/human IgG1 Fc fusion protein. [Mimura et al. 2018]. The core complex biantennary heptasaccharide attached to the purified nivolumab Fc region is GlcNAc2Man3GlcNAc2. Previously mentioned heptascchride can be attached to G0, G1 or G2 saccharide according to the number of galactose residues.G0 has no galactose residue, and G1 has one galactose terminal, while G2 has two galactose residues. [Mimura et al. 2018]. 6-glucosyl-transferase enzyme [CaUGT3] can elongate the heptasacchride G0/G1/G2 of nivolumab as a sugar acceptor to ß-D-glucopyranose of solamargine. [Masada et al. 2009]. The enzymatic assay is used with the purified nivolumab using quercetin 3-O-glucoside as an acceptor substrate in the presence of UDP-glucose. The same retention time and UV absorption of quercetin 3-O–gentiobioside result in nivolumab [one side Fc region] with solamargine polymer [Masada et al. 2009]. [fig.7]
4. Solamargine rhamnose moiety is transferred to one A5 complex molecule of cisplatin [attached to STAT1] by rhamnosyltransferases besides the nucleotide diphosphate-sugar UDP-rhamnose [UDP-Rha] as a substrate to result in STAT1-Pt-Nivolumab molecule [Lairson et al. 2008] [fig.3].

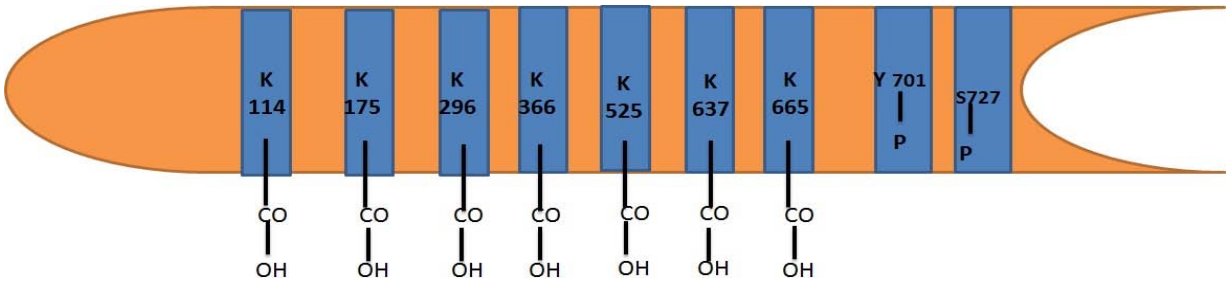


Figure (5): Structure of purified STAT1 that contains phosphorylated tyrosine 701, phosphorylated serine 727 and seven (114, 175, 296, 366, 525, 637, 665) deaminated lysine residues

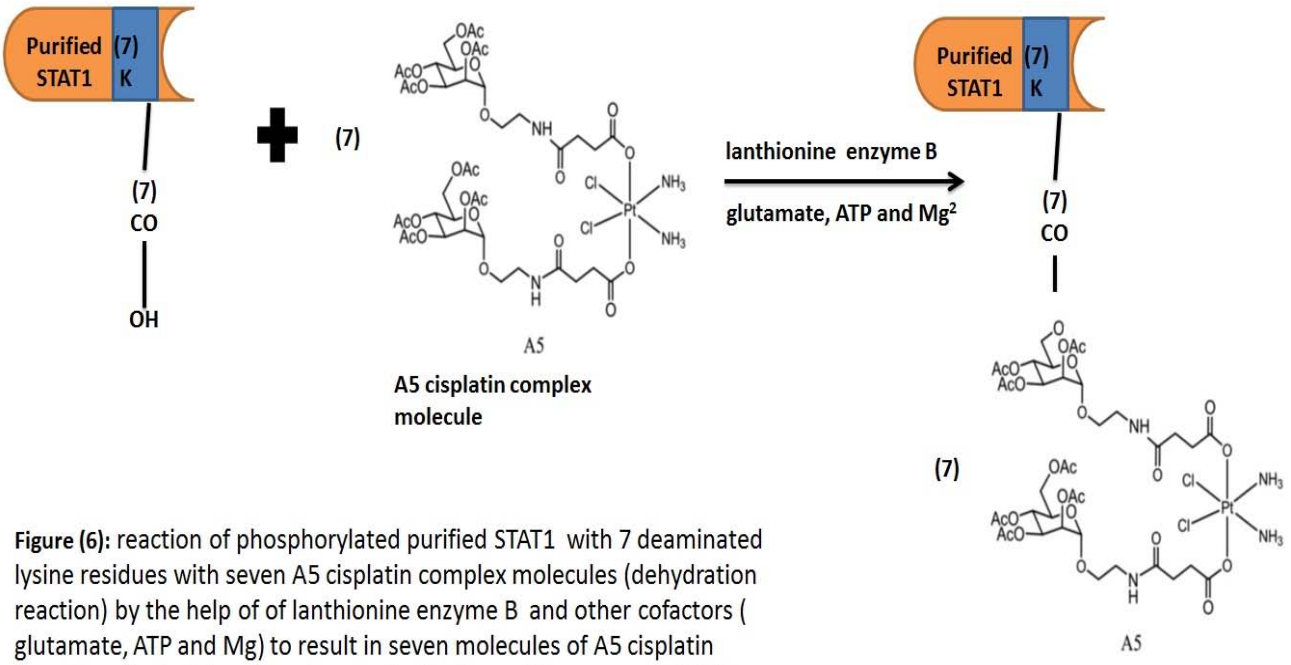
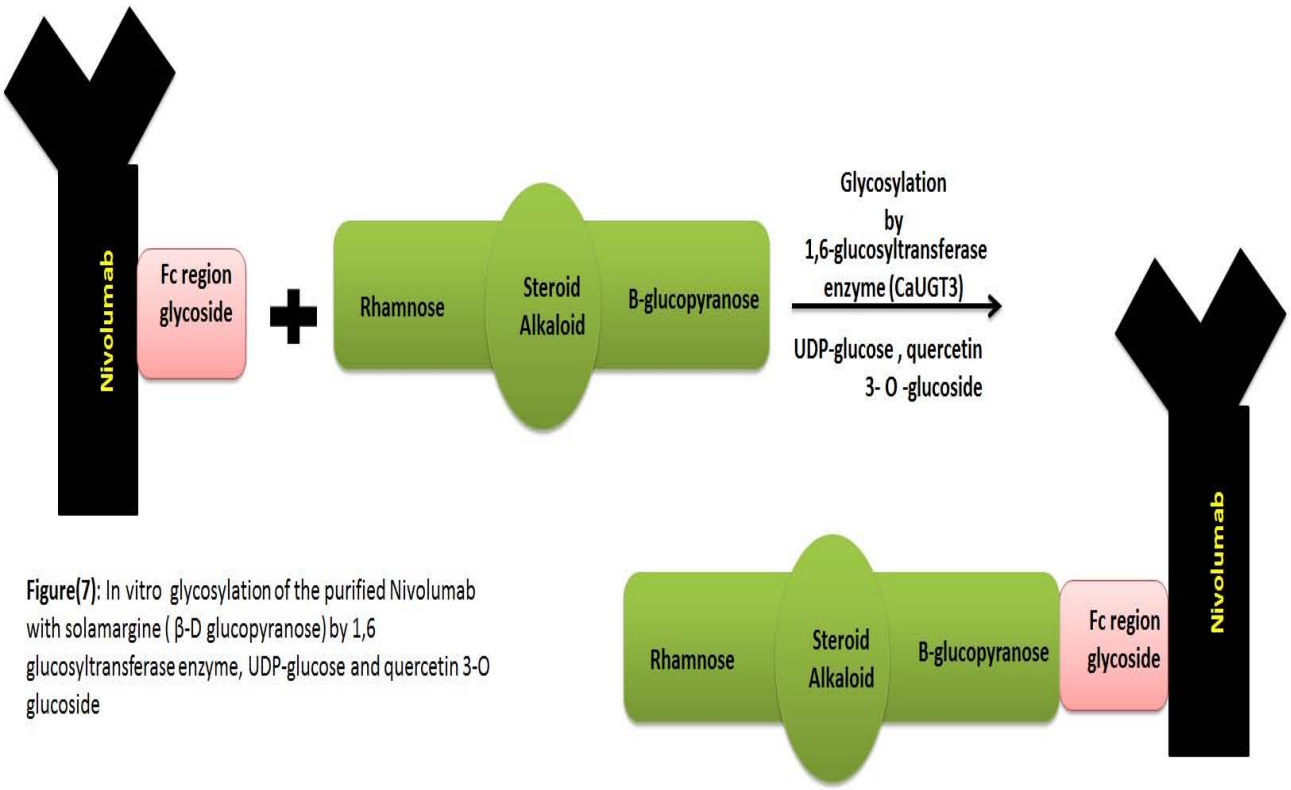


Figure (6): reaction of phosphorylated purified STAT1 with 7 deaminated lysine residues with seven A5 cisplatin complex molecules (dehydration reaction) by the help of of lanthionine enzyme B and other cofactors (glutamate, ATP and Mg) to result in seven molecules of A5 cisplatin complex attached to seven deaminated lysine residues on one STAT1 molecule.



Figure(7): In vitro glycosylation of the purified Nivolumab with solamargine (β-D glucopyranose) by 1,6 glucosyltransferase enzyme, UDP-glucose and quercetin 3-O glucoside

II. Conclusion

STAT1-Pt nivolumab molecules are targeted towards malignant cells only by the bridging solamargine glycoside. After binding nivolumab to PD-1 expressing malignant cell, endocytosis occurs. Here, glycosidic bonds of solamargine - nivolumab and solamargine- A5 cisplatin STAT1 are hydrolyzed by malignant cell glycosidase enzymes. STAT1- Pt [A5 cisplatin molecules] are transported to the nucleus and seven molecules of platinum IV of A5 cisplatin complex molecule are reduced by malignant cell reductants [glutathione and ascorbic acid] to functioning cytotoxic platinum II. The active form of the used STAT1 in the therapeutic molecule is essential because it does facilitate its nuclear translocation upon different cytokines and IFN-γ secretion by malignant cells [malignant cells use those mediators for over recruitment PD-1/PD-L1 genes to resist nivolumab]. So the more mediators secretion, the more STAT1-A5 cisplatin movement to the malignant cell nucleus. In the end, the aim of the molecule is reached, which is damaging PD-1/PD-L1 genetic promoter regions by multiple concentrated platinum containing STAT1 molecules. Also anti-tumor role of endocytosed solamargine is not forgotten as it becomes free after glycosidic bonds hydrolysis. While nivoulmab exerts anti PD-1 signaling pathway, it can be considered a targeting molecule besides solamargine towards PD-1 expressing malignant cell for initiating the cytotoxic reactions of the innovated therapeutic molecule.

Conflict of Interest

Authors have no conflict of interest.

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Abbreviations

STAT1: Signal transducer and activator of transcription 1.
PD-1: Programmed death 1
PD-L1: Programmed death ligand 1
NK: natural killer
NKT: Natural killer T cells
DC: Dendretic cells
TGF-b: cytokines transforming growth factor-b
IL-10: interleukin-10
TAMs: tumor associated macrophages
JANs: Janus Kinases
IFN- γ: interferon γ
ISGs: interferon stimulated genes

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Introducing the Subject into Primary Care - Person-Centeredness in Primary Care Needs Participatory Research

By Ottomar Bahrs
University of Duesseldorf

Abstract- Our health care system is considered to lack comprehensible orientation. As for the specific field of action in family medicine, it is hard to convince medical students and graduates of its positive aspects. People often criticize that general practitioners act very differently and that there is a lack of a shared professional self-image. However, there is unanimous agreement that family medicine relates to people in their health and illness, i.e. it is person-centered.

The present paper assumes that a "hermeneutical approach to the case" could create common ground for a more explicit profession-related self-concept. According to the German Society for General and Family Medicine (DEGAM), the hermeneutic understanding of cases is characteristic of general practice. However, there is no operational definition, so this is not part of education and training. The presentation refers to the discussion of "The Patient as Text", which is put up, especially in English-speaking and Scandinavian countries.

Keywords: hermeneutic understanding of the case; general practice; dialogue-based medicine; introduction of the subject; person-centeredness; participatory research.

GJMR-B Classification: DDC Code: 331.11 LCC Code: RA410.7

Strictly as per the compliance and regulations of:

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Ottomar Bahrs

Abstract- Our health care system is considered to lack comprehensible orientation. As for the specific field of action in family medicine, it is hard to convince medical students and graduates of its positive aspects. People often criticize that general practitioners act very differently and that there is a lack of a shared professional self-image. However, there is unanimous agreement that family medicine relates to people in their health and illness, i.e. it is person-centered.

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Family medicine can be understood as a series of interpretative actions. Thus, family medicine is part of a hermeneutical venture. One of the cases that need to be understood is the doctor himself.

Our analysis depicts tacit knowledge in (social and medical) practice based on the individual experiences of those involved and their ancestors. These prior experiences may have settled into routines and institutional rules. Turning tacit knowledge into explicit knowledge would encourage a common ground for a clearer self-concept of family practice both as a practice and as a science. Scientifically accompanied (interdisciplinary) quality circles are suitable for this. They function as further training and research instruments in participatory research and implementation. Quality circles contribute to making hermeneutic case understanding teachable and learnable.

Person-centered medicine - understood as care of persons for persons by persons and with persons - requires that all participants engage as subjects. In this participatory process, traditional roles can and must be redefined. Health and illness are, at the same time, profoundly personal and social affairs.

Keywords: hermeneutic understanding of the case; general practice; dialogue-based medicine; introduction of the subject; person-centeredness; participatory research.

Author: Umbrella Organisation Salutogenesis, Göttingen, Institute of General Practice, University of Duesseldorf, Germany.
e-mail: obahrs@gwdg.de

I. Introduction

According to their self-image as generalists, G.P.s specialize in the whole person (1) and thereby apply a biopsychosocial approach to health and illness (2). However, to synthesize the various aspects in a case-specific way, G.P.s - and other health professionals - must apply their specialist knowledge to specific situations and cases. For this, they need a "*hermeneutic understanding of the case*" (3, 4): "General Practice takes somatic, psycho-social, socio-cultural and ecological aspects into account. When interpreting symptoms and findings, it is particularly important to appreciate the importance of the patient's understanding of his disease, his environment, and personal history (hermeneutic approach). (3)

However, physicians do not learn these basic skills in education, training and continuing education. Fortunately, most of them already have some of the relevant competencies based on their pre- and non-professional experience and continue to develop them in their everyday practice. Ortmann, therefore aptly speaks of the "*family doctor as an inventor*" (5). Accordingly, GP's action varies with the practitioner and the respective contexts, which are given, among other things, by the respective biographical experiences, the current life situation, the framework conditions of the treatments, and the relationship of the participants. Person-centered medicine becomes possible by encountering "whole people" in a specific setting. So far, this core of GP activity has received little attention in the science of general practice, which tends to focus on the formality of medical roles and the rule-based nature of procedures.

Viktor von Weizsäcker, the founder of the Heidelberg School of Anthropological Medicine, had already called for the "introduction of the subject into medicine" in the first third of the 20th century and justified this epistemologically with reference to the findings of physics, morally against the background of human rights and clinically under the aspect of value-guided coordination processes on treatment goals (6, 7). Furthermore, Weizsäcker underlined that the relationship between the sick person (or person seeking help) and his or her helper is a companionship, independent of professional specialization, and should

be the basis of general (or anthropological) medicine (6). More recently, similar considerations have been taken up again under the aspect of person-centeredness.

The characteristic features of person-centeredness are conceptually derived from the findings of various sciences (such as philosophy, psychiatry, pedagogy, sociology, systems theory, neuroscience, and linguistics, for example) on the one hand and on the experiences of practicing health professionals ("reflective practice") on the other. Mezzich names the following principles in his overview:

- Ethical commitment
- Cultural awareness and responsiveness
- Holistic approach, relational focus
- Individualization of care
- Collaborative diagnosis and shared decision-making
- People-centered organization of services
- And person-centered education and research (8).

Thus, person-centered medicine can be defined in summary by Mezzich et al. as *"promoting the care of the person* (of the totality of the person's health, including its ill and positive aspects), *for the person* (promoting the fulfillment of the person's life project), *by the person* (with clinicians extending themselves as full human beings with high ethical aspirations) *and with the person* (working respectfully, in collaboration and in an empowering manner)". (9, emphasis mine, OB)

II. From "Patient as Text" to Dialogue-based Medicine

In an essay published in 1986, Daniels emphasizes that medical action has always implied interpretative acts (10). However, the art of interpretation ("hermeneutics"), anchored in the humanities, receives little attention as a methodological procedure in medicine. He proposes to understand the patient as a "text" analogous to literary works, distinguishing four levels:

- The primary text refers to bodily layered life experiences that are expressed, among other things, in symptom formations.
- For these to become legible, a (spoken and written) medical history must be collected and, thus, a secondary text generated.
- The physician's task is then to interpret these two texts, which leads to a diagnosis and, using documentation, to a third-order text.
- Therapeutic action constitutes the fourth text by (re)interpreting the patient's problem regarding the patient's social context. To do this, the therapist must empathically engage with the patient and use a prior understanding of what is meaningful for the patient ("hermeneutic circle"). However, the practitioner is not unaffected by this, so the consultation extends into the lifeworld of both participants.

Daniels' proposal was taken up, critically discussed, and expanded. Not only does the doctor decode the messages, but this takes place constantly in everyday life in concrete interactions and self-reflexive acts and is also embedded as professional action in dialogues. At least the following dimensions can be distinguished:

- Patient as "text" - doctor as "interpreter" (10)
- Patient as "text" and as "interpreter" (11)
- Patient as "text" and as "interpreter" - doctor as "interpreter" against the background of pre-existing "scripts" ("illness is between people", (6))
- Patient as "text" - patient and doctor as joint interpreters and authors of a modified "text" (12)
- Patient as "text", doctor as "con-text" - patient and doctor as collaborative interpreters and authors of modified "texts" (13)
- Patients and physicians as individual and collaborative readers and texters against the background of existing and changing scripts.

III. The Layering of Meaning

The layering of meaning is a process (table 1): The members of a society are born into a field of meaning that has materialized in symbols and is constantly produced and expanded in concrete interactions [1]. This social body of knowledge contains ideas about health and illness and the appropriate way to deal with them. On an individual level, life-world experiences [2] form the background for (possibly crisis-like) (bodily) experiences of people who are/will potentially become patients [3] and evaluate their experiences themselves against the background of their concepts of health and illness [4]. If those affected - or their relevant reference group - ascribe disease value to the phenomena, this can lead to medical care utilization. The patient's experiences and self-interpretations thus become symptoms [5], perceived by the physician [6], and recorded in the form of findings [7]. The doctor synthesizes them into diagnoses [9] against the background of his or her individually and professionally developed health/illness and treatment concepts [8]. The doctor's own crisis management experiences [10], the shared treatment history [11] as well as the patient's readiness for treatment and possibility of change [12] frame - in addition to the inherent dynamics of the processes to be interpreted and the resources available in the social environment - the prognosis [13], based on which the therapy plan [14] is developed. Whether the therapy is helpful or not [16] is ultimately determined by whether the patient (and his or her reference system) can successfully cope with (modified) everyday life [15].

Defining "success" is ultimately left to the patient [17] and is done about a future-oriented concept of a meaningful life. However, these assessments are potentially in competition with interpretations made from other perspectives (in particular, the doctor, social reference system, and funding agency) [18].

Table 1: The layering of meaning

1. prior social production of meaning fields and symbols	
Patient's lifeworld	2. individual experiences of crisis and coping
	3. current (crisis) (bodily) experiences of (potential) patients
	4. health and illness concepts of (potential) patients
	5. complaints and symptoms
Medical world	6. perceptionsofthedoctor
	7. findings
	8. health/disease and treatment concepts of the doctor
	9. diagnoses
	10. crisis management experiences of the doctor
	11. joint treatment experiences of patient and doctor
	12. the patient's willingness to change/treat
	13. prognosis
	14. therapy plan
Patient's lifeworld	15. patient's willingness and ability to implement treatment
	16. changed everyday coping
	17. patient'sfeelingofrecovery
18. evaluation as therapy (failure) success	

The question is no longer whether medical action is (also) a hermeneutic endeavor but how this is done. In particular, one has to clarify which "case" is to be understood in the sense of the GP's way of working.

IV. The View of the Case

According to a widespread understanding in medicine, patients represent individual manifestations of a case of something, which is to be elicited and treated according to the appropriate guideline. From a time perspective, a case can denote a single consultation, a treatment episode, or also the development in the course. The developmental dynamics come to the fore concerning the disease entity. If one focuses on the doctor-patient interaction, their relationship and the course of treatment can become a case (13-15). If the patient's life world and biography are taken into account, the respective way of coping with oneself and the world ("staging") can be determined and processed as a case. Finally, the rules that become effective in the respective social reference group (family, work, community) can be considered. So, the social system becomes a case. Other possible case levels concern professional action. Thus, the personal style of the individual doctor, performed in different interactions, can be questioned in supervision concerning its patient-related appropriateness and traced in a biographical perspective in its life-historical shaping (16, 17). In a comparative analysis, the modes of action common to different doctors and their structural foundations point to the respective professional profile. Differences between other practitioners can become an occasion for reflection on what is appropriate. The case can be constructed at each level of meaning stratification described above and can be understood in the tradition of structural hermeneutics (18) in terms of the "structuring law of a social practice". Understanding means becoming aware of its latent social meaning.

At which level(s) of trapping the definition of the professional society DEGAM is aimed is unclear. A personal understanding subject is almost entirely missing. The patient, who is to be understood holistically, is confronted with "general practice". Thus, the discipline does not perceive its subject basis in its self-definition. This is all the more astonishing since, in statistical analyses, "the doctor factor" is attributed to the most significant part in explaining the large differences in process and outcome quality in primary care (19).

Wanting to treat the whole person corresponds to the idealized professional self-image of many health professionals and the wishes of many patients (20 - 22). However, comprehensive care is rarely realized. As can be seen from well-documented individual cases, person-centeredness is nevertheless possible (e.g. 4, 23). Suppose (general) practitioners - and their patients who co-create their treatment - are "inventors". In that case, it should be possible to make person-centeredness comprehensible by reconstructing their interactions and shaping everyday life that precedes or follows them. In particular, it seems useful to analyze specific cases of "positive deviant behavior" (24) in the sense of salutogenesis to elaborate on conditions for a successful person-centered practice.

V. The Introduction of the Subject

Health-related processes and health-related actions are permanently embedded in the social actions of concrete persons and are inevitably interpreted in terms of their social meaning. Even if the interpretation concepts of participants, social environment, and professional helpers may differ, they are always attributions by persons who should be aware of their subjectivity and make it comprehensible. Mezzich's definition of person-centeredness is also applicable to health-related research (9). It, too, needs to be person-centered and framed as a collaborative interpretive effort. Therefore, health researchers are in need of a specific basic attitude, methodological competencies, and the willingness of all actors to cooperate (25).

Person-centered health research presupposes humility, self-reflexivity, respect for participants, a desire to understand others, and interpretive communities grounded in mutual connectedness as human beings (26). These principles are claimed for health-related research and practice similarly. The concrete design varies depending on the directness of the interactions - and thus on the respective case level. As this is a highly complex process (27, 28), it is impossible to consider the case levels outlined here simultaneously. I will limit to exemplary hints from my research practice or that of colleagues I know personally (as a first approximation, see Table 2).

Table 2: The reconstruction of meaning in the research process

Dimension	Expression of affected person's competence	Researchers's Subjectivity	Methodology
The patient's life world	Narration and self-reflection Facial expression, gestures Pictures, Photos	Active listening and self-reflection Facial expressions, gestures, and counter-transference analysis Self-insertion	Biographical interviews, narratives Document analysis (e.g. diary, photos) Participatory observation Interaction Analysis
Medical world	Narration and self-reflection Design of the setting	Active listening and self-reflection Facial expressions, gestures, and counter-transference analysis Self-insertion Creativity Gestalt perception	(Professional) biographical interviews, narratives Document analysis (e.g. diary, files) Participatory observation Video-assisted recall Transcript analysis Analysis in the group

a) The patient's life world (levels 2-5 as well as 15-17)

The social and biographical background against which problems acquire illness value and the person concerned becomes a patient can best be explained by the person himself and, if necessary, by his significant others (family, friends). For this purpose, I conducted biographical interviews. I gave the interviewees much space for narration and took on the role of an active listener who influenced the narrative flow as little as possible and, if necessary, tried to contribute to clarification and deepening by asking questions. Where the flow of the story got bogged down, I also gave impulses through my own examples in the sense of self-input. In doing so, I also paid attention to the accompanying gestures and facial expressions and, in the sense of Devereux's theorem that countertransference is the most relevant datum of the behavioral sciences (29), I made sure the feelings triggered in me in order to guess what might be going on in the interlocutor. I remember a particularly impressive conversation in which a woman of about 45 years of age talked for several minutes without a break, weighing up the pros and cons of a dilemmatic decision-making situation, while her face showed her being torn back and forth. Suddenly she broke off and abruptly asked me what I thought. I was completely overwhelmed, as I could not and would not decide for her. However, at this moment I now understood that she urgently needed a counterpart and why her GP described the conversations with her as "mirror fencing", in which he felt powerless and at the same time indispensable for the patient. To my relief, I could ascertain that my acknowledgment of her dilemma was sufficient for the interviewee: she had not sought advice but understanding, which was also allowed to express itself in more in-depth questions.

Narrative interviews can also provide access to central experiences in coping with health and illness. For example, they can clarify how patients experience the process of treatment as a whole, what is particularly important to them, how they take up recommendations made by their helpers, how successful this is, and how they interpret this themselves (see e.g. 30).

b) The doctor's life world (levels 8 and 10)

When I began my studies on doctor-patient communication, I assumed, in accordance with the

prevailing model of Talcott Parsons (31), that patient and doctor encounter each other as role bearers and that the doctor's task was to take appropriate account of the individual characteristics of each patient. After I had presented an interaction analysis at a congress using the means of structural hermeneutics with which I was familiar, a man approached me who bore an astonishing resemblance to the colleague I had presented. Moreover: it was him too - and he commented that *his wife*, a musician, could undoubtedly have done much with my interpretation. Left unsaid was what he had missed, but I immediately understood that I had objectified him and not taken any notice at all of the terms of his doctoring. We subsequently interpreted some of his conversations together - a cross-professional quality circle emerged from this, and I learned that the doctor also acts in a lifeworld context (32). Another family doctor pronouncedly drew attention to the subjectivity of the doctor as a resource:

"When you observe doctor-patient conversations, the doctor typically remains as a professional and not as a symmetrical partner who brings in his problems. Well, that's what we are taught, and that's what psychotherapists teach us, to use the conversation as an instrument and not as a *personal* expression of *personal* concern. I am of a completely different opinion, namely that this is not possible as a family doctor. This psychotherapeutic attitude is not a family doctor's attitude. I am rather of the opinion that we have to deal with our very personal life stories, which the patients also experience! We not only experience the patient's life story, but we also experience our own, so that we also have to be doctors with it." (Family doctor, 50 years)

Accordingly, I have been interested in how one becomes the doctor he or she is and which lifeworld and institutional conditions influence their actions. Biographical interviews have also proved helpful for this purpose (16, 33).

c) Medical world (levels 6,7, 9, 11-14)

I then approached the (general) medical world through the analysis of video-documented doctor-patient interactions, which dedicated teaching physicians had already produced with the consent of the participants. I was particularly interested in how the different worlds can be mediated in dialogue in such a way that a joint understanding can emerge and the patient can speak of "his family doctor" and the doctor of "his patient" (34). For this purpose, selected interaction sequences were interpreted in detail, partly directly on the video material (video-assisted recall; (35)) and partly additionally based on transcripts. Ideally, this was done in an interdisciplinary group, involving researchers, practitioners from different health care professions, and occasionally patients. Patterns of interaction could be worked out in contrast to possibilities imaginable in thought experiments without yet being realized.

Since the consultations are typically shaped in the context of long-term treatments (14), we extended our analyses and, for example, reconstructed courses of illness and treatment based on information from the medical records. These individual case analyses allowed typifications but no indications of frequencies. I could gain practical epidemiological insights through targeted documentation. I was impressed by a 2-day observation visit, during which conversations were recorded by video, and I could be present in the consulting room. I experienced the challenge of holistically adjusting to very different people every 10 minutes. Furthermore, I got to know the extraordinary willingness of all those involved to support such practical research after the objective had become apparent and the participants had a sense of the researcher. My colleague Vera Kalitzkus was even able to observe during her observation how the consultation hour takes on an overall shape and how earlier ones could influence later conversations even if other patients were involved (36).

In the EUROCOM study (37), we documented consultations in 6 countries by video and evaluated a total of more than 3000 consultations. For this purpose, 190 physicians recorded 20 conversations consecutively by video and documented additional information on the reasons for encounters and diagnoses. The patients answered the same questions through questionnaires and provided insights into their expectations and satisfaction with the consultations. Unfortunately, the extensive data collection was carried out in a division of labor, and the researchers rarely interacted with the "researched". Therefore this limited insight into the contexts.

Moreover, the researchers' relationship-building with the patients and clinicians involved was restricted. While willingness to participate was still high, it was lower than in studies where my colleagues and I had face-to-face contact. However, the statistical analyses did provide information on, among other things, interview duration and workload. Overall, the country- and health system-related differences were remarkable 37). Even more important to us seemed to be the sizeable physician-related variance, which was evident in the style of interviewing and documentation and pointed to the influence of the physician as a subject. In order to be able to examine the interrelationships in an exemplary manner, we prepared case studies in Germany in cooperation with some physicians on issues of particular interest to us, which were later used in the training of medical students (as an example: 38).

d) Multi-perspective view

In the doctor-patient conversation, the life worlds of the participants and the "institutional world" overlap. This consultation, located in the institutional framework, is referred to as a "medical conversation", which is misleading insofar as a co-creative

performance takes place to which all interlocutors contribute.

To examine this process, we made multi-perspective observations in the project "Health Promoting Practices" (39). Step by step, we retraced how the field of meaning was enriched on a case-related basis. For this purpose, two scientifically accompanied interprofessional quality circles were formed, which explored possibilities of strengthening self-help potentials in patients with chronic diseases using the example of video-documented consultations of the participants. The 30 participants worked in different primary care professions; two were affected patients and represented self-help groups. In almost each of the 50 2-3 hour group meetings, one of the participants presented one of their conversations for discussion and provided supplementary contextual information about the working methods and focus of their practice, their clientele, and the treatment process to date. The conversations were initially discussed as a whole, with group participants drawing different facets of a conversation depending on their qualifications, age, gender, and biographical experiences, which then gradually took on a coherent shape.

Furthermore, there were as many counter-transferences offered as participants. These counter-transferences referred to the patient's subjectivity and showed possible relationship constellations available to him. In detailed analyses of selected conversation sequences, patterns could then be worked out, in structural hermeneutics (18), for how patient and doctor systematically *failed* to perceive possible interaction alternatives. The joint analysis aimed at revealing meaningful but unspoken goals. Finally, we considered how to raise the treasure of emerging unused self-help potentials. Each participant thus took away concrete suggestions for treating the patients he or she presented and gained cross-case indications for a more flexible approach to patients with chronic illnesses.

In an interim assessment after one year, it was noted as a shared result that ingrained routines could stand in the way of the perception of specific problem situations and that doctor and patient often talked past each other despite knowing each other well from the "experienced anamnesis". The groups suggested deliberately abandoning the standard procedure to conduct a conversation with their long-term patients as if the participants were meeting for the first time. Instead, the way should be opened for an unprejudiced discussion of the patient's central life and treatment goals by excluding previous knowledge.

This modified procedure was tested in the second year. The video-documented conversations with the patients already presented in the first project year were discussed in the manner described above. As a rule, the patients could use the extended conversation space. The participants thus achieved a new understanding and rated this as enriching and relieving. In individual cases, however, the possible freedom also proved to be threatening, and further need for support became apparent.

After completing the work phase of the quality circles, we interviewed the primary care providers involved and those patients whose conversations had been discussed and analyzed. Again, we used guideline-supported open and biographically directed interviews so that a multi-layered, multi-perspective view was possible for each "case" (cf. figure).[1]

[1] The project duration was insufficient to also specifically evaluate the interviews with the treatment providers within the framework of the project. These became the subject of separate psychological qualification work. (43, 44)

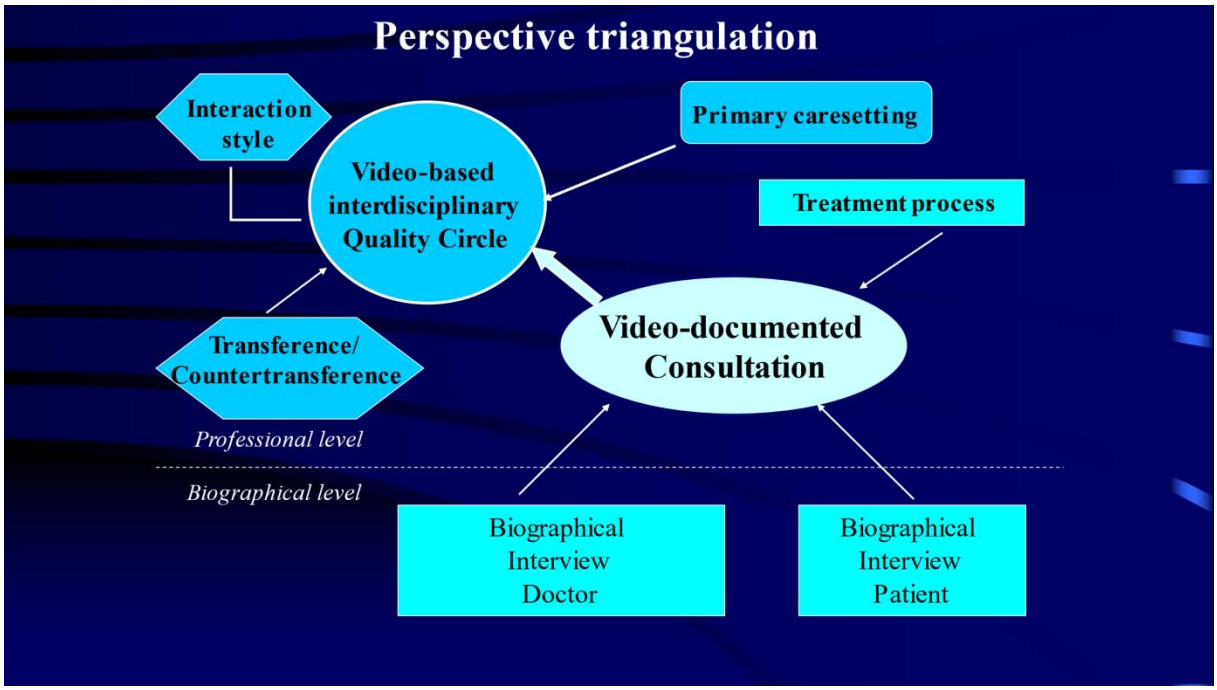

Figure 1: Multi-perspective consideration of the doctor-patient interaction (according to Bahrs & Matthiessen 2007)

We evaluated and modified the jointly developed conversation type of the review dialogue in a follow-up project with other stakeholders (40, 41). However, the approach was similar, so I will refrain from further elaboration here.

With the development of the review interview, I got an idea of answers to the questions that led me to family medicine. Accordingly, as a sociologist initially from outside the field, I could now participate in recommendations for the design of initial interviews in GP practices (42) and promote the fact that the proposal was developed in the unity of research and professional training. The - also biographically based - diversity of competencies thus becomes an opportunity for collaborative learning, and the limit of equal participation depends on the conditions of the division of labor.

Participatory research with people with whom I had come into contact in their role as patients had so far only been possible to a limited extent. In the conversation, I respected their expert status as a matter of course. In individual cases, I obtained and gave feedback on research results in the sense of communicative validation. Increasingly, I involved patients as experts in their own right in the quality circle discussions, and they were involved in the process of generating the results (39). However, I have not yet been able to take the step towards citizen research, in which potentially all participants become researchers. Presumably, this also presupposes that the participants transcend the framework of health care with its strongly differing role offerings and seek common ground in solving questions that affect all participants (45).

e) Where is the researcher as the subject?

In the general understanding, scientificity is equated with objectivity, as if the practically active cognizing subject were unimportant. However, even the *choice of topic* is value-driven and an expression of interest in knowledge. In continuously developing the topic, a research program characteristic of the person can reveal itself. For example, through a friend, I was allowed to work on a project with people with chronic illnesses - and this world was very familiar to me because I had experienced vital and vulnerable, chronically ill people in my family from birth. In the process, I grew up with a flexible way of dealing with being stigmatized, self-stigmatization, and passive as well as active stigmatization. Thus I approached my counterparts with a pre-concept of salutogenesis, curious about the specific ways in which they were involved and found solutions. This attitude was palpable to my counterparts, whether they were more in the role of help-seeker or helper at the time. However, I addressed my background of experience only occasionally and when explicitly asked. Such questions were rare and mostly came at the end of the conversation. Perhaps one can also say: that with the transgression of the roles we had met, they simultaneously prepared the return to their respective worlds.

Empathy is required of the researcher in health and social care professionals in the *data collection* phase. Being able to speak out may occasionally also have a quasi-therapeutic effect in the context of research interviews. People do remarkable developmental work and are capable of fundamental

self-corrections in a stimulated self-reflection if the interlocutor lets them and, at most, asks follow-up questions that enable further clarification. The same is true in group discussions, in which new perspectives for action are constantly emerging. My turn was to enable autopoietic processes, but not as an advisor. In accompanying the group discussions, I functioned as a representative of the group memory and as the person ultimately responsible for the products that emerged (protocols, case studies, project reports, concept of the review dialogue).

In the *texting and reporting,* the person of the researcher seems to be erased. Processes congeal into structures, which in the German language becomes clear in passively constructed sentences and in the renunciation of acting subjects. The "I" is replaced by a "man", perhaps also "the scientist". If I do not follow these rules, my scientific community reminds me of them. However, a personal relationship to the research "object" usually develops in a research process that lasts several years. I see this as a specific creative achievement to be made transparent in its development process, not merely a subjective deformation (bias). Thus, in my opinion, Sartre could rightly say about his portrait of Gustave Flaubert that he was not interested in whether the other had been as Sartre described him - what was important was whether Flaubert *could* have been like that. "I would like my study of Flaubert to be read like a novel because it is the story of an apprenticeship (...). However, I would also like people to take it for the truth when they read it, for a true novel. Throughout the book, it is *Flaubert as I imagine him (*emphasis mine, O.B.), but since I have methods that seem to me stringent, I also think that it is Flaubert as he is, as he has been." (46)

Wheeler is one of the few to reflect in her excellent doctoral thesis on the particular contribution she made as a clinically untrained sociologist in researching the management of medically unexplained symptoms (MUS) (47). On the one hand, she lacked specific expertise that she could organize for herself by employing support from trained supervisors and drawing on the expertise of practitioners involved in the project. On the other hand, she was freed from seemingly shared self-understanding and could legitimately ask questions that seemed outlandish at first but went further - because they enabled a new framing. Familiar with the subject matter to be researched, also from her own experience, she was able to act as an interpreter between the internal and external viewpoints, developing her terminology in the process. Wheeler reflects excellently on how conceptual presuppositions had a structuring - and in some cases limiting - effect on data collection and analysis and how she was able to correct the limitations partly based on contrasting findings of her own. Finally, she describes a research process that culminates in a condensed result ("bricolage"). The result is a personally formed Gesamtkunstwerk in which the author expresses his or herself with a specific attitude to the world and a characteristic style, which at the same time bears witness to a social practice shaped by many people.

VI. Summary

Our health care system is considered to lack comprehensible orientation. Instead of a fundamental orientation towards enabling health, the defense, administration, and alleviation of illness dominate our actions. The fundamental question should relate to what it is worth to be "healthy" and to what our health-related actions may once have been good.

As for the specific field of action in family medicine, it is tough to convince medical students and graduates of its positive aspects. People often criticize that general practitioners act very differently and that there is a lack of a shared professional self-image. However, there is unanimous agreement that family medicine relates to people in their health and illness, i.e. it is person-centered.

The present paper assumes that a "hermeneutical approach to the case" could create common ground for a more explicit profession-related self-concept. According to the German Society for General and Family Medicine (DEGAM), the hermeneutic understanding of cases is characteristic of general practice. However, there is no operational definition, so this is not part of education and training. The presentation refers to the discussion of "The Patient as Text", which is put up, especially in English-speaking and Scandinavian countries.

Family medicine is relation-based and can be understood as a series of interpretative actions. Thus, family medicine is part of a hermeneutical venture. Consultation is based on a dialogue - not always formulated in language - and thus a co-creative act in which something new can be generated. One of the cases that need to be understood is the doctor himself.
Our analysis depicts tacit knowledge in (social and medical) practice based on the individual experiences of those involved and their ancestors. These prior experiences may have settled into routines and institutional rules. Turning tacit knowledge into explicit knowledge would encourage a common ground for a clearer self-concept of family practice both as a practice and as a science. Scientifically accompanied (interdisciplinary) quality circles are suitable for this. They function as further training and research instruments in participatory research and implementation. Quality circles contribute to making hermeneutic case understanding teachable and learnable.

Person-centered medicine - understood as care of persons for persons by persons and with persons -

requires that all participants engage as subjects. In this participatory process, traditional roles can and must be redefined. This also concerns researchers.

Regarding my work, I give examples of person-centered research and the difficulties concerning implementing the desiderata of subject-centeredness and participation in institutional contexts.

Health and illness are, at the same time, profoundly personal and social affairs and require a dialogue-mediated self-assurance of the members of society about their central values.

Conflicts of interest: Nothing to declare.

Acknowledgments

My comments draw on experiences from several medical sociology and general practice projects. I sincerely thank the more than 100 primary care providers and more than 2000 patients involved, without whose trusting support this would not have been possible. I would also like to thank my colleagues of many years standing, with whom I have been able to walk parts of the path together and learn with them. I would like to make special mention of (in alphabetical order) Heinz-Harald Abholz, Martin Beyer, Ralf in der Beek, Atie van den Brink-Muinen, Ferdinand Gerlach, Susanne Heim, Karl-Heinz Henze, Eberhard Hesse, Vera Kalitzkus, Anja Klingenberg, Michael Köhle, Martin Konitzer, Peter Matthiessen, Helmut Müller, Michael Peltenburg, Thomas Ripke, Joachim Szecsenyi, Gernot Rüter, Iris Veit, Peter Verhaak, Stefan Wilm, and Georg Bernhard Wüstenfeld. I owe many suggestions to my advisors (in chronological order) Hannes Friedrich, Jürgen Wilhelm, Eckart Sturm, Wolfram Fischer, Gisela Fischer, and Bruno Hildenbrand.

The studies were partly self-financed and made possible by specific project funding programs. I would like to thank

- The European Commission (Eurocommunication study; BIOMED-II- research pro of the European Commission, contract no BMH4-CT96-1515);
- The AOK Bundesverband (promoting the model project Salutogenetic Orientation in Family Practice - Health Promoting Practices) and
- The Federal Ministry of Education and Research (funding the project Review dialogues to promote patient orientation and improve the quality of primary care treatment for people in chronic conditions (BILANZ); grant number 01GX1030A-C).

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Development of Formulation and Technology of Combined Generic Powder for Oral Solution in Sachets based on the Quality by Design Approach

By Oksana Panysheva

Abstract- Using quality by design approaches (dispersion analysis and random balance method), the formulation and technology of powder for oral solution in sachetswere developed. It includes paracetamol, ascorbic acid, phenylephrine hydrochloride, and pheniramine maleate. The influence of 27 excipients from 5 functional groups on 11 quality attributes was established by the dispersion analysis. The preferred excipients were selected using the utility function. The quantities of preferable excipients (7 quantitative factors at three levels) were researched by the random balance method. Their influence on 11 pharmaco-technological parameters of the powder and the physical properties of the solution studied. The optimal composition of the powder for the oral solution established.9 sequences of introducing components were analyzed by dispersion analysis. The optimal technology of the powderfor oral solution in sachets byroll compaction is established.

Keywords: *chemistry, pharmaceutical; drug development; powders; drugs, generic; risk assessment; quality indicators, health care; research design; excipients; algorithms; technology.*

GJMR-B Classification: *DDC Code: 615.783 LCC Code: RM666.A19*

Strictly as per the compliance and regulations of:

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I. Introduction

Today the topical issue of pharmaceutical development is the search for new objects of research and the use of science-based approaches.

Effervescent drugs are becoming increasingly popular with consumers, clinicians,and technologists yearly.

This is due to several of essential advantages. They provide a faster release of active pharmaceutical ingredients from the dosage form. As a result, high speed and completeness of absorption are ensured. This increases bioavailability and accelerates the onset of therapeutic action, reduces the irritating effect on the mucous membrane of the gastrointestinal tract, and increases the amount of fluid consumed. Effervescent drugs are well received by patients because they combine the advantages of tablets (portability, accurate dosage) and the possibility of easier administration (no need to swallow the pill) [1, 2]. They make it possible to convert difficult-to-dissolve compounds into solution by the salt formation and solubilization with carbon dioxide, correction of unpleasant organoleptic properties of active substances. Effervescent dosage forms allow you to combine components that react with each other [3, 4]. They are promise dosage forms for cardiovascular, antispasmodic, analgesic, expectorant, and other pharmacological groups, as well as for pediatric and geriatric practice [5].

The main components of effervescent dosage forms are a gas-forming mixture of acidic and alkaline fractions. They are selected individually for each drug. In most cases, they make up 80-95% of the total mass [6]. Dry organic acids with carbonates and, or bicarbonates of alkali and alkaline earth metals are most often used so, effervescent substances, choose citric acid and sodium hydrogen carbonate [4].

The most common acid component is anhydrous citric acid (E 330). Sometimes it can be replaced by ascorbic or adipic acids [7]. Isolated cases of fumaric, malic, and tartaric acids were observed. Fumaric acid can be used in the form of more soluble salts, in particular sodium and potassium fumarates [6]. The acid source can be organic acids - tartaric, succinic, and acid anhydrides - mostly citric, succinic, and glutamic.Tartaric, adipic, and fumaric acids are usually used in small quantities due to their low solubility in water. The acid fraction can also be represented by salts of acids, such as sodium dihydrogen phosphate, sodium dihydrogen citrate, sodium dihydrogen tartrate, disodium dihydrogen pyrophosphate, and sodium sulfite [3, 6].

Currently, carbonates or bicarbonates of alkaline and alkaline earth metals or their mixtures are used as the carbonate fraction.The alkaline fraction of the gas-forming mix is most often formed by sodium bicarbonate (E 500) alone or with anhydrous sodium carbonate [7]. Hydrocarbons and potassium carbonates can be used as an alternative.As an alkaline components, calcium carbonates, magnesium, sodium glycine carbonate, sodium lysine carbonate, sodium arginine carbonate, potassium acesulfame, and others are also used [3].

Sodium salts (sodium citrate dihydrate and monosodium citrate anhydrous) are an antioxidant synergist, buffer, acidity stabilizer, and carbon dioxide retainer. Sodium hydrogen phosphate anhydrous is added as an acidity regulator. Anhydrous sodium sulfate acts as a humidity regulator. The composition also includes sodium benzoate as a preservative. Sodium

Author: Farmak JSC. e-mail: oksanatryhubchak@gmail.com

docusate and sodium chloride are also used to obtain effervescent dosage forms [7].

Taking into account the fact that the mass of effervescent dosage forms is quite large and varies between 2-4 g, an essential step in the development stage is the choice of filler. It has specific requirements, the main of which are: high solubility in water, low hygroscopicity values, good compressibility, and satisfactory flowability. According to the literature, sugars (dextrose, glucose, lactose) and polyols (sorbitol, mannitol) are used as fillers in the production of the effervescent dosage forms. Sugars play the role not only of structure-formers but also of taste correctors. It is noted that the effervescent dosage form includes sorbitol, anhydrous lactose, lactose monohydrate, mannitol, glycine, sucrose, anhydrous glucose, and maltodextrin. Pregelatinized starch was used as a filler [8].

Effervescent drugsare a solution after dissolving in water. Therefore, one of the essential issues is the choice of optimal correctors of taste, smell, and color. Thus, natural water-soluble substances are used as taste correctors - sucrose, lactose, xylitol, D-glucose, sorbitol, mannitol, and glycine. However, most effervescent drugs formulations contain artificial sweeteners, including aspartame, cyclamates, and sodium, or potassium salts of saccharin. The leaders among taste correctorsare sodium saccharin, aspartame, sodium cyclamate, lemon flavoring BSL code 119. The last includes natural lemon oil, natural/identical to natural lime flavoring, mannitol, maltodextrin, gluconolactone, sorbitol, acacia. So the bitter taste of ciprofloxacin, and paracetamol is masked by sodium saccharin. In addition, the effervescent effect of citric acid, tartaric acid, and sodium hydrogen carbonate leads to an improvement in the taste of the drug [9, 10]. Aspartame was most effective in masking the bitter taste of ranitidine hydrochloride [2]. The use of a complex of 2-hydroxypropyl-β-cyclodextrin and mannitol with inclusion in a solid dispersion allows you to hide the bitter and sour taste of levocetirizine hydrochloride [5].

In order to improve organoleptic properties, natural and artificial fruit flavors in dry forms (orange, lemon, pineapple, etc.) are added to the composition of effervescent dosage forms.The most common flavoring is lemon. Aromas close to it are characterized by orange, tangerine, and lime.Fruit scents are popular: blackberry flavoring B, aromatic fruit additives of raspberry, grapefruit, red fruits, and mixed fruit flavoring. They are unique for each drug. For example, vanillin flavor increases sensitivity, when taking ciprofloxacin effervescent tablets [10]. For ranitidine hydrochloride effervescent tablets, mint and orange flavors were the most effective [2]. Strawberry and banana flavoring enhances the palatability of paracetamol effervescent tablets [9]. In the test of potassium citrate effervescent tablets, the combinations of flavors orange - lemon and strawberry - raspberry were acceptable [11].

The ability of dyes to change color under the influence of pH of the solution should be taken into account when choosing color correctors. Preference is given to natural pigments (carotene and chlorophyll)for the production of effervescent dosage forms [6]. Unique red AC, quinoline yellow lake, and red beet juice powder are also used [7].

Therefore, 5 to 15 components must be used to obtain an effervescent dosage form [12].This involves the study of multifactorial dependencies, especially when it comes to active pharmaceutical ingredients with specific pharmaco-technological properties. Another aspect that requires significant amounts of experimental research is the creation of particular types of solid dosage forms. Currently, research on the outcome of effervescent dosage forms is rarely conducted. They require more significant of experimental studies due to the lack of thorough research on their development and the lack of experience in their production [13].

During the development of optimal formulations and drug technology, the experience gained in using the introductory provisions of mathematical planning of the experiment (quality by design). Depending on the physical and technological properties of active pharmaceutical ingredients, a research design has been developed that allows for a comparative evaluation of excipients, to establish a possible interaction between them, select the most rational ones, and establish their optimal quantities in the composition. Before starting experimental work, it is reasonable to use a priori ranking, especially when the first steps are taken to create drugs.

The beginning of experimental studies includes the selection of rational excipients for the created solid dosage form. A significant list of excipients available on the pharmaceutical market prompts the use of unique dispersion analysis plans. The developed algorithm for choosing a rational method of the experiment and its statistical processing makes it possible not only to reduce the number of experimental studies but also to obtain information about the interaction between the levels of the studied factors.It is practically impossible to get with a traditional single-factor study [13, 14]. The influence of excipients from different functional groups on the pharmaco-technological indicators of tablets was studied using the method of dispersion analysis [15-17]. Based on the results of the experiment, a ranked series of advantages constructed, significant factors determined, and the best excipients were selected [18].

Then the quantitative excipients influence on the pharmaco-technological properties of the solid dosage form is studied [14]. For experimental studies, it is rational to use the method of random balance, with the help of which it can significantly reduce the number of factors and select the most significant ones for further

research [13]. Analyzis using quality by design is successfully used to optimize various technological processes. The main advantage of mathematical planning of the experiment compared to classical research methods is the possibility of the simultaneous study of a large number of factors [18]. The influence of quantitative factors on the leading quality indicators of the drug was studied, using the approaches of mathematical planning of the experiment, namely, the random balance method. The most significant factors affecting formulation and leading quality tablets indicators have been identified [19-21].

The purpose work was to develop the formulation and technology of a powder for oral solution in sachets, which includes 325 mg of paracetamol, 50 mg of ascorbic acid, 10 mg of phenylephrine hydrochloride, 20 mg of pheniramine maleate.

II. Material and Methods

The objects of the study were powders with paracetamol, phenylephrine hydrochloride, pheniramine maleate, ascorbic acid, and excipients. Paracetamol is manufactured by Hebei Jiheng (Group) Pharmaceutical Co. Ltd, China. Phenylephrine hydrochloride is manufactured by Unichem Laboratories, India. Pheniramine maleate is manufactured by Supria Litescience Ltd., India.Ascorbic acid is manufactured by Swiss DSM Nutritional Products. All excipients used in the development of the drug are generally accepted substances that are widely used in the production of powders for internal use. Theraflu, powder for oral solution in sachets No. 10 of the company Novartis Pharmaceuticals Canada Inc., Canada/Switzerland was used as a reference drug.

In the process of pharmaceutical development, guidelines ICH Q8, ICH Q9, ICH Q10, ICH Q11, and normative documents of CQA, CMA, and CPP were guided, ultimately increasing the effectiveness of research in the creation of the solid dosage form and its subsequent registration [13].

The following equipment used to produce of powders: electronic balanceMettler Toledo PB8001-S, rotor sieve GSF 60, container blender Glatt CML, roll compactor Alexandwerden WP120.

The following research methods used: technological, organoleptic, analytical, and statistical.

Bulk density was calculated by determining the book that has the fixed weight of powder. Tapped density was calculated by determining the volume that holds the set weight of powder after 1250 taps on tapped density testerERWEKA SVM 202. Carr's index calculated by the equation:

$$Carr's\ index = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100$$

Carr's index is an indication of the compressibility of a powder [22]. The resulting index from 12 to 15 shows good compressibility, 16-24 – fair to passable, 25-35 – poor, 36-39 – very poor, and more than 40 – extremely poor. Flowability tested by flow tester ERWEKA GTB. Flowability is the speed of rash powder through a funnel with an opening diameter of 10 mm. The obtained results are the average for threesizes.This device also allows measuring the angle of repose by a laser. The obtained results are the average for 3 measurements.Loss on drying of the granulate was determinedusing a moisture analyzer Mettler Toledo HB 43according to the Ph.Eur.method. The powder of 1 sachet was dissolved in 200 ml of purified water and the pH of the solution was tested using a pH meter Mettler Toledo S220 Seven Compact according to the pharmacopoeial method [23].

Organoleptic properties (appearance of powdersandsolutions, smell, and taste of the solution) were evaluated using a 5-score scale.

Analytical properties of the powder (assay, content uniformity) were tested according to pharmacopoeia[23]. The composition includes four active pharmaceutical ingredients with different physicochemical properties. Liquid chromatography with detection at wavelength 273 nmwas usedfor quantitative determination of paracetamol, phenylephrine hydrochloride, and pheniramine maleate. Titration method by direct iodometrywas used for quantitative determination ascorbic acid.

Methods of mathematical and statistical planning of the experiment (dispersion analysis, random balance method) were used for setting up and conducting experiments [18]. Statistical processing of experimental results was conducted according to generally accepted methods using standard Excel computer programs [24].

In the course of pharmaceutical development, the excipientseffect on the quality indicators of the powder and its solution is studied. For this purpose, excipients were grouped into five factors: alkaline fraction (factorA): a_1 – sodium bicarbonate, a_2 – calcium carbonate, a_3 – calcium phosphate; acid fraction (factorB): b_1 – citric acid anhydrous, b_2 – a mixture of citric acid anhydrous andmalic acid (5:1), b_3 – malic acid; coloring (factorC): c_1 – iron oxide, c_2 – curcumin, c_3 – riboflavin; flavoring (factorD): d_1 – lemon, d_2 – lemon-lime, d_3 – orange, d_4 – grapefruit, d_5 – blackberry, d_6 – raspberry, d_7 – strawberries, d_8 – apple, d_9 – chocolate; filler (factorE): e_1 – fructose, e_2 – sorbitol 60, e_3 – sorbitol 450, e_4 – mannitol, e_5 – a mixture of sugarand xylitol (3:2), e_6 – dextrose hydrate, e_7 – sugar powder, e_8 – lactose monohydrate 200, e_9 – maltitol.

Experimental studies were carried out using the Latin cube of the second order (Table 1).

Table 1: Five-factor plan based on the Latin cube of the second order

Batch	A	B	C	D	E
1	a_1	b_1	c_1	d_1	e_1
2	a_1	b_2	c_1	d_5	e_2
3	a_1	b_3	c_1	d_9	e_3
4	a_2	b_1	c_1	d_2	e_4
5	a_2	b_2	c_1	d_6	e_5
6	a_2	b_3	c_1	d_7	e_6
7	a_3	b_1	c_1	d_3	e_7
8	a_3	b_2	c_1	d_4	e_8
9	a_3	b_3	c_1	d_8	e_9
10	a_1	b_1	c_2	d_4	e_9
11	a_1	b_2	c_2	d_8	e_7
12	a_1	b_3	c_2	d_3	e_8
13	a_2	b_1	c_2	d_5	e_3
14	a_2	b_2	c_2	d_9	e_1
15	a_2	b_3	c_2	d_1	e_2
16	a_3	b_1	c_2	d_6	e_6
17	a_3	b_2	c_2	d_7	e_4
18	a_3	b_3	c_2	d_2	e_5
19	a_1	b_1	c_3	d_7	e_5
20	a_1	b_2	c_3	d_2	e_6
21	a_1	b_3	c_3	d_6	e_4
22	a_2	b_1	c_3	d_8	e_8
23	a_2	b_2	c_3	d_3	e_9
24	a_2	b_3	c_3	d_4	e_7
25	a_3	b_1	c_3	d_9	e_2
26	a_3	b_2	c_3	d_1	e_3
27	a_3	b_3	c_3	d_5	e_1

In addition, 0.01% titanium dioxide was added to the composition, which provides anextended stay of substances in the stomach and increases their bioavailability [25].

The powder technology consisted in mixing by the trituration method 1/5 filler withtitanium dioxide, phenylephrine hydrochloride, pheniramine maleate, and coloring. Ascorbic acid, acid fraction, flavoring, paracetamol, and alkaline fraction gradually added to the trituration mixture. It mixed and added the restof filler. The mixture passed through a sieve with a hole diameter of 1 mm.

The obtained mass tested twice according to pharmaco-technological indicators, the solution characteristics of the sachet in 200 ml of purified water studied.The experimental data subjected to statistical processing by the method of dispersion analysis and determined the influence of the nature of the investigated excipients on quality indicators.

The results of the dispersion analysis were summarized using the utility function. The best substances in the drug composition determined according to the most signifcant sum of the combined data of the ordinal numbers of the factor levels in the ranked series of advantages.

At the next stage of the research,amounts of selected excipients studied. For regulate the medium pH, sodium citrate was additionally introduced into the experimental plan. To choose the optimal content of titanium dioxide, its amount also studied. The list of factors and their levels in the study of the quantitative excipientscharacteristics in the formulation is given in Table 2.

Table 2: Factors and their levels in the study of the quantitative excipientscharacteristics in the formulation

Marking	Factor	Factor level		
		Lower(–)	Basic (0)	Upper (+)
x_1	Quantity of calcium phosphate, g	0.0820	0.7000	1.3180
x_2	Quantity of citric acid anhydrous, g	0.6500	0.9360	1.2220
x_3	Quantity of malic acid, g	0.0500	0.0900	0.1300
x_4	Quantity of sodium citrate, g	0	0.0605	0.1210
x_5	Quantity of curcumin, g	0.0213	0.0383	0.0400
x_6	Quantity of lemon-lime flavoring, g	0.1915	0.2000	0.2085
x_7	Quantity of titanium dioxide, g	0.0014	0.0032	0.0050

A research plan drawed using the random balance method (Table 3). The average weight of 22.13 g adjusted by the amount of sugar powder. Technological properties of the powder and organoleptic characteristics of solution from the powder in 200 ml of purified water studied.

Table 3: Planning matrix of the experiment of studying quantitative factors

Batch	x_1	x_2	x_3	x_4	x_5	x_6	x_7
28	-	-	-	+	+	+	-
29	-	+	-	+	-	+	-
30	+	-	-	-	-	-	+
31	+	+	-	-	+	-	+
32	-	-	+	+	-	-	+
33	-	+	+	-	+	+	-
34	+	-	+	+	+	-	-
35	+	+	+	-	-	+	+
36	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0

Each batch of the drug were studied according to the pharmaco-technological and organoleptic indicators of the quality of powder masses and their solution. Scatter diagrams constructed based on the experimental results.Significant factors are taken from scatter diagrams, and their selection is proved by calculations [26, 27]. This made it possible to determine the influence of investigated quantitative factors on quality indicators.

The algorithm of the researched technologies is shown in Table 4. Schemes for the ingredients introduction differed in the components set for mixing and compaction and consisted of a stages different number (from 2 to 9).

Table 4: Researched technologies of powder for oral solution

Factor	Batch	Factor level
A–product introduction scheme	38	a_1 – compacting of the mixture 1: paracetamol + phenylephrine hydrochloride + pheniramine maleate + ascorbic acid + sugarpowder; compacting of the mixture 2: sodium citrate + malic acid + citric acid anhydrous + calcium phosphate + curcumin + titanium dioxide + lemon-limeflavoring + sugarpowder; compacting of sugar and their mixing
	39	a_2 – compacting of the mixture 1: paracetamol + calcium phosphate + curcumin + titanium dioxide + lemon-lime flavoring + sugar powder; compacting of sugar powder and their mixing with mixture 2: phenylephrine hydrochloride + pheniramine maleate + ascorbic acid + sodium citrate + malic acid + citric acidanhydrous
	40	a_3 – compacting of the mixture: phenylephrine hydrochloride + pheniramine maleate + ascorbic acid + paracetamol + sodium citrate + malic acid + citric acid anhydrous + calcium phosphate + curcumin + titanium dioxide + lemon-lime flavoring + sugar powder
	41	a_4 – compacting of the mixture 1: paracetamol + sodium citrate + malic acid + citric acid anhydrous + calcium phosphate + curcumin + titanium dioxide + lemon-lime flavoring + sugar powderand mixing with mixture 2: phenylephrine hydrochloride + pheniramine maleate + ascorbic acid + sugar powder
	42	a_5 – compacting of the mixture1: phenylephrinehydrochloride+ pheniramine maleate + ascorbic acid + sugar powder+ paracetamol;compacting of the mixture2: sodium citrate + malic acid + citric acid anhydrous + calcium phosphate + curcumin + titanium dioxide + lemon-lime flavoring + sugar powder; mixing them with sugarpowder
	43	a_6 – compacting of the mixture 1: paracetamol + sugarpowder;compacting of the mixture2: phenylephrinehydrochloride+ sugar powder;compacting of the mixture 3: pheniramine maleate + sugar powder;compacting of the mixture 4: sodium citrate + malic acid + citric acidanhydrous + calcium phosphate + sugar powder + curcumin + titanium dioxide + lemon-lime flavoring and mixing them with ascorbic acid
	44	a_7 – mixture 1: paracetamol + sugar powder; mixture 2: phenylephrine hydrochloride + pheniramine maleate + curcumin + titanium dioxide + sugar powder; mixing them with calcium phosphate and lemon-lime flavoring; compacting and mixing with mixture 3: ascorbic acid + sodium citrate + malic acid + citric acidanhydrous
	45	a_8 – compacting of the mixture1: paracetamol + curcumin + titanium dioxide + sugar powder + lemon-lime flavoring; compacting of the mixture 2: phenylephrine hydrochloride + pheniramine maleate + sugar powder + calcium phosphate; mixing them with mixture 3: ascorbic acid + sodium citrate + malic acid + citric acidanhydrous
	46	a_9 – compacting of the mixture1: paracetamol + curcumin + titanium dioxide + sugar powder + calcium phosphate + lemon-lime flavoring; mixing with mixture 2: phenylephrine hydrochloride + pheniramine maleate + sugar powder and mixture 3: ascorbic acid + sodium citrate + malic acid + citric acid anhydrous

Each batch analyzed according to pharmaco-technological and analytical indicators. Experimental data were subjected to dispersion analysis and the optimal sequence of introducing the components is selected.

The powder is packed in sachets made of foil with a height of 11 cm and a width of 6 cm.

III. Results

The reference drug Theraflu for cold and flu with lemon flavor, and powder for oral solution is presented in the form of free-flowing, large granules of white color with yellow inclusions and the smell of citrus fruits. One sachet contains paracetamol 0.325 g, pheniramine maleate 0.02 g, phenylephrine hydrochloride 0.01 g, ascorbic acid 0.05 g andexcipients: sucrose, citric acid anhydrous, natural lemon flavoring, sodium citrate dihydrate, calcium phosphate, malic acid, titanium dioxide (E 171), sunset yellow dye (E 110), quinoline yellow tint (E 104). The drug is used for treating flu and cold symptoms and has an antipyretic, analgesic, and anti-allergic effect [28].

The drug Paracetamol 325 mg + phenylephrine 10 mg + pheniramine 20 mg + ascorbic acid 50 mg, powder for oral solution is developed as a generic to the reference listed drug Theraflu for cold and flu with lemon flavor, powder for oral solution, Famar Orleans, France. The pharmaceutical form of the developed drug is identical to the reference one - powder for oral solution and has the same introduction method. The nature and number of the active pharmaceutical ingredients are the same for the developed and reference medicines.

When creating the developed drug in the form of a powder for the oral solution from 325 mg of paracetamol, 10 mg of phenylephrine hydrochloride, 20 mg of pheniramine maleate, and 50 mg of ascorbic acid, the ratio of active pharmaceutical ingredients and their physicochemical properties primarily taked into account. A small content of phenylephrine hydrochloride and pheniramine maleate can cause inhomogeneity of their distribution in the powder mixture, which requires their introduction by trituration. Paracetamol is an amorphous powder with poor flowability.So it is advisable to add a large number of fillers and use additional technological methods, which will prevent the caking of sachets [29, 30]. A feature of ascorbic acid is its high ability to oxidize at temperature.

During the development of the drug, the impact of the excipients physico-chemical characteristics included in the composition, their quantity on the critical quality indicators of the drug compared with the reference drug, and the regulatory requirements for this dosage form also studied.

Therefore, taking into account the above information and the results of the study of the reference drug Theraflu for cold and flu with lemon flavor, powder for oral solution, a target quality profile (QTPP) has been determined for generic paracetamol 325 mg, phenylephrine hydrochloride 10 mg, pheniramine maleate 20 mg, ascorbic acid 50 mg, powder for oral solution in the sachet. It is given in table 5.

Table 5: Quality Target Product Profile (QTPP) of the drug

QTPP element	Purpose	Justification
Dosage form	Powder for oral solution	Pharmaceutical equivalence requirement: same dosage form
Route of administration	The oral route	Pharmaceutical equivalence requirement: same route of administration
Dose	1 sachetcontains paracetamol 325 mg, phenylephrine hydrochloride 10 mg, pheniramine maleate 20 mg, ascorbic acid 50 mg	Pharmaceutical equivalence requirement: same strength
Stability	Shelf life is 2 years. Keep outin original packagingat a temperature not exceeding 25°C	Equivalent to or better than the reference listed drug shelf-life
Drug product quality attributes	Appearance	Pharmaceutical equivalence requirement: must meet the same Compendial or other applicable (quality) standards (i.e., identity, assay, purity)
	Identification *	
	Average weight of the contents of the sachet	
	pH	
	Uniformity of dosage units	
	Loss on drying	
	Assay	
	Microbial purity *	
Container/closure system	Sachet bag made of material similar to the reference listed drug	Necessary to achieve the target expiration date and ensure the stability of the drug during transportation
Administration	According to the SmPC for the reference listed drug	Equivalent to or better than the reference listed drug
Alternative method of use	Preliminary data is absent	Not applicable
*Note: * - formulation and process variables are unlikely to impact the CQA. However, the CQA remains a target element of drug product profile and should be addressed accordingly*		

To investigate the variability of the formulation and technology in the context of further studies, a risk assessment conducted. It includes previously acquired knowledge and experience in the development of similar drugs.

The initial risk assessment of formulation variables reflects the possible impact of the product formulation and the technological process of manufacturing the finished medicinal product on the established quality attributes.

In the process of risk assessment, quantitative factors were divided into three categories (high, medium, and low). The results of the initial risk assessment of composition variables are presented in table 6.

Table 6: Initial risk assessment of pharmaceutical development

Drug Product quality attributes	Formulation variability											Technology variability
	Nature of alkaline fraction	Nature of acid fraction	Nature of coloring	Nature of flavoring	Nature of filler	Quantity of alkaline fraction	Quantity of acid fraction	Quantity of sodium citrate	Quantity of coloring	Quantity of flavoring	Quantity of titanium dioxide	Sequence of introduction of components
Appearance	Medium	Medium	High	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Low
pH	High	High	Low	Medium	Medium	High	High	Medium	Low	Medium	Medium	Low
Uniformity of dosage units	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	High
Loss on drying	Low	Low	Low	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Low	Low
Assay	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	High

The results of powder research when choosing excipients are shown in table 7.

Table 7: Results of studies of powder for the oral solution when studying the nature of excipients

Batch	y_1	y_1'	y_2	y_2'	y_3	y_3'	y_4	y_4'	y_5	y_5'	y_6	y_6'	y_7	y_7'	y_8	y_8'	y_9	y_9'	y_{10}	y_{10}'	y_{11}	y_{11}'
1	4	3	0.5696	0.5729	0.6617	0.6627	13.92	13.54	9.1	9.1	37.8	38.0	0.95	0.95	1	1	5	4	4	5	5.57	5.57
2	2	2	0.6745	0.6735	0.7856	0.7765	14.14	13.27	10.7	10.3	38.0	38.0	0.70	1.24	2	2	4	5	5	4	6.83	6.83
3	4	5	0.4777	0.4725	0.5442	0.5443	12.22	13.19	13.7	12.6	40.0	41.7	0.91	0.91	2	2	4	3	4	5	4.93	4.93
4	5	5	0.5068	0.5054	0.7141	0.7121	29.03	29.03	25.3	28.3	45.6	45.4	0.37	0.37	2	2	5	5	5	5	5.00	5.01
5	5	5	0.8641	0.8632	0.9377	0.9318	7.85	7.37	6.6	5.9	37.1	38.3	0.73	0.73	2	2	5	4	5	5	5.98	5.98
6	3	4	0.6936	0.6907	0.8010	0.7976	13.40	13.40	8.3	8.6	36.2	34.8	7.29	7.29	2	2	4	4	5	4	4.83	4.83
7	5	5	0.7760	0.7742	1.0165	1.0141	23.66	23.66	39.4	40.0	36.9	34.8	0.51	0.51	3	2	5	5	5	5	3.89	3.89
8	5	5	0.5987	0.6000	0.8618	0.8636	30.53	30.53	114.5	110.0	45.5	47.1	0.38	0.38	3	3	4	3	4	4	4.67	4.67
9	4	5	0.6071	0.6087	0.7058	0.7000	13.98	13.04	9.7	10.0	38.2	37.6	0.75	0.75	2	2	3	3	5	5	4.02	4.02
10	5	5	0.5697	0.5657	0.7121	0.7000	20.00	19.19	10.0	10.1	37.7	37.4	0.61	0.61	5	5	4	3	4	5	5.44	5.44
11	5	5	0.6159	0.6134	0.8596	0.8623	28.35	28.87	39.0	46.0	44.9	45.1	0.47	0.47	5	5	4	4	5	5	6.75	6.75
12	5	5	0.5711	0.5684	0.8346	0.8308	31.58	31.58	22.6	21.4	46.8	47.5	0.56	0.56	3	4	4	5	4	5	4.64	4.64
13	5	5	0.4519	0.4516	0.5417	0.5385	16.58	16.13	14.6	14.8	39.3	39.3	1.23	1.23	3	3	4	5	5	4	4.98	4.98
14	5	5	0.5696	0.5670	0.6766	0.6707	15.82	15.46	9.9	9.7	38.5	38.0	0.98	0.98	3	2	4	3	4	3	5.99	5.99
15	5	5	0.6592	0.6563	0.7812	0.7778	15.63	15.63	10.4	10.1	38.2	37.4	1.14	1.14	3	4	5	4	5	5	4.94	4.94
16	5	5	0.7000	0.6979	0.8048	0.8072	13.02	13.54	15.9	9.0	35.9	36.1	7.47	7.47	4	3	5	5	5	4	3.86	3.86
17	5	5	0.5322	0.5269	0.7332	0.7313	27.42	27.96	54.8	79.0	42.5	40.1	0.37	0.37	3	4	5	5	5	4	4.79	4.79
18	5	5	0.9160	0.9140	1.0142	1.0119	9.68	9.68	6.7	6.8	38.6	35.2	0.32	0.32	3	4	5	5	5	5	3.96	3.96
19	5	5	0.8250	0.8191	0.9124	0.9059	9.57	9.57	7.3	7.4	39.2	39.5	0.73	0.73	5	5	4	5	5	5	5.42	5.42
20	5	4	0.6928	0.6947	0.7918	0.7857	12.50	11.58	9.9	10.0	35.8	35.0	7.65	7.65	5	5	5	4	5	5	6.64	6.64
21	5	5	0.4715	0.4688	0.6891	0.6923	31.58	32.29	19.1	21.3	48.7	48.8	0.41	0.41	5	5	5	4	5	4	4.69	4.69
22	5	5	0.6091	0.6044	0.8798	0.8730	30.77	30.77	98.2	109.6	47.7	47.8	0.35	0.35	3	3	4	5	4	3	4.78	4.78
23	5	5	0.5786	0.5745	0.6799	0.6750	14.89	14.89	10.2	10.6	37.7	36.9	0.65	0.65	3	3	5	5	4	3	5.91	5.91
24	5	5	0.6162	0.6105	0.8870	0.8788	30.53	30.53	52.6	61.4	42.4	44.8	0.58	0.58	3	3	4	3	5	4	4.67	4.67
25	4	4	0.7006	0.7021	0.8131	0.8148	13.83	13.83	11.7	11.8	36.1	36.9	0.78	0.78	3	4	4	5	3	4	3.99	3.99
26	4	5	0.4727	0.4731	0.5636	0.5641	16.13	16.13	14.1	14.2	42.1	41.8	0.79	0.79	3	3	5	5	3	4	4.86	4.86
27	5	5	0.5888	0.5938	0.6840	0.6867	13.92	13.54	9.7	10.1	37.3	37.8	1.16	1.16	3	4	5	5	5	4	3.94	3.94

Notes: y_1, y_1' – appearance of the powder of the first and second replicates, score; y_2, y_2' – bulk density of the first and second replicates, g/ml; y_3, y_3' – tapped densityof the first and second replicates, g/ml; y_4, y_4' – Carr's index of the first and second replicates, %; y_5, y_5' – flowabilityof the first and second replicates, s/100 g; y_6, y_6' – angle of repose of the first and second replicates, °; y_7, y_7' – loss on drying of the first and second replicates, %; y_8, y_8' – appearance of the solutionof the first and second replicates, score; y_9, y_9' – smell of the solution of the first and second replicates, score; y_{10}, y_{10}' – taste of the solution of the first and second replicates, score; y_{11}, y_{11}' – pH of the solution of the first and second replicates

Summary data of ordinal numbers of factor levels in the ranked series of advantages are given in table 8.

Table 8: Summary table of ranked series of advantages

Factor / indicator	a_1	a_2	a_3	b_1	b_2	b_3	c_1	c_2	c_3	d_1	d_2	d_3	d_4	d_5	d_6	d_7	d_8	d_9	e_1	e_2	e_3	e_4	e_5	e_6	e_7	e_8	e_9
y_1	3	1	2	0	0	0	3	1	2	8	4.5	2	2	9	2	6.5	4.5	6.5	7	9	6	2.5	2.5	8	2.5	2.8	5
y_2	3	2	1	1	2	3	1	2	3	9	1	4	6	8	3	2	5	7	7	3	9	8	1	2	4	5	6
y_3	3	2	1	1	3	2	1	2	3	9	2	1	3	8	6	4	5	7	8	5	9	6	1	4	2	3	7
y_4	2	3	1	2	1	3	1	3	2	3	5	7	9	2	6	4	8	1	3	4	5	8	1	2	7	9	6
y_5	1	2	3	2	3	1	2	1	3	1	5	6	9	3	4	7	8	2	2	5	6	7	1	4	8	9	3
y_6	1	2	3	3	2	1	3	2	1	6	5	4	2	9	3	7	1	8	6	8	4	2	5	9	3	1	7
y_7	2	3	1	0	0	0	0	0	0	5	7	3	1.5	6	9	8	1.5	4	8	6	7	1	4	9	3	2	5
y_8	1	3	2	0	0	0	3	2	1	9	3	6	1	7	3	3	5	8	9	7	8	2.5	2.5	2.5	2.5	6	5
y_9	3	2	1	1	2	3	3	2	1	4	1.5	1.5	9	4	4	6	7.5	7.5	5.5	3.5	5.5	1	2	3.5	7.5	7.5	9
y_{10}	1	3	2	2	3	1	1	2	3	7	1	7	7	4.5	2.5	2.5	4.5	9	7.5	5.5	7.5	3.5	1	3.5	2	9	5.5
y_{11}	3	2	1	2	3	1	3	2	1	6	8	1	3	9	2	5	7	4	8	9	3	2	6	5	4	1	7
Total	2.09	2.27	1.64	1.27	1.73	1.36	1.91	1.73	1.82	6.09	3.91	3.86	4.77	6.32	4.05	5	5.18	5.82	6.45	5.91	6.36	3.95	2.45	4.77	4.14	5.03	5.95

The utility function shows that the ranked number of advantages the studied alkaline fraction is as follows: $a_3 > a_1 > a_2$, so calcium phosphate received the advantages. The best-generalized indicators were observed when using citric acid anhydrous (b_1) among the excipients group of the acid fraction. However, the lowest pH is provided by a mixture of citric acid anhydrous and malic acid (5:1) (b_2).So a decision was made to study their optimal ratio further. Curcumin (c_2) was the unequivocal leader among colorings (factor C). There is the advantages of excipients of natural origin over yellow iron oxide:$c_2 > c_3 > c_1$. The ranked order of preference for factor D can be represented as an inequality: orange (3.86) > lemon-lime (3.91) > raspberry (4.05) > grapefruit (4.77) > strawberry (5.00) > apple (5.18) > chocolate (5.82) > lemon (6.09) > blackcurrant (6.32). Orange and lemon-lime flavorings have close overall results. Still, according to the main indicator for this factor (y_{10} – thetaste of the solution), advantages were obtained by lemon-lime (d_2), which was selected for further research. The generalized inequality of the advantages of factor E is as follows: a mixture of sucrose with xylitol (2.45) > mannitol (3.95) > powdered sugar (4.14) > dextrose hydrate (4.77) > lactose monohydrate 200 (5.03) > sorbitol 60 (5.91) > maltitol (5.95) > sorbitol 450 (6.36) > fructose (6.45). Among the studied levels of factor A,a mixture of sucrose and xylitol (e_5) was preferred. Considering the economic costs, other excipients based on sucrose should be considered as a filler, in particular, powdered sugar (e_7), with the use of additional technological techniques.

Therefore, according to the results of the trough function, it is advisable to introduce calcium phosphate (a_3), anhydrous citric acid (b_1), malic acid (b_3), curcumin (c_2), lemon-lime flavoring (d_2), and powdered sugar into the composition of the powder for oral solution (e_7).

The results of the tests in the study of the excipients quantities are given in table 9.

Table 9: The results of the study of the number of excipients

Batch	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}
28	5	0.6086	0.8844	31.18	56.8	48.3	0.30	5	5	4	3.05
29	4	0.6403	0.8658	26.05	44.9	47.5	0.31	5	4	5	2.78
30	5	0.7403	1.0193	27.37	52.7	41.0	0.32	4	4	3	4.01
31	5	0.7393	1.0071	26.59	50.1	40.5	0.46	4	4	5	3.74
32	4	0.6502	0.8824	26.31	17.5	46.9	0.37	5	4	4	3.10
33	5	0.6187	0.8517	27.36	18.4	46.6	0.46	5	5	5	2.58
34	5	0.7246	1.0016	27.66	69.1	41.7	0.46	4	4	3	3.98
35	4	0.7444	1.0066	26.05	66.6	41.4	0.39	4	5	3	3.70
36	5	0.6944	0.9742	28.72	62.6	43.6	0.50	4	4	5	3.60
37	5	0.7075	0.9988	29.16	57.6	45.2	0.42	4	4	4	3.60

Notes: y_1– appearance of the powder, score; y_2– bulk density, g/ml; y_3– tapped density, g/ml; y_4– Carr`s index, %; y_5– flowability, s/100 g; y_6– angle of repose, °; y_7– loss on drying, %; y_8– appearance of the solution, score; y_9– smell of the solution, score; y_{10}– taste of the solution, score; y_{11}– pH of the solution

To summarize the experimental data obtained, a table 10 was constructed.

Table 10: The results of the experiment are summarized in the development of the composition of the powder in sachets packages

Factor/ indicator	x_1	x_2	x_3	x_4	x_5	x_6	x_7
y_1	+*	0	0	-*	+*	-*	0
y_2	+*	+	-	-*	-	-*	+*
y_3	+*	-	-	-*	-	-*	+*
y_4	-	+*	+	-	-*	+	+*
y_5	-*	+	+*	+	-	+	-
y_6	-*	-	0	+*	+	+*	-*
y_7	-*	-*	-*	+*	-*	+*	+
y_8	-*	0	0	+*	0	+*	-*
y_9	-	+	+	-	+	+*	-
y_{10}	-*	+*	-*	+	+*	+*	+
y_{11}	-*	+	0	+*	+	+*	-*
Total	-	+	-	+	0	+	0
*Note: * - a significant factor*							

Based on the total values of the generalized results, the levels of the studied factors selected: lower for factors x_1, x_3, upper for factors x_2, x_4, x_6, andbasic for factors $x_5$and x_7.

The conducted studies made it possible to establish the optimal composition of the powder for oral solution (Table 11).

Table 11: Optimal composition of the drug powder for oral solution per 1 sachet pack

Ingredient	Quantity, g/sashet	Quantity, %
Paracetamol	0.3250	1.47
Ascorbic acid	0.0500	0.23
Phenylephrine hydrochloride	0.0100	0.05
Pheniramine maleate	0.0200	0.09
Calcium phosphate	0.0820	0.37
Sodium citrate	0.1210	0.55
Citric acid anhydrous	1.2220	5.52
Malic acid	0.0500	0.23
Curcumin	0.0383	0.17
Lemon-lime flavoring	0.2085	0.94
Titanium dioxide	0.0032	0.01
Sugar powder	20.0000	90.38
Total	22.1300	100.00

The proposed composition of the powder for the oral solution had the following characteristics: bulk density 0.59 g/ml, tapped density of 0.75 g/ml, Carrs index 21.0 %, flowability 4.3 g/s, angle of repose 41.3 ° pH of the solution 2.81 and satisfactory organoleptic properties.

Poor powder flowability can cause problems in the operation of the machine during packaging and inhomogeneity of dosage in sachets.

For improve the properties of the powder, the sequence of the introduction of the ingredients studied, and the optimal technology selected. A modern solution to the technical task is using the roller compaction method [31-35]. The results of the study of the pharmaco-technological and analytical indicators of the powder obtained by the proposed technologies are shown in table 12.

Table 12: Results of the study of quality parameters during technology development

Batch	y_1	y_1'	y_2	y_2'	y_3	y_3'	y_4	y_4'	y_5	y_5'	y_6	y_6'	y_7	y_7'	y_8	y_8'	y_9	y_9'	y_{10}	y_{10}'	y_{11}	y_{11}'	y_{12}	y_{12}'	y_{13}	y_{13}'
38	0.8225	0.8201	1.0204	1.0196	19.39	19.57	5.3	5.5	38.2	37.9	97.35	101.54	3.02	4.16	99.28	103.18	2.50	3.85	93.35	109.63	9.34	6.53	96.00	103.51	5.64	7.41
39	0.7702	0.7712	1.0197	1.0203	24.47	24.41	6.3	5.9	39.3	40.0	102.81	98.76	2.12	3.48	103.47	96.85	3.40	4.12	74.55	121.04	8.59	9.25	105.30	103.84	13.74	18.29
40	0.7833	0.7859	1.0195	1.0205	23.17	22.99	5.5	5.7	39.4	38.6	97.02	103.63	11.08	13.21	104.80	96.74	2.20	3.72	98.47	103.45	6.93	8.31	96.20	104.90	6.28	7.27
41	0.7828	0.7745	0.9823	0.9761	20.31	20.65	4.8	5.6	41.3	39.2	102.88	101.49	1.49	2.84	100.94	100.55	2.20	1.60	102.62	99.26	5.33	3.63	102.80	103.10	3.62	3.63
42	0.7882	0.7874	1.0197	1.0183	22.70	22.68	6.1	5.9	40.9	40.3	96.49	104.93	22.07	26.58	104.43	97.16	1.10	2.47	97.51	104.81	8.28	7.63	97.60	102.76	15.63	18.47
43	0.7809	0.7817	1.0517	1.0530	25.75	25.76	6.3	6.0	38.7	39.2	94.10	97.26	4.43	3.37	106.12	99.63	2.00	3.41	110.98	105.79	3.77	4.12	99.80	104.42	3.59	4.15
44	0.8055	0.8043	1.0170	1.0142	20.80	20.70	7.2	6.9	37.2	38.5	100.63	103.97	1.42	2.14	101.36	103.61	2.50	3.18	100.94	103.41	4.23	2.61	101.50	104.69	1.86	3.02
45	0.7792	0.7818	1.0169	1.0149	23.37	22.97	6.8	6.5	38.7	39.4	100.80	104.59	1.79	2.81	101.70	102.93	2.20	2.16	101.21	104.53	4.48	3.04	101.50	103.15	1.43	2.45
46	0.8173	0.8151	1.0801	1.0771	24.33	24.32	5.2	4.9	38.4	39.7	100.47	102.38	2.47	1.65	102.60	97.37	2.80	3.98	100.40	103.18	4.23	2.31	103.00	99.06	4.03	4.62

Notes: y_1, y_1' – bulk density of the first and second replicates, g/ml; y_2, y_2' – tapped density of the first and second replicates, g/ml; y_3, y_3'– Carr`s index of the first and second replicates, %; y_4, y_4'–flowability of the first and second replicates, g/s; y_5, y_5'–angle of repose of the first and second replicates, °; y_6, y_6' – assay of paracetamol of the first and second replicates, %; y_7, y_7' – content uniformity of paracetamol of the first and second replicates, %; y_8, y_8' – assay of ascorbic acid of the first and second replicates, %; y_9, y_9' – content uniformity of ascorbic acid of the first and second replicates, %; y_{10}, y_{10}' – assay of phenylephrine hydrochloride of the first and second replicates, %; y_{11}, y_{11}' – content uniformity of phenylephrine hydrochloride of the first and second replicates, %; y_{12}, y_{12}' – assay of pheniramine maleate of the first and second replicates, %; y_{13}, y_{13}' – content uniformity of pheniramine maleate of the first and second replicates, %

The results of the dispersion analysis of the research data on the technologies of the powder for oral solution show deviations of the pharmaco-technological indicators within the measurement error.They do not depend on the technology used. According to the results of the analysis of the quantitative content of the active pharmaceutical ingredients and the uniformity of the dosage units, it established that the a_9 technology meets the pharmacopoeial requirements, therefore, this method of introducing the ingredients chosen as optimal. It included the following stages. Compacting of the mixture 1: paracetamol + curcumin + titanium dioxide + sugar powder + calcium phosphate + lemon-lime flavoring; mixing with mixture 2: phenylephrine hydrochloride + pheniramine maleate + sugar powder and mixture 3: ascorbic acid + sodium citrate + malic acid + citric acid anhydrous.

Based on study results, the risk assessment ofvariability of the formulation and technology was revised.

The risk of all variables belong to the low risk.

IV. Discussion

a) Selection of the nature of excipients

The results of the dispersion analysis showed that the influence of the factors on the appearance of the powder (y_1, y_1') is expressed as follows: C > E > A > D. It was experimentally confirmed that dyes have the most significant influence on the appearance of the powder. With the addition of curcumin, the powders had a uniform yellowish color, and their appearance was rated at 5 points.

The use of riboflavin provided an intense yellow-hot homogeneous color (4.78 points), and the introduction of iron oxide was accompanied by non-uniform distribution in the mass and obtaining powders with dark brown spots on a white background (4.22 points). The ranked series for factor E (fillers) is as follows: powdered sugar = lactose monohydrate 200 = mannitol = mixture of sucrose with xylitol (5 points) > maltitol (4.83 points) > sorbitol 450 (4.67 points) > fructose (4.5 points) > dextrose hydrate (4.33 points) > sorbitol 60 (3.67 points).Among the excipients of the alkaline fraction, calciumphosphate is slightly inferior to calcium carbonate in terms of its effect on the appearance of the powder (4.78 points versus 4.83 points, respectively). With the addition of sodium bicarbonate, the powders were rated at an average of 4.39 points. The ranked order for flavors is as follows: d_3 (d_4; d_6) (5 points) > d_2 (d_8) (4.83 points) > d_7 (d_9) (4.5 points) > d_1 (4.33 points) > d_5 (4 points). The greatest value of the appearance of the powder was obtained when using orange, grapefruit, and raspberry flavorings.

The results of data on bulk density (y_2, y_2') showed the influence of factors that were ranked as follows: E > D > A > C > B. The ranked series for factor E looks as follows: $e_5 > e_6 > e_2 > e_7 > e_8 > e_9 > e_1 > e_4 > e_3$ (Fig. 1).

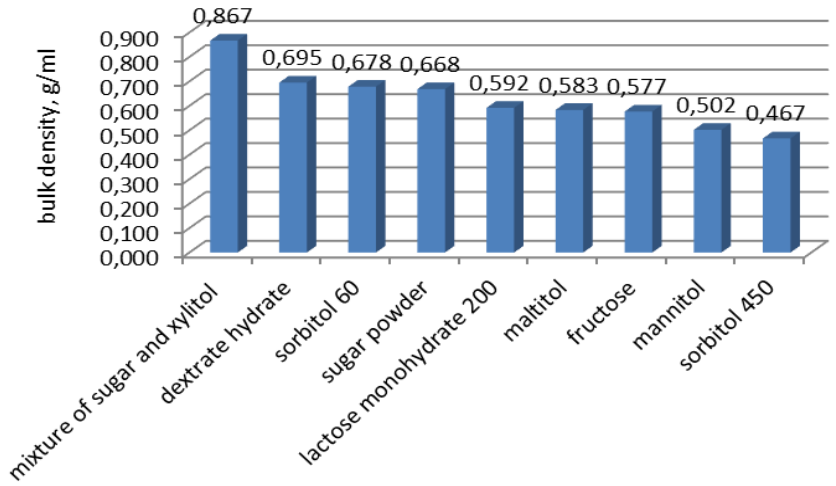

Fig. 1: The influence of fillers on bulk density of the powder

The ranked series for flavors is as follows: lemon-lime (0.7050 g/ml) > strawberry (0.6817 g/ml) > raspberry (0.6783 g/ml) > orange (0.6400 g/ml) > apple (0.6100 g/ml) > grapefruit (0.5933 g/ml) > chocolate (0.5817 g/ml) > black currant (0.5717 g/ml) > lemon (0.5667 g/ml). Calcium phosphate (0.6544 g/ml) had the greatest impact on bulk density, calcium carbonate (0.6150 g/ml) differed significantly in its positive influence, masses containing sodium hydrogen carbonate (0.6067 g/ml) had the worst bulk density ml). The ranked series for dyes is as follows: c_1 (0.6406 g/ml) > c_2 (0.6194 g/ml) > c_3 (0.6167 g/ml). The anhydrous citric acid (0.6333 g/ml) is the leader among excipients of the acid fraction in terms of bulk density. A mixture of anhydrous citric and malic acids (5:1) has slight advantages over malic acid (0.6217 g/ml and 0.6211 g/ml, respectively).

The results of the variance analysis showed that the influence of the factors on tapped density (y_3, y_3') is expressed as follows: E > D > A > B > C. The ranked series for factor C looks as follows: $e_5 > e_7 > e_8 > e_6 > e_2 > e_4 > e_9 > e_1 > e_3$ (Fig. 2).

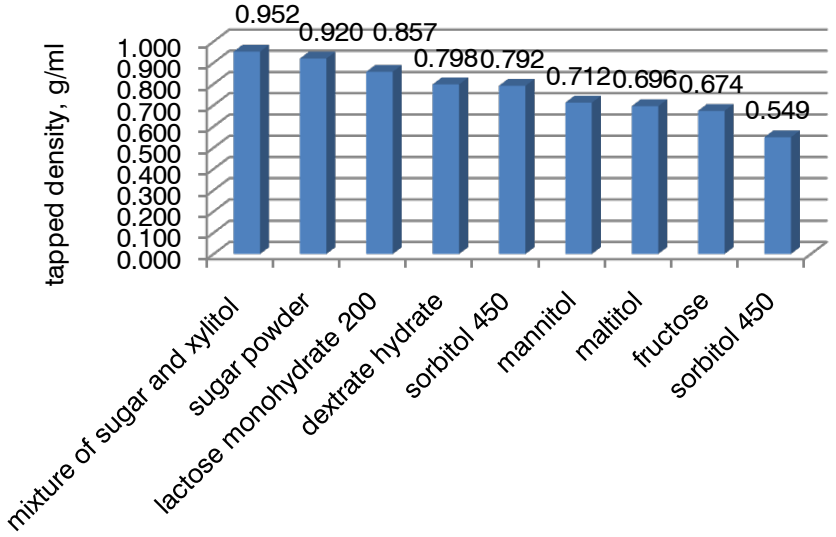

Fig. 2: Diagram of the influence of fillers on tapped density of the powder

The rank order for factor D is as follows: orange (0.8418 g/ml) > lemon-lime (0.8383 g/ml) > grapefruit (0.8172 g/ml) > strawberry (0.8136 g/ml) > apple (0.8134 g/ml) > raspberry (0.8105 g/ml) > chocolate (0.6773 g/ml) > black currant (0.6688 g/ml) > lemon (0.6685 g/ml). Calcium phosphate (0.7995 g/ml) was preferred among the excipients of the alkaline fraction in terms of tapped density. It is inferior to calcium carbonate (0.7641 g/ml) and sodium bicarbonate (0.7529 g/ml). The influence of the acid fraction on tapped densityis expressed by the following inequality: b_1 (0.7825 g/ml) > b_3 (0.7701 g/ml) > b_2 (0.7639 g/ml). So, anhydrous citric acid has advantages. The ranked order of preference for factor C illustrates the ranked order of preference: ferric oxide (0.7795 g/ml) > curcumin (0.7716 g/ml) > riboflavin (0.7654 g/ml).

The flowability of the powder was expressed using Carr's index (y_4, y_4'). The results of dispersion

analysis showed that the influence of factors on this indicator is expressed as follows: E > D > C > A > B. Fillers can be ranked in the following sequence by their influence on Carr`s index:e_5 (8.95 %) >e_6 (12.91 %) >e_1 (14.37 %) >e_2 (14.39 %) >e_3 (15.06 %) >e_9 (16.00 %) >e_7 (27.60 %) >e_4 (29.55 %) >e_8 (30.96 %). It shows the advantages of amixture of sucrose and xylitol on Carr's index of the powder. The ranked series for flavorings looks like this:d_9 (14.06 %) >d_5 (14.60 %) >d_1 (15.16 %) >d_7 (16.89 %) >d_2 (16.92 %) >d_6 (17.61 %) >d_3 (23.38 %) >d_8 (24.30 %) >d_4 (26.89 %). So, chocolate flavoring has the greatest influence on flowability. Among colorings, iron oxide provides an average Carr's index of 17.54%, which is dominated by riboflavin (19.27%) and curcumin (19.78%). The leader of the alkaline fraction in terms of influence on Carr's index is calcium phosphate (18.00%), followed by sodium bicarbonate (19.27%) and calcium carbonate (19.32%). The mixture of citric acidanhydrous and malic acid (18.54%) exhibits dominant properties on Carr'sindex among the acid fraction. The use of citric acid anhydrous providesCarr's index of 18.87% and malic acid - 19.19 %.

The results of flowability data (y_5, y_5') showed the influence of the factors that ranked in this way: E>D>A>B>C. The influence of the investigated fillers on flowability reflects inequality: a mixture of sucrose with xylitol (6.8 s/100 g) > fructose (9.6 s/100 g) > maltitol (10.1 s/100 g) > dextrose hydrate (10.3 s/100 g) > sorbitol 60 (10.8 s/100 g) > sorbitol 450 (14.0 s/100 g) > mannitol (38.0 s/100 g) > powdered sugar (46.4 s/100 g) > lactose monohydrate 200 (79.4 s/100 g). The ranked series of advantages of flavorings on flowability has the following form: d_1 (11.2 s/100 g) > d_9 (11.6 s/100 g) > d_5 (11.7 s/100 g) > d_6 (13.0 s/ 100 g) > d_2 (14.5 s/100 g) > d_3 (24.0 s/100 g) > d_7 (27.6 s/100 g) > d_8 (52.1 s/100 g) > d_4 (59.8 s/100 g). Lemon flavoring preferred. Sodium bicarbonate (16.1 s/100 g) has the dominant flowability properties. It is inferior to calcium carbonate (27.5 s/100 g) and calcium phosphate (31.5 s/100 g). Based on the results of statistical processing of the experimental data, a ranked series of the acid fraction for flowability obtained: b_3 (17.5 s/100 g) > b_1 (26.2 s/100 g) > b_2 (31.4 s/100 g), which shows the advantage malic acid. Curcumin (21.7 s/100 g) is the leader among colorings in flowability. According to this indicator, iron oxide has slight advantages over riboflavin (26.2 s/100 g and 27.2 s/100 g, respectively).

When studying the angle of repose (y_6, y_6'), the influence of all the studied factors were established, which were ranked as follows:E>A>D>C>B. Among the fillers, powdered sugar provided the optimal values of this indicator at the level of 41.48°. High angle of repose results were characterized by mannitol (45.18 °) and lactose monohydrate 200 (47.07°). A decrease in the values of angle of repose was observed in the powder, which included dextrose hydrate (35.63°), sorbitol 60 (37.43°), maltitol (37.58 °), fructose (37.90°), a mixture of sucrose with xylitol (37.98°) and sorbitol 450 (40.70°). Among alkaline fraction, calcium phosphate is the leader (38.92°) and has advantages over calcium carbonate (40.30°) and sodium bicarbonate (41.11°). The ranked order for flavorings is as follows: d_5 (38.28°) > d_9 (38.53°) > d_7 (38.72°) > d_1 (39.22°) > d_2 (39.27°) > d_3 (40.10°) > d_6 (40.82°) > d_4 (42.48°) > d_8 (43.55°). According to the effect on the angle of repose, the colorings were placed in the following sequence: iron oxide (39.50°), curcumin (39.92°), and riboflavin (40.91°). Among the acid fraction, citric acid anhydrous (39.52°) has advantages over a mixture of citric acid anhydrous and malic acid (40.13°) and malic acid (40.67°).

An important indicator of the quality of the powder, which can affect the stability of the drug, is the loss of drying (y_7, y_7'). The influence of the studied factors on this indicator reflects the inequality: E > D > A. The ranked number of advantages of fillers for weight loss during drying is as follows: e_4 >e_8 >e_7 >e_5 >e_9 >e_2 >e_3 >e_1 >e_6 (Fig. 3).

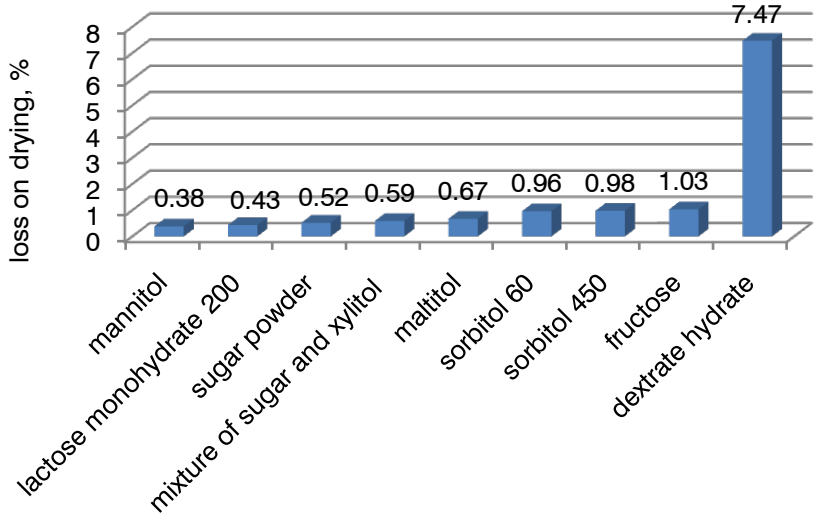

Fig. 3: Effect of fillers on loss on dryingof the powder

The dependence of flavorings on the loss on drying illustrates the inequality: grapefruit (apple) (0.52 %) > orange (0.57 %) > chocolate (0.89 %) > lemon (0.96 %) > black currant (1.12 %) > lemon-lime (2.78 %) > strawberry (2.80 %) > raspberry (2.87 %). Calcium phosphate (1.39%) has the leading properties among alkaline fraction, which is dominated by sodium bicarbonate (1.47%) and calcium carbonate (1.48%).

The powder for the oral solution as a dosage form must meet not only pharmacopoeial requirements but also consumer characteristics. Since patients take the powder in dissolved form, the organoleptic characteristics of the solution, which evaluated on a 5-point scale, were studied during development. According to the appearance of the solution (y_8, y_8'), the tested mixtures differed in transparency and color intensity. The influence of experimental factors on the appearance of the solution can be ranked as follows: C > A > D (E).On average, riboflavin and curcumin provided the appearance of the solution with 3.78 points and 3.67 points, respectively, compared to iron oxide - 2.06 points. Among the alkaline fraction, sodium bicarbonate provided the best results of this indicator (3.72 points). It was inferior to calcium phosphate (3.11 points) and calcium carbonate (2.67 points). The ranked number offlavoring advantages is as follows: d_4 (3.67 points) > d_2 (d_6; d_7) (3.50 points) > d_8 (3.33 points) > d_3 (3.00 points) > d_5 (2.83 points) > d_9 (2.67 points) > d_1 (2.50 points). The influence of fillers on the appearance of the solution reflects inequality: e_4 (e_5; e_6; e_7) (3.50 points) > e_9 (3.33 points) > e_8 (3.17 points) > e_2 (3.00 points) > e_3 (2.67 points) > e_1 (2.33 points).

The results of statistical processing of experimental data show that not only flavorings affect the smell of the solution (y_9, y_9'). According to the impact on this indicator, the investigated factors can placed in the following sequence: D>B (C) >E>A. The ranked number of preferences of flavorings for smell of the solution is as follows: lemon-lime (orange) (4.83 points) > lemon (blackcurrant; raspberry) (4.67 points) > strawberry (4.50 points) > apple (chocolate) (3.83 points) > grapefruit (3.50 points). Among the acid fraction, citric acid anhydrous improves the odor of the solution by an average of 4.56 points, a mixture of citric acid anhydrous and malic acid by 4.39 points, and malic acid by 4.17 points. Riboflavin (4.56 points) is the leader among colorings in terms of impact on the smell of the solution. Curcumin (4.39 points) has advantages over iron oxide (4.17 points). The dependence of the studied indicator on the nature of the filler reflects a ranked number of advantages: e_4 (4.83 points) >e_5 (4.67 points) > e_2 (e_6) (4.50 points) >e_1(e_3) (4.33 points) > e_7 (e_8) (4.17 points) > e_9 (3.83 points). In terms of impact on the smell of the solution, calcium phosphate exhibits dominant properties (4.56 points). It is somewhat inferior to calcium carbonate (4.33 points) and sodium bicarbonate (4.22 points).

The taste characteristics of the solution (y_{10}, y_{10}') made it possible to reflect the influence of factors in the form of the following inequality: C > E > B > D > A. Among the dyes, the leader was iron oxide (4.67 points), which had slight advantages over curcumin (4, 56 points) and riboflavin (4.17 points). The ranked number of advantages of fillers is as follows: e_5 (5.00 points) > e_7 (4.83 points) > e_4 (e_6) (4.67 points) > e_2 (e_9) (4.33 points) > e_1 (e_3) (4.17 points) > e_8 (4.00 points). The effect of the acid fraction on the taste of the solution can be represented as follows: malic acid (4.67 points) > citric acid anhydrous (4.44 points) > mixture of citric acid anhydrous and malic acid (4.28 points). The advantages of the lemon-lime flavoring are shown by inequality: d_2 (5.00 points) > d_6 (d_7) (4.67 points) >d_5 (d_8) (4.50 points) >d_1 (d_3; d_4) (4.33 points) >d_9 (3.83 points). The dependence of the taste of the solution on the alkaline fraction shown by the expression: sodium bicarbonate (4.67 points) > calcium phosphate (4.39 points) > calcium carbonate (4.33 points).

One of the most important indicators of the powder was the pH of the solution (y_{11}, y_{11}'). The solubility of the dosage form depended on it. At low pH values, the powder wholly dissolved and did not leave a residue. The influence of the investigated factors on the pH of the solution was significant for all factors, which were ranked as follows: A > B > E > D > C. Fig. 4 shows the advantages of calcium phosphate over calcium carbonate and sodium bicarbonate.

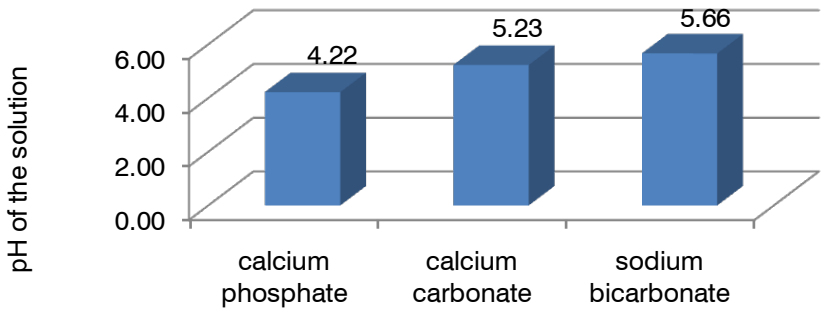

Fig. 4: Effect of alkaline fraction excipients on pH of the solution

Among acid fraction excipients, malic acid shows dominant properties in the pH of the solution (Fig. 5).

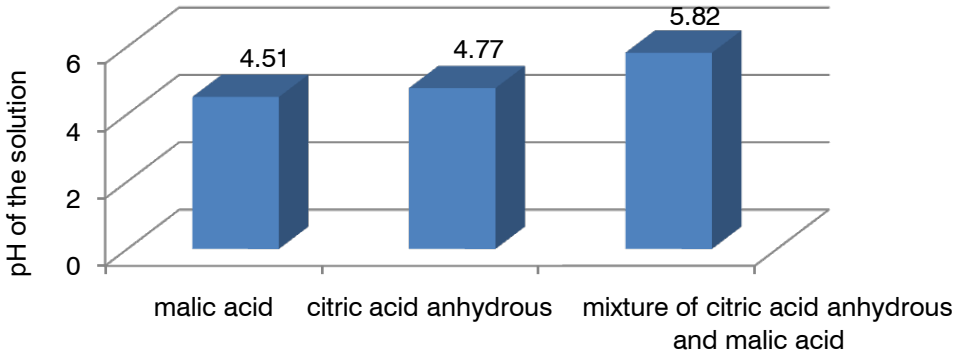

Fig. 5: Diagram of the influence of acid fraction excipients on pH of the solution

The ranked number of advantages of fillers on pH of the solution is as follows: $e_8 > e_4 > e_3 > e_7 > e_6 > e_5$ (e_9) $> e_1 > e_2$ (Fig. 6).

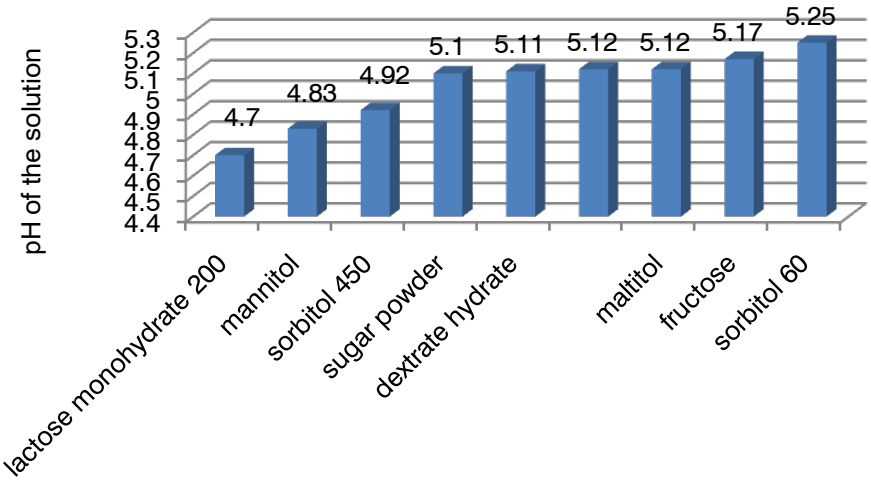

Fig. 6: Dependence of pH of the solution on nature of thefiller

The effect of flavorings on pH of the solution reflects inequality: $d_3 > d_6 > d_4 > d_9 > d_7 > d_1 > d_8 > d_2 > d_5$ (Fig. 7).

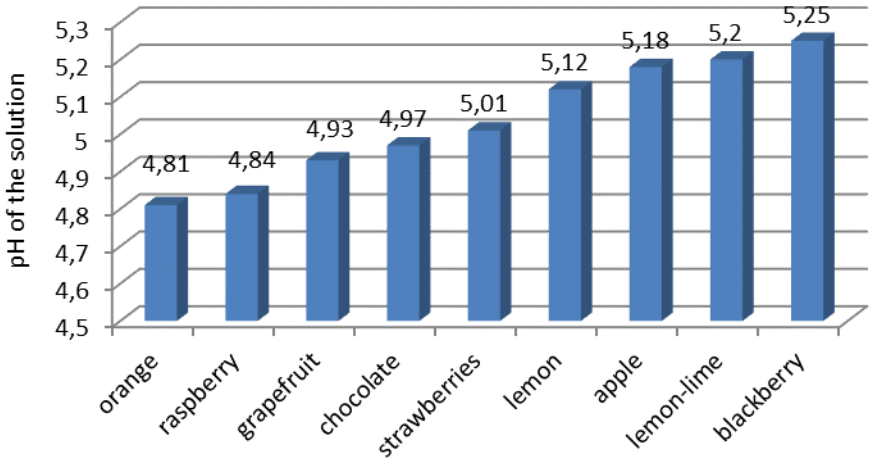

Fig. 7: The effect of flavorings on the pH of the solution

Figure 8 shows the dependence of the pH of the solution on the nature of coloring. Natural colorings have advantages over synthetic iron oxide[36].

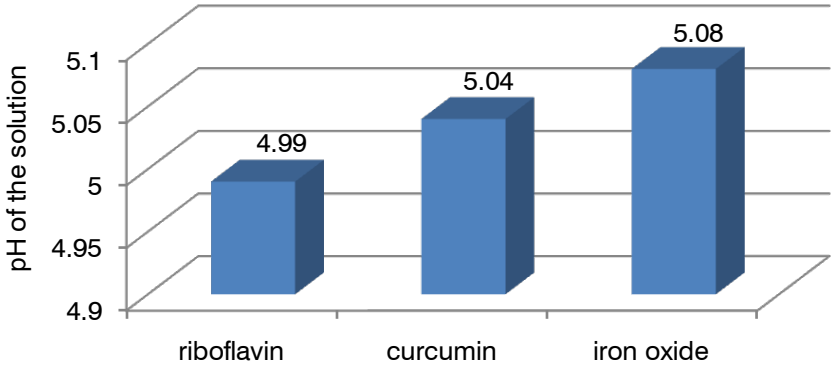

Fig. 8: Dependence of pH of the solution on the nature of the coloring

b) Study of the quantities of excipients

The influence of quantitative factors on the appearance of the powder (y1) is shown in Figure 9.

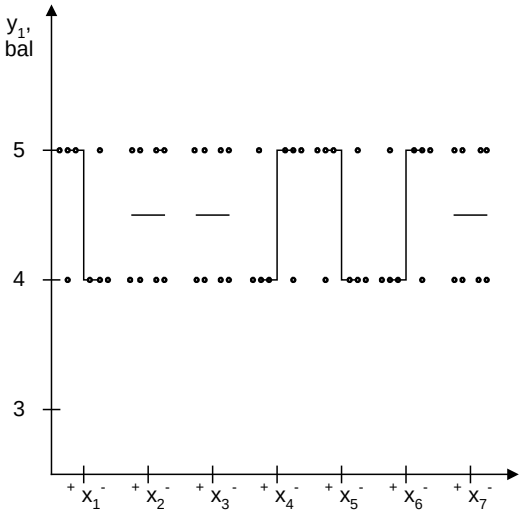

Fig. 9: Scattering diagram of the effect of quantitative factors on the appearance of the powder

The analysis of the scattering diagram of the study of the influence of quantitative factors on the appearance of the powder showed that the most significant of this indicator is infused with calcium phosphate, sodium citrate, curcumin, and lemon-lime flavor. The intensity of color and appearance of the powder is significantly increased, with an increase in the quantity of calcium phosphateand curcumin, and the study of the lower levels of factors $x_4$and x_6. The analysis of the influence of other factors showed their insignificant effect on this indicator.

The influence of quantitative factors on the bulk density (y_2) and the density after shrinkage (y_3) is given in Fig. 10.

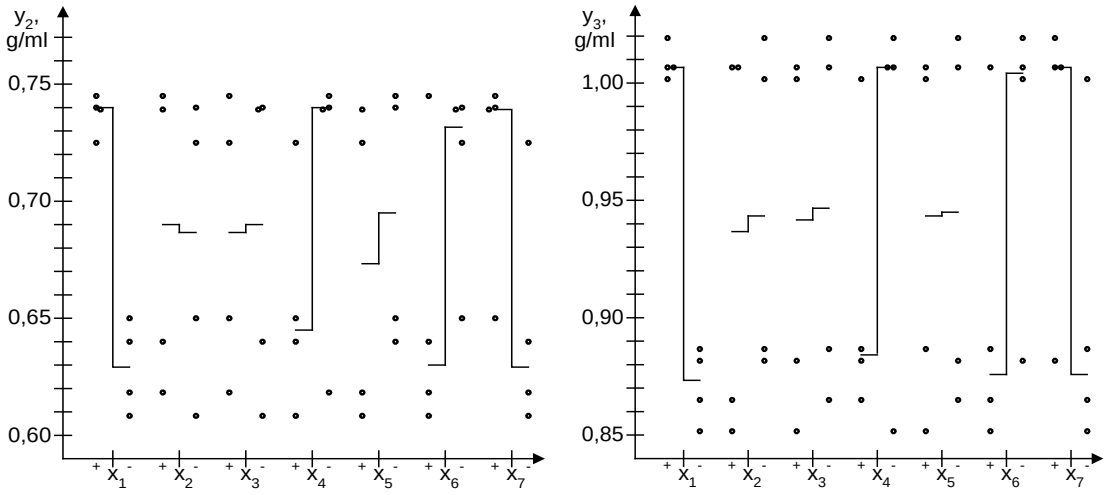

Fig. 10: Diagram of dispersion of the quantitative factors influence on the density of the powder: a – on the bulk density; b – on the density after shrinkage

The diagrams show that the significant factors are x_1, x_4, x_6, and x_7. Increasing the quantities of calcium phosphate and titanium dioxide, as well as reducing the content of sodium citrate and flavor of lemon-lime, is accompanied by an improvement in the studied parameters.

Analysis of the scattering diagram of Carr's index (y_4) showed that the results of the study are most influenced by the factors x_2, x_7, and x_5. With an increase in the content of lemon acid anhydrous and titanium dioxide, the bulk density of the powder mass improves from 27.5 % to 26.3 %. The adding greater quantity of curcumin is accompanied by an increase in Carr's index from 26 % to 27 %, which indicates deterioration of mass flowability and may cause damage of filling in the packaging stage.

An illustration of the influence of quantitative factors on flowability (y_5) is shown in Figure 11.

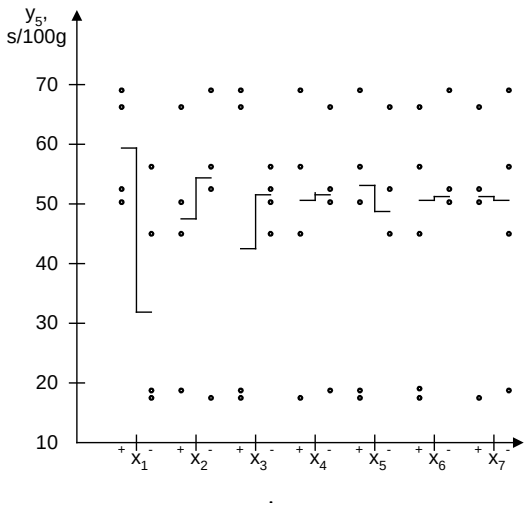

Fig. 11: Scattering diagram of the influence of quantitative factors on the flowability of the powder

The analysis of the scattering diagram of the study of the effect of quantitative factors on flowability showed that the experimental values most significantly depend on the quantity of calcium phosphate. The increase in the factor x_1 is accompanied by a deterioration of the powder flowability from 32.0 s/100 g to 59.0 s/100 g. This indicator is somewhat improved (varies in the range from 51.0 s/100 g to 42.0 s/100 g) with the administration of a greater quantity of malic acid due to the crystalline structure of its particles.

The scatter diagram of the influence of quantitative factors on the angle ofrepose (y_6) is shown in Fig. 12.

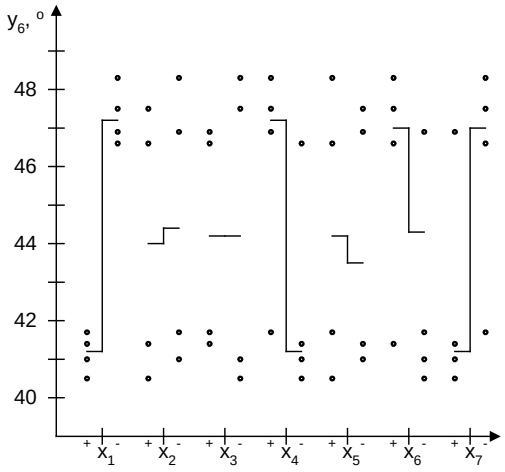

Fig. 12: Scattering diagram of the influence of quantitative factors on the powder angle ofrepose

In assessing the influence of quantitative factors on the angle ofrepose, using the scatter diagram, it noted that the factors x_1, x_4, and x_7 are the most significant. At the same time, when the quantity of calcium phosphate and titanium dioxide decreases, the difference in the average is from 47° to 41°. However, the introduction of more sodium citrate is accompanied by opposite changes. It should noted that the increase in the quantity of lemon-lime flavor improves the angle of repose from 44° to 47°.

Loss in mass during drying is an essential indicator of quality, especially in storing powder in sachet packs. High moisture content can cause the powders to soften and impair their characteristics. The influence of quantitative factors on mass loss during drying (y_7) is shown in Fig. 13.

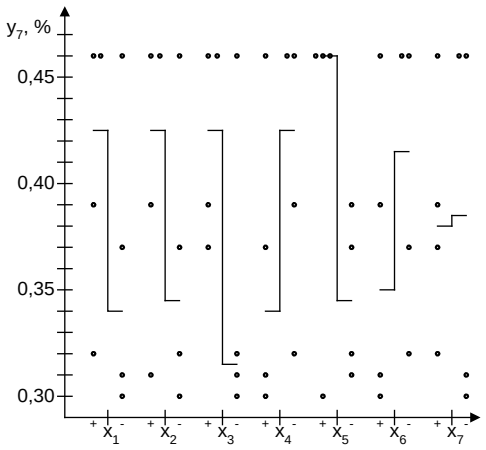

Fig. 13: Scattering diagram of the influence of quantitative factors on mass loss during drying

It can seen from the diagram that the factors x_5 and x_3 show the most significant influence on the mass loss index during drying. The lowest values of this index were observed with the addition of 0.0213 g of curcumin. The introduction of malic acid at the lower investigated level also significantly reduces the moisture content of the powder. Reducing the quantities of calcium phosphate and anhydrous citric acid leads to an improvement in the investigated index. However, an increase in sodium citrate content and a lemon-lime flavor can reduce the loss of mass in mass during drying.

For the powder for oral solution, essential parameters are also organoleptic characteristics of the solution from the content of the sachet pack. To quantify their contents, one packet of powder was dissolved in 200 ml of purified water, and the solution analyzed in terms of appearance, smell, taste, and pH. The appearance of the obtained solutions (y_8) is shown in Figure 14.

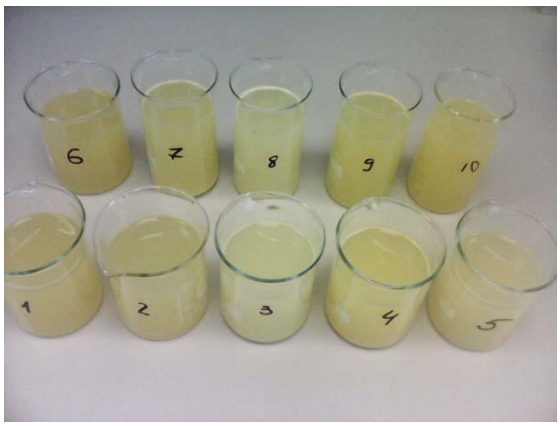

Fig. 14: The appearance of the solutions when studying the quantitative factors of the powder

It can seen from the figure that the solutions differed in color intensity and transparency. The influence of quantitative factors on the appearance of the solution is shown in Fig. 15.

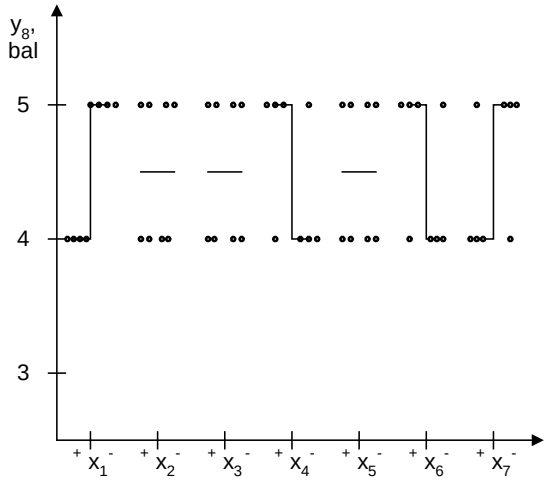

Fig. 15: Scattering diagram of the influence of quantitative factors on the appearance of the solution

Based on of the scattering diagram of the appearance of the solution, the determining influence of the factors x_1, x_4, x_6, and x_7 is established. With increasing quantities of sodium citrate and lemon-lime flavor, transparent solution with intense coloration obtained. Administration of higher contents of calcium phosphate and titanium dioxide causes turbidity of the solution.

The influence of quantitative factors on the smell of the solution (y_9) is shown in Figure 16.

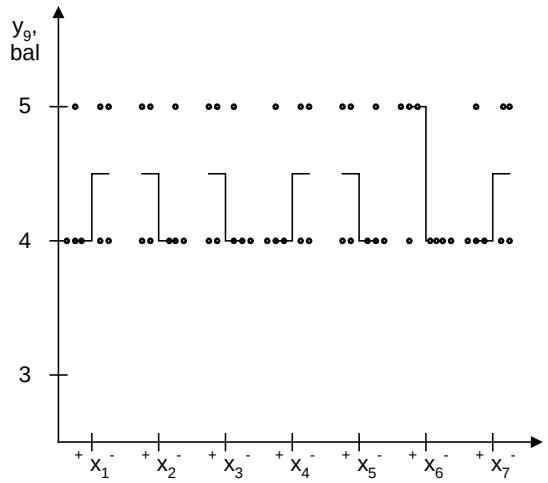

Fig. 16: Scattering diagram of the influence of quantitative factors on the smell of the solution

The significance of the quantity of lemon-lime flavor on the smell of the solution, and with the increase in its content, the results improve. There is also an insignificant influence of other factors on the smell of the solution.

The influence of quantitative factors on the taste of the solution (y_{10}) is shown in fig.17.

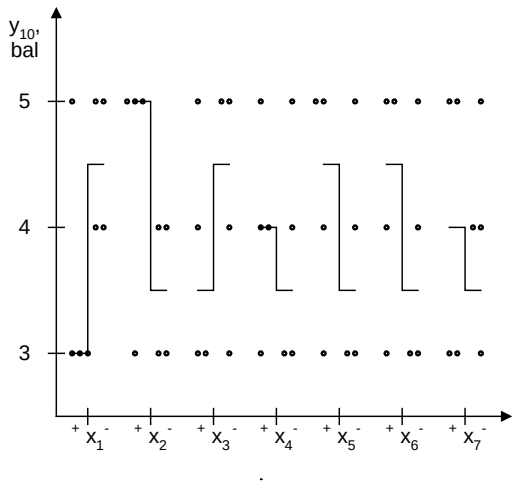

Fig. 17: Scattering diagram of the influence of quantitative factors on the taste of the solution

Analysis of the scattering diagram of the taste of the solution showed that the most significant are the factors x_1 and x_2. With an increase in the quantity of acid citric anhydrous, there is a pleasant sour taste, and an increase in calcium phosphate contentcontrary effect on the taste of the solution. Adding more malic acid also causes unpleasant taste sensations. An increase in the quantities of curcumin and lemon-lime flavor addsa sour taste, allowing some improvement in the investigated parameter.

One of the most important characteristics was the pH of the solution (y_{11}). It depended on the solubility of the content of the sachet package. At low values, the pH of the powder was dissolved entirely and left no residue. The influence of quantitative factors on the pH of the solution is shown in Fig. 18.

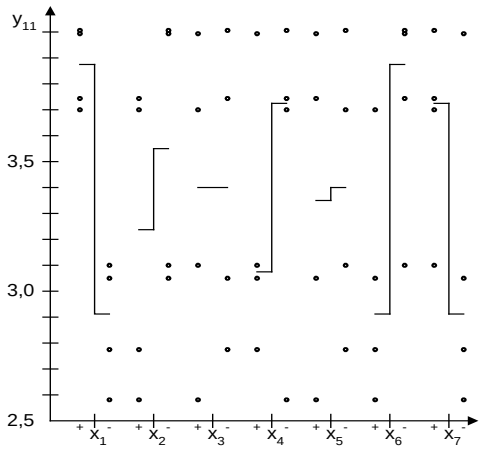

Fig. 18: Scattering diagram of the influence of quantitative factors on the pH of the solution

The diagram shows that the most significant factors are x_1 and x_6. Significant influence is demonstrated by the factors x_7 and x_4. The lowest pH values were observed with the addition of 0.0820 g of calcium phosphate, 0.1210 g of sodium citrate, 0.2085 g of lemon-lime flavour, and 0.0014 g of titanium dioxide[37].

V. Conclusions

1. A combined generic drug in the form of a powder for oral solution in sachets has been developed.It includes paracetamol, ascorbic acid, phenylephrine hydrochloride, and pheniramine maleate.
2. The quality target product profile described; the critical quality attributes defined. A risk assessment of formulation and technology variability was performed. Considering the development study results, the risks of all variables were reducedto the low risk.

3. The influence of 27 excipients from 5 functional groups on 11 quality attributes studied for the production ofpowder for oral solution. Statistical processing of the experiment results performed by the dispersion analysis. A significant influence of the pH of the solution on the solubility of the investigated powder was noted. Excipients were selected by using the utility function from the groups: alkaline and acid fractions, coloring, flavoring, and filler.
4. The influence of the quantities of 7 excipients on the pharmaco-technological properties of the powder and the physical characteristics of the solution was studied using the random balance method. A scatter plot plotted using the study results; significant factors were identified on as the basis of median values. Amounts of excipients that ensure the best quality attributes were selected. The optimal composition of the powder for oral solution is proposed: 0.325 g of paracetamol, 0.05 g of ascorbic acid, 0.01 g of phenylephrine hydrochloride, 0.02 g of pheniramine maleate, 0.082 g of calcium phosphate, 0.121 g of sodium citrate, 1.222 g of anhydrous citric acid, 0 .05 g of malic acid, 0.0383 g of curcumin, 0.2085 g of lemon-lime flavoring, 0.0032 g of titanium dioxide and 20 g of powdered sugar.
5. For the preparation of powder for oral usetechnology the method of roll compaction is proposed. The sequence of input of ingredients by nine algorithms was studied using dispersion analysis. The optimal component input scheme is selected: compacting of the mixture 1: paracetamol + curcumin + titanium dioxide + sugar powder + calcium phosphate + lemon-lime flavoring; mixing with mixture 2: phenylephrine hydrochloride + pheniramine maleate + sugar powder and mixture 3: ascorbic acid + sodium citrate + malic acid + citric acid anhydrous.

Gratitude

Grateful to Farmak Joint-Stock Company for providing raw materials and equipment for experimental researches.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Quantitative Determination of Ethyl Methyl Hydroxypyridina Succinate in the Preparation Seroxidol and its Bioequivalence

By Dilfuza Sarvarova & Nodira Yunuskhodzhaeva

Tashkent Pharmaceutical Institute

Abstract- Nowadays, the most common spread diseases are cerebral circulation disorders, including ischaemic stroke and its consequences, such as dyscirculatory encephalopathy, vegetative-vascular dystonia, neurotic and neurosis-like disorders, memory and attention disorders or mental impairment, and atherosclerosis of the cerebral vessels.

Research on the need for new drugs for those neurological disorders in Uzbekistan has been done and successfully produced an injectable solution of Seroxidol.

This drug is an inhibitor of free radical processes and a membrane protector with antihypoxic, stress-protective, nootropic, anticonvulsant and anxiolytic effects. Seroxidol increases the resistance to the effects of various damaging factors (shock, hypoxia and ischaemia, cerebral circulation disorders, alcohol intoxication and antipsychotic drugs with neuroleptics) and improves the functional state of the ischaemic myocardium. It effectively restores myocardial contractility in cases of reversible cardiac dysfunction. Seroxidol contains ethyl methyl hydroxypyridine succinate and sodium metabisulfite.

Keywords: *seroxidol, factor, antioxidant, toxicity, diuresis, blockade, intensity.*

GJMR-B Classification: *DDC Code: 616.105 LCC Code: RC691*

Strictly as per the compliance and regulations of:

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Keywords: seroxidol, factor, antioxidant, toxicity, diuresis, blockade, intensity.

I. Introduction

Modern world requires people to adapt to the increased stress on the psyche associated with economic and political instability, social problems, and man-made and environmental factors, which together lead to the development of urban stress, accompanied by fatigue, irritability, tension and even unmotivated hatred and aggression [1].

When the body is under the influence of extreme environmental factors, both physiological shifts and psychological changes of varying degrees of severity can occur, with a pattern of manifestations commonly including a kind of "blockade" of cognitive processes, in which the volume of perception narrows, synthesis processes in thinking are disrupted, and purposeful behaviour becomes disorganized [2].

Accordingly, the discovery, development and use of drugs that increase stress resistance, resistance to pathogenic factors and work capacity and that activate mental activity, the ability to concentrate and learn, with minimal side effects, is an urgent task in pharmacology[8].

The domestic drug Seroxidol, injected as a 50 mg/ml solution, has pronounced antioxidant, antihypoxic and membranotropic effects.

Purpose of the study: This study pursued the development of a method for the quantitative determination of ethyl methyl hydroxypyridine succinate by UV spectrophotometry of the drug Seroxidol in a 50 mg/ml solution for injection and its bioequivalence.

II. Materials and Methods

Class A volumetric glassware was used in the work: conical flasks of 50 ml and 100 ml, volumetric pipettes, an AS-220/X ser # B635963283 analytical balance (Ohaus, Germany), a SHIMADZU UV-1900UV spectrophotometer, and cuvettes with a thickness of 10 mm.

The object of the study was injection solutions corresponding to the TPA Seroxidol, a 50 mg/ml solution for injection. Determination was carried out by UV spectrophotometry.

The acute toxicity of the preparations was studied in 60 white mice of both sexes, weighing 19-21 g. From the compared preparations Seroxidol produced by LLC "MEDIOFARM", Uzbekistan and "Mexidol®" produced by LLC "Ellara", Russia, a 2.5% solution was prepared and administered to the mice once intravenously at doses of 150 mg/kg, 175 mg/kg, 200 mg/kg, 225 mg/kg and 250 mg/kg (0.12-0.2 ml) [3].

The animals were kept under continuous observation during the first hour, then under hourly observation throughout the first day of the experiment and once a day for the next 13 days of the experiment.

As indicators of the functional state of animals, the general condition of the mice and their behaviour, the intensity and nature of the motor activity, the presence of seizures, coordination of movements, reaction to external stimuli and tone of skeletal muscles, appetite, body weight, and number and consistency of faecal masses were monitored[7].

Author α: Teaching assistant of the Department of Organization of Pharmaceutical Production and Quality Management of the Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan.
e-mail: author.uzb@mail.ru
Author σ: Associate Professor, DSc of the Department of Organization of Pharmaceutical Production and Quality Management of the Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan.

During the experiment, the clinical state of the animals was monitored: the presence/absence of signs of poisoning, the time of their appearance, and death.

All experimental animals were kept under standard conditions on a common diet with free access to water and food [3].

After completion of the experiment, the average lethal doses (LD50) were determined [5].

III. Results and Discussion

Two millilitres of the medicine were placed in a volumetric flask with a capacity of 100 ml, and the volume of the solution was brought up to the mark with 0.01 mol/l hydrochloric acid and stirred.

Next, 1.0 ml of the resulting solution was placed in a 100 ml volumetric flask, and the volume of the solution was brought to the mark with 0.01 mol/L hydrochloric acid and mixed (test solution).

The optical density of the resulting solution was measured on a spectrophotometer at the absorption maximum at a wavelength of 297 nm in a cuvette with a layer thickness of 10 mm, using 0.01 mol/L hydrochloric acid as a reference solution.

$$X = \frac{D_1 x a_0 x\ 1\ x\ 100\ x\ 100\ x\ P\ x\ (100 - W)}{D_0 x\ 2\ x\ 1\ x\ 100\ x\ 1\ x\ 100\ x\ 100\ x\ 100} = \frac{D_1 x a_0 x\ P\ x\ (100 - W)}{D_0 x\ 20000},$$

where:

D_1- optical density of the test solution;

D_0- optical density of the working standard solution of ethyl methyl hydroxypyridine succinate;

A_0- weighed portion of WS of ethyl methyl hydroxypyridine succinate, g;

P- content of the main ingredient ethyl methyl hydroxypyridine succinate in the WS of ethyl methyl hydroxypyridine succinate, %;

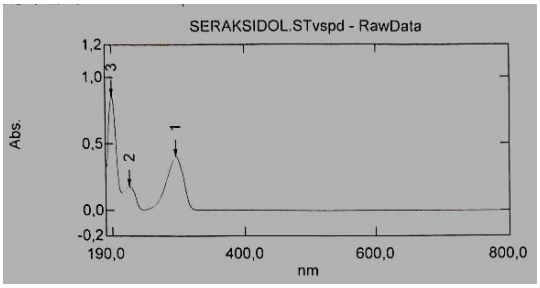
W- moisture content in the WS of ethyl methyl hydroxypyridine succinate, in %.

The content of C12H47NO5 (ethyl methyl hydroxypyridine succinate) in 1 ml of the preparation should be from 0.045 to 0.055 g.

Preparation of WS of ethyl methyl hydroxypyridine succinate: Approximately 0.1 g (accurately weighed) of ethyl methyl hydroxypyridine succinate was placed in a volumetric flask with a capacity of 100 ml, and 50.0 ml of 0.01 mol/l hydrochloric acid solution was added.

After the complete dissolution of the sample, the volume of the solution was brought to the mark with the same solvent and mixed. Then, 1.0 ml of the resulting solution was placed in a 100 ml volumetric flask, the volume of the solution was brought to the mark with the same solvent, and the solution was mixed. One millilitre of WS contained approximately 0.00001 g of ethyl methyl hydroxypyridine succinate.

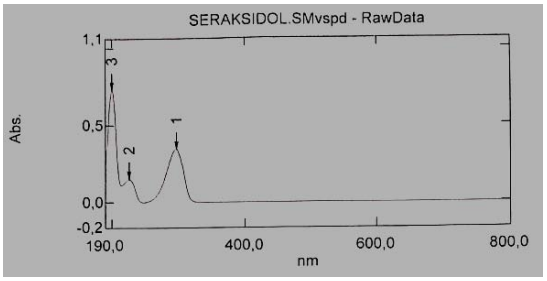
The solution must be freshly prepared. The data obtained are presented in Figures 1, 2 and 3.



[Peak Pick Table]
Threshold: 0,001000
Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	296,0	0,403	
2	↑	226,0	0,179	
3	↑	200,0	0,855	
4	↓	247,0	0,006	
5	↓	214,0	0,133	

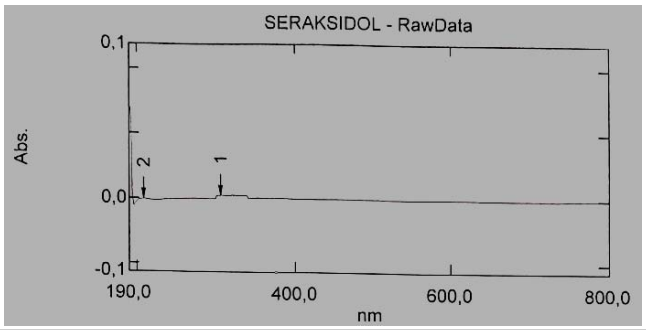
Fig. 1: Typical spectrum of a standard sample of the tested succinate



[Peak Pick Table]
Threshold: 0,001000
Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	297,0	0,345	
2	↑	226,0	0,148	
3	↑	200,0	0,731	
4	↓	350,0	-0,003	
5	↓	247,0	-0,001	
6	↓	214,0	0,109	

Fig. 2: Spectrum of the product ethyl methyl hydroxypyridine, Seroxidol



[Peak Pick Table]
Threshold: 0,001000
Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	306,0	0,003	
2	↑	209,0	-0,000	
3	↓	225,0	-0,001	
4	↓	196,0	-0,006	

Fig. 3: Spectrum of the "placebo" solution

Table 1: The results of the quantitative determination of the medicine Seroxidol

X_i, %	$\overline{X}$,%	f	S^2	S	$\Delta\overline{X}$	$\overline{\varepsilon}$,%
X_1=0.049 X_2=0.047 X_3=0.051 X_4=0.050 X_5=0.050	0.049	4	0.0000023	0.00152	0.0042	3.812

Experiments have shown that after a single intravenous administration of the Seroxidol preparation, produced by MEDIOFARM LLC, Uzbekistan, and Mexidol®, produced by Ellara LLC, Russia, at a dose of 150 mg/kg, no visible changes were observed in the behaviour and functional state of the animals.

All mice were active and responded to external stimuli, and food and water consumption was normal. No pathological changes in the hair and skin, diuresis, no diuresis, and no changes in the consistency and amount of faeces were observed. No signs of intoxication were observed. In this group, until the end of the experiment, no deaths were observed among the animals.

When the drugs were administered at a dose of 175 mg/kg, the mice developed clonic-tonic seizures, 1 mouse died in the generic group, and 2 mice died in the comparison group. [4]

When the drugs were administered at a dose of 200 mg/kg, a decrease in motor activity, rapid breathing, impaired coordination of movements, a weakening of the reaction to external stimuli, and a decrease in food and water consumption were observed in experimental animals. Three mice died in the comparison group.

At a dose of 225 mg/kg, the animals ceased to respond to external stimuli, and no food or water consumption was observed. During the experiment, 5 individuals in each group died [4].

The administration of a dose of 250 mg/kg of either Seroxidol produced by LLC "MEDIOFARM", Uzbekistan, or "Mexidol®" produced by LLC "Ellara", Russia, caused the immediate death of some animals after the administration of the medicine. By the end of the experiment, the condition of the surviving animals returned to normal as the signs of intoxication decreased. The LD50 of the drug Seroxidol produced by LLC "MEDIOFARM", Uzbekistan, was 200.0 (188.7 ÷ 211.1) mg/kg. The LD50 of the drug "Mexidol®" produced by LLC "Ellara", Russia, was 193.9 (178.5 ÷ 209.6) mg/kg. The acute toxicities of the compared drugs are shown in Table 2.

Table 2: Determination of the acute toxicity (LD50) of Seroxidol preparations produced by MEDIOFARM LLC, Uzbekistan, and Mexidol® manufactured by Ellara LLC, Russia

№ animals	Seroxidol produced by LLC "MEDIOFARM", Uzbekistan					"Mexidol®" produced by LLC "Ellara", Russia				
	weight, g	dose		method of administration	lethality	weight, g	dose		way introduction	lethality
		mg/kg	ml				mg /kg	ml		
1 2 3 4 5 6	21 20 21 20 20 21	150	0.13 0.12 0.13 0.12 0.12 0.13	i/v	No No No No No No	21 20 19 21 21 19	150	0.13 0.12 0.11 0.13 0.13 0.11	i/v	No No No No No No
1 2 3 4 5 6	20 19 19 19 20 21	175	0.14 0.13 0.13 0.13 0.14 0.15	i/v	No death No No No No	20 20 21 19 20 19	175	0.14 0.14 0.15 0.13 0.14 0.13	i/v	No death No death No No
1 2 3 4 5 6	21 20 19 19 20 20	200	0.17 0.16 0.15 0.15 0.16 0.16	i/v	death death No death No No	21 20 21 19 21 21	200	0.17 0.16 0.17 0.15 0.17 0.17	i/v	death No death No No death
1 2 3 4 5 6	19 19 20 20 21 21	225	0.17 0.17 0.18 0.18 0.19 0.19	i/v	death No death death death death	20 21 20 21 19 21	225	0.18 0.19 0.18 0.19 0.17 0.19	i/v	death death death No death death
1 2 3 4 5 6	20 1921 20 2019	250	0.20 0.19 0.21 0.20 0.20 0.19	i/Av	death death death death death death	21 2120 19 2121	250	0.21 0.21 0.20 0.19 0.21 0.21	i/v	death death death death death death
LD_{50}		200.0 (188.7÷211.1) mg/kg				193.9 (178.5÷209.6) mg/kg				

IV. Conclusion

This method was carried out in accordance with the requirements of the TPA. One millilitre of the drug Seroxidol contained 0.049 mg of ethyl methyl hydroxypyridine succinate.

Thus, the data obtained show that the preparations Seroxidol (50 mg/ml solution for injection) produced by "MEDIOFARM" LLC (Uzbekistan) and "Mexidol®" (50 mg/ml solution for injection)produced by "Ellara" LLC, (Russia) at 5 ml each are biologically equivalent in terms of acute toxicity.

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10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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Global Journal of Medical Research

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ISSN 9755896