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Chiral Inversion of Ibuprofen after an Oral Administration under Complete Fasting and Fed Conditions in Caucasian Healthy Subjects

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Abstract- *Background:* The aim was to determine the effect of feeding on chiral inversion of Ibuprofen isomers.

Method: Six healthy volunteers participated in a two-treatment crossover study where a single dose of Ibuprofen racemate was given. One stage consisted in an 8-hour interval of fasting, while in the other, the drug was administered with a solution of saccharose and a standard food intake regimen was implemented.

Keywords: *ibuprofen chiral inversion, stereos elective pharmacokinetics.*

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Chiral Inversion of Ibuprofen after an Oral Administration under Complete Fasting and Fed Conditions in Caucasian Healthy Subjects

Marianela Lorier [α], Pietro Fagiolino [σ], Marta Vázquez [ρ], Manuel Ibarra [ω] & Natalia Guevara [¥]

Abstract- ***Background:*** The aim was to determine the effect of feeding on chiral inversion of Ibuprofen isomers.

Method: Six healthy volunteers participated in a two-treatment crossover study where a single dose of Ibuprofen racemate was given. One stage consisted in an 8-hour interval of fasting, while in the other, the drug was administered with a solution of saccharose and a standard food intake regimen was implemented.

Results: Higher ($p<0.01$) S/R area under concentration-time curve ratios were achieved in the Fed stage (1.44) compared to the Fasting stage (0.976). Half-life of R-ibuprofen was significantly diminished ($p<0.001$) under fed conditions.

Conclusions: Clearance of R-ibuprofen increased following the ingestion of saccharose and food, however, the increased bioavailability of S-ibuprofen due to R-to-S chiral inversion overrode its increased clearance. This increased inversion might be explained by the supplementary amount of drug molecules that reaches the enter ocytes through pancreatic/intestinal juice secretion following the ingestion of food.

Keywords: ibuprofen chiral inversion, stereos elective pharmacokinetics.

I. Introduction

Nonsteroidal anti- inflammatory drugs (NSAIDs) are widely used for pain, fever and inflammation treatment for its inhibition of prostaglandin synthesis. NSAIDs can be administrated intravenously to achieve a more intense and rapid effect while avoiding the most common adverse effects, or orally. The oral presentations are varied and include immediate release formulations as soft gelatin capsules or tablets and modified release formulations.

NSAIDs are almost completely absorbed after the oral administration of an immediate release formulation despite their acidic properties which could lead to a certain absorption window, especially for extended-release formulations, due to the gradual increase of pH observed through the intestinal tract.[1] At higher pH values, these drugs ionize and their capacity to permeate membranes is diminished. NSAIDs show high plasma protein binding, liposolubility and are eliminated by metabolism and posterior glucuronidation.[2],[3]

Some NSAIDs, as Ketoprofen and Ibuprofen, present one chiral center. Although physicochemically identical, isomers can exhibit different pharmacokinetic and pharmacodynamic properties.[4],[5] These drugs are clinically administered as a racemic mixture though its anti-inflammatory activity is attributed almost entirely to the S- enantiomer. One of the possible metabolism routes for these drugs is the conversion of the R to S enantiomer.[6],[7],[8] The process occurs through an Acyl-CoA Thioester intermediate that can either undergo an epimerization reaction or transform into the parent drug by hydrolysis. Since only an R-Acyl-CoA Thioester intermediate has been observed *in-vitro,* the conversion has proven to be unidirectional. This has also been demonstrated *in-vivo* by oral administration of the enantiomers separately. [9], [10]

The epimerization reaction is mediated by an enzyme found mainly in liver but also in intestine and other organs.[11],[12],[13]It has been reported that the mean residence time at the gastro intestinal tract affects the conversion rate between isomers. Slow absorption rates lead to higher conversion ratios.[8],[14],[15] However, it is systematically observed in different published studies that the phenomenon occurs after the ingestion of a meal.

This has been previously reported by our group for Ketoprofen[16]. Although only 10% of the administered Ketoprofen dose undergoes chiral inversion[17], an increase in S/R isomers concentration ratio could be observed after food ingestion. Given its pKa, Ketoprofen could be secreted with pancreatic and intestinal juice in agreement with the pH-partition theory, following a driving force given by the blood-juice difference of pHs. [18],[19],[20] R-to-S conversion of secreted drug following meal intakes with subsequent reabsorption into the systemic circulation could explain the evident S/R ratio increase.

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Ibuprofen, the most widely consumed NSAID, on the other hand, exhibits a much higher chiral inversion ratio of 63% of the oral administered drug and was therefore chosen for the present study. [21], [22] The experimental design of the study was based on a previously reported study. [23] In this study, although both involved formulations showed an increase in the S/R isomer ratio after lunch, an unexpected increase of R-to-S conversion ratio was early observed for the faster-absorbed formulation that could not be associated with food administration. Saccharose was one of the excipients in this formulation, a powerful stimulator of pancreatic secretion even in cephalic phase.[24]

The aim of this investigation was to determine the effect of food in the absorption and enteric reabsorption process of Ibuprofen, particularly saccharose as a stimulator of pancreatic juice secretion, in addition to studying the effect that it could have on the chiral inversion rate.

II. Materials and Methods

a) Subjects and study design

Six healthy Caucasian volunteers (3 women and 3 men) between 22 and 31 years old with mean body weight of 61 and 92 kg, respectively, were enrolled in a two-treatment (fasting and fed), two-period (first and second week) and two-sequence (fasting-fed and fed-fasting) crossover study where a single dose of 600 mg of Ibuprofen was given.

An immediate release formulation was selected for a rapid drug release, avoiding any possible excipient-related interference with drug pancreatic secretion, important to achieve the aim of the study. The chosen formulation was a soft gelatin capsule (Actron®, Bayer), since pancreatic secretion is not stimulated by gelatin.[25]

The study was carried out administering the Ibuprofen capsule with only 200 mL of water at one period (Fasting stage) and with 20 g of saccharose in 200 mL of water at the other period (Fed stage). During the Fedstage, another 20 g of saccharose in 200 mL of water were given two hours post dose and 4 hours post dose, lunch was ingested. For the Fasting stage, fasting was prolonged throughout the whole 8-hour study. Subjects maintained an eight-hour overnight fasting period before each stage but were given a light breakfast three hours before drug administration in order to disrupt the prolonged fasting and ensure the safety of the volunteers. One-week washout interval was kept between each period. The present study distinguishes from previously reported ones due to this absolute fasting stage that allows a purer comparison between fed and fasting conditions.

The study protocol was designed according to the clinical research guidelines and was approved by the Institutional Ethics Review Committee of the Faculty of Chemistry (Uruguay). Written informed consent was obtained from all subjects before their entry in the study. The study was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation (*CEBIOBE* as abbreviation of its Spanish spelling), situated in "Dr. Juan J. Crottogini" Hospital (Montevideo, Uruguay).

b) Sampling and chemical analysis

Blood samples were drawn from the antecubital vein through cannulation and immediately placed into heparinized tubes. The samples were scheduled at 0 (before dose intake) and 20, 40, 60, 90, 120, 140, 160, 180, 210, 240, 360 and 480 minutes after dosing. Plasma was separated by centrifugation and stored at -25°C until analysis. Stability of the samples was proven for the storage period. Sample preparation involved extraction of Ibuprofen with a mixture of hexane and ethyl acetate from 0.5 mL of acidified plasma, evaporation of the organic phase under a stream of nitrogen and reconstitution of the residue with mobile phase. Fifty microliters of a Furosemide solution (3000 µg/mL) were used as internal standard. Drug quantification in plasma was performed using a validated HPLC-UV chiral method, which was an adaptation of a previously published methodology. [26] Mobile phase consisted of 20 mM phosphate buffer at pH 7.0 and acetonitrile in the v/v ratio of 99/1 and the flow rate was 0.5 mL/min. CHIRALPACK AGP® column (5μ, 100 x 4,0 mm) with silica guard column was used. The detector was set at 220 nm. The analysis was carried out at 15°C and the injection volume was 20 µL. The lower limit of quantification was 0.6029 mg/L, and linearity was proven up to 40.23 mg/L. For concentrations located at the lower, middle, and higher portions of the calibration curve, intra and inter-day coefficients of variation (precision) and relative errors (accuracy) were below 13%. An 83% recovery from plasma samples was achieved.

c) Pharmacokinetic and statistical analysis

Concentration values were obtained for the 6 volunteers. Mean concentration-time profile of ibuprofen enantiomers in plasma after the administration of the drug under fed and fasting conditions, were constructed.

Maximum R- and S-ibuprofen plasma concentration (C_{MAX}) and their respective time-to-peak (T_{MAX}) were obtained from the experimental concentration-vs-time profile in each individual. Area under the plasma concentration-time curve (AUC) from 0 to the last quantifiable concentration for each volunteer and each enantiomer was calculated using the trapezoidal rule. Elimination half-life ($t_{½}$) for the R-enantiomer was calculated in each individual as the ratio between Ln (2) and the slope of the best fit line for the terminal Ln-concentration-vs-time decay. Since R-to-S

conversion might lead to altered S-enantiomer´s elimination half-life, this was not calculated. Mean C_{MAX}, AUC and $t_{½}$ (± standard deviation) and median T_{MAX} (range) were calculated.

Mean C_{MAX}, AUC and $t_{½}$ were compared via paired Student's t-test between fasting and fed administration. Also mean C_{MAX} and AUC were compared between R- and S-ibuprofen in both fasting and fed states. Statistical significance to reject the null hypothesis of equality is assumed when the p-value is less than 0.05

In addition, means of S/R concentration ratios were calculated at each sampling time as an indicator of the conversion rate of the R enantiomer to S enantiomer and S/R area under concentration-time curves (S/R AUC) were compared via paired Student's t-test between fasting and fed administration.

III. Results

Table 1: Summarizes the pharmacokinetic results obtained

	$t_{½}$ (min) ± SD	AUC (mg.min/L) ± SD		$C_{MÁX}$ (mg/L) ± SD		$T_{MÁX}$ (min) (range)		S/R AUC ratio ± SD
	R	R	S	R	S	R	S	
Mean								
Fasting	147,2± 22,6[a]	4828± 1279[a]	4502± 670	28,78± 6,52	27,54± 4,56	75 (20-160)	75 (20-160)	0,9761± 0,2523[a]
Fed	78,82± 7,68[a]	3077± 342[a,b]	4432± 1020[b]	23,38± 6,83	28,29± 4,68	90 (90-140)	90 (90-140)	1,441± 0,277[a]

[a] *significant differences between fed and fasting conditions via paired Student's t-test ($p=0.0002$ for $t_{1/2}$, $p=0.022$ for AUC and $p=0.006$ for S/R AUC)*

[b] *significant differences between R and S isomers via paired Student's t-test ($p=0.014$)*

Table 1. Mean or median pharmacokinetic parameters (± standard deviation or range) after the oral administration of an immediate release formulation containing 600 mg of Ibuprofen under a complete fasting regimen and fed conditions.

Figures 1 showsmean concentration-time profile of Ibuprofen enantiomers in plasma after the administration of the drug under fed and fasting conditions. Almost complete elimination of the administered drug is achieved after 8 hours.

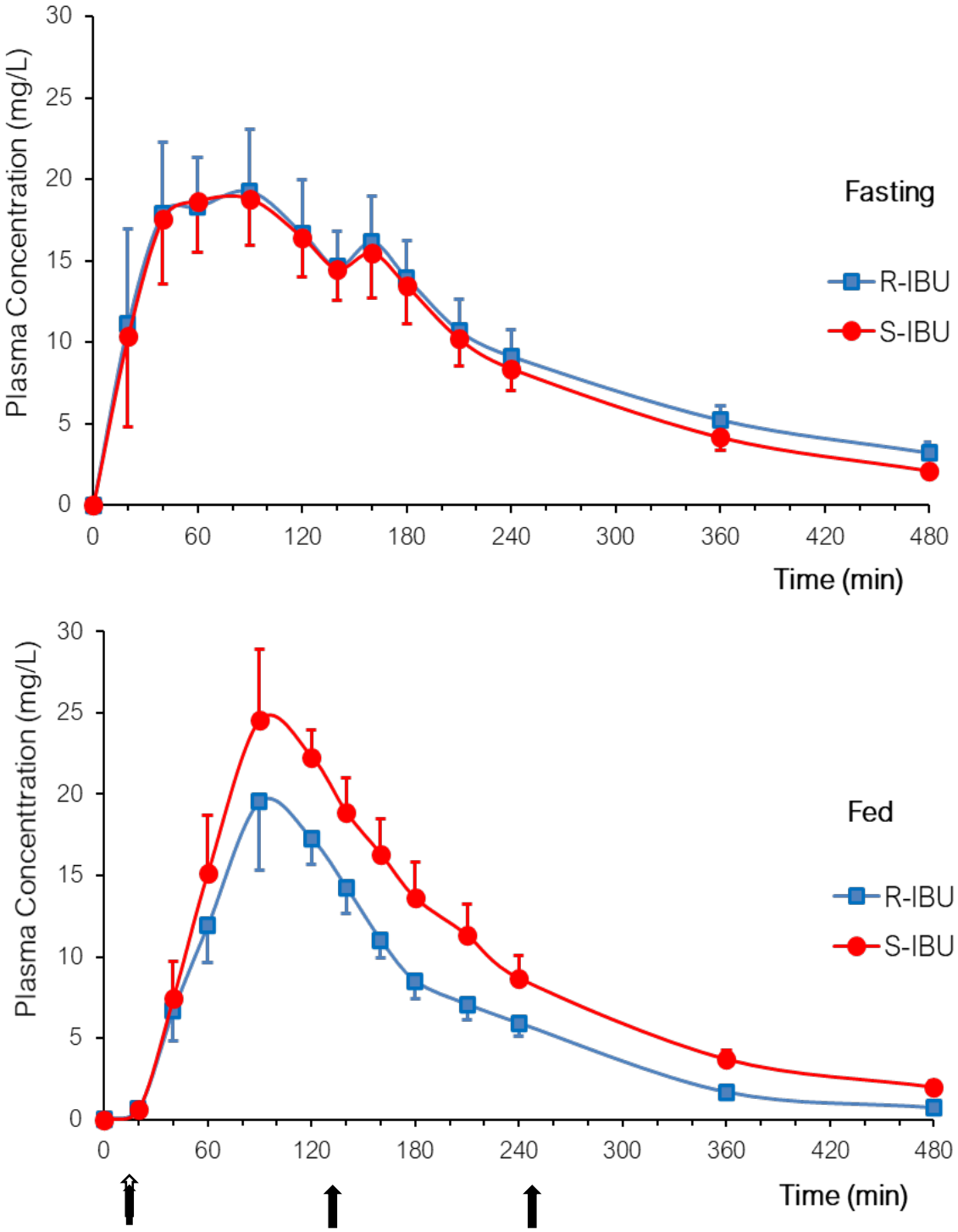

Figure 1: Mean concentration-time profile of R and S ibuprofen isomers (n=6) after the administration of an immediate release formulation containing 600 mg of a racemic mixture under a complete fasting regimen and fed conditions. Black arrows indicate the ingestion of saccharose or food

S isomer concentrations are consistently lower than R isomer concentrations in the Fasting stage and higher in the Fed stage. Mean S/R AUC ratio in the Fasting stage was 0.976 while in the Fed stage a 1.44 ratio was achieved ($p<0.01$).In men, S/R ratio was always above 1 (R-Ibuprofen plasma concentrations were always lower than for S-Ibuprofen concentrations) but higher in the Fed stage compared to the complete fasting conditions. However, in women, S/R plasma concentration ratios were opposite for the different stages (below 1 for Fasting stage and over 1 for Fed stage).

Figure 2 shows S/R plasma concentration ratio progress throughout time for both administration conditions. S/R plasma concentration ratios diminished throughout time for the Fasting stage while these ratios increased under fed condition, becoming significantly higher from those observed in fasting stage after 2 h post-dose.

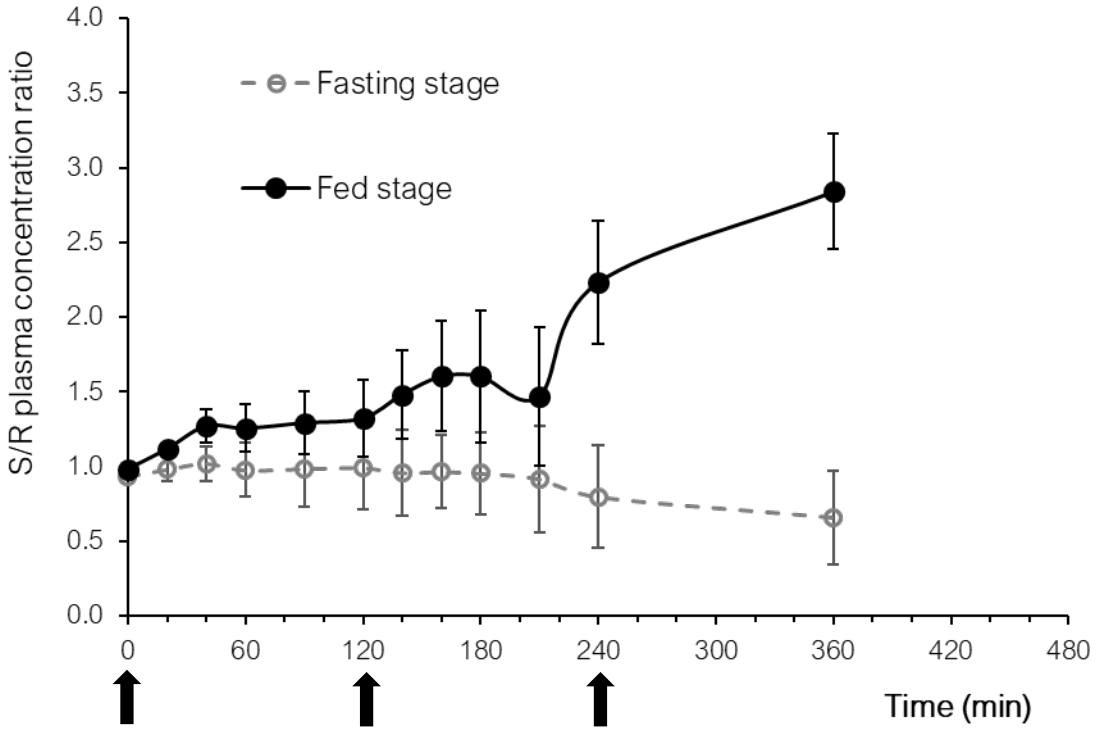

Figure 2: Mean S/R (±SD) plasma concentration ratio progress throughout time after the administration of an immediate release formulation containing 600 mg of Ibuprofen under fed and fasting conditions. Black arrows indicate the ingestion of saccharose or food for the Fed stage

IV. Discussion

On one hand, AUC for the R-isomer obtained during the Fed stage denoted a significant reduction in bioavailability, or clearance augmentation, or both, compared to the Fasting stage. The diminished $t_{½}$of the R-isomer during Fed stage suggests that, at least, an increase in systemic clearance has occurred.

In the Fed stage, by administering the dose with saccharose, repeating the ingestion at 2 hours after dosing and the lunch ingestion 4 hours after dose, the cardiac output fraction delivered to the splanchnic region was favored throughout the whole period. Thus, intestinal and hepatic clearance increased. It has been reported that cardiac output fraction to pancreas and intestine increases up to 50% after food ingestion. [27]

Intestinal clearance might have increased even more than the given by the increase of the cardiac output fraction. As mentioned previously, given Ibuprofen´s acidic properties, the pH difference between blood and pancreatic/intestinal juice, may induce drug transfer to these fluids. Ibuprofen could then be secreted to the intestinal lumen, stimulated by the ingestion of food or saccharose, returning to blood stream by absorption at this level. A supplementary amount of drug molecules reaches the enterocytes, which might have increased the intestinal clearance above expected. However, the lower number of molecules that reach the liver through the pancreatic vein, might result in a lower-than-expected increase of hepatic clearance in Fed stage.

On the other hand, AUC for the S-isomer remained constant during both stages. It would be rare to assume that systemic clearance remained unchanged for S-isomer under both administration conditions but not for R-isomer. Therefore, a countervailing increase in bioavailability must have occurred. Since the only reported difference between the enantiomer´s metabolizing pathways is the unidirectional R-to-S conversion, only an augmented bioinversion can explain these results. If intestinal clearance was indeed the most affected, then chiral inversion could be situated, not only in liver, but also in intestine. It should be noticed on figure 2 that S/R concentration ratio increases after the ingestion of saccharose or food, revealing the importance of the intestinal site for the R-to-S conversion.

Pre systemic chiral inversion has been discarded by other authors due to the high bioavailability that R-Ibuprofen exhibits.[21] However,

this is not sufficient to conclude that bio inversion does not occur at this level. Enzyme saturation during drug absorption could explain the low R-to-S pre systemic conversion. Chiral inversion at the enterocyte could be favored when Ibuprofen absorption is achieved through (reabsorption processes) or followed by (administration with saccharose) pancreatic/intestinal juice secretion. This high pH secretion might spread partially ionized molecules of ibuprofen across the intestine, diminishing its absorption rate, and hence, avoiding enzyme saturation.

Although few subjects were enrolled in this study, a significant difference between isomer´s plasma exposition was evident between both administration conditions, Fasting and Fed stage. To our knowledge, there are no other reported stereoselective pharmacokinetic analysis with a complete fasting regimen as

Sex differences seemed to be present, however, confirmation of this trend should be made in a trial with a larger number of subjects. Isomer plasma levels were similar for men and women except for R isomer levels in the Fasting stage where women achieved a 60% higher R-AUC.R-to-S basal conversion rate in men, which normally have higher body masses than women, may be increased by avoidance of enzyme saturation therefore exhibiting higher S/R ratios in Fasting stage compared to women.

V. Conclusions

The study allowed a clear comparison between fasting and fed administration conditions due to the complete fasting regimen that characterized one of the study's stages.

R-and S-Ibuprofen exhibited different disposition characteristics. S/R concentration ratios were much higher during Fed stage compared to Fasting stage.

By administering saccharose or food, cardiac output fraction delivered to the splanchnic region is favored and thus, systemic clearance increased. R-isomer concentrations decrease as expected but S-isomer levels remained unchanged compared to the Fasting stage, possibly due to a countervailing increase in bioavailability given by a higher R-to-S conversion rate during Fed stage. The increased chiral inversion might be explained by the supplementary amount of drug molecules that reaches the enterocytes through pancreatic/intestinal secretions stimulated by food ingestion.

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Authors' contributions:

Marianela Lorier: Designed study/Performed research/Analyzed data/Wrote Paper

Marta Vázquez: Designed and supervised study/Analyzed data

Pietro Fagiolino: Designed and supervised study/Analyzed data

Manuel Ibarra: Supervised analytical research

Natalia Guevara: Performed research

References Références Referencias

1. Nugent SG, Kumar D, Rampton DS, Evans DF. Intestinal luminal pH in inflammatory bowel disease : possible determinants and implications for therapy with aminosalicylates and other drugs. Gut. 2001;48:571–7.
2. Grosser T, Smyth E, FitzGerald GA. Analgesic-Antipyretic and Anti-Inflammatory agents: Pharmacotherapy of Gout. In: Brunton LL., Chabner BA. Knollmann BC editors,Goodman & Gilman's the Pharmacological Basis of Therapeutics. Mc Graw-Hill Professional; 2010, p. 434–6.
3. Negm A., Furst DE. Nonsteroidal Anti-Inflammatory Drugs, Disease-Modifying Antirheumatic Drugs, Nonopioid Analgesics, & Drugs Used in Gout. In: Katzung B.G. editor, Basic and Clinical Pharmacology. Mc Graw-Hill Professional; 2012, p. 636–8.
4. Tucker GT, Lennard MS. Enantiomer specific pharmacokinetics. Pharmacol Ther 1990;45:309–29.
5. Nguyen L, He H, Pham-Huy C. Chiral Drugs: An Overview. Int J Biomed Sci 2006;2:85–100.
6. Jamali F, Brocks DR. Clinical pharmacokinetics of ketoprofen and its enantiomers. Clin Pharmacokinet 1990;19:197–217. https://doi.org/10.2165/00003088-199019030-00004.
7. Mehvar R, Jamali F. Pharmacokinetic Analysis of the Enantiomeric Inversion of Chiral Nonsteroidal Antiinflamatory Drugs. Pharm Res 1988;5:76–9.
8. Jamali F, Mehvar R, Russell AS, Sattari S, Yakimets WW, Koo J. Human pharmacokinetics of ibuprofen enantiomers following different doses and formulations: Intestinal chiral inversion. J Pharm Sci 1992;81:221–5. https://doi.org/10.1002/jps.2600810306.
9. Geisslinger G, Schuster O, Stock KP, Loew D, Bach GL, Brune K. Pharmacokinetics of S(+)- and R(-)-

ibuprofen in volunteers and first clinical experience of S(+)-ibuprofen in rheumatoid arthritis. Eur J Clin Pharmacol 1990;38:493–7. https://doi.org/10.1007/BF02336690.
10. Wechter WJ. Drug chirality: On the mechanism of R-aryl propionic acid class NSAIDs. Epimerization in humans and the clinical implications for the use of racemates. J Clin Pharmacol 1994;34:1036–42. https://doi.org/10.1002/j.1552-4604.1994.tb01977.x.
11. Wsól V, Skálová L, Szotáková B. Chiral inversion of drugs: coincidence or principle? Curr Drug Metab 2004;5:517–33.
12. Yen CLE, Nelson DW, Yen MI. Intestinal triacylglycerol synthesis in fat absorption and systemic energy metabolism. J Lipid Res 2015;56:489–501. https://doi.org/10.1194/jlr.R052902.
13. Selkälä E. Role of α-methylacyl-CoA racemase in lipid metabolism. In: Vuolteenaho O. editor. Acta universitatis ouluensis D Medica 1355. 2016.
14. Jamali F, Singh NN, Pasutto FM, Russell AS, Coutts RT.Pharmacokinetics of Ibuprofen Enantiomers in Humans Following Oral Administration of Tablets with Different Absorption Rates. Pharm Res An Off J Am Assoc Pharm Sci 1988;5:40–3. https://doi.org/10.1023/A:1015811428066.
15. Ding G, Liu Y, Sun J, Takeuchi Y, Toda T, Hayakawa T, et al. Effect of absorption rate on pharmacokinetics of ibuprofen in relation to chiral inversion in humans. J Pharm Pharmacol 2007;59:1509–13. https://doi.org/10.1211/jpp.59.11.0007.
16. Lorier M, Magallanes L, Ibarra M, Guevara N, Vázquez M, Fagiolino P. Stereoselective Pharmacokinetics of Ketoprofen After Oral Administration of Modified-Release Formulations in Caucasian Healthy Subjects. Eur J Drug Metab Pharmacokinet 2016;41:787–93. https://doi.org/10.1007/s13318-015-0313-2.
17. Rudy C, Liu Y, Craig D, Hall D. Stereoselective Inversion Pharmacokinetics of (R) - Ketoprofen Healthy Volunteers in. J Clin Pharmacol 1998; 38:3–10.
18. Milne M., Scribner BH, Crawford MA. Non ionic Diffusion and the excretion of weak acids and bases. Am J Med 1958: 709-729.
19. Hall JE. Secretory Functions of the Alimentary Tract. In: Hall JE. editor. Guyton and Hall Textbook of Medical Physiology. Elsevier;2012. https://doi.org/10.1016/b978-1-4160-5451-1.00064-5.
20. Fagiolino P, Vazquez M, Alvariza S, Maldonado C, Ibarra M, Olano I. Antiepileptic drugs: Energy-consuming processes governing drug disposition. Front Biosci2014; 6: 387-396. https://doi.org/10.2741/e714.
21. Cheng H, Rogers JD, Demetriades JL, Holland SD, Seibold JR, Depuy E. Pharmacokinetics and Bioinversion of Ibuprofen Enantiomers in Humans. Pharm Res An Off J Am Assoc Pharm Sci 1994;11:824–30. https://doi.org/10.1023/A:1018969506143.
22. Lee EJD, Williams K, Day R, Graham G, Champion D. Stereoselective disposition of ibuprofen enantiomers in man. Br J Clin Pharmacol 2004;19:669–74.
23. Torrado JJ, Blanco M, Farré M, Roset P, García-Arieta A. Rationale and conditions for the requirement of chiral bioanalytical methods in bioequivalence studies. Eur J Clin Pharmacol 2010;66:599–604. https://doi.org/10.1007/s00228-010-0792-7.
24. Ohara I, Otsuka SI, Yugari Y. Cephalic phase response of pancreatic exocrine secretion in conscious dogs. Am J Physiol - Endocrinol Metab 1988;254. https://doi.org/10.1152/ajpgi.1988.254.3.g424.
25. Chandra R, Liddle RA. Regulation of Pancreatic Secretion 2 . Phases of Meal Response. Am Pancreat Assoc 2015:1–40. https://doi.org/10.3998/panc.2015.38.
26. Pettersson KJ, Olsson A. Liquid chromatographic determination of the enantiomers of ibuprofen in plasma using a chiral AGP column. J Chromatogr B Biomed Sci Appl 1991;563:414–8. https://doi.org/10.1016/0378-4347(91)80050-M.
27. Koffert J, Honka H, Teuho J, Kauhanen S, Hurme S, Parkkola R, et al. Effects of meal and incretins in the regulation of splanchnic blood flow. Endocr Connect 2017;6:179–87. https://doi.org/10.1530/EC-17-0015.

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Synthesis, Crystal Structure, Hirshfeld Surface, Energy Framework and Molecular Docking of 2-(((6-Methoxy Pyridin-3-Yl) Imino) Methyl)Phenol

By M. Singh, S. Anthal, P. Akhileshwari, M. A. Sridhar, H. M. Vinusha, S. Bindya, M. Begum, R. K. Chandrasekaran, M. Saminathan & R. Kant

University of Jammu

Abstract- The structure of 2-(((6-methoxypyridin-3-yl)imino)methyl)phenol (MPIMP) ($C_{13}H_{12}N_2O_2$) has been determined by X-ray diffraction methods. It crystallizes in the tetragonal crystal system with space group $P4_2/n$ and unit cell dimensions a = 14.2958(3) Å, b = 14.2958(3) Å, c = 11.0179(3) Å, V = 2251.73(12) $Å^3$, Z = 8. The structure has been refined by full-matrix least square procedure to a final R-value of 0.0518(wR_2 = 0.1312) for 1709 observed reflections. The molecules linked via two intermolecular (C-H…N and C-H…O) hydrogen bonds. The crystal structure was further stabilized by a strong intramolecular N-H…O hydrogen bond. The Hirshfeld surface analysis reveals the interaction contacts of the molecule and the strength of molecular packing in the crystal. The energy framework has been performed through different intermolecular interaction energies for structural stability. The molecular docking of MPIMP was performed against tuberculosis enzyme Decaprenyl-phosphoryl-b-Dribose 20-epimerase (DprE1, PDB code: 4KW5) to reconnoiter the binding interactions at the active sites.

Keywords: crystal structure, x-ray diffraction, direct methods, intermolecular interactions, and molecular docking.

GJSFR-B Classification: FOR Code: 260101

SYNTHESISCRYSTALSTRUCTUREHIRSHFELDSURFACEENERGYFRAMEWORKANDMOLECULARDOCKINGOF2METHOXYPYRIDIN3YLIMINOMETHYLPHENOL

Strictly as per the compliance and regulations of:

Synthesis, Crystal Structure, Hirshfeld Surface, Energy Framework and Molecular Docking of 2-(((6-Methoxy Pyridin-3-Yl)Imino)Methyl)Phenol

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Abstract- The structure of 2-(((6-methoxypyridin-3-yl)imino)methyl)phenol (MPIMP) ($C_{13}H_{12}N_2O_2$) has been determined by X-ray diffraction methods. It crystallizes in the tetragonal crystal system with space group $P4_2/n$ and unit cell dimensions a = 14.2958(3) Å, b = 14.2958(3) Å, c = 11.0179(3) Å, V = 2251.73(12) Å^3, Z = 8. The structure has been refined by full-matrix least square procedure to a final R-value of 0.0518(wR_2 = 0.1312) for 1709 observed reflections. The molecules linked via two intermolecular (C-H…N and C-H…O) hydrogen bonds. The crystal structure was further stabilized by a strong intramolecular N-H…O hydrogen bond. The Hirshfeld surface analysis reveals the interaction contacts of the molecule and the strength of molecular packing in the crystal. The energy framework has been performed through different intermolecular interaction energies for structural stability. The molecular docking of MPIMP was performed against tuberculosis enzyme Decaprenyl-phosphoryl-b-Dribose 20-epimerase (DprE1, PDB code: 4KW5) to reconnoiter the binding interactions at the active sites.

Keywords: crystal structure, x-ray diffraction, direct methods, intermolecular interactions, and molecular docking.

I. Introduction

The pyridine Schiff bases form one of the most significant classes of chemical compounds in organic chemistry and are generally characterized by the presence of pyridine and a phenolic ring connected by an azomethine group. The nitrogen present in the pyridine is usually responsible for the antifungal effects, while the phenolic ring participates in the bioactivity [1]. Schiff bases, being synthetically available and structurally varied, are obtained by replacing the carbonyl group (>C=O) present in the aldehyde or ketone with imine or azomethine group (>C=N) by reacting the carbonyl group with primary amines (-NH_2) [2].

As the Schiff bases with an imine group in its structure exhibit a wide range of potential for biological activities like antifungal, antibacterial, anticancer, antidepressant, anticonvulsant, anti-inflammatory, antiviral, antitumor, antioxidant, etc. [3-15], we present synthesis and single crystal X-ray (SCXR) structure and related spectroscopic characterization of a new pyridine Schiff base MPIMP. The SCXR molecular structure has been obtained by using direct methods, and Hirshfeld surfaces (HS) and Energy framework calculations have been performed by using Crystal Explorer (17.5) [16]. Since pyridine derivatives are capable of inhibiting Decaprenylphosphoryl-b-Dribose 20-epimerase (DprE1) [17, 18], *in silico* molecular docking of MPIMP has also been reported.

II. Experimental Section

All the chemicals of analytical grade required for our present research work were purchased from Sigma Aldrich and utilized without any purification.

The Schiff base, 2-(((6-methoxypyridin-3-yl)imino)methyl)phenol has been synthesized by condensation reaction between 6-methoxypyridin-3-amine with 2-hydroxybenzaldehyde. 6-methoxypyridin-3-amine (1.24g, 0.01mol) has been mixed with 2-hydroxybenzaldehyde (1.22g, 0.01mol) and 10 ml of methanol as solvent. The mixture was refluxed with 2-3 drops of glacial acetic acid at 60-70°C for 6hrs. The reaction progress was monitored by using TLC. The reaction mixture kept for overnight evaporation after the completion of the reaction. The reddish-brown colored crystals have been recrystallized using methanol. The Reaction scheme of the compound is presented in Fig. 1.

The intensity data of MPIMP were collected using Super Nova, Single source at offset/far, HyPix3000 diffractometer equipped with graphite monochromatic MoK_α radiation (λ =0.71073 Å). Using Olex2 [19], the structure was solved using SHELXT [20] software routine with Intrinsic Phasing. Five cycles of full-matrix least-squares refinement have been performed, and the final

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R-factor (0.0518) yielded the best possible atomic peaks. All non-hydrogen atoms of the molecule were located from the E-map and refined in anisotropic approximation using SHELXL [21]. The Crystallographic data are summarized in Table I. Hydrogen atoms bonded to C atoms were geometrically fixed and allowed to ride on the corresponding non-H atoms [C-H = 0.93-0.96 Å, and U_{iso}(H) =1.5 U_{eq} of the attached C atoms for methyl groups and 1.2 U_{eq}(C) for other H atoms]. Residual electron density ranges from -0.27 to 0.15 e.Å^{-3}. The atomic scattering factors were taken from the International Tables for X-ray Crystallography (1992, Vol C, Tables 4.2.6.8 and 6.1.1.4). Olex2 [19], PLATON [22], PARST [23], and Mercury [24] defines the molecular geometry. The crystallographic information file (CIF) of the compound has been deposited at Cambridge Crystallographic Data centre (CCDC number 2091399). This CIF file can be accessed free of cost from Cambridge Crystallographic Data Center via https://www.ccdc.cam.ac.uk/structures.

The Hirshfeld surface (HS) mapped plots (d_{norm}, 2-D fingerprint, electrostatic potential, shape-index, and curvedness) and energy framework has been generated using Crystal-Explorer (version 17.5) with B3LYP function and 6-31G (d, p) basic set [16]. The atomic coordinates have been imported from the final validated CIF to Crystal Explorer (17.5). HS has been drawn for the asymmetric unit using high resolution of three-dimensional pictures of intermolecular close contacts in a crystal.

The molecular docking studies have been executed using AutoDock Vina software [25] which is a suite of automated docking tools (ADT). The target enzyme Decaprenylphosphoryl-b-Dribose 20-epimerase (4KW5) has been obtained from the protein data bank (www.rcsb.org/pdb). The coordinates of the grid center are fixed at X= 6.47, Y= -8.61, Z= 39.07, and grid sphere radius is 42. The conformational protein structure is modeled and visualized using Discovery Studio Visualizer [26].

III. Result and Discussion

The X-ray crystallographic analysis of MPIMP reveals its asymmetric unit consists of a benzene and pyridine ring. An ORTEP plot of the molecular structure is shown in Fig. 2 [24]. The bond distances, bond angles, and torsion angles between the non-H atoms are contained in Table II. All the bond distances and bond angles are within the normal range and are comparable with the related structures [27-28]. A C=N group seems to have a strong electron-withdrawing character in the azomethine group of the structure. Thus, the C1-O1 bond distance of 1.3490(17) Å is reliable with C-O single bond length; similarly, the C7=N1 distance of 1.2834(16) agrees well with the standard value [28]. The benzene and pyridine ring systems [having a dihedral angle of 7.53 (5)^{o}] are planar.

In the crystal structure, strong N-H…O intramolecular interactions are responsible for the stability of molecules within the unit cell. This intramolecular interaction leads to the formation of a virtual six-membered ring forming the S6 graph set motif [29]. In the crystal structure, there exist two intermolecular hydrogen bonds [C7-H7…N2 and C13-H13A…O1] that link the molecules into chains along the c-axis, as shown in Fig. 3 (Mercury) [24]. A summary of intra and inter-molecular hydrogen bonding are listed in Table III. The packing of molecules in the crystal viewed along the c-axis is visualized in Fig. 4, (PLATON) [22].

Hirshfeld surface (HS) analysis was carried out to explore the intermolecular interactions responsible for molecular packing in the crystal. Fig. 5(a) shows the 2D fingerprint plot of overall contribution from all the interactions to the total HS area, whereas Fig.5 (a*) represents the HS over normalized contact distance d_{norm}; where d_{norm} gives the distance between two atoms across the surface to the combined van der Waals radii of the atoms [30]. The d_{norm} value is either positive or negative, depending upon the longer or shorter intermolecular contacts than the van de Waals radii, respectively. The red-colored region represents shorter contacts with negative d_{norm} value, blue regions correspond to longer contacts with positive d_{norm} value, and the white region represent the distance of contacts is exactly equal to the van der Waals separation with a d_{norm} value of zero [31]. The three red spots shown on the HS represent the shortest hydrogen bond interactions due to strong C-H…O, C-H…N, and O-H…N contacts shorter than van der Waals radii as shown in Fig. 5(a*).

Fig. 5(b-f) represents the 2D fingerprint plots showing H-H, C-H/H-C, O-H/H-O, C-C, N-H/H-N contacts with the percentages contribution and (b*), (c*), (d*), (e*), (f*) represents the associated d_{norm} HS, respectively. These plots have been generated in the d_{norm} range -0.1466Å to 1.7068 Å. The d_i and d_e on the plot are the distance from the HS to the nearest nuclei inside and outside the surface, respectively. For any given d_i and d_e pairs on the 2D plot, white color represents no occurrence, blue color shows some occurrence, and green indicates more frequent occurrence. These 2D fingerprint plots enable us to determine the percentage contribution of each type of contact to the total HS area. The major contribution to the total Hireshfeld area is H-H contacts with 50.9%, while the remaining significant contribution is from C-H/H-C (17.1%), O-H/H-O(12.8%), C-C (6.2%), and N-H/H-N (5.8%), as shown in Fig. 6. The d_i and d_e combination in the 2D fingerprint plot provides an outline of intermolecular contacts in the crystal, where one molecule acts as an acceptor ($d_e < d_i$) and the other as a donor ($d_e > d_i$). The O…H/H…O and

N…H/H…N molecular interactions represent by two distinct spikes in the bottom left and right region of almost equal length in 2D plots with $(d_i + d_e) \approx 2.50$Å and 2.45Å, respectively, as shown in Fig. 5(d) and 5(f).

The Hirshfeld surface (HS) mapped over the calculated electrostatic potential for the title compound MPIMP as shown in Fig.7 (a). This mapping gives direct insight into the intermolecular interaction, which is responsible for molecular packing in the crystal [32]. The electrostatic potential map has been generated by using the B3LYP function with 6-31G (d) basis set [16]. The blue-colored area on the map is the electropositive region (i.e., the region around hydrogen bond donor) and the red-colored area is the electronegative region (i.e., the region around hydrogen bond acceptor). The electropositive blue region near C13-H13A atoms is complementary to the electronegative red region around the O1 atom, as shown in Fig. 7(a).

Fig. 7(b) shows the molecular HS mapped over the shape index for the compound. This mapping of shape index gives us the visual identification of the regions where the two molecular HS touch each other, and uses complementary pairs of red and blue colored schemes [30]. The concave red-colored region on the shape index represents the cluster of the surface around the acceptor atoms. In contrast, the blue-colored bumps regions represent the cluster of the surface around the donor atoms.

The curvedness map displays large regions of green (relatively flat) separated by dark blue boundaries (large positive curvatures), as shown in Fig.7(c). The plot shows no flat surface patches. Consequently, there is no indication of planner stacking between the molecules.

The energy frameworks give information on accurate inter-molecular interaction energies, which are responsible for the supramolecular construction of molecules in the crystal [33]. These calculations has been carried out using the Crystal Explorer (17.5) program based on 6-31G (d, p) basic set [16], where interaction energies have been calculated within a radius of 3.8Å cluster around a single molecule of the title compound MPIMP. The scale factors used for the construction of energy framework for B3LYP/6-31G (d, p) electron densities are k_ele = 1.057, k_pol = 0.740, k_disp = 0.871, k_rep = 0.618 [34], respectively. Table IV contains the interaction energies, viz. electrostatic, polarization, dispersion, and repulsive energies. Fig. 8 represents the molecular pairs in which few molecules are separated by respective radial distance (as shown in Table IV) from the centroid molecule involved in the calculation of interaction energies along the c-axis.

The images of different interactions energies – coulomb interaction energy (red), dispersion energy (green), and total energy (blue) of the title compound MPIMP along a, b, and c-axes are illustrated in Fig. 9. The cylinders represent the energies between the molecular pairs joining with the center of mass of the molecules, and the radius of the cylinder is proportional to the magnitude of the interaction energy. The framework of the cylinders represents the relative strengths of molecular packing in different directions. An overall scale factor is applied to expand or contract the size of the cylinders in the framework [33]. To avoid very crowded structures, weaker interactions have been omitted below a certain threshold energy, due to which there is an absence of the cylinders in a particular direction. The calculated energies for electrostatic, polarization, dispersion, and repulsion are -34.5 KJ/mol, -8.5 KJ/mol, -170.1 KJ/mol, and 94.9 KJ/mol, respectively. The total interaction energy is -132.5 KJ/mol. The dispersion energy dominates over the electrostatics Coulomb energy in the title compound MPIMP.

The molecular docking has done performed against DprE1(PDB code: 4KW5), and its resolve is to calculate the binding modalities of MPIMP. Since the hydrogen bond is an essential key factor in the structure and function of biological compounds, the ligand-receptor interactions have been inspected based on the hydrogen bonding. The molecular binding site of compound MPIMP and pyrazinamide interaction into DprE1 enzyme is present in Fig.10. The binding energy, distance and bonding type of compound MPIMP, and standard drug pyrazinamide with DprE1are listed in Table V. In Compound MPIMP-4KW5 complex, the oxygen (O) atom of MPIMP has branched H-bond polar interaction with the atom NE2 and OE1 of residue HIS123 and GLN336 at a distance 2.506 and 2.204 Å, respectively. Moreover, the oxygen atom of MPIMP interacts with atom HZ1 of LYS418 at a separation of 2.530Å. Three hydrophobic Alkyl- π interactions can be noticed between atoms of residue (CYS387, VAL365 & PRO116) and rings of compound MPIMP.

In Pyrazinamide -4KW5 complex, the oxygen (O) atom of pyrazinamide has interaction with the atom O of residue ALA53 at a distance of 2.027 Å, and the hydrogen (H) atom of pyrazinamide has interaction with the atom N of residue GLY55 at a distance of 2.262 Å. Moreover, the ring of pyrazinamide Hydrophobic interacts with atom N of GLY125 and atom CD of ALA128 at a separation of 3.800 and 4.847 Å, respectively. The outcome predicts that the compound MPIMP could strongly fix in the active site of DprE1, with binding energy of 7.4 kcal/mol, compared to 5.4 kcal/mol of pyrazinamide.

IV. Conclusions

2-(((6-methoxypyridin-3-yl)imino)methyl)phenol crystallizes in tetragonal crystal system with $P4_2/n$ space group. The crystallographic analysis shows the presence of different inter and intramolecular interactions, which aids the crystal packing. The

Hirshfeld surface analysis has been carried out to understand the intermolecular interaction contacts and the percentage contribution of each type of contact. The major contribution is from H-H contacts with 50.9%. The energy framework has also been carried out to calculate the different interaction energies viz., electrostatic, dispersion, polarization, and repulsion between the molecular pairs in the crystal, where dispersion energy was the dominant value among all the interaction energies. The molecular docking analysis has been performed. The molecular docking results recommend that the compound MPIMP might exhibit strong inhibitory activity against DprE1 enzymes compare to the available pyrazinamide drug. It may result in the development of the new antituberculosis drug.

Acknowledgments

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Table I: Crystal data and experimental details

Empirical formula	$C_{13}H_{12}N_2O_2$
CCDC No.	2091399
Formula weight	228.25
Temperature (K)	293(2)
Crystal system	Tetragonal
Space group	$P4_2/n$
a (Å)	14.2958 (3)
b (Å)	14.2958 (3)
c (Å)	11.0179 (3)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å^3)	2251.73 (12)
Z	8
ρ calc (g/cm^3)	1.347
μ (mm-1)	0.093
F(000)	960.0
Crystal size (mm^3)	0.12 × 0.08 × 0.06
Radiation	MoKα (λ = 0.71073 Å)
2Θ range for data collection (°)	6.794 to 54.774
Index ranges	-17 ≤ h ≤ 17, -17 ≤ k ≤ 16, -13 ≤ l ≤ 14
Reflections collected	17000
Independent reflections	2430 [Rint = 0.0311, Rsigma = 0.0227]
Data / restraints / parameters	2430/0/156
Goodness-of-fit on F^2	1.059
Final R indexes [I≥2σ (I)]	R1 = 0.0518, wR2 = 0.1312
Final R indexes [all data]	R1 = 0.0723, wR2 = 0.1456
Largest diff. peak /hole (e.Å^{-3})	0.15/-0.27

Table II: Bond distances (Å), Bond angles (o) and Torsion angles (o)

Bond	Distance (Å)	Bond	Distance (Å)
O2-C10	1.3454(17)	C6-C7	1.4401(19)
O2-C13	1.4326(17)	C6-C1	1.4053(19)
O1-C1	1.3490(17)	C6-C5	1.3997(19)
N1-C8	1.4122(18)	C10-C11	1.3934(19)
N1-C7	1.2834(16)	C1-C2	1.380(2)
N2-C10	1.3130(16)	C5-C4	1.364(2)
N2-C9	1.3394(17)	C12-C11	1.3611(19)
C8-C9	1.3715(18)	C4-C3	1.384(2)
C8-C12	1.3967(18)	C3-C2	1.377(2)
Bond	**Angles (°)**	**Bond**	**Angles (°)**
C10-O2-C13	117.21(11)	N1-C7-C6	122.52(12)
C7-N1-C8	121.51(11)	O1-C1-C6	120.94(14)
C10-N2-C9	116.74(11)	O1-C1-C2	119.33(13)
C9-C8-N1	117.25(11)	C2-C1-C6	119.73(13)
C9-C8-C12	116.60(13)	N2-C9-C8	125.05(12)

C12-C8-N1	126.14(11)	C4-C5-C6	121.65(13)
C1-C6-C7	121.97(12)	C11-C12-C8	119.55(12)
C5-C6-C7	119.82(12)	C12-C11-C10	118.66(12)
C5-C6-C1	118.21(13)	C5-C4-C3	119.34(14)
O2-C10-C11	116.88(11)	C2-C3-C4	120.51(15)
N2-C10-O2	119.74(12)	C3-C2-C1	120.56(15)
N2-C10-C11	123.38(14)		
Bond	Torsion Angles (°)	Bond	Torsion Angles (°)
C13-O2-C10-N2	-2.66 (19)	C7-C6-C1-O1	0.86 (21)
C13-O2-C10-C11	176.99 (13)	C7-C6-C1-C2	-178.90 (14)
C7-N1-C8-C9	172.36 (13)	C5-C6-C1-O1	-179.45 (13)
C7-N1-C8-C12	-8.87 (20)	C5-C6-C1-C2	0.79 (21)
C8-N1-C7-C6	178.37 (12)	C7-C6-C5-C4	178.79 (14)
C9-N2-C10-O2	179.26 (12)	C1-C6-C5-C4	-0.91 (21)
C9-N2-C10-C11	-0.36 (20)	O2-C10-C11-C12	-178.76 (13)
C10-N2-C9-C8	-0.57 (21)	N2-C10-C11-C12	0.88 (22)
N1-C8-C9-N2	179.81 (13)	O1 -C1-C2-C3	-179.97 (14)
C12-C8-C9-N2	0.92 (21)	C6- C1-C2-C3	-0.21 (23)
N1-C8-C12-C11	-179.12 (13)	C6-C5-C4-C3	0.43 (23)
C9-C8-C12-C11	-0.34 (20)	C8-C12-C11-C10	-0.48 (21)
C1-C6-C7-N1	1.94 (21)	C5-C4-C3-C2	0.19 (24)
C5-C6-C7-N1	-177.75 (13)	C4-C3-C2-C1	-0.29 (25)

Table III: Hydrogen bonding geometry (e.s.d.`s in parentheses)

D–H…A	D–H(Å)	H…A(Å)	D…A(Å)	D–H…A(°)
O1-H1…N1	0.82	1.88	2.6137(15)	148
C7-H7…N2[(i)]	0.93	2.57	3.4273(17)	153
C13- H13A…O1[(ii)]	0.96	2.58	3.384(2)	141

Symmetry codes: *(i) 1/2 - x, 3/2- y, z (ii) 1- x, -1/2 + y, 1/2 + z*

Table IV: Different interaction energies of the molecular pairs in KJ/mol.

N	Symmetry operation	Radial Distance	Electron Density	E_ele	E_pol	E_dis	E_rep	E_tot
1	-x+1/2, -y+1/2, z	4.46	B3LYP/6-31G(d,p)	-7.7	-2.0	-54.8	28.5	-39.8
1	-x, -y, -z	5.77	B3LYP/6-31G(d,p)	-5.7	-1.5	-48.3	19.7	-37.0
2	-y+1/2, x, -z+1/2	9.32	B3LYP/6-31G(d,p)	-4.5	-0.6	-10.4	6.1	-10.5
1	-x, -y, -z	10.34	B3LYP/6-31G(d,p)	-0.6	-0.3	-17.8	8.1	-11.3
2	y+1/2, -x, z+1/2	6.35	B3LYP/6-31G(d,p)	-14.2	-3.1	-24.1	24.3	-23.3
2	-y, x+1/2, z+1/2	6.35	B3LYP/6-31G(d,p)	-1.8	-1.0	-14.7	7.7	-10.6

Table V: Binding energy, hydrogen bond and hydrophobic contacts of *2*-Methoxy 4-(2-hydroxybenzylidene) amino pyridine (MPIMP) and pyrazinamide with 4WK5.

Inhibitor	Binding Energy (Kcal m^{-1})	Interactions	Distance Å	Bonding	Bonding Types	Binding Site of Protein	Binding Site of Ligand
MPIMP	7.4	HIS123[NE2…H-O]	2.506	Hydrogen	H-bond	NE2	O
		GLN336[OE1…H-O]	2.204	Hydrogen	H-bond	OE1	O
		LYS418[HZ1…O]	2.530	Hydrogen	H-bond	HZ1	O
		VAL365[VA…π]	4.833	Hydrophobic	Alkyl- π	VA	Methoxy ring
		PRO116[PR… π]	5.445	Hydrophobic	Alkyl- π	PR	Pyridine ring
		CYS387[CY… π]	5.019	Hydrophobic	Alkyl- π	CY	Methoxy Ring
pyrazinamide	5.4	ALA53144[O…H-O]	2.027	Hydrogen	H-bond	O	O
		GLY55[N-H..O]	2.262	Hydrogen	H-bong	N	O
		GLY125[N…π]	3.800	Hydrophobic	Amide-π	N	Ring
		ALA128[CD…π]	4.847	Hydrophobic	Alkyl-π	CD	Ring

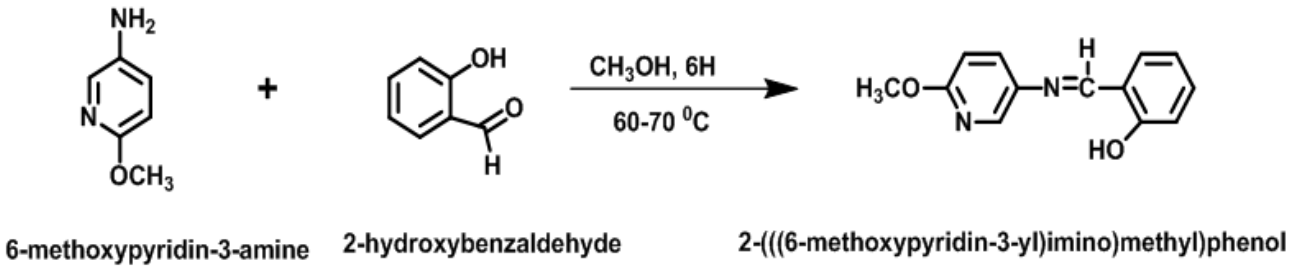


Fig. 1: Reaction scheme of 2-(((6-methoxypyridin-3-yl)imino)methyl)phenol.

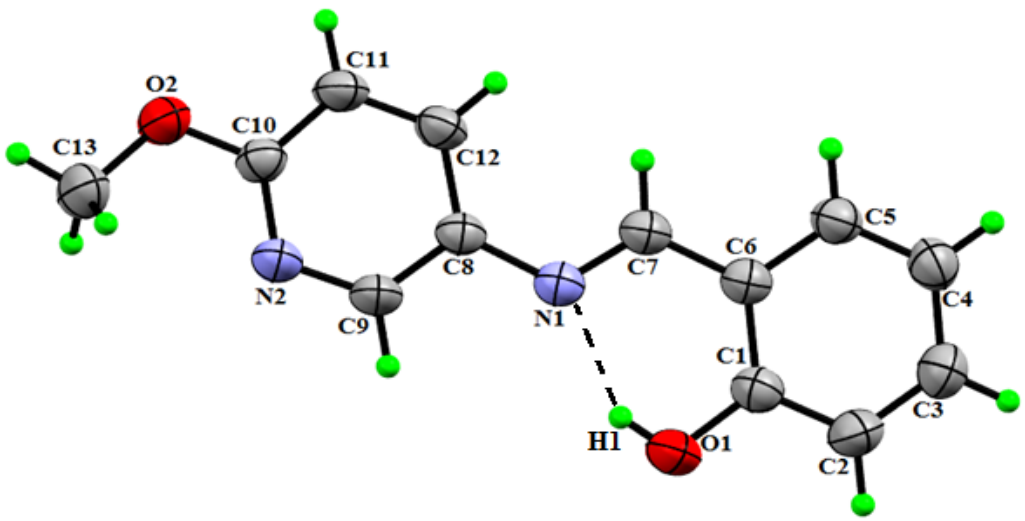


Fig. 2: ORTEP view of molecule with displacement ellipsoids drawn at 40% probability level (H atoms are shown as small arbitrary radius). The dotted line shows the intramolecular hydrogen bond.

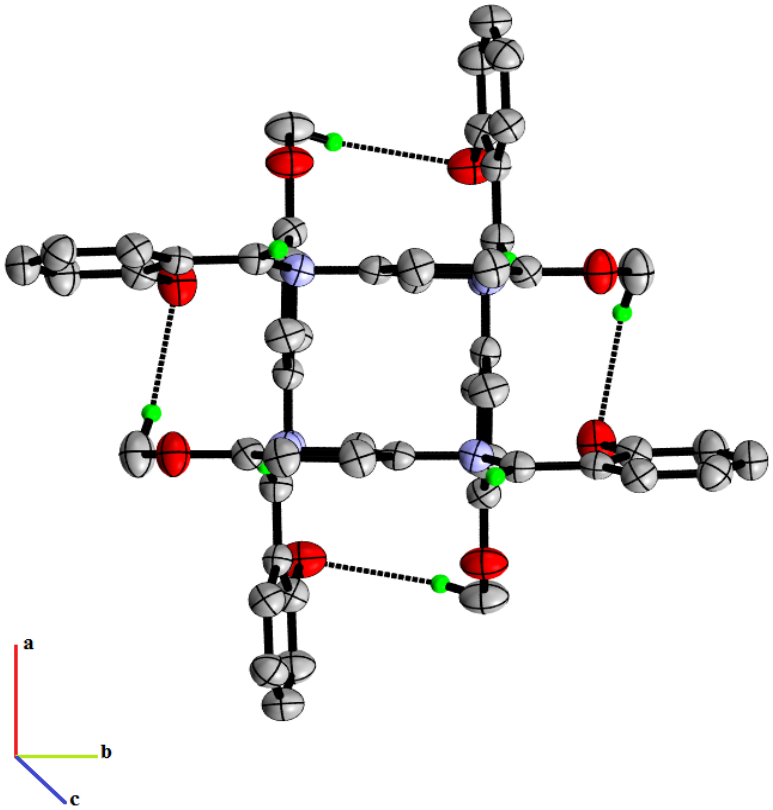


Fig. 3: Packing of crystal structure view along c-axis (dashed lines represents H-bonded interactions)

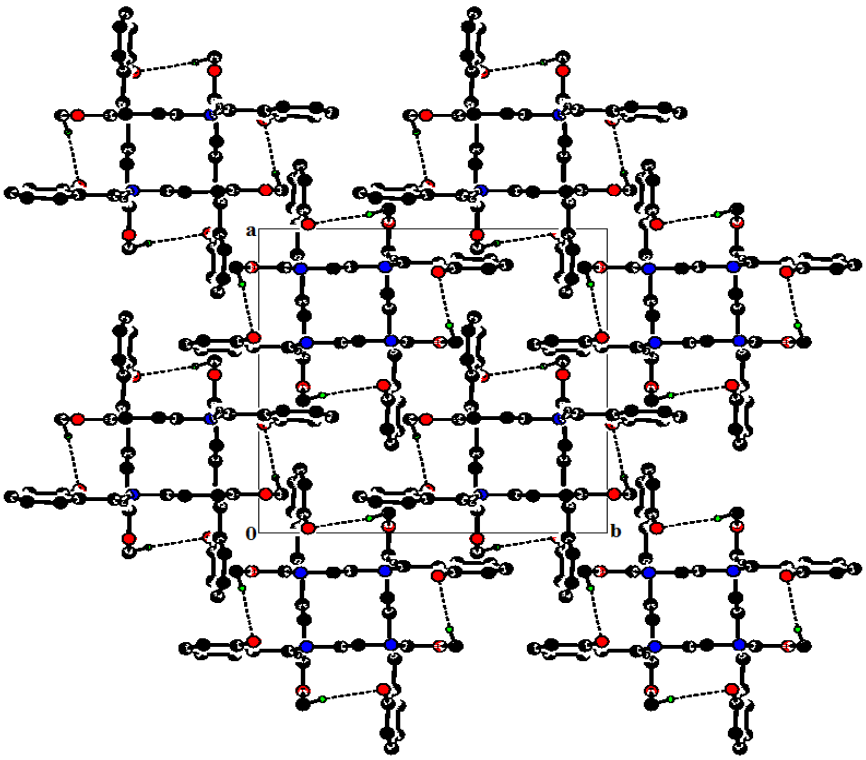
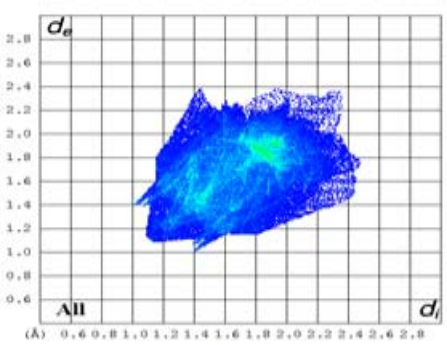
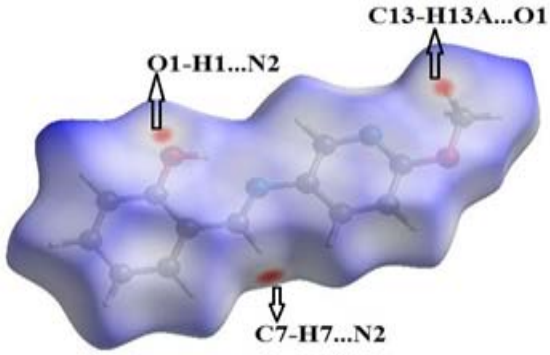


Fig. 4: Packing of crystal structure view along c-axis (dashed lines represents H-bonded interactions).



(a)



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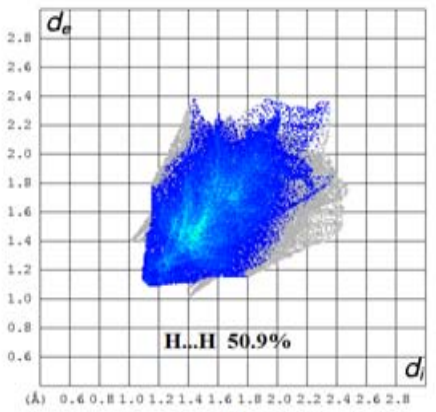

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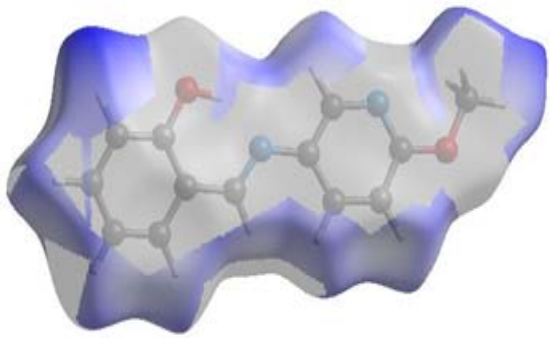
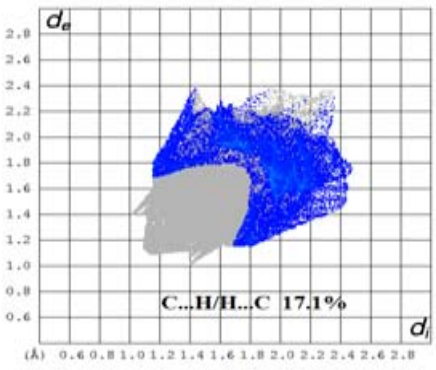
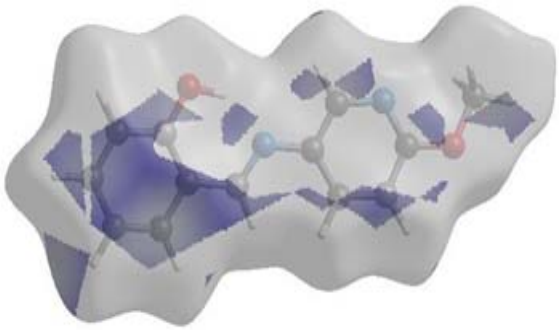


(b*)



(c)



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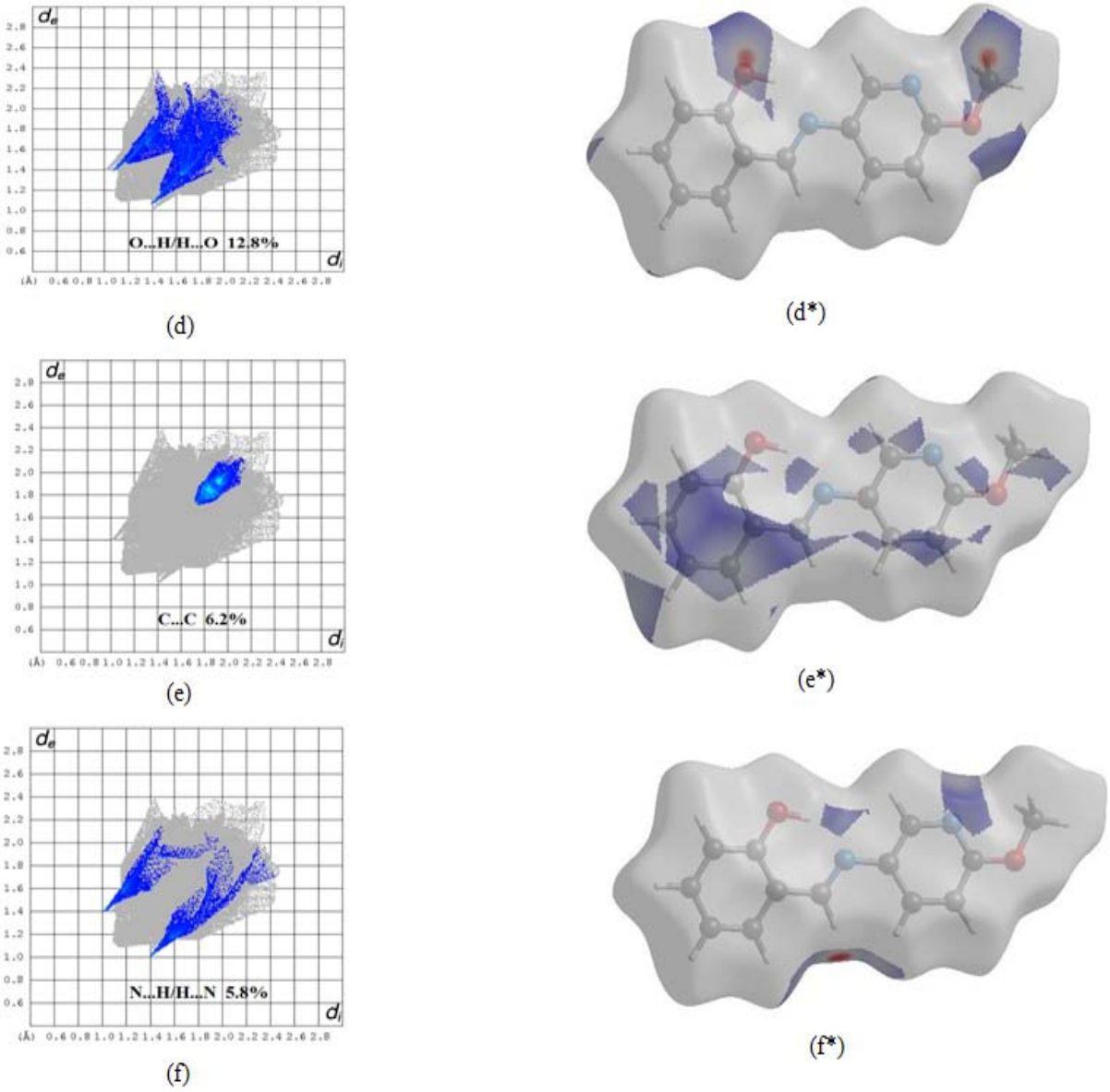

Fig. 5: Fingerprint plots of the compound from all the intermolecular contacts, (a*) Hireshfeld surface mapped over d_{norm}, (b), (c), (d), (e) and (f) are the 2D fingerprint plots showing H-H, C-H/H-C, O-H/H-O, C-C, N-H/H-N contacts with the percentages contribution and (b*), (c*), (d*), (e*) and (f*) represent the associated d_{norm} Hireshfeld surfaces respectively

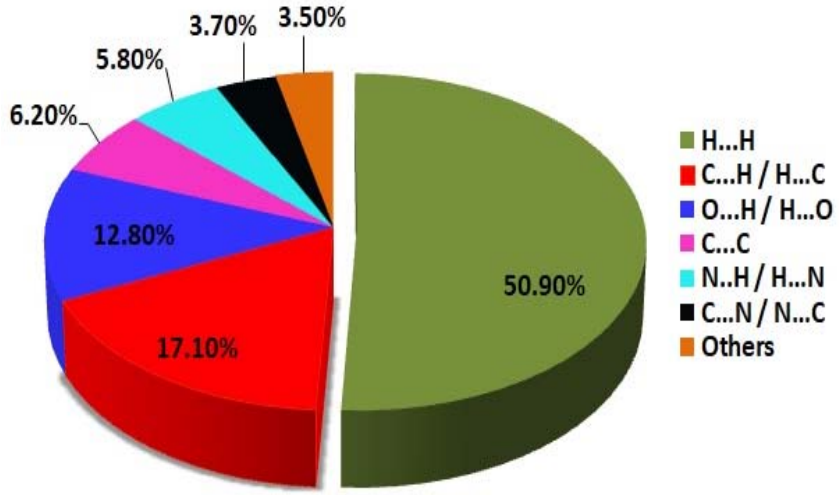

Fig. 6: Relative contributions of various intermolecular interactions to the Hirshfeld surface area.

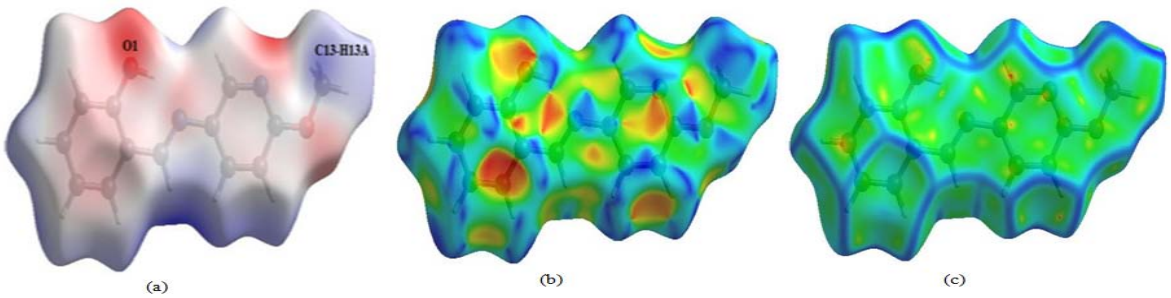

Fig. 7.(a): Electrostatic potential map (b) Shape index mapped on Hirshfeld surface (c) Curvedness plot mapped on Hirshfeld surface

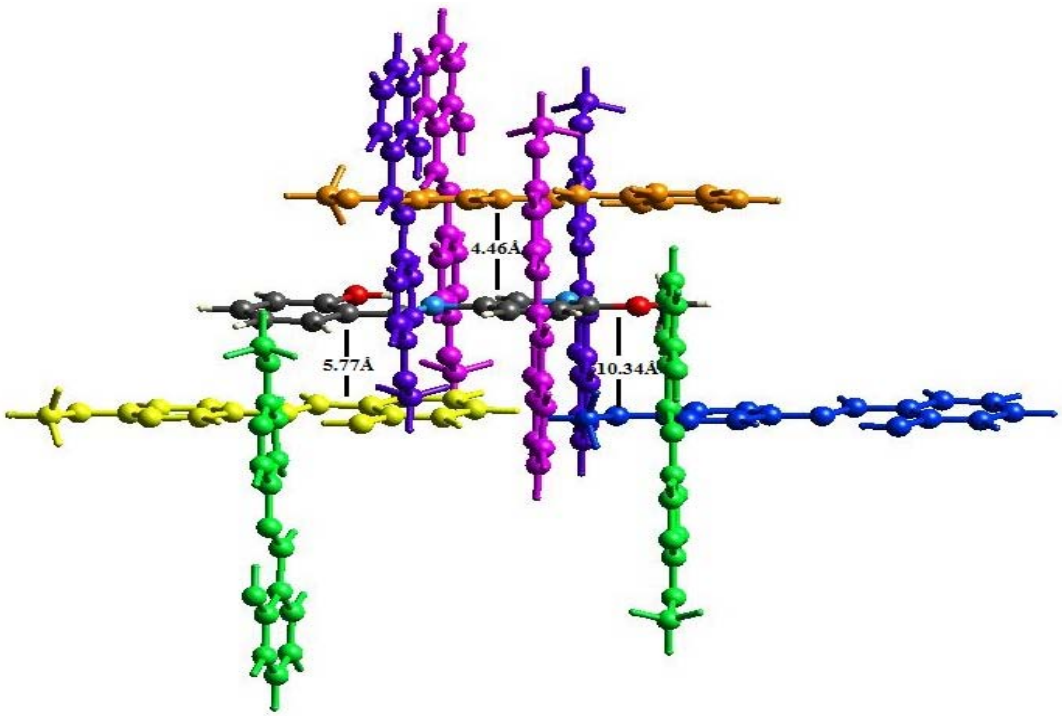

Fig. 8: Molecular pairs separated by respective radial distance from the centriod molecule involved in the calculation of interaction energies along c-axis

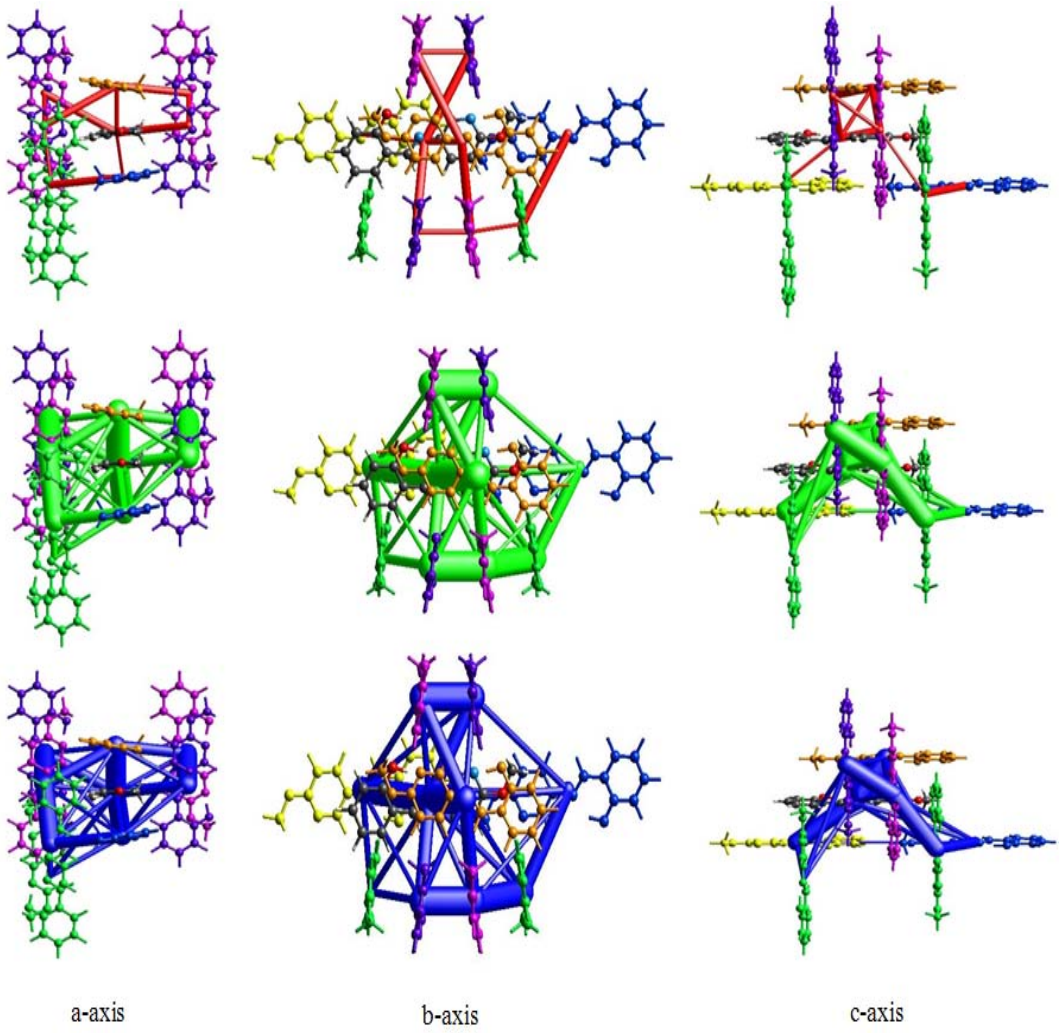

Fig. 9: The graphical representation of electrostatic interactions *viz*. coulomb interaction energy (red), dispersion energy (green), total interaction energy (blue) of the compound along different axes

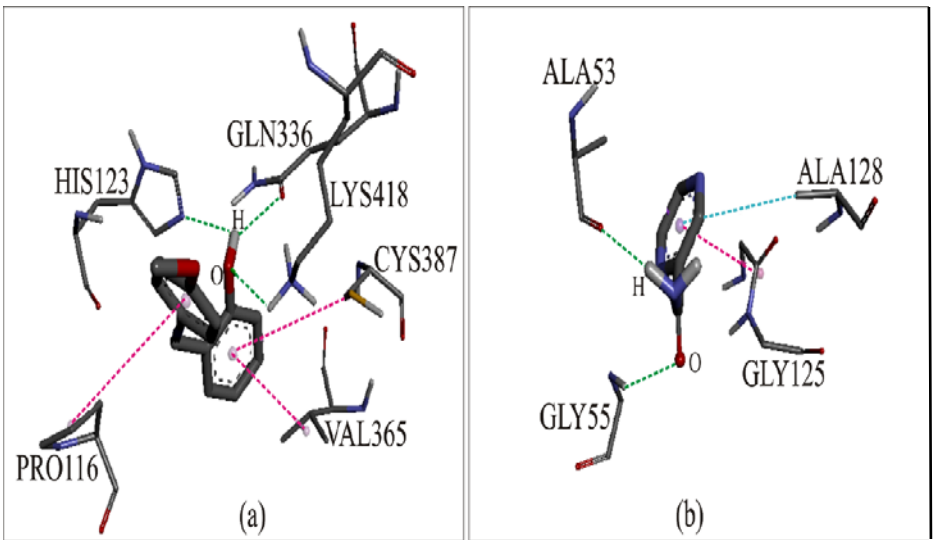

Fig. 10: Interaction of (a) MPIMP and (b) pyrazinamide to 4KW5 binding site

References Références Referencias

1. A. Carreño, L. Rodríguez, D. Páez-Hernández, R. Martin-Trasanco, C. Zúñiga, D. P. Oyarzún, M. Gacitúa, E. Schott, R. Arratia-Pérez and J. A. Fuentes, Front. Chem. 6, 312 (2018).
2. A. E. Parlak, H. Cakmak, S. Sandal, B. Yılmaz, M. Sekerci, Z. KaragözGenc, M. Tuzcu and M. Karatepe, J. Biochem. Mol. Toxicol. 33 (2), e22247 (2019).
3. E. Yousif, A. Majeed, K. Al-Sammarrae, N. Salih, J. Salimon, and B. Abdullah, Arabian J. Chem. 10, S1639 (2017).
4. M. A. Malik, O. A. Dar, P. Gull, M. Y. Waniand and A. A. Hashmi, Med. Chem. Commun. 9 (3), 409 (2018).
5. A. B. Thomas, R. K. Nanda, L. P. Kothapalli, and S. C. Hamane, Arab. J. Chem. 9, S79 (2016).
6. M. B. Gawande, P. R. Nilkanth, S. Ghorai, K. Dhawale, S. Shelke and T. Ahamad, Chem. Biodivers. 17 (9), e2000342 (2020).
7. S. Murtaza, M. S. Akhtar, F. Kanwal, A. Abbas, S. Ashiq and S. Shamim, J. Saudi Chem. Soc. 21, S359 (2017).
8. D. Dewangan, K. T. Nakhate, V. S. Verma, K. Nagori, and D. K. Tripathi, J. Heterocycl. Chem. 54 (6), 3187 (2017).
9. Y. Y. Wang, F. Z. Xu, Y. Y. Zhu, B. Song, D. Luo, G. Yu, S. Chen, W. Xue and J. Wu, Bioorg. Med. Chem. Lett. 28 (17), 2979 (2018).
10. A. S. Hassan, H. M. Awad, A. A. Magd-El-Din and T. S. Hafez, Med. Chem. Res. 27 (3), 915 (2018).
11. D. A. Sabbah, F. Al-Tarawneh, W. H. Talib, K. Sweidan, S. K. Bardaweel, E. Al-Shalabi and M. S. Mubarak, Med Chem. 14 (7), 695 (2018).
12. A. A. Shanty, and P. V. Mohanan, Spectrochim. Acta A, 192, 181 (2018).
13. K. Karrouchi, L. Chemlal, J. Taoufik, Y. Cherrah, S. Radi, M. E. A. Faouzi, and M. Ansar, Ann. Pharm. Fr. 74 (6), 431 (2016).
14. M. S. More, P. G. Joshi, Y. K. Mishra and P. K. Khanna, Mater. Today Chem. 14, 100195 (2019).
15. A. M. Abu-Dief and I. M. Mohamed, Beni Suef Univ J Basic Appl Sci. 4 (2), 119 (2015).
16. M. J. Turner, J. J. Mckinnon, S. K. Wolff, D. J. Grimwood, P. R. Spackman, D. Jayatilaka, and M. A. Spackman, *Crystal Explorer 17.5* (The University of Western Australia, 2017).
17. S. Bodige, P. Ravula, K. C. Gulipalli, S. Endoori, P. K. R. Cherukumalli, N. S. C. JN and N. Seelam, Synth. Commun. 49 (17), 2219 (2019).
18. J. Gawad and C. Bonde, Chem. Cent. J. 12, 138 (2018).
19. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, and H. Puschmann, J. Appl. Crystallogr. 42, 339 (2009).
20. G. M. Sheldrick, Acta Cryst. A71, 3 (2015).
21. G. M. Sheldrick, Acta Cryst. C71, 3 (2015).
22. L. Spek, Acta Crystallogr. D65, 148 (2009).
23. M. Nardelli, J. Appl. Crystallogr. 28, 659 (1995).
24. C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. Van De Streek and P. A. Wood, J. Appl. Cryst. 41, 466 (2008).
25. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, J. Comput. Chem. (2009).
26. Discovery studio visualizer: http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization.html.
27. Z-L. Jing, R-N. Li, N and Yang, Acta Cryst. E63, o3001 (2007).
28. H. Unver, B. Boyacioglu, C. T. Zeyrek, D. Yolal, M. Yildiz, N. Yildinm, N. Demir, A. Kiraz and A. Elmali, J. Appl. Crystallogr. 48 (1), 32 (2018).
29. J. Bernstein, R. E. Davis, L. Shimoni and N. L. Chang, Angew. Chem. Int. Ed. Engl. 34, 1555 (1995).
30. M. A. Spackman and D. Jayatilaka, CrystEngComm. 11, 19 (2009).
31. J. J. McKinnon, D. Jayatilaka and M. A. Spackman, Chem. Commun. 3814 (2007).
32. M. J. Turner, S. Grabowsky, D. Jayatilaka and M. A. Spackman, J. Phys. Chem. Lett. 5 (24), 4249 (2014).
33. M. J. Turner, S. P. Thomas, M. W. Shi, D. Jayatilaka and M. A. Spackman, Chem. Commun. 51 (18), 3735 (2015).
34. A. J. Edwards, C. F. Mackenzie, P. R. Spackman, D. Jayatilaka and M. A. Spackman, Faraday Discuss. 203, 93 (2017).

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Thermodynamic and Kinetic Regularities of the Swelling of Edible Gelatin in Water Irradiated with an Electromagnetic Field at Various Values of the Medium Acidity

By I. E. Stas & S. S. Pavlova

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Keywords: deionized water, gelatin, swelling rate constant, degree of swelling, heat of swelling, calorimetry, degree of ionization, isoelectric point of protein, electromagnetic field, frequency.

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I. Introduction

Currently a large amount of experimental data has been accumulated, indicating the efficiency of physical fields of various nature uses in the physical and chemical processes implementation in an aqueous medium. It is assumed that the effect of magnetic, electric and electromagnetic fields of different frequency ranges leads to a reorganization of the water structure and, as a consequence, to a change in the nature and strength of its interaction with molecules or ions of the solute [1 – 3]. The optical, electrical, rheological and other properties of aqueous solutions, the rate of chemical reactions in it change as a result [1 – 9].

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Water is very sensitive to the influence of an electromagnetic field (EMF) of ultra-high frequencies (30 – 300 MHz). An increase in its surface tension and heat of evaporation, a decrease in the rate of evaporation, and deterioration of the wetting ability have been determined as a result of the field action, which indicates an increase in intermolecular force in an aqueous medium [10–12]. The main factors determining the efficiency of field action are the EMF frequency and the time of its action [11]. It means that we can talk about the selectivity of the EMF effect on the water system, because the effects are revealed either only in its specific frequencies, or at certain frequencies expressed to the maximum extent. The frequencies corresponding to the maximum change in the property depend on the nature of the substance dissolved in the aqueous medium [13 – 16]. An increase in the action time leads to a gradual increase in the property up to a certain limit. Water and polymer solutions retain their changed properties throughout the observation period (more than six months), and electrolyte solutions gradually return to their initial state after the termination of the field action [10].

The modern theory of solutions of high molecular weight compounds considers the polymers swelling and dissolution as a mixing liquids process [17, 18]. Being dissolved, polymers molecules of a low molecular weight liquid penetrate into the polymer immersed in it. This is possible because chain high molecular mass compound molecules are flexible: their bonds, bending, create a poor packing of macromolecules. The liquid absorbed at the first stage of swelling has been used for salvation of the polymer polar groups.

The heat of swelling Q (the total amount of heat released during the swelling of 1 g of dry polymer) is the sum of the following values

$$Q = - Q_1 - Q_2 + Q_3$$

where Q_1 is the heat corresponding to the work of separation of macromolecules $(Q_1<0)$; Q_2– heat corresponding to the separation of solvent molecules $(Q_2<0)$; Q_3 is the heat released as a result of the

interaction of the solvent and polymer molecules ($Q_3 > 0$ is solvation) [19].

An increase in intermolecular force in an aqueous medium due to the electromagnetic field influence should lead to an increase in Q_2 and a decrease in Q_3, as a result of which a decrease in the total thermal effect of swelling can be observed. In addition, it is possible to slow down the diffusion of solvent molecules into the bulk of the polymer. This hypothesis can be confirmed by a change in the rate and degree of swelling, as well as the process heat effect.

The main task of this work was to determine the rate and degree of gelatin swelling at various values of the medium acidity, to determine the value of the isoelectric point (IEP), as well as the degree and heat of gelatin swelling in water exposed to EMF of various frequencies.

The object of research was gelatin – a protein material, a polydisperse mixture of proteins. Due to the presence of ionogenic groups, gelatin swells limitedly in cold water and unlimitedly –in hot water. Gelatin is used for preparation of hard and soft capsules, in the production of plasma substitute solutions, for preparation of hemostatic wound dressings in the pharmaceutical industry.

a) Experimental

Deionized water, purified by using membrane distillation DME-1/B (Russia)has been used for the experiments. The specific conductivity of the water was 1.1 μS / cm. The required pH values were obtained by adding HCl solution (chemically pure).

The source of the electromagnetic field was a Г3-19A high-frequency signal generator (Russia) with a frequency in the range of 30 – 200 MHz and an output power of 1 Watt. A 100 ml capacitive cell was used to expose water with EM field. The voltage on the high-frequency electrodes was 20 – 22 V. The water irradiation lasted 3 hours. As shown by previous studies, water exposed to EMF retains its changed properties for a long time – up to a year or more. Therefore, the timing of subsequent experiments could not affect the results obtained.

We used powdered (particle size is less than or equivalent to 0.25 mm.) edible gelatin produced by JSC MOZhELIT (Belarus) with a moisture content of no more than 16%. Before the experiment, the gelatin powder was dried to constant mass.

A quantitative determination of swelling is the degree of swellingα, equals to

$$\alpha = \frac{V - V_0}{V_0},$$

where V and V_0 are the volumes of the swollen and initial polymer.

In the case of limited swelling, the degree of swelling reaches the limiting value α_{max} [7].

Since the rate of swelling is determined by the rate of solvent molecules diffusion into the polymer, then the swelling process can be considered as a reaction of the first order in the simplest case, and the rate constant of the process is usually calculated using the equation

$$k = \frac{1}{t} \ln \frac{\alpha_{max}}{\alpha_{max} - \alpha_t}$$

Swelling kinetics was studied by measuring the volume of swollen polymer every 5 minutes.

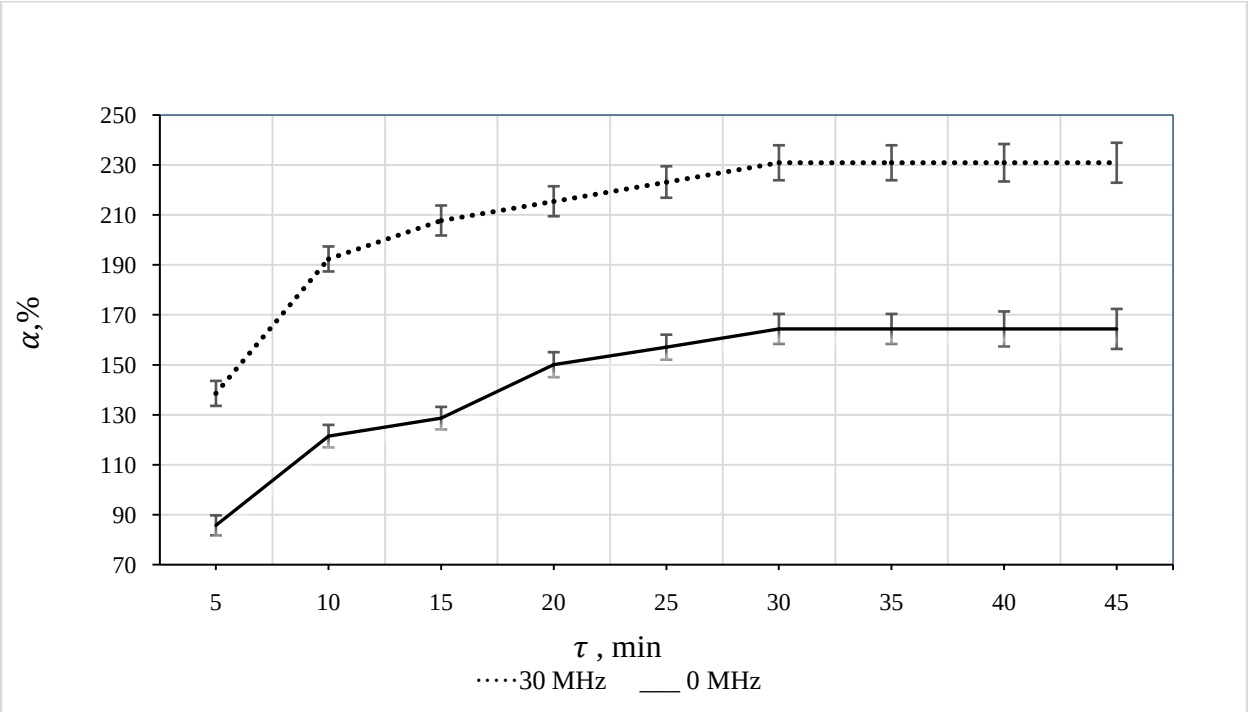
100 ml of activated or non-activated water was poured into a porcelain beaker and placed in a calorimeter, a test tube containing 4 g of powdered gelatin was placed there too to determine the heat of swelling. A Beckmann thermometer (temperature variation accuracy is ± 0.01°) and a stirrer were placed in the beaker with water. The temperature of the calorimetric system was measured for 15 min with continuous stirring. After reaching stable temperature, a sample weight of gelatin was poured from the test tube into a glass with water and the temperature change was recorded every minute for 15 – 20 minutes. The heat of swelling of the polymer per 1 g was calculated by the values of Δt. The calorimeter constant was determined from the heat of KCl dissolution. All experiments were carried out 3 times.

II. Results and Discussion

The change in the degree of swelling of gelatin (α) in water exposed to the electromagnetic field of ultra-high frequencies in the range of 30 – 190 MHz was established. Both its increase, most pronounced for water irradiated by a field of 30 and 90 MHz, and a decrease, for frequencies of 110, 150 and 170 MHz (pH = 6.3) are observed. The increase in the degree of swelling amounted to a maximum 67 – 119%, the decrease – 31 – 49% in absolute units. The rate of the process changes respectively, as evidenced by the calculated swelling rate constants (k) of gelatin (Table 1). The rate constants increase to varying degrees depending on the field frequency; however, there is no clear correlation between the degree and rate of swelling. Figure 1 shows the kinetic curves of the gelatin swelling in water exposed to EMF with frequencies of 30 and 90 and 170 MHz.

Table 1: Values of the degree, rate constant, and time of gelatin maximum swelling in water exposed to EMF of various frequencies (T = 24 °C, pH = 6.3)

f, MHz	α_{max}, %	$\Delta\alpha$,%	$\alpha^f_{max}/\alpha^0_{max}$	k, min^{-1}	t, min
0	164±6	-	1.00	0.14	30
30	231±6	67	1.40	0.19	30
50	193±5	29	1.20	0.15	30
70	193±8	29	1.20	0.15	35
90	283±6	119	1.70	0.16	30
110	146±4	-20	0.89	0.18	30
130	177±5	13	1.10	0.16	30
150	115±2	-49	0.70	0.22	20
170	133±3	-31	0.81	0.19	25
190	140±6	-24	1.10	0,18	30



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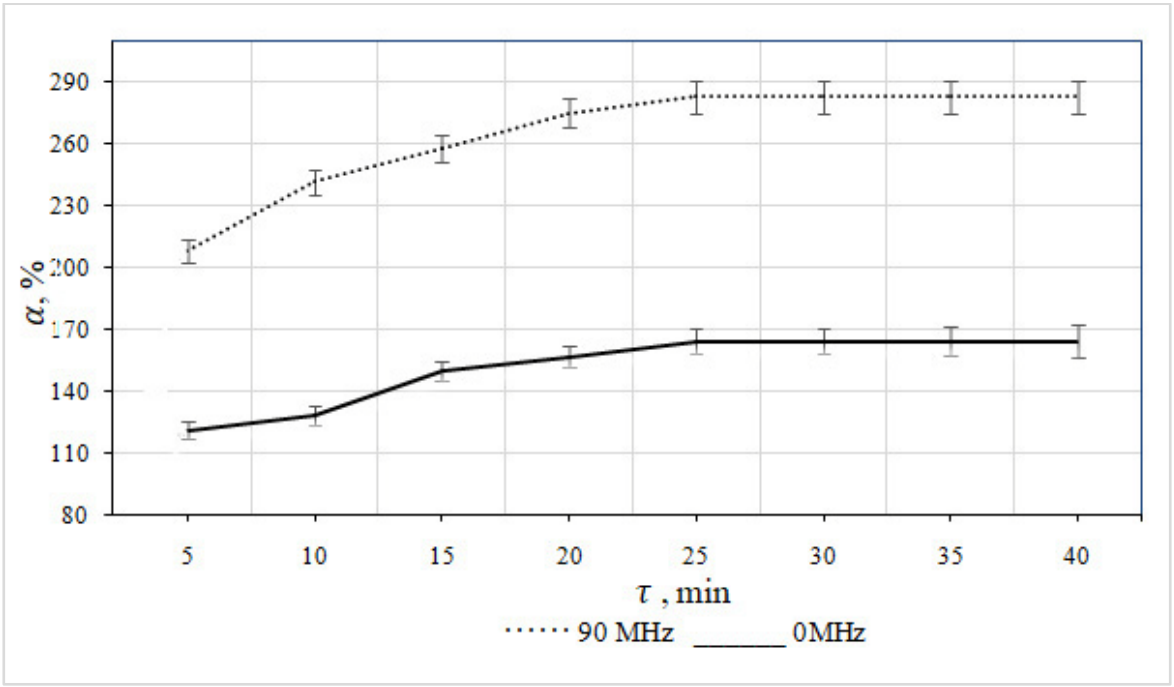

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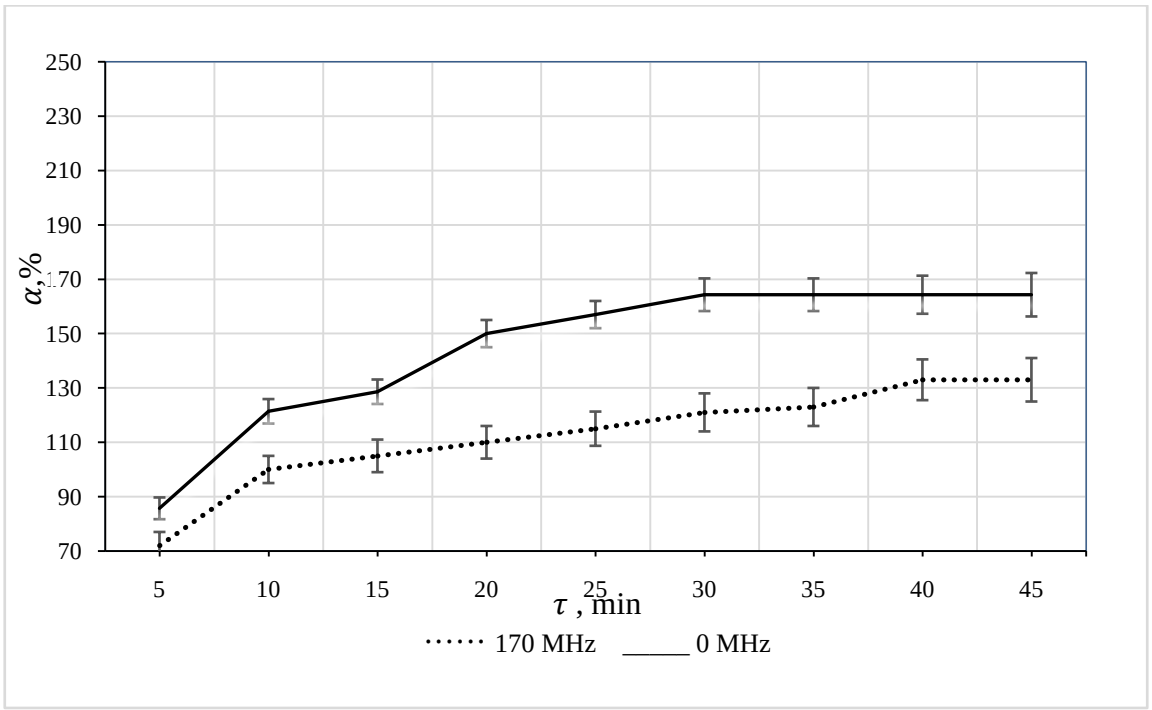

c

Figure 1: Kinetic curves of gelatin swelling in non-activated and activated water (T = 24 °C pH = 6.3): a) 30 MHz; b) 90 MHz; c) 170 MHz

Swelling is accompanied by the heat release, which is revealed in a temperature increase when dry gelatin is mixed with water. Calorimetric measurements showed that when gelatin swells in water exposed to EMF, the value of the thermal effect of this process decreases. The most significant decrease in the swelling heat is observed when using water exposed to EMF of 70 MHz frequency – by a maximum of 23%. When using water exposed to EMF of 190 MHz frequency, the swelling heat increases sharply – by 77.8% (table 2).

Table 2: Temperature change during the gelatin swelling and the specific heat capacity of its swelling in water exposed to EMF of various frequencies (T = 24 °C, pH = 6.3)

f, MHz	0	30	50	70	90
Δt^0	0.23±0.01	0.19±0.01	0.24±0.03	0.17±0.02	0.20±0.01
Q, J/g	96±4	81±3	98±5	75±2	85±3
ΔQ, %	–	-16	2,8	-23	-11
f, MHz	**110**	**130**	**150**	**170**	**190**
Δt, °C	0.20±0.02	0.21±0.03	0.19±0.02	0.22±0.02	0.40±0.06
Q, J/g	85±3	89±4	81±3	94±4	170±9
ΔQ, %	-11.0	-6.7	-16.0	-2.2	78.0
Δt° – temperature change during swelling of 4 g of gelatin in 100 g of water					

The swelling parameters for substances of protein nature depend on the pH of the medium, since the charge characteristics of macromolecules change when the pH changes. They are negatively charged in an alkaline medium, and they are positively charged in an acidic medium. The total charge of a protein molecule is zero in IEP. Having chosen two frequencies corresponding to the maximum decrease and maximum increase in the heat of gelatin swelling, we studied the parameters of polymer swelling depending on the pH of the medium. Table 3 shows the values of the maximum degree and rate constants of gelatin swelling at different pH values of non-activated and activated(70 and 190 MHz) water.

Table 3: Dependence of the gelatin swelling degree and rate constant on pH (T = 24°C)

pH	4.0	4.4	4.5	4.6	4.7	4.8	4.9	5.0	6.3
f = 0 MHz									
α_{max}, %	185±10	171±9	158±7	150±5	143±5	127±3	186±10	169±8	164±7
k, s^{-1}	0.30	0.32	0.32	0.30	0.27	0.21	0.21	0.18	0.14
f = 70 MHz									
α_{max}, %	184± 9	157±8	154±7	267±9	275± 8	250±9	229± 11	229±9	193±9
k, s^{-1}	0.28	0.28	0.20	0.21	0.24	0.26	0.26	0.26	0.21
f = 190 MHz									
α_{max}, %	181±10	178± 7	175±8	170±9	167±10	123±3	150±8	146±6	140±6
k, s^{-1}	0.35	0.37	0.44	0.52	0.24	0.31	0.28	0.40	0.33

We observe a shift of IEP to the acidic region in water exposed by a field of 70 MHz, – a minimum of the degree of swelling is observed at pH = 4.5, while IEP corresponds to pH = 4.8in non-activated water. At the same time, the degree and rate of gelatin swelling is lower for pH <IEP values in comparison with non-activated water, and it is higher at pH> IEP. The maximum difference α_{max} corresponds to pH = 4.7 and amounts to 132% in absolute units. IEP does not shift at a frequency of 190 MHz, the degree of swelling exceeds α_{max} at pH <IEP, and lower values are observed at pH> IEP. The results obtained indicate a change in the charge characteristics of macroions in activated water.

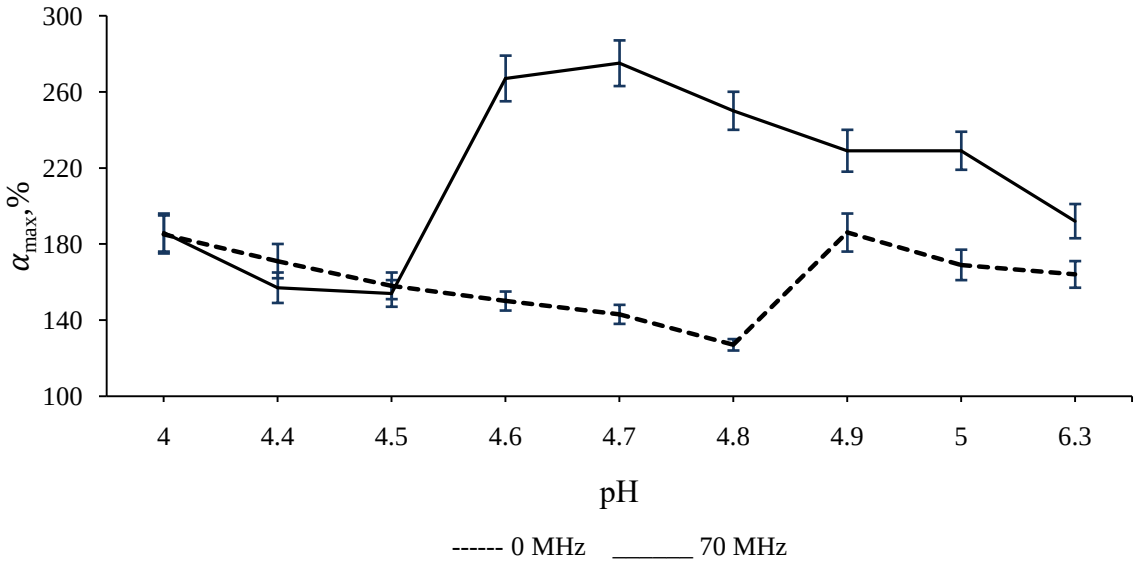

Figure 2: Dependence of the swelling degree of gelatin in non-activated and activated (70 MHz) water exposed to EMF on pH (T = 24°C)

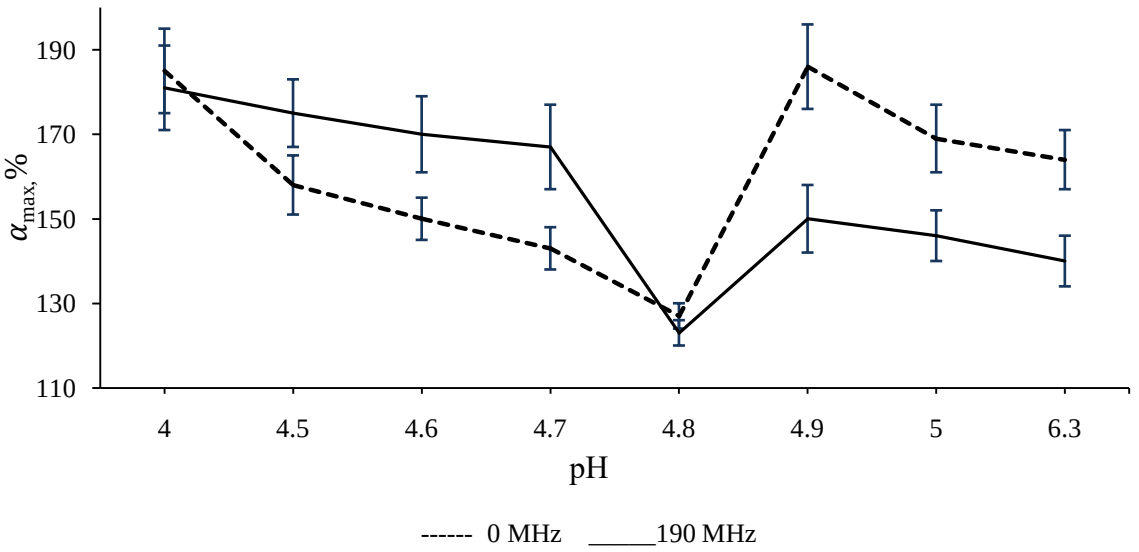

Figure 3: Dependence of the swelling degree of gelatin in non-activated and activated (190 MHz) water exposed to EMF on pH (T = 24°C)

It was shown that the heat of gelatin swelling also significantly depends on pH. Figures 4 and 5 show the corresponding dependences. The minimum of specific swelling heat corresponds to pH = 4.9fornon-activated water. Two minima are observed on the curve – at pH 4.5 and 4.9 at a frequency of 70 MHz. In this case, the swelling heat of the polymer is lower in comparison with non-activated water (the degree of swelling in this range is also lower)in the range of pH = 4.0 – 4.5,andit is higher at pH> 4.5. The minimum value of Q is observed at pH = 4.8 at a frequency of 190 MHz, i.e. as well as for the degree of swelling. The heat of gelatin swelling is higher (except for pH = 4.8) in almost the total pH range in activated water (190 MHz). The maximum differences in *Q* values are achieved at pH = 4.9 and 5.0, reaching 93 and 84%, respectively.

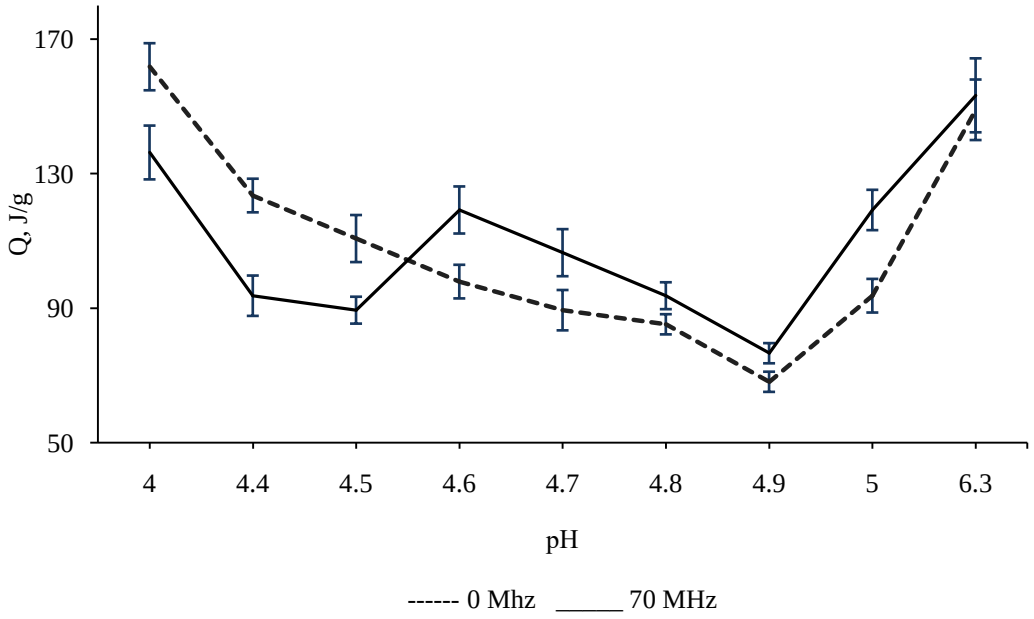

Figure 4: Dependence of the specific swelling heat of gelatin in non-activated and activated (70 MHz) water exposed to EMF on pH (T = 24°C)

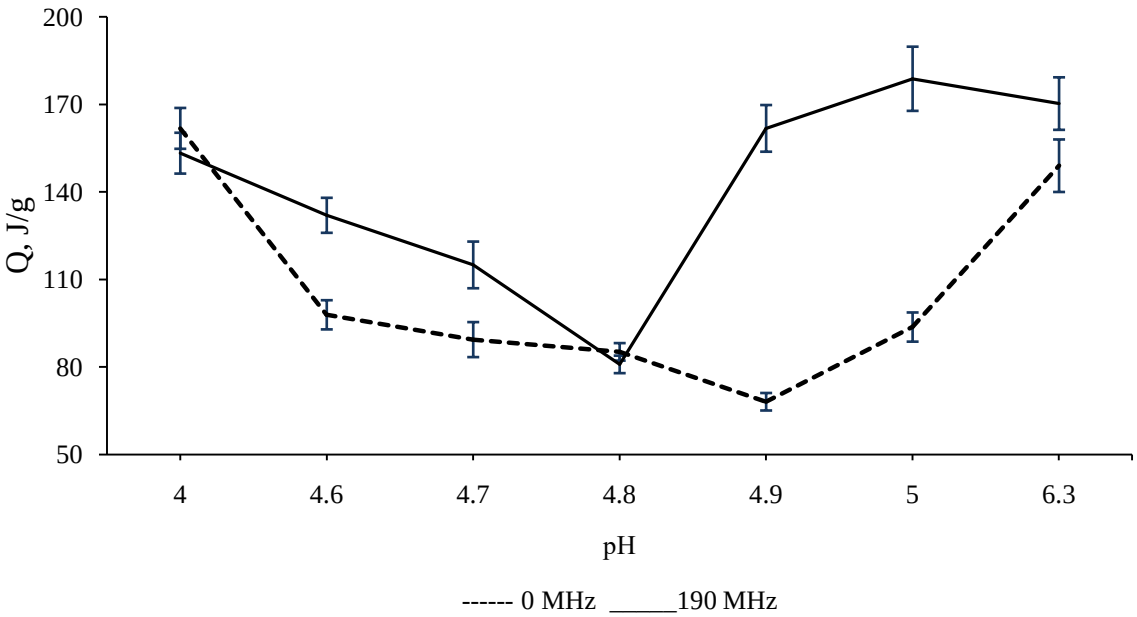

Figure 5: Dependence of the specific heat of swelling of gelatin in non-activated and activated (190 MHz) water exposed to EMF on pH (T = 24°C)

Based on the hypothesis of the strengthening of the supra molecular structure of water as a result of electromagnetic effect, it can be concluded that intermolecular forces strengthen in an aqueous medium (the value of Q_2 increases) in exposed water (with the exception of the 190 MHz frequency), and the solvation of proteins macroions composing gelatin (the Q_3value decreases) weakens. As a result, the specific heat of gelatin swelling decreases. It is obvious that the reverse processes occur at a frequency of 190 MHz. It was shown earlier [20] that during the general decrease in the heat of wetting of powdered Al_2O_3 with water irradiated by a field of different frequencies, its increase is observed for frequencies of 150 and 190 MHz, which also indicates the destruction of the structure of the aqueous medium.

An increase in the degree and rate constant of the gelatin swelling in activated water would seem to contradict the hypothesis of a decrease in the mobility of water molecules due to an increase in intermolecular

forces and a decrease in its ability to solvation. However, it is known [18] that the solvation of the polar proteins groups composing gelatin leads to an increase in the rigidity of the chain molecules, which decreases the mobility of the polymer chain regions, hinders the penetration of the solvent among them, and slows down the swelling. Therefore, the gelatin swelling in activated water, due to the lower rigidity of the polymer chains, proceeds faster and is accompanied by higher values of the limiting degree of swelling. At the same time, it should be noted that the process of polymer swelling consists of two stages [21]. The first stage is the diffusion of the solvent into the polymer and the solvation of the polymer molecules. In this case, the swelling heat is released. The second stage is characterized by an almost complete cessation of heat release, but entropy increases, since the loosening of the spatial polymer network and the associated partial release of macromolecules increases the number of configurations. Thus, the second stage of swelling is due to the entropy effect. It is at this stage that the main increase in the volume and mass of the polymer occurs. Based on this, we can talk about the effect of water irradiation with an electromagnetic field on both stages of the swelling process – there is a decrease in the solvation of macromolecules by activated water (decrease in *Q*)at the first stage, an increase in the degree of gelatin swelling due to an increase in the mobility of less solvated molecules at the second stage. When determining the rate constant, the total swelling effect is recorded. At the same time, it can be argued that the first stage of the process in activated water proceeds much slower, as evidenced by the curves of the dependence of the temperature change during the gelatin swelling on time during calorimetric measurements (Figure 6). The first section of the curve corresponds to the change in the temperature of the calorimetric system over time, the second - to the change in temperature when gelatin swells. Heat is released within 5 minutes in non-activated water, and 15 minutes in activated water. The same occurs at other frequencies, including 190 MHz.

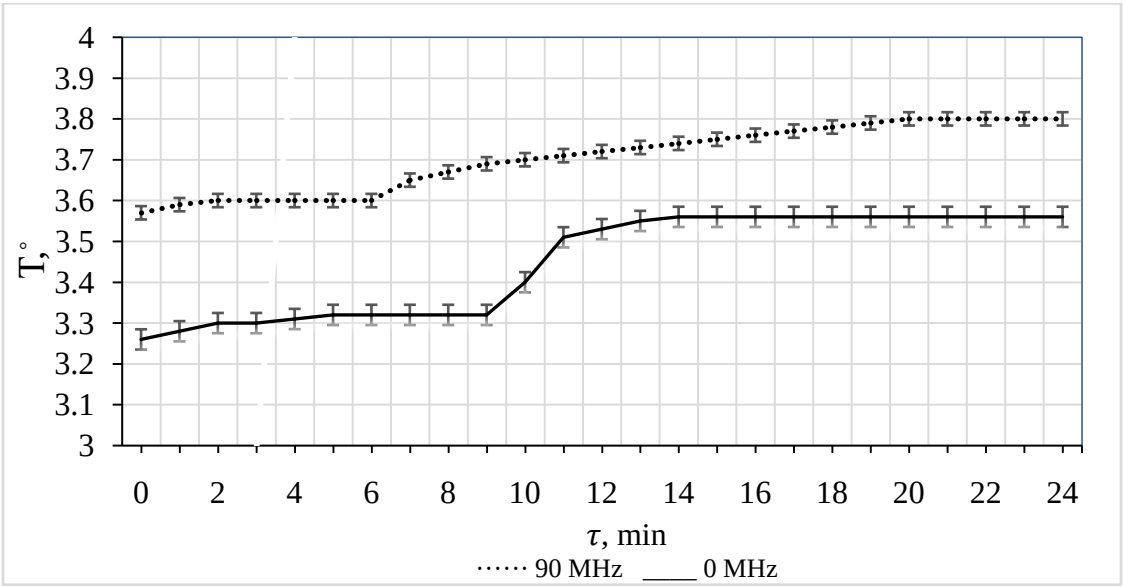

Figure 6: Dependence of temperature change during gelatin swelling in water exposed to EMF (90 MHz), on time (T_{init} = 24 ± 0.3°C)

A shift of IEP in activated water and a significant change in the thermal effect depending on pH indicate a change in the degree of ionization of protein molecules ionogenic groups, which affects the forces of their mutual repulsion or attraction and, as a consequence, the swelling degree. Moreover, gelatin is not an individual polymer, but a mixture of proteins. The electromagnetic field can affect the charge magnitude of individual protein macromolecules to a different extent, which complicates the correlation of data by the degree, rate, and heat of swelling. Nevertheless, it can be stated that the electromagnetic treatment of water significantly affects both the kinetic and thermodynamic parameters of swelling – i.e., using water exposed to an electromagnetic field at ultrahigh frequencies as a solvent; it is possible to control the polymers swelling, which is a process task in many industries.

References Références Referencias

1. De Ninno A. & Castellano A. C. (2014). Influence of magnetic fields on the hydration process of aminoacids: Vibrational spectroscopy study of L-phenylalanine and L-glutamine. *Bioelectro-magnetics*. 35(2), 129-135
2. Quickenden T.I., Betts D.M., Cole B. & Noble M. (2017). The effect of magnetic fields on the pH of

water. *Journal of Physical Chemistry*. 75(18), 2830-2831.

3. Musienko K. S., Ignatova T. M. & Glazkova V. V. (2014). Izuchenievliyaniyafizicheskihpolejnafiziko-himicheskiesvojstvavody. [Studying the influence of physical fields on the physical and chemical properties of water] *Biomedicinskaya inzheneriya i elektronika – Biomedical engineering and electronics*. 6(2), 1-7 [In Russian].
4. Vadsa J.S. (1997). Influence of a high-frequency electromagnetic field on change of volume properties of solutions electrolits. *Journal of Electrochemistry*. 9(3), 54-65.
5. Krasikov N. N. (2000). Dejstvieelektromag nitnogopolyanazhidkosti, osushchestvlyaemoe bezkontakta s potencialozadayushchimielektrodami. [Action of the electromagnetic field on the liquid, carried out without contact with potential-setting electrodes] ZHurnal fizicheskoj himii – *Journal of physical chemistry*. 74(6), 1133 – 1135[In Russian].
6. Vanger G.S. (2001). Studying of dependence of properties solutions from frequency of an imposed field. *Journal of Physical Chemistry*. 10(25), 87-99.
7. Trukhan E. M. (2010 Nekotorye fiziko-himicheskie harakteristiki slabyh elektromagnitnyh vozdejstvij na vodnuyu sredu. [Some physical and chemical characteristics of weak electromagnetic effects on the water environment]. *Ekologicheskij vestnik – Ecological bulletin.* 12(2), 66-72 [InRussian].
8. Suslyaev V.I. Mongolina N.A. & Pavlov A.A. (2006). Changing the conductivity of distilled water under the influence of a constant magnetic field. *Proceedings of the universities.Physics.*9, 127-128.
9. Yamashita M., &Duffield C. (2003). Direct current magnetic field and electromagnetic field effects on the pH and oxidation–reduction potential equilibration rates of water. *Langmuir*. 19(12), 6851-6856.
10. Stas I. E., Chirkova V. Yu. & Shtobbe I. A. (2015). Fiziko-himicheskie processy v elektromagnitnom pole ul'travysokih chastot. [Physical and chemical processes in the electromagnetic field of ultrahigh frequencies], Barnaul: Izd-vo Alt. un-ta – Barnaul: Publishing house Alt. University press. 101 [In Russian].
11. Chirkova V. Yu., Sharlaeva E. A. & Stas I. E. (2019). Izmenenie kogezionnyh I adgezionnyh harakteristik vody kak rezul'tatel ektromagnitnogo vozdejstviya. [Change of cohesive and adhesive characteristics of water as a result of electromagnetic influence] Izvestiya vuzov. Prikladnaya himiya i biotekhnologiya – *News of universities. Applied chemistry and biotechnology*. 9(2), 222–231 . [InRussian].
12. Chirkova V.Yu., Sharlayeva Ye. A. & Stas I.Ye. (2019). Boiling temperature and the enthalpy of water vaporization exposed to high frequency electromagnetic field. *Bulletin of the university of Karaganda. Chemistry*. 94(2), 51-55.
13. SHipunov B.P. & Ryabyh A.V. (2019). Vliyanie vysokochastotnogo elektromagnitnogo polya na mutarotaciyu vodnyh rastvorov glyukozy i fruktozy [Influence of high-frequency electromagnetic field on mutarotation of aqueous solutions of glucose and fructose] *Himiya rastitel'nogo syr'ya – Chemistry of plant raw materials*.3. 335-240. [InRussian].
14. Shipunov B. P. & Ryabykh A. V. (2020). Change in the heat of D-glucose dissolution in water exposed to electromagnetic field. *Bulletin of the Karaganda University. Chemistry Series*.1(97), 83-89.
15. SHipunov B.P. & Markin V.I. (2020). Anomal'nay areologiya rastvorov agar-agara, prigotovlennyh s ispol'zovaniem vody podvergshejsya vozdejstviyu elektromagnitnogo polya [Abnormal rheology of agar-agar solutions prepared using water exposed to an electromagnetic field] *Himiya rastitel 'nogo syr'ya – Chemistry of plantraw.*1. 73–80. [InRussian].
16. Stas I. E., Chirkova V. Yu. & Minin M. I (2016). Vyazkost' rastvorov zhelatina, prigotovlennyh na obluchennoj elektromagnitnym polem vode. [Viscosity of gelatin solutions prepare don water irradiated by an electromagnetic field] *Vestnik Voronezhskogo gosudarstvennogo universiteta. Himiya. Biologiya. Farmaciya – Bulletin of the Voronezh State University. Chemistry. Biology. Pharmacy.* 2.32-36[InRussian].
17. Kireev V.V. (2013). Vysokomolekulyarnyesoe dineniya [High molecular weight compounds] M.:Yurite, [InRussian].
18. Tager, A. A. (2007). Fiziko-khimiiapolimerov [Physical Chemistry of Polymers]. M.: Nauchnyimir. [In Russian].
19. Stas' I. E. (2006). Dispersnyesistemy v prirode i tekhnike: Uchebnoeposobie. [Disperse systems in nature and technology: textbook.] Barnaul: Izd-vo Alt. Un-ta. [InRussian].
20. Stas' I. E. (2020). Vliyanieobrabotkivodyvysoko chastotnymelektromagnitnympolemnaintegral'nuyut eplotusmachivaniyaporoshkoobraznogooksidaalyu miniya [Influence of water treatment by a high-frequency electromagnetic field on the integral heat of wetting of powdered aluminum oxide], *Polzunovskijvestnik* – Polzunovsky Bulletin.2.124-128. [In Russian].
21. Voyuckij S.S. (1976). Kurskolloidnojhimii [Course of colloidal chemistry]. 2-e izd-e. M.: Himiya, [In Russian].

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Evaluation of Invitro Anti-Inflammatory Potential of Aqueous *Solanum Aethiopicum* (Garden Egg) Leaf Extract

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Abstract- Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. This study is aimed at evaluating the invitro anti-inflammatory potential of aqueous leaves extract of Solanum aethiopicum (Family: Solanaceae). In vitro anti-inflammatory potentials were evaluated using standard experimental protocols such as Inhibition of albumin denaturation, Anti-lipoxygenase activity, Membrane stabilization and Proteinase inhibitory action at different concentrations with aspirin and diclofenac used as the standard drug. Aqueous extract of S.aethiopicum leaves were tested for the presence of alkaloid, flavonoid, tannin, glycoside, steroids, phenol and terpenoids. The total phenol and flavonoid content were evaluated as well. The total phenol and flavonoid content were found to be (132±0.13 mg of Gallic acid/g of equivalent) and (146±1.12mg of Quercetin/g of equivalent).The IC_{50} values of the extract, diclofenac and aspirin (standard drug) in inhibition of albumin denaturation were 50.20 μg/ml and 31.54μg/ml; in Anti-lipoxygenase activity were 199μg/ml and 28.2μg/ml; in Membrane stabilization were 9.36μg/ml and 19.85μg/ml and Protein Denaturation 714μg/ml and 23.5 μg/ml.

Keywords: inflammation, solanum aethiopicum, antilipoxygenase, membrane stabilization, albumin denaturation.

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Evaluation of Invitro Anti-Inflammatory Potential of Aqueous *Solanum Aethiopicum* (Garden Egg) Leaf Extract

Olasunkanmi A. A. [α] & Afuye O. O. [σ]

Abstract- Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. This study is aimed at evaluating the invitro anti-inflammatory potential of aqueous leaves extract of Solanum aethiopicum (Family: Solanaceae). In vitro anti-inflammatory potentials were evaluated using standard experimental protocols such as Inhibition of albumin denaturation, Anti-lipoxygenase activity, Membrane stabilization and Proteinase inhibitory action at different concentrations with aspirin and diclofenac used as the standard drug. Aqueous extract of S.aethiopicum leaves were tested for the presence of alkaloid, flavonoid, tannin, glycoside, steroids, phenol and terpenoids. The total phenol and flavonoid content were evaluated as well. The total phenol and flavonoid content were found to be (132±0.13 mg of Gallic acid/g of equivalent) and (146±1.12mg of Quercetin/g of equivalent).The IC_{50} values of the extract, diclofenac and aspirin (standard drug) in inhibition of albumin denaturation were 50.20 µg/ml and 31.54µg/ml; in Anti-lipoxygenase activity were 199µg/ml and 28.2µg/ml; in Membrane stabilization were 9.36µg/ml and 19.85µg/ml and Protein Denaturation 714µg/ml and 23.5 µg/ml. The Inhibition of membrane stabilization might be the possible mechanism by which the extract elicits its anti-inflammatory effect. The results obtained in the present study indicate that the aqueous extract of Solanum aethiopicum is a potent source of anti-inflammatory agents and this justified its uses in the treatment of various infections.

Keywords: inflammation, solanum aethiopicum, antilipoxy genase, membrane stabilization, albumin denaturation.

I. Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain (Palladino et al.,2003). Inflammation is either acute or chronic inflammation. Acute inflammation, with exudation of fluid and plasma proteins as its main features, occurs very rapidly, and the process can last for few or several minutes to several days. Chronic inflammation occurs when the acute inflammatory process occurs repeatedly or continuously, with the process lasting for several weeks to months and even years (Paramita et al., 2017). The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. It is metabolized by the Cyclooxygenase (COX) pathway to prostaglandins and thromboxane A2, whereas the 5-lipoxygenase (5-LOX) pathway to eicosanoids and leukotrienes (LT's), which are known to act as chemical mediators in a variety of inflammatory events (Anoop and Bindu. 2015).Currently available anti- inflammatory drugs block both enzyme activities and relief symptoms but they have serious side effects (Verma, 2016).Therefore it is essential to administer anti-inflammatory drugs with lesser side effects.

Medicinal plants have the ability to synthesize a wide variety of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used to effectively treat the various ailments for mankind. Plants have a great potential for producing new drugs and are used in traditional medicine to treat chronic and even infectious diseases. In the present review an attempt has been made to investigate the anti-inflammatory activity of some medicinal plants (Panda et al., 2009).

Garden egg (*S. aethiopicum*) also known as African eggplant, Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family *Solanaceae.* The genus *Solanum* includes both the edible and non-edible species. The family is one of the largest and most important families of vegetables grown for their edible fruits (Prohens, 2005).They are native to sub-Saharan Africa and are essentially tropical in origin. *S. aethiopicum* is of high edible quality. The fruits can be eaten fresh without cooking and have a long history of consumption in West Africa (Schippers, 2000). Report has shown that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcers in rats. They are used to treat colic; severe pain resulting in periodic spasm in an abdominal organ and blood pressure (Grubben and Denton.2004).Other reports on the pharmacological activity of the plant show that it has purgative(Saba et al., 2003), sedative and anti-diabetic effects (Ezeugwu et al., 2004), but none have reported its anti-inflammatory activity.

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II. Materials and Methods

a) Collection and authentication of plant Leaves of S. aethiopicum

Leaves of *S. aethiopicum* were purchased from Owode market in Ogun state, Southwest Nigeria. The leaves were identified and authenticated in the Department of Botany of University of Lagos, Lagos state by a botanist, Mr. G.I Nodza. With Voucher specimens No 8381 were deposited at the herbarium of Federal Polytechnic Ilaro,Ogun state.

b) Preparation of leave extract

The leaves were selected and thoroughly washed in water to remove dirt and unwanted particles. It was air dried at room temperature for a month and reduced to coarse powder by grinding with a grinder. 200g of the sample was measured and macerated into 2000litres of distilled water and was transferred into a standard flask. It was shaken thoroughly and kept in fume cupboard for five days. The sample solution was filtered with a Whitman No 1.filter paper. The filtrate was freeze dried prior to analysis.

i. *Preparation of Extracts*

Into a conical flask 2gm of the crude extract and 50 ml of solvent (distil water) was added. The flask was labeled and allowed to stand for 1hr, filtered using Watmann No.1 filter paper. Phytochemical analysis extract was carried out using the method described by Odebiyi and Sofowora (1978)

ii. *Test for Phenol*

To 1cm³ of the extracts 2 drops of 5% $FeCl_3$ was added in a test tube. A greenish precipitate indicates the presence of phenol.

iii. *Test for Flavonoid*

To 3cm³ of the extract, 1cm³ of 10% NaOH was added. A yellow colouration indicates the presence of flavonoids.

iv. *Test for Sterols*

Into a test tube 5 drops of concentrated H_2SO_4 was added to 1cm³ of the extracts. Red colouration indicates the presence of steroids

v. *Test of Alkaloids*

In a test tube1cm³ of 1%HCl was added to 3cm³ of the extracts. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in the following tests: 2 drops of Wagner's reagent was added to 1cm³ of the extracts. A reddish brown precipitate indicates the presence of alkaloids

vi. *Test for Glycosides*

To 1cm³ of the extracts10cm³ of 50% H_2SO_4 was added, the mixture was heated in boiling water for 15 minutes. 10cm³ of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.

vi. Test for Tannins

1cm³ of freshly prepared 10% KOH was added to 1cm³of the extracts. A dirty white precipitate indicates the presence of tannins.

vii. *Test for Saponins*

Frothing test: 2cm³ of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.

viii. *Test for Terpenoids*

Into a test tube, 5ml of extract of the sample is mixed with 2ml of CHCl3 in a test tube, 3ml of con. H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

c) Total Phenol and Total Flavonoid Content

i. *Estimation of Total Phenolic Content*

The total phenolic content of sample was estimated according to the method of Makkar et al. (1997). The aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 μg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

ii. *Estimation of Total Flavonoid Content*

Total flavonoid content was measured by aluminium chloride colorimetric assay. 1ml of extracts or standard solution of Quercetin (500μg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO2 was added. After 5 minutes, 0.3 ml of 10% AlCl3 was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of the flower was expressed as percentage of Quarcetin equivalent per 100 g of fresh mass.

d) Invitro Anti Inflammatory Assay

The plant extract was subjected to preliminary assessments using standard procedures to detect its anti-inflammatory activities.

i. *Preparation of Extract*

5mg of the plant extract was dissolved in distilled water to produce a solution of concentration 5mg/ml. The following assays were done according to

the method of Tappel (1962) with slight modifications (Wallace and Wheeler, 1975).

ii. *Antilipoxygenase Activity*

A total volume of 200 µl assay mixture contained, 160 µl sodium phosphate buffer (100 mM, pH 8.0), 10 µl test extract (50 to 250 µg extracted material in 100 mM Tris buffer pH 7.4) and 20 µl lipoxygenase enzyme. The contents were preincubated for 10 min at 25°C. The reaction was initiated by the addition of 10 µl linoleic acid solution as substrate. The change in absorbance was observed after 6 min at 234 nm. All reactions were performed in triplicates in 96-well microplate reader Spectra Max 190 (Molecular Devices, USA). The positive and negative controls were included in the assay. The percentage inhibition (%) was calculated by the following formula:

$$\text{Inhibition (\%)} = [(\text{Abs of control} - \text{Abs of test sample})/\text{Abs of control}] \times 100$$

iii. *Protein Inhibitory Action*

The test will be performed according to the method of Oyedepo et al. (1995) and Sakat et al. (2010) with modifications. The reaction mixture (1.5ml) containing 0.06mg trypsin, 0.5ml of 20mM TrisHCl buffer (pH7.4) and 0.5ml test sample of different concentrations of different solvents. The reaction mixture will be incubated at 37^0C for 5min and then 0.3ml of 1.5% (W/V) casein will be added. The mixture will be incubated for an additional 20 min, 0.2ml of 70% perchloric acid will be added to terminate the reaction. Cloudy suspension will be centrifuged and the absorbance of the supernatant read at 210nm against buffer as blank. The experiment will be performed in triplicate. The percentage of inhibition of proteinase inhibitory activity will be calculated;

The % inhibition of the protein denaturation will be calculated by

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

e) Membrane Stabilization Activity

i. *Preparation of Red Blood Cells (RBCs)*

Suspension Fresh whole mammalian blood (10ml) was collected and transferred to heparinzed centrifuge tubes. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10%v/v suspension with normal saline.

ii. *Heat Induced haemolysis*

The 2ml reaction mixture is consisted of 1ml of test extract at various concentrations and 1ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 560C for 30min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicate. % of membrane stabilization activity was calculated by the formula mentioned below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

iii. *Inhibition of Albumin Denaturation*

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37 °C in incubator for about 15mins and then heated at 70 °C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

f) Statistical Analysis

The experimental data were expressed as mean ±SEM. The difference between the control and extract were compared using one-way analysis of variance (ANOVA) followed by Duncan (control vs test) using the SPSS software version 20.0. $P<0.05$ was considered statistically significant. The IC_{50} value was calculated using Microsoft Excel version 2016.

III. Results

Table 1: Qualitative phytochemical screening of *Solanum aethiopicum*

Phytochemical test	*S. aethiopicum* leave extract
Flavonoids	+
Steroids	+
Tannins	–
Alkaloids	+
Glycosides	+
Saponins	+
Terpenoids	+

Key= + (present) - (absent)

The result of qualitative phytochemical screening of *S.aethiopicum*is presented in the table above. The leaves of *S.aethiopicum* were found to contain phenol, flavonoid, steroid and glycoside while tannin was found absent.

a) Total Phenolic and Total Flavonoid Content

Plant Material	Total Phenolic Content	Total Flavonoid Content
S. aethiopicum	132 ± 0.13	146 ± 1.12

The total phenolicand flavonoid contents of the leaf extract were expressed as mg of garlic acid (GAE)/g of extract. The total phenolic content in Aqueous *S.aethiopicum* leaves extract was 132± 0.13 GAE/g of the extract and total flavonoid content was 146±1.12GAE/g of extract.

b) Invitro Anti-Inflammatory Activity

i. *Anti-lipoxygenase activity*

Table 2: Effect of aqueous *S.aethiopicum* leaves extract on anti-lipoxygenase

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	19.6172^{a} ±0.419	82.75629^{c} ±0.450
40	18.48356^{a} ± 0.432	67.40437^{c} ±0.348
30	16.15015^{a} ±0.168	49.72505^{b} ±0.120
20	15.37209^{a} ± 0.408	36.35356^{b} ±0.024
10	11.17095^{a} ±0.01	26.49731^{b} ±0.418
IC_{50}	199	28.2

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Diclofenac). $p < 0.05$

Anti-lipoxygenase activity of the aqueous leaf extract of *S.aethiopicum* at a concentration of 50µg/ml demonstrated a stronger lipoxygenase inhibition of 19.6172±0.05% with an IC_{50} value of 199µg/ml. The standard drug (diclofenac) also demonstrated the highest lipoxygenase inhibition with an IC50 value of 28.2µg/ml when compared to leaf extract.

ii. *Membrane stabilization activity*

Table 3: Effect of aqueous *S.aethiopicum* leaves extract on membrane stabilization

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	69.127315^{b}±0.269	82.51644^{c}±0.269
40	63.47878^{a} ±0.	72.38494^{b} ±0.179
30	62.044235^{a} ±0.956	69.33652^{a} ±0.06
20	58.93604^{a} ±0.	63.269575^{a} ±0.69
10	58.87059^{a} ±0.006	61.835025^{a} ±1.225
IC_{50}	19.85	9.35

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p < 0.05$.

The plant extract and standard drug indicated that the high percentage erythrocyte stabilization was observed at a concentration 50µg/ml with a percentage inhibition of 82.5±0.269 also with an IC_{50} value of 9.35µg/ml compared to leaf extract 19.85µg/ml.

iii. *Inhibition of Albumin denaturation*

Table 4: Effect of aqueous *S.aethiopicum* leaves extract on inhibition of albumin denaturation

Concentration (μg/ml)	Percentage inhibition	
	Sample	Standard
50	55.826495[a] ±0.680	66.87573[b] ±0.451
40	35.410315[b] ±2.691	54.790155[c] ±0.124
30	45.720985[a] ±1.641	49.231535[a] ±0.228
20	34.58382[b] ±3.165	44.97304[c] ±0.307
10	34.46659[a] ±7.386	27.239155[a] ±2.421
IC_{50}	50.2	31.5

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$

The result of inhibition of albumin denaturation indicated that the IC_{50} values of the standard were significantly higher than that of the extract which are 50.2μg/ml and 31.5μg/ml respectively. The standard drug significantly inhibited the activity of Albumin denaturation. However, it was discovered that at a concentration of 50μg/ml, the standard drug, aspirin, exhibited the highest percentage inhibition of Albumin denaturation at 66.86± 0.451.

iv. *Proteinase inhibitory action*

Table 5: Effect of aqueous *S.aethiopicum* leaves extract on inhibition on proteinase inhibitory action

Concentration (μg/ml)	Percentage inhibition	
	Sample	Standard
50	22.52099[a] ±0.283	65.49651[c] ±0.112
40	50.36014[b]±0.409	59.22728[c] ±0.213
30	50.22378[a] ±0.014	52.0979[a]±0.350
20	38.98602[a] ±0.385	50.3989[b] ±0.091
10	27.32168[a] ±0.091	41.34965[b] ±0.301
IC_{50}	714	23.5

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$.

From the result of proteinase inhibitory Action, the standard drug (aspirin) exhibited a higher percentage inhibition of 65.496± 0.112 at a concentration of 50μg/ml with an IC_{50} value of 23.4μg/ml compared to that of the extract 714μg/ml. It was also observed that the standard shows a higher proteinase inhibition.

c) *Invitro Anti-Inflammatory Activity*

i. *Anti-lipoxygenase activity*

Table 2: Effect of aqueous *S.aethiopicum* leaves extract on anti-lipoxygenase

Concentration (μg/ml)	Percentage inhibition	
	Sample	Standard
50	19.6172[a] ±0.419	82.75629[c] ±0.450
40	18.48356[a] ± 0.432	67.40437[c] ±0.348
30	16.15015[a] ±0.168	49.72505[b] ±0.120
20	15.37209[a] ± 0.408	36.35356[b] ±0.024
10	11.17095[a] ±0.01	26.49731[b] ±0.418
IC_{50}	199	28.2

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Diclofenac). $p< 0.05$

ii. *Membrane stabilization activity*

Table 3: Effect of aqueous *S.aethiopicum* leaves extract on membrane stabilization

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	69.127315[b]±0.269	82.51644[c]±0.269
40	63.47878[a] ±0.897	72.38494[b] ±0.179
30	62.044235[a] ±0.956	69.33652[a] ±0.06
20	58.93604[a] ±0.598	63.269575[a] ±0.69
10	58.87059[a] ±0.006	61.835025[a] ±1.225
IC_{50}	19.85	9.35

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$

iii. *Inhibition of Albumin denaturation*

Table 4: Effect of aqueous *S.aethiopicum* leaves extract on inhibition of albumin denaturation

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	55.826495[a] ±0.680	66.87573[b] ±0.451
40	35.410315[b] ±2.691	54.790155[c] ±0.124
30	45.720985[a] ±1.641	49.231535[a] ±0.228
20	34.58382[b] ±3.165	44.97304[c] ±0.307
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IC_{50}	50.2	31.5

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$

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IC_{50}	714	23.5

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$.

IV. Discussion

Plant based drugs used in the practice of traditional treatment of diseases including inflammation have become the focus of current research because they are cheap and have great therapeutic potential without much of the side effects associated with synthetic drugs (Okwu and Uchenna. 2009). The present study evaluated the anti-inflammatory activity of the aqueous extract of *S. aethiopicum and* showed its effectiveness in reducing inflammation in in-vitro inflammatory models. The analysis of the phytochemical constituents of plants aids the screening of their biological activities and has great interest in pharmaceutical companies for the production of new drugs.

The phytochemicals are the plants' secondary metabolites that help the plant to combat competitors, predators or pathogens (Kennedy, 2011).The previous phytochemical studies showed that leaves of *S.aethiopicum* are rich on flavonoids especially Quercetin. Quercetin relaxes intestinal smooth muscle and inhibits the bowel contraction leading to anti-diarrheal effect (Joseph, 2011) and reduces the capillary permeability in the abdominal cavity which promotes medicinal applications *of S.aethiopicum* leaves (Choudhury, 2012). Further, Quercetin will contribute to anti-inflammatory activity (Metwally et al., 2010). Other than that, flavonoids have biological activities such as anti-oxidant, anti-apoptotic, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerosis, cardiovascular protection and improvement of

endothelial function, inhibition for angiogenesis and cell proliferation activities (Rahman et al., 2015). Phenolic compounds contribute to analgesic, anti-inflammatory, anti-microbial, hepato-protective and antioxidant activities (Choudhu, 2012). Moreover, phenols such as catechin and epichatechin decrease the cholesterol level prevent type 2 diabetes and act as anti-oxidants (Biswas et al*.,* 2013). The presence of steroids increases the pharmaceutical value of garden egg leaves in such a way that the steroids increase the protein synthesis and thus promotes the growth of muscle and bones (Offor, 2015). Further, Glycosides such as Saponins can reduce the cholesterol levels in the body (Barbalho et al*.,* 2012). Phytochemical screening of the plants revealed that the fruits contained alkaloids, flavonoids, sterols, saponins, cardiac glycosides. Further it has been reported that flavonoids and saponins exerted profound stabilizing effect on lysosomal membrane both in vitro and in vivo while tannins and saponins possess ability to bind cations there by stabilizing erythrocyte membranes and other macromolecules (Anilkumar and Johny. 2015).

The denaturation of proteins is one of the causes of inflammation. In certain rheumatic diseases, the production ofauto-antigens may be due to denaturation of proteins (Zhao et al*.,* 2008). Anti-inflammatory drugs are known to inhibit the denaturation of proteins (Chatterjee et al*.,* 2012). Non-steroidal anti-inflammatory drugs are the major pharmacological agents used for the anti-inflammatory and pain-relief management due to their capacity in inhibiting protein denaturation (Saso et al*.,* 2001). Denaturation of the protein involves the disruption of secondary, tertiary and quaternary structure of the molecules and finally leads to cell death. It occurs due to stress such as high level of salt, high temperature and high level of acidity. From the findings, there was significant ($p<0.05$) inhibition of protein denaturation in standard at concentration of 50μg/ml showed better activity than extract. From the results of the present study it can be stated that *S.aethiopicum* may control the production of auto-antigens by preventing in-vitro denaturation of proteins in rheumatic diseases and the IC_{50} value obtained at the inhibition of protein denaturation, indicated that the standard has higher inhibitory effect than the aqueous extract. This result is supported by the findings of Mizushina and Kobayashi (1986) on the in vitro anti-inflammatory potential of pharmacophores.

Stabilizing effect of heat and saline induced erythrocyte lysis is a very good index of anti-inflammatory activity. The membrane of RBC is similar to that of lysosomal membrane. In inflammatory condition, stabilizing the lysosomal membrane helps to prevent the release of lysosomal constituents (Vallabh et al., 2009) of activated neutrophil such as proteases and bactericidal enzymes which cause further tissue inflammation and damage upon extra cellular release. In the study there was significant ($p<0.05$) membrane stabilization effect of both standard and extract and percentage inhibition of membrane stabilization produced by standard concentrations50μg/ml showed better activity than extract with an IC_{50} of 9.35 and 19.85.Activities of flavonoids and alkaloids have also been reported to modulate cellular activities of inflammatory related cells by stabilizing their membranes, thus preventing de-gradation and therefore impairing lysosomal enzyme release of arachidonic acid, elactase and glucoronidase (Kanashiro et al*.,* 2007).

Lipoxygenases (LOXs) are a family of non-heme iron-containing enzymes that have been implicated in the metabolism of arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites which are known mediators of inflammatory and immune response. The aqueous extract of the leaves showed a lesser Anti-lipo-oxygenase ability when compared with the standard drug diclofenac. The IC_{50} of anti-lipoxygenase of the extract *S. aethiopicum* was found to be 199 and the result of standard diclofenac 28.2 which showed that the standard drug inhibit compared to extract. The result of this study revealed that Inhibition of Anti-Lipoxygenase enzyme was concentration dependent and also suggest that *S. aethiopicum* has a significant anti-inflammatory activity.

V. Conclusion

From this study, results indicate that the aqueous extract of S. aethiopicum leaves have high potent anti-inflammatory activities. The extract was able to reduce the activities of stabilizing membrane of erythrocyte. These activities may be due to the strong occurrence of secondary metabolite such as alkaloids, flavonoids, terpenoids, steroids and phenols.

References Références Referencias

1. Anilkumar, M., Johny, J. (2015). Evaluation of in vitro anti-inflammatory activity of the methanolic extract of Litse aquinque flora (Dennst) Suresh. *Journal of Pharmacy and Biological Sciences*.; 10(2):32-36.
2. Anoop, M.V, Bindu, A.R.(2015). *International Journal of pharma research & review* 4(8), 18-27.
3. Barbalho, S.M., Farinazzi, M.V., Goulart, R.A., Brunnati, A.S., Ottoboni, A.M, Nicolau, C.C. (2012). P. guajava (Guava): A plant multipurpose medicinal-applications. *Medicinal and Aromatic Plants*.; 1(2):1-4.
4. Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., Yadav, A. (2013). Antimicrobial activities of leaf extract of Guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. *International Journal of Microbiology*., 1-7.
5. Chatterjee, P., Chanda, S., Dev, P., Bhattacharya, S. (2012). Evaluation of anti-inflammatory effects of

green tea and black tea: a comparative in vitro study. *Journal of Advanced Pharmaceutical Technology and Research.*; 3(2):136-138.
6. Choudhury, S., Sharan, L., Sinha, M.P. (2012). Phytochemical and antimicrobial screening of P. guajava L. Leaf extracts against clinically important gastrointestinal pathogens. *Journal of Natural Product Plant Resources*.; 2(4):524-529.
7. Ezeugwu, C.O., Okonta, J.M., Nwodo, N.J.(2004). Antidiabetic properties of ethanolic fruit extract of Solanum aethiopicum L. *Res J Pharmaceut Allied Sci*; 2(2): 251-254.
8. Grubben, G.J., Denton, O.A. (2004).Plant resources of tropical Africa 2: vegetables. *Wageningen: PROTA Publication*, 253-254
9. Joseph, B., Minipriya, R. (2011). Review on nutritional, medicinal and pharmacological properties of Guava (P. guajavaLinn). *International Journal of Pharmacy and Biological Sciences*.; 2(1):53-69.
10. Kanashiro, A.,Souza, J.G., Kabeya, L.M., Azzolini, A.E., LucisanoValim, Y.M.(2007). Stimulet
11. neutrophils inhibited by flavonoids: Importance of the catechol group.*Natureforsch*.;62:357–361.
12. Kennedy, Wightman. (2011). Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and
13. the Enhancement of Human Brain Function1. *Advance in Nutrition*.; 2:32-50.
14. Makkar H. P. S., and Becker, K. (1997). Nutrients and anti-quality factors in different morphological parts of *Moringa oleifera* tree. J.Ag. Sci.:128,pp.311-322.
15. Mizushima Y. and Kobayashi M (1968); Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. *J of Pharma Pharmacol* 20:169- 173.
16. Odebiyi, A., sofowora, E. A (1978). phytochemical screening of Nigerian medicinal plant. Part II, Liyodia. Vol. 403, 1978, pp. 234-246.
17. Offor, C.E. (2015). Phyto-chemical and proximate analysis of P. guajava leaves. *Journal of Research in Pharmaceutical Science*.; 2(6):5-7.
18. Okwu D.E, Uchenna N.F. (2009). "Exotic multifaceted medicinal plants of drugs and pharmaceutical industries." *African Journal of Biotechnology*; vol 8(25):7271-7282.
19. Oyedepo, O.O, Femurewa, A.J. (1995). "Anti-Protease And Membrane Stabilizing Activities Of Extracts Of *Fagraz anthoxiloides, Olax subscorpioides* And *Tetrapleura tetraptera"*. *International Journal of Pharmacology*; 33:65-69.
20. Palladino, M.A., Bahjat, F.R., Theodorakis, E.A., Moldawer, L.L.(2003). Anti-TNF-α therapies: the next generation. *Nat Rev Drug Discovery*; 2: 736-746.
21. Paramita, S., Kosala, K., Dzulkifli, D., Saputri, D.I., Wijayanti, E.(2017). Anti-inflammatory activities of ethnomedicinal plants from Dayak Abai in North Kalimantan, Indonesia. *Biodiversitas*; 18(4): 1556-1561.
22. Panda, S.K., Thatoi, H.N., Dutta, S.K.(2009). Antibacterial activity and phytochemical screening of leaf and bark extracts of Vitex negundo from Similipal biosphere reserve Orissa. *J Med Plant Res*; 3(4):294-300.
23. Prohens, J., Blanca, J.M., Neuz, F.(2005). Morphological and molecular-variation in a collection of eggplant from a secondary centre of diversity: implications for conservation and breeding. *J Am Soc Horticul Sci*; 130: 54-63.
24. Rahman, H., Eswaraiah, M.C., Dutta, A.M.(2015). In-vitro anti-inflammatory and anti-arthritic activity of Oriza sativaVar. Joha Rice (An Aromatic Indigenous Rice of Assam).*American-Eurasian Journal of Agricultural and Environmental Sciences*.; 15(1): 115-121.
25. Saba A.B, Dina O.A, Adedapo A.A, Akhiromen I. O (2003). Effect of aqueous leaf extract of Solanum aethiopicum on isolated guinea pig ileum. African Journal of Biomedical research.
26. Sakat S, Juvekar AR, Gambhire MN. (2010). In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharma and Pharmacological Sciences 2(1):146-155.
27. Saso, .L, Valentini, G., Casini, M.L., Grippa, E., Gatto, M.T., Silvestrini, B.(2001). Inhibition of heat-induced denaturation of non-steroidal anti-inflammatry drugs (NSAIDs): pharmacological implications. *Archives of Pharmacal Research*.; 24(2):150-158.
28. Schippers, R.R. (2000). African indigenous vegetables: an overview of the cultivated species. London: Natural Resources Institute/Africa Caribbean and Pacific-European Union Technical Centre for Agricultural and Rural Cooperation: 214.
29. Vallabh-deshpande, Varsha-Jadav, M., Kadam, V. (2009). In-vitro anti-arthritic activity of Abuti lamindicum (Linn.) sweet. *J Pharm Res*.; 2(4): 644-645.
30. Verma S (2016). Medicinal plants with anti-inflammatory activity. J Phytopharmacol; 5(4): 157-159.
31. Wallace J.M, Wheeler E.L (1975). Lipoxygenase from wheat an examination of its reaction characteristics. *Journal of Agriculture and Food Chemistry*; 23:146-150.
32. Zhao, H.L., Harding, S.V., Marinangeli, CP., Kim, Y.S., Jones, P.J. (2008). Hypo-cholesterolemic and anti-obesity effects of saponins from Platycodon-grandiflorum in hamsters fed atherogenic diets. *Journal of Food Science.*; 73(8):195-200.

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Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

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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