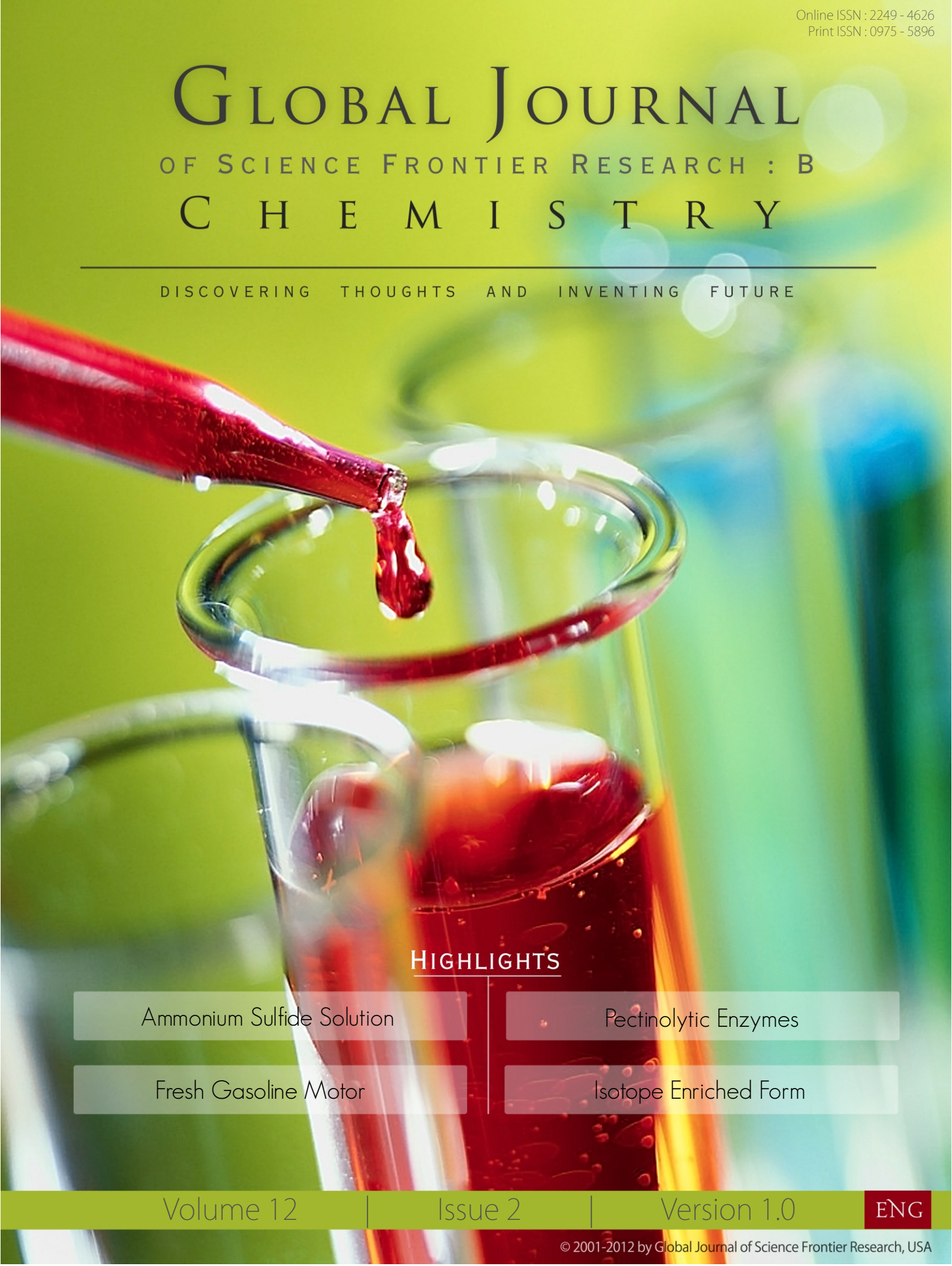
Online ISSN : 2249 - 4626
Print ISSN : 0975 - 5896

GLOBAL JOURNAL
OF SCIENCE FRONTIER RESEARCH : B
CHEMISTRY

DISCOVERING THOUGHTS AND INVENTING FUTURE



HIGHLIGHTS

- Ammonium Sulfide Solution
- Pectinolytic Enzymes
- Fresh Gasoline Motor
- Isotope Enriched Form

Volume 12 | Issue 2 | Version 1.0 ENG

Global Journal of Science Frontier Research: B
Chemistry

Global Journal of Science Frontier Research: B
Chemistry
Volume 12 Issue 2 (Ver. 1.0)

Open Association of Research Society

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

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals Inc., Headquarters Corporate Office,
Cambridge Office Center, II Canal Park, Floor No. 5th, ***Cambridge (Massachusetts)***, Pin: MA 02141
United States
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Open Association of Research Society, Marsh Road, Rainham, Essex, London RM13 8EU
United Kingdom.

Packaging & Continental Dispatching

Global Journals, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investers@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Editorial Board Members (HON.)

Dr. Bart Lambrecht
Director of Research in Accounting and FinanceProfessor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of ReginaPh.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and FinanceLancaster University Management SchoolPh.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research
Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical NeuroscienceNorthwestern University
Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh

Contents of the Volume

Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Electrochemical Behaviour of a Copper Electrode in Ammonium Sulfide Solution

By I. Zaafarany & Herbert Boller

Umm Al-Qura University, Makkah Al-Mukaramha,Saudia Aabia

Abstract - The electrochemical behaviour of a copper electrode in ammonium sulphide solutions was studied using cyclic voltammogrammetry and potentiostatic and galvanostatic polarization techniques. The morphology and composition of the layers formed on the copper electrode were studied by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) and X-ray powder diffractometry. Besides different binary copper sulphide phases the ternary phases $NH_4Cu_{7-x}S_4$ and NH_4CuS_4 were observed, $NH_4Cu_{7-x}S_4$ crystallizing as needles perpendicular to the electrode surface.

Keywords : Electrochemistry, copper, ammonium sulphide, copper sulphides, ammonium thiocuprates.

GJSFR-B Classification: FOR Code: 030604,030304

Strictly as per the compliance and regulations of:

Electrochemical Behaviour of a Copper Electrode in Ammonium Sulfide Solution

I. Zaafarany [α] & Herbert Boller [σ]

Abstract - The electrochemical behaviour of a copper electrode in ammonium sulphide solutions was studied using cyclic voltammogrammetry and potentiostatic and galvanostatic polarization techniques. The morphology and composition of the layers formed on the copper electrode were studied by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) and X-ray powder diffractometry. Besides different binary copper sulphide phases the ternary phases $NH_4Cu_{7-x}S_4$ and NH_4CuS_4 were observed, $NH_4Cu_{7-x}S_4$ crystallizing as needles perpendicular to the electrode surface.

Keywords : Electrochemistry, copper, ammonium sulphide, copper sulphides, ammonium thiocuprates.

I. Introduction

Copper combines corrosion resistance with high electrical and heat conductivity, formability, machinability and strength. It has good resistance to urban, marine and industrial atmosphere and water. Being a noble metal it is not corroded by acids unless oxygen or another oxidizing agent is present. Copper and its alloys are also resistant to slightly alkaline solutions. Several authors studied the electrochemical behaviour of copper in alkaline solutions [1-5]. The presence of sulphide ions, however, enhances its corrosion. When soluble sulphides are present in potable water or sea water, a thick black, poorly adherent scale forms on a copper or brass surface [6]. This scale is composed mainly of Cu_2S although copper deficient $Cu_{1-x}S$, CuS and Cu_2O have also been reported [7].There is a lack of studies of the electrochemical behaviour of copper in ammonium sulphide solutions.

There are several binary copper sulphides also occurring as minerals: Cu_2S (Chalcocite), $Cu_{1.97}S$ (Djurleite), $Cu_{1.8}S$ (Digenite), $Cu_{1.74–1.82}S$ (Roxbyite), $Cu_{1.4}S$ (Anilite) and CuS (Covellite) [8].

Two ammonium thiocuprates $NH_4Cu_7S_4$ [9] and $NH_4Cu_4S_3$ [10] and one polysulphide NH_4CuS_4 [11] have been reported and the formation of $NH_4Cu_{7-x}S_4$ and of the new compound $NH_4Cu_4S_3$ by reaction of copper with yellow ammonium sulphide solutions has been studied [10].

The present work aims to study the electrochemical behaviour of a copper electrode in ammonium sulphide solutions using cyclic voltammograms, potentiostatic and galvanostatic polarization techniques with special emphasis on the morphology, chemical and phase composition of the layers and scales formed.

II. Experimental

Cyclic voltammograms (CVs), potentiostatic and galvanostatic polarization techniques were performed with the computer-controlled electrochemical measurement system Autolab (ECO Chemie) combined with the software package GPES (General Purpose Electrochemical System). A standard electrochemical cell with five holes - three holes for the electrodes and two for nitrogen inlet and outlet - was used. A commercial Ag/AgCl electrode was used as reference electrode. The counter electrode was a platinum wire. The working copper electrode was prepared from a high purity (99.98%) copper rod. A small piece of copper rod - diameter 6 mm, length 6 mm - was placed in a "Kel-F" shield, secured by epoxy resin. The bottom electrode was screwed onto a polyethylene holder with a contact wire in order to obtain good electrical contact. The electrodes were successively abraded with finest grade emery paper and degreased with acetone. Complete wetting of the surface was taken as indication of its cleanliness when rinsed with bi-distilled water. All chemicals used were of A.R. quality. The solutions were prepared using bi-distilled water; no attempts were made to de-aerate them. Most experiments were done in relatively concentrated 2.9M ($p_H \approx 9$) ammonium sulphide solutions.

Cyclovoltammetric sweeping was generally between -2.0V (hydrogen evolution) to +1.0 V (oxygen evolution). All measurements were taken at 25 ±1 °C.

The surface of the electrode and the scales formed at the electrode were investigated by scanning electron microscopy (SEM) and energy dispersive X-ray elemental analysis (EDAX). Guinier powder photographs were used for the crystallographic phase analysis of the products.

III. Results And Discussion

a) Cyclic Voltammogram Curves

Figure 1 represents the cyclic voltammogram of a Cu electrode in 2.9M ammonium sulphide solutions between -2.0V and 1.0V at a voltage scan rate 50mV sec^{-1}. Inspection of this figure reveals that there are three

Author α : Chem. Dept, Faculty of Appl. Sci, Umm Al-Qura University, Makkah Al-Mukaramha,Saudia Aabia.
E-mail : ishaq_zaafarany@yahoo.com
Author σ : Institut für Anorganische Chemie, Johannes-Kepler-Universität Linz, Austria.

anodic peaks (A, B, C). A corresponds to the formation of the copper sulphide layer $Cu_{2-x}S$ while the peaks B and C are related to the formation of CuS and some Cu_2O [12]. The reverse scanning shows three cathodic peaks namely, D, E and F. The cathodic peak D corresponds to the partial reduction of CuS, while the cathodic peak E is associated with the reduction of the sulphide layer formed in region A - B. The study was then restricted to the potential range where the processes associated with A, B, and E take place, since the aim of this work was to elucidate the formation of the sulphide layer. Such a voltammogram is shown in Figure 2. The layer does not passivate the surface, and the fact that the ratio of Q_a/Q_c was found to be close to 1 (where Q_a and Q_c are the anodic and cathodic charges) indicates that no appreciable dissolution of the electrode or of the layer takes places in this potential region.

b) Potentiostatic Polarization Measurements

Figure 3 (a, b and c) shows the potentiostatic polarization (i - t) curves for the copper electrode in 2.9M ammonium sulphide solution at different potentials. The general shape of the transients is characteristic of a nucleation and growth process. At very short times double layer charging takes place, followed by an increase of current due to the formation and growth of nuclei. The current reaches a maximum and starts to decrease rapidly because of the layer growth controlled by mass transport till a steady state is reached. Although the initial current increases are usually too fast to be seen in the potentiostatic current-time curves, it is clearly shown in Figure 3b.

c) Galvanostatic Polarization Measurements

Figure 4a represents the galvanostatic polarization curves of the copper electrode in 2.9M ammonium sulphide solution at zero current. The shape of the transient is characteristic of a nucleation process. Sand's equation seems to be obeyed in the region between the minimum (1sec) till the end of the experiment

The main contribution of the peak observed during the galvanostatic polarization experiment is that of the nucleation process. The potential deceases rapidly to less negative potentials arriving at a steady state at -0.975V probably corresponding to chalcocite formation.

Figure 4b represents the galvanostatic polarization curves of a copper electrode in 2.9M of ammonium sulphide at current = 1A. No steady state is reached within the measuring time. The discontinuities in the transient indicate mechanical ruptures during layer formation.

d) The Composition And Nature Of Anodically Formed Layers

The layers formed on the copper electrode in 2.9M ammonium sulphide solution under potentiostatic conditions at different potentials were investigated by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX) and X-ray powder diffraction.

Dependent on the experimental conditions layers with different morphologies and compositions are observed (Table 1). EDAX analyses indicate that oxygen contents are generally very low. Besides binary copper sulphide phases two ternary compounds, $NH_4Cu_{7-x}S_4$ and NH_4CuS_4, are formed. The morphology of $NH_4Cu_{7-x}S_4$ is drastically different from that of Chalcocite. The layer is felt-like with thin packages of needles growing perpendicularly to the electrode surface (Figure 5a). By increasing time the needles become a little thicker and longer without changing their orientation.

Two reaction paths are feasible:

a) in situ reaction of anodically formed Cu^+ with S^{2-} and NH_4^+
b) consecutive reaction of anodically formed Cu_2S with S^{2-} and NH_4^+

Reaction path a) appears to be more probable in view of the oriented crystal growth. Cu^+ ions are supposed to travel through the channels existing in the quasi-one-dimensional crystal structure of $NH_4Cu_{7-x}S_4$ along the needle axis (c-axis). Hollow needles were also observed (Figure 5b). In this case the Cu^+ ions can also travel along the macroscopic channels inside the needles.

Figure 5c shows a typical micrograph of apparently well crystallized NH_4CuS_4.

Scales formed under cyclo-voltammetric conditions correspond to irreversible processes. Therefore they are different from those formed under potentiostatic conditions. Thus by cycling between -0.8 V- to positive potentials of +0.2 to 0.8 V dark grey layers of Djurelite ($Cu_{\sim 1.96}S$) were obtained, while under potentiostatic conditions at comparable positive potentials dark blue layers of Covellite (CuS) are formed.

IV. Conclusions

The electrochemical processes in the studied system are complex. Scale formation is governed by nucleation and growth and is characterized by concurrent reversible and irreversible reactions. Scales formed at cyclo-voltammetric conditions are different from those formed under potentiostatic conditions, where also the ternary phases $NH_4Cu_{7-x}S_4$ and NH_4CuS_4 are formed. $NH_4Cu_{7-x}S_4$ is growing as fine needles perpendicular to the electrode surface suggesting an in situ reaction of anodically formed Cu^+ ions travelling from the electrode through the scale with $[NH_4]^+$ and S^{2-} ions in the solution.

References Références Referencias

1. J.M.M. Droog, C.A. Abler Liester, P.T. Alderliesten, G.A. Bootsma, J. Electroanal. Chem. 111(1980), 61.

2. H.H. Trehblow and B. Tilze, Electrochim Acta, 25(1980), 839.
3. L.D. Burke, M.L.G. Ahern, T.G. Ryan, J. Electrochem. Soc. 137(1990), 553.
4. C.H. Pyum, S.M. Park, J. Electrochem. Soc., 133(1986), 2024.
5. S.M. Abd El-Haleem, E.E. Abd El-Aal, Corrosion, 62(2)(2006), 121.
6. S. Jakobs, M. Edwards, Water Res., 34(2000), 2998
7. M. B. McNeil, A. L. Anos, T. L. Woods, Corrosion 49(1993), 755.
8. A. F. Wells, Structural Inorganic Chemistry, 5th ed.,pp.1142, Clarendon Press Oxford 1987.
9. J. Gattow, Acta Cryst. 10(1957), 549.
10. H. Boller, M. Sing, Solid State Ionics, 101-103(1997), 1287.
11. C. Burschka, Z. Naturforsch, 35 B(1980), 1511.
12. V. Ashworth, J. Electrochem. Soc. 124(1977), 506.

Table 1

Anodically formed layers under potentiostatic conditions
(electrolyte concentration 2.9M)

-0.73 to -0.53V	dark grey layer	chalcocite (Cu_2S)
-0.67 to -0.60V	needles + polycrystalline substrate	$NH_4Cu_7S_4$ + chalcocite(Cu_2S)
-0,4 to -0.3V	black layer	digenite ($Cu_{1-x}S$)
-0.3 to +0.2V	brown layer	NH_4CuS_4 + chalcocite
+0.4 to +0.6V	black blue layer	Covellite (CuS)

Figure Captions

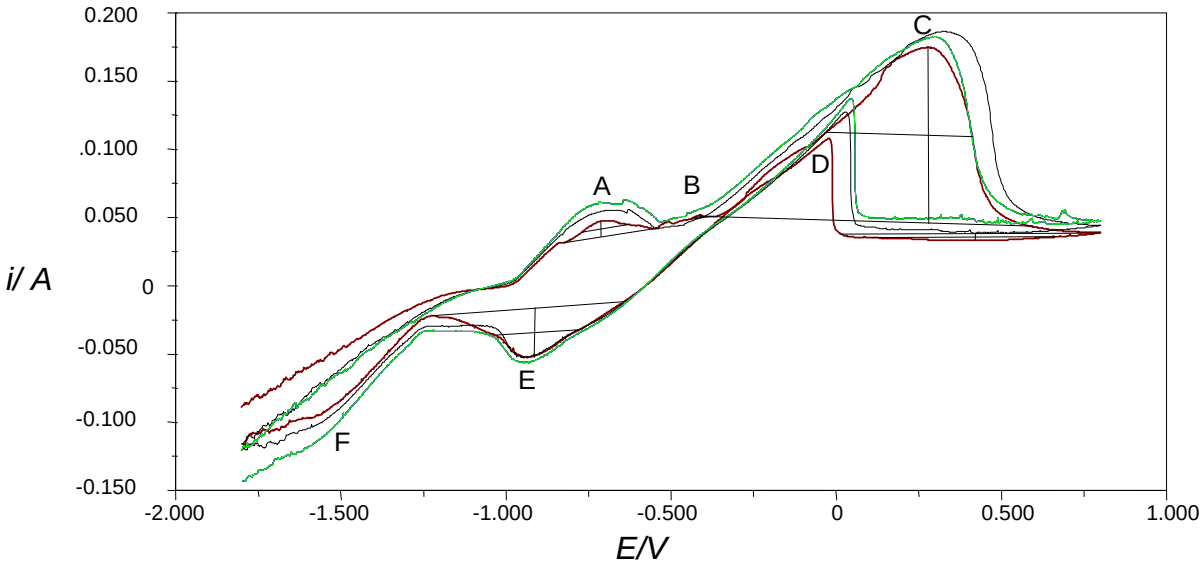


Figure 1 : Cyclic voltammogram of copper in 2.9 M ammonium sulphide solution at a sweep rate of 50mV/s.

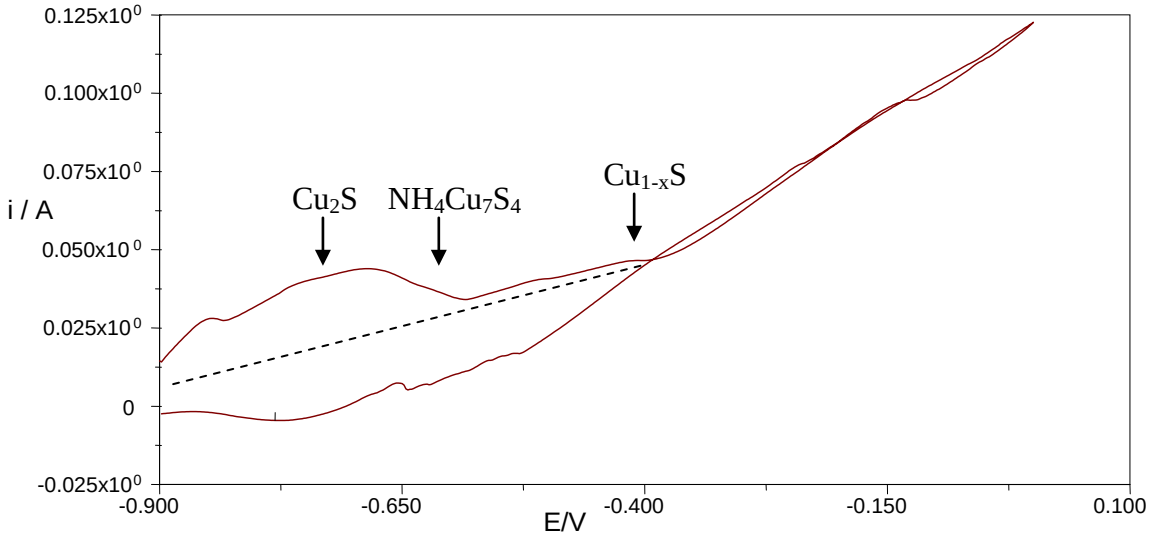
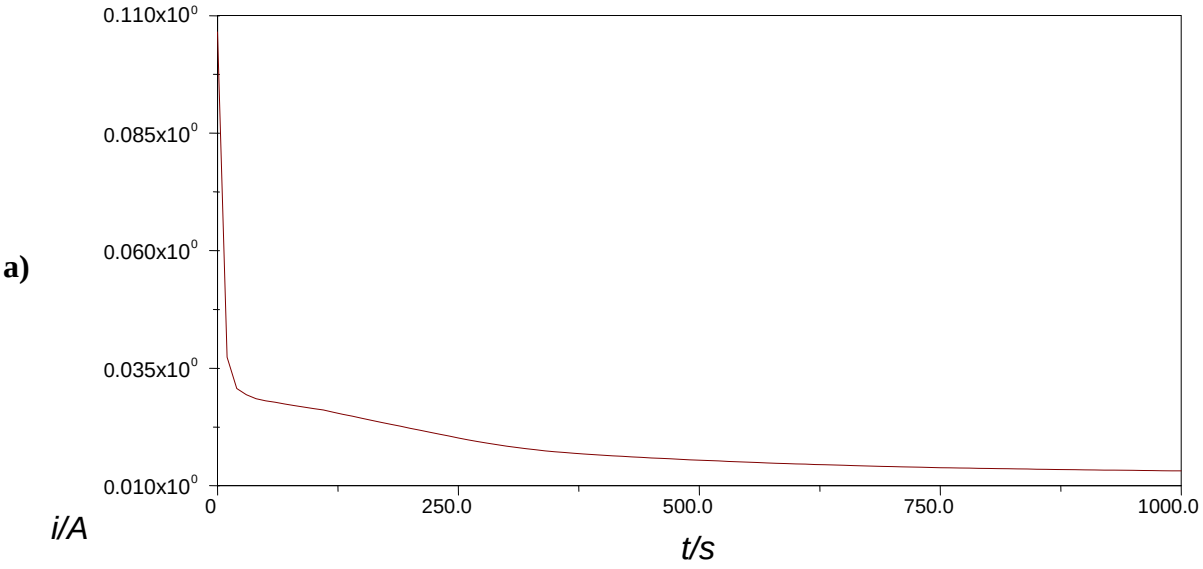
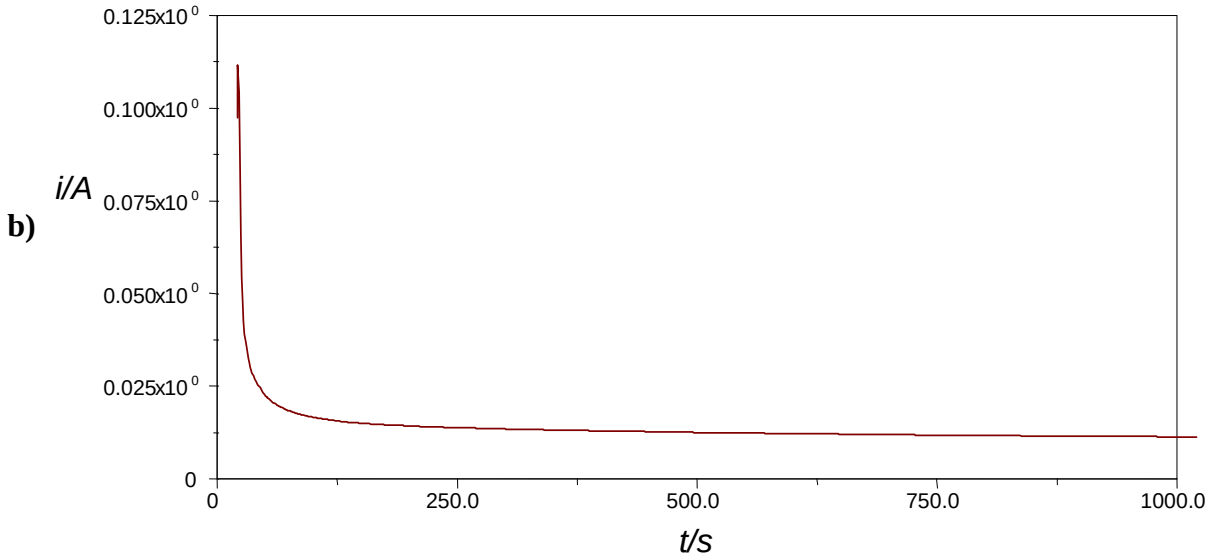


Figure 2 : Cyclic voltammogram of copper in 2.9 M ammonium sulfide solution in the range of $Cu_{2-x}S$ layer formation at sweep rate 50mV/s.





c)

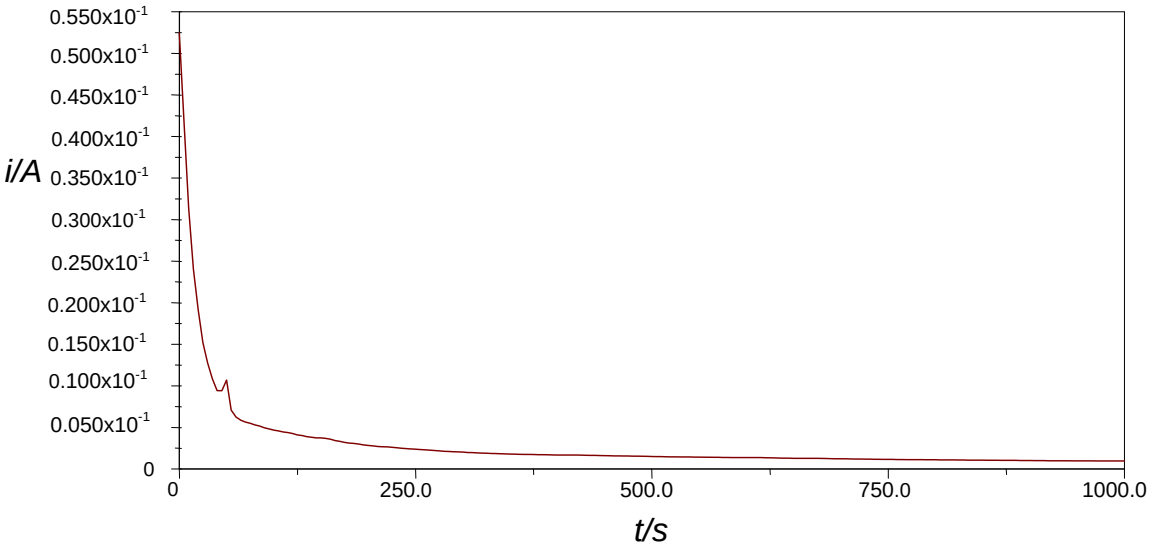
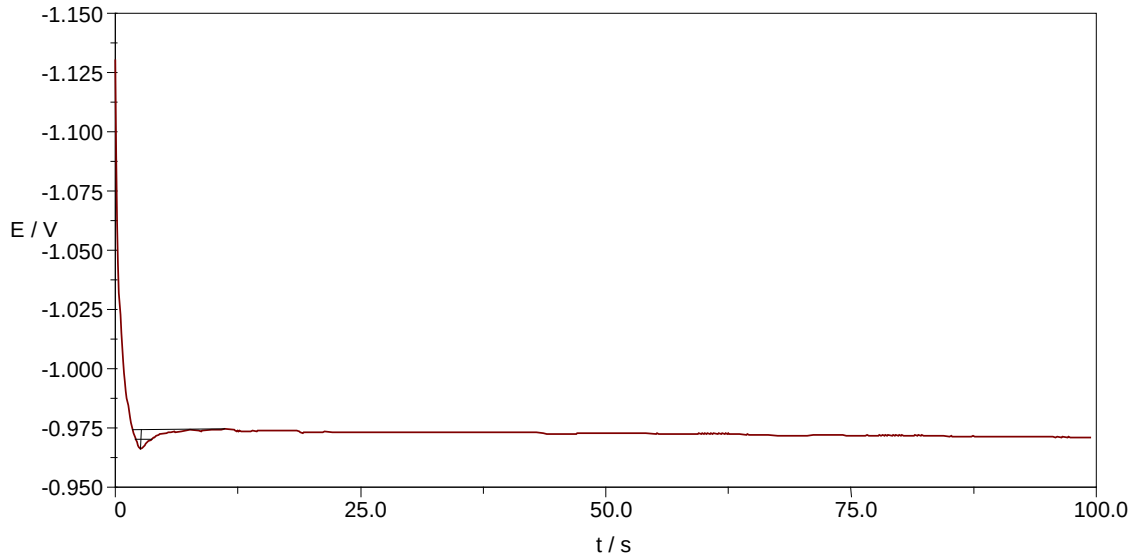


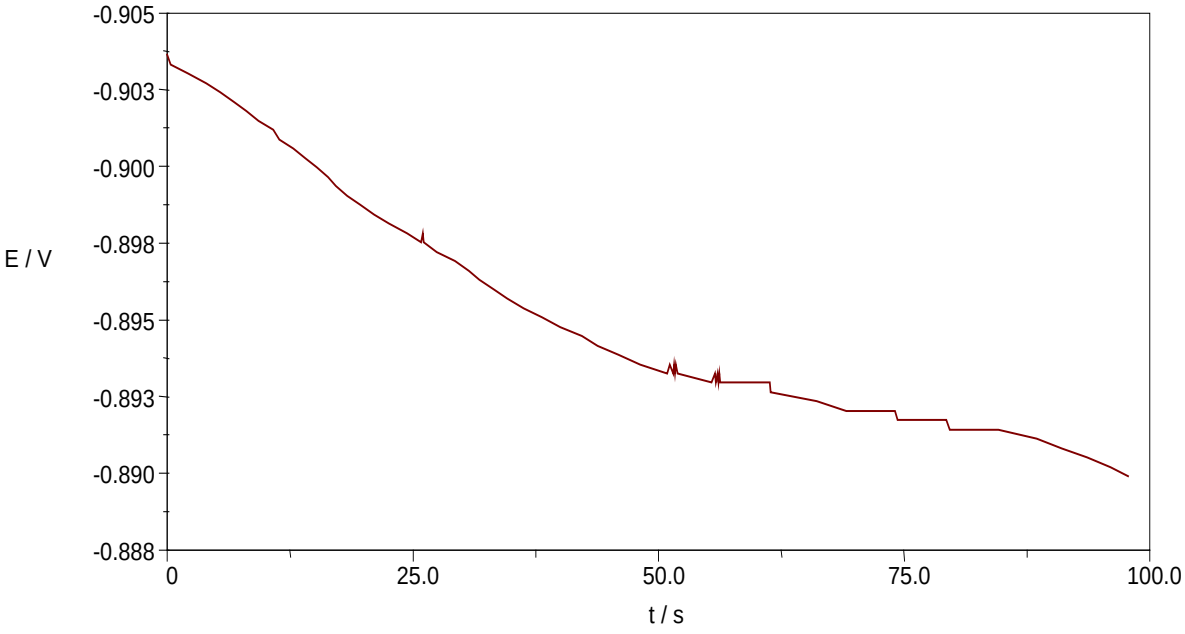
Figure 3 : Potentiostatic current-time curve of copper electrode in 2.9M ammonium sulfide solution at

a) potential at -0.463V (formation of Cu_2S, Digenite).
b) potential at -0.568V (formation of $NH_4Cu_{7-x}S_4$).
c) potential at -0.704V (formation of Cu_2S, Chalcocite).



a

Galvanostatic curve recorded for copper electrode in 2.9M ammoniumsulfide solution at current = 0

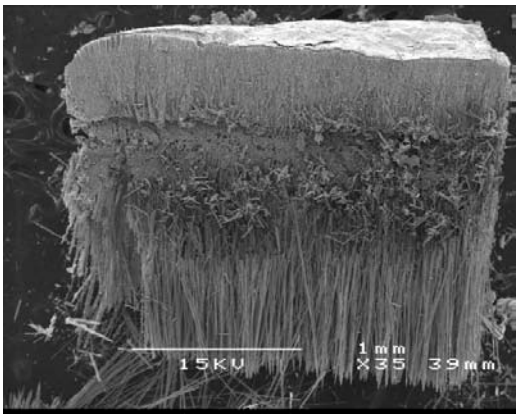


b

Galvanostatic curve recorded for copper electrode in 2.935M ammonium sulfide solution at current = 1A.

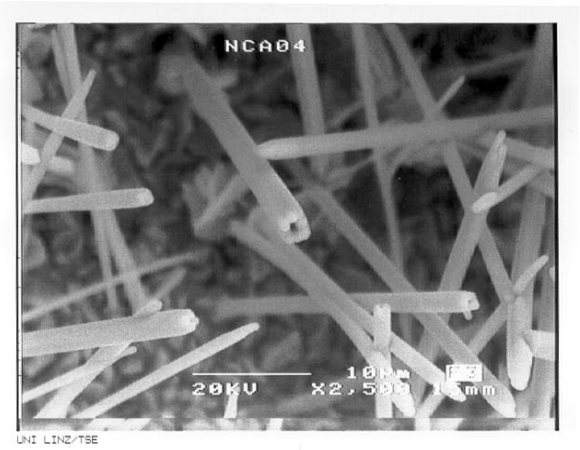
Figure 4 : Galvanostatic curve recorded for a copper electrode in 2.9M ammonium sulfide solution at

a) current = 0
b) current = 1A



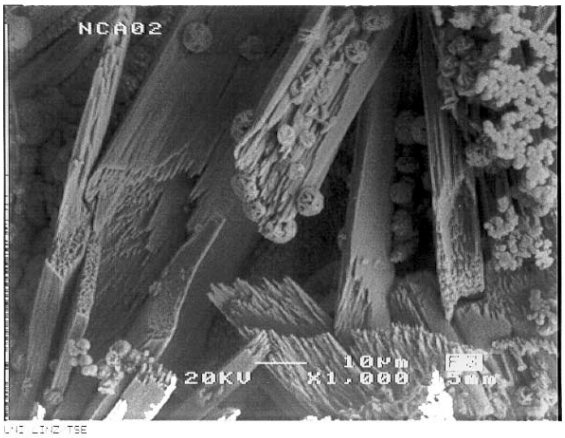
a

Scanning electron micrograph of a layer formed in 2.9M ammonium sulfide solution at potential –0.63V (*$NH_4Cu_7S_4$*).



b

Scanning electron micrograph of $NH_4Cu_7S_4$ needles formed at the same conditions.



c

Scanning electron micrograph of a layer formed in 2.935M ammonium sulfide solution at potential 0V (*NH_4CuS_4*).

Figure 5 : SEM micrographs of electro crystallized $NH_4Cu_7S_4$ and NH_4CuS_4

This page is intentionally left blank

Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Application of ICP-OES in the Comparative Analysis of a Used and Fresh Gasoline Motor Oil

By Behnam Rahimi, Abolfazl Semnani, Alireza Nezamzade Ejhieh, Hamid Shakoori Langeroodi, & Massoud Hakim Davood

Islamic Azad University, Shareza, Ifahan, IRAN

Abstract - In a fresh and 14500 km used oil, viscosity, viscosity index, flash point, pour point, specific gravity, color, total acid number, total base number, water content, as well as concentrations of twenty four elements, were determined. The mineral baesd, gasoline motor oil Speedy SL, from Sepahan Oil Company was chosen for study. The physical properties were characterized by ASTM protocols. The elemental analysis was performed by inductively coupled plasma- optical emission spectroscopy (ICP-OES) . The results indicate that after the application of fresh oil, both of the physical properties and elemental concentrations have been changed significantly. Possible reasons for the observed variations have been discussed.

Keywords : *Oil Analysis, ICP-OES, mineral oils, gasoline oil,used oil.*

GJSFR-B Classification: *FOR Code: 030503*

Strictly as per the compliance and regulations of:

Application of ICP-OES in the Comparative Analysis of a Used and Fresh Gasoline Motor Oil

Behnam Rahimi[α], Abolfazl Semnani[σ], Alireza Nezamzade Ejhieh[α], Hamid Shakoori Langeroodi[ρ] & Massoud Hakim Davood[ω]

Abstract - In a fresh and 14500 km used oil, viscosity, viscosity index, flash point, pour point, specific gravity, color, total acid number, total base number, water content, as well as concentrations of twenty four elements, were determined. The mineral baesd, gasoline motor oil Speedy SL, from Sepahan Oil Company was chosen for study. The physical properties were characterized by ASTM protocols. The elemental analysis was performed by inductively coupled plasma- optical emission spectroscopy (ICP-OES) . The results indicate that after the application of fresh oil, both of the physical properties and elemental concentrations have been changed significantly. Possible reasons for the observed variations have been discussed

Keywords : Oil Analysis, ICP-OES, mineral oils, gasoline oil,used oil.

I. Introduction

Oil analysis (OA) is the sampling and laboratory analysis of a lubricant's properties, suspended contaminants, and wears debris. OA is performed during routine preventive maintenance to provide meaningful and accurate information on lubricant and machine condition. By tracking oil analysis sample results over the life of a particular machine, trends can be established which can help eliminate costly repairs [1-5].

OA was first used after World War II by the US railroad industry to monitor the health of locomotives. In 1946, the Denver and Rio Grande Railroad's research laboratory successfully detected diesel engine problems through wear metal analysis of used oils. A key factor in their success was the development of the spectrograph, an instrument that replaced several wet chemical methods for detecting and measuring individual chemical element such as iron or copper. This practice was soon accepted and used extensively throughout the railroad industry [5-9].

Used oil analysis is comparable to a medical analysis with a blood test. Like blood, lubricating oil contains a good deal of information about the envelope in which it circulates. Wear of metallic parts, for example, produces many minute particles, which are carried by the lubricant. These small metal particles can give information about the machine elements that are wearing. In addition, variations of physical properties such as viscosity, viscosity index, flash point and etc, can give valuable information about engine and lubricant performance [5-9].

A number of techniques such as inductively coupled plasma [10] , Fourier transformation infrared spectroscopy [11,12], atomic absorption spectroscopy [13], , differential scanning colorimetry [14] , x-ray fluorescence spectroscopy [15], laser induced break down spectroscopy [16], spectrography [17], ferrography [18], mass spectrometry [19], and chromatography [20] have been applied for the oil analysis.

We have been recently involved in the investigation of lubrication oils [21-23]. In this paper, we report the results of the comparative physical and chemical analysis of fresh and used gasoline engine oil with mineral base, by ICP-OES and some other techniques.

Author[α] : Department of Chemistry, Shahreza Branch, Islamic Azad University, Shareza, Ifahan, IRAN.
Author[σ] : Department of Chemistry, Faculty of Sciences, University of Shahrekord, P. O. Box 115, Shahrekord, IRAN.
E-mail : a.semnani1341@gmail.com
Author[ρ] : Barzin Sepand Sepahan Company, Dehagh Industrial City, Isfahan, IRAN.
Author[ω] : Sepahan Oil Company, Isfahan, IRAN.

Experimental

a) Materials

All of the materials were used directly without any further processing. The employed materials, as well as the name of the corresponding manufacturer are as follows:

Material	Manufacturer	Material	Manufacturer
Gasoline oil Speedy SL	Sepahan Oil Company	Potassium Hydroxide	Merck
Ethanol	Merck	Acetic acid	Merck
Hydrochloric acid	Merck	Acetic anhydride	Merck
Lithium chloride	Merck	Chlorobenzene	Merck
Methanol	Merck	Sodium perchlorate	Merck
Propane-2-ol	Merck	Sodium carbonate	Merck
Buffer	Merck	Xylene	Merck
Chloroform	Merck	Acetone	Merck
Perchloric acid	Merck	Solid carbon dioxide	Merck

b) Test Methods

The test methods were followed as: ASTM D-445 for viscosity @ 40^0c and 100^0c, ASTM D-2270 for viscosity index, ASTM D- 92 for flash point, ASTM D-97 for pour point, ASTM D-1298 for specific gravity, ASTM D-1500 for color, ASTM D-664 for total acid number, ASTM D-6304 for water content,

c) Instrumental

All of the viscosities, viscosity indices and specific gravities were determined by viscometer Anton Paar model SVM 3000. Flash points were evaluated by flash point tester Herzog model HC 852. Pour points were determined by pour point tester Herzog model HC 852. The colors were determined by Dr. Long instrument. TBNs were determined by robotic titro sampler Metrohm model Dosiono 800. TANs were determined by titrator Methrohm model Titrino MPT 789. FTIR spectrum was recorded on a FTIR spectrum Perkin Elmer model Spectrum 65 using KBr pellet. The elemental analysis were performed by ICP-OES Perkin Elmer model Optima 5300V

d) Sampling

The sampling was performed immediately after turning off the car. An adequate amount of oil sample was taken by 100 mL syringe.

II. Results And Discussion

Typical properties such as vis@40°c, vis@100°c, viscosity index, flash point and etc of fresh and used oil are compared in Table 1. As it can be seen, all of the properties have been changed approximately. This means that upon the usage of motor oil and during the performance of the oil several changes has been occurred in the oil composition [24].

Consistency, flow properties, or viscosity in the case of oils, are key parameters to create lubrication efficiency and the application of lubricants [25]. The viscosity of used engine oil can drop for reasons of fuel dilution, or as a result of high water content and/or shearing of the VI improver [1]. Viscosity can increase because of heavy contamination of the oil by soot, polymerization, vaporization losses, and emulsions due to water contamination and/or oxidation of the oil [1].

Obviously, the final status of the oil viscosity depends on the combination effects of decreasing and increasing factors. If the falling factors overcome to the rising ones, the drop in viscosity will happen. An increase in the property will be observed, in the reverse conditions.

There is no signal of fuel dilution. Therefore, the appearance of water in the used oil and the drop of viscosity index (as a result of shearing of the VI improver) are the main viscosity reducing agents.

It is well known that heavy contamination of the oil by soot causes the appearance of a band at 2000 cm-1 in the IR spectrum of corresponding oil [1]. Because of the absence of such band in the spectrum of used oil (Fig. 1) viscosity increase by soot formation is discarded. On the other hand, considerable reduction in the volume of the oil was not observed. Which means that the evaporation loses through usage of the oil are minimal. Furthermore, there is no evident of polymerization. Thus, among the viscosity increasing factors the main role can be attributed to the oxidation. This is further confirmed by the increase of TAN, density and color, as some of the oxidation signals.

The data in Table 1 indicate that vis@40°c and vis@100°c have been increased. Based on the discussion on previous paragraph it can be claimed that viscosity-increasing factors have been overcome to the decreasing ones.

The flash point is the lowest temperature at which an ignition source causes the vapors of the specimen (lubricant) to ignite under specified conditions [2]. Like viscosity, the flash point test has always been a standard part of a lubricant's specification. Because of the low flash points of most fuels, a sudden drop in flash temperature in crankcase oil can usually be relied upon as an indication of dilution. Occasionally, very high, localized temperatures can lead to thermal cracking within the oil. As there is no evident of fuel dilution, the observed reduction (Table 1) can be attributed to thermal cracking. Because of lower flammability of lighter molecules, the flash point has been reduced. The production of lighter molecules is further confirmed by decreasing of pour point. As it can be seen, the pour point has been reduced for 4 degrees of celicious.

The total acid number is a measure of the acidic constituents in petroleum products. The acidity of unused oils and fluids is normally derived from the type and concentration of specific additive material whereas the acidity of used oil is of interest to measure the degree of oxidation of the fluid. The total base number (TBN) characterizes the alkaline reserve in petroleum products [24, 25]. It is particularly used for engine oils where by acidic combustion products use up the alkaline reserve. Both TAN and TBN can be obtained by acid base titration. Sample titration curve is shown in Fig. 2. The resulting data indicate that TAN of used oil has been increased relative to fresh oil. In contrast, TBN has been decreased. The increasing of TAN can be attributed to production of acidic products due to oil oxidation. In fact, in high temperature of motor conditions some of the oil constituents oxidize. The carboxylic acid is produced. The reduction of TBN can be assigned to depletion of additives, which mostly do have basic character. The appearance of carbonyl band in IR spectrum of used oil (Fig. 1) further confirms the production of acidic adducts.

The concentration of different elements was measured by ICP-OES. The final results are given in Table 2. According to the data, different elements can be categorized to three groups: (i) the elements, which have high concentration in fresh oil, (ii) elements, which have low concentration in fresh oil, and (iii) elements that do not exist in fresh oil. Based on this categorization sulfur, zinc, phosphorus, magnesium, silicon, calcium and barium can be located in first group. Bohr molybdenum, aluminum, silver, chromium, nickel and sodium in second group and manganese, iron, copper, tin, titanium, vanadium, led, cadmium, antimony and potassium in third group.

Among the elements of the first group, the highest concentration belongs to sulfur. Keeping in mind that the examined motor oil do have mineral character and mineral base oils do have relatively high sulfur content [25], such an observation is not unexpected. On the other hand, the decrease in the concentration of this element in the used oil can be attributed to oxidation followed by evaporization of some of the sulfur containing constituents.

The high concentration of other elements such as Zinc, Phosphorous, magnesium, silicon and barium indicate that these elements are the constituents of employed additives. The existence of these elements can be attributed to ZDDP, barium sulfonate, and other additives. The decrease in the concentration of these elements indicates that during the performance of the oil the additives have been depleted.

The low concentration elements (group (ii)) origin from the additives which are used in very low concentration or the elements which in the process of oil production have been produced in the oil.

The encounter with the elements which initially are absent in the fresh oil but appear in the used oil (group (iii)) can be attributed to the wearing of motor parts during the oil performance, contamination or both o them. Equipment as it operates will deposit microscopic amounts of wear metals in the lubricant. For example iron originates from the wearing of cylinders, copper from bushings, bearings, cam bushings, oil coolers, valve train bushings, thrust washers, oil pumps, aluminum from pistons, bearings, blocks (some), bushings, housing, oil pumps, blowers, thrust bearings, cam bearings/bushings, chromium from rings, roller/taper bearings (some), liners, exhaust valves, wear treatment, lead from bearings, gasoline, octane improver .Molybdenum from plating or surface hardening agent in certain bearings, rings.

Under normal conditions, wear will be very low and under abnormal conditions, the wear will be high Among these elements the concentration of iron and manganese show drastic change, which indicate drastic wear of iron alloy occurs such as. Because the concentration of wear elements is not very high, it can be concluded that considerable wearing has not occurred.

III. Conclusions

Based on obtained results it can be concluded that due to depletion of additives, oxidation, thermal cracking and wear, some of the physical and chemical properties of the selected oil have been changed. Minor wearing has been occurred. It general it can be claimed that the oil shows acceptable performance characteristics.

IV. Acknowledgements

The financial support of this works by Azad University of Shahreza is highly appreciated. The authors also respect from Sepahan oil Company due to lab support of this work

References Références Referencias

1. D. Troyer, & J. Fitch, Oil analysis basics, Noria Corporation, (2001).
2. R. M. Mortier, M. F. Fox and Stefan T. Orszulik, Chemistry and technology of lubricants, Springer, (2010).
3. J. S. Evans, Oil analysis handbook (Coxmoor's Machine & Systems Condition Monitoring) Coxmoor Publishing Co., (2008).
4. B. J. Roylance and T. M. Hunt, The wear debris analysis handbook (Coxmoor's machine & systems condition monitoring) Coxmoor Publishing Co., (1999).
5. J. Fitch, Source book for used oil elements, Noria Corporation, (2001).
6. L. A. Toms, Allison M. Toms, Machinery oil analysis - methods, automation & benefits, 3rd Edition, Noria Corporation, (2008).
7. R. F. Haycock, Arthur J. Caines, John E. Hillier, Automotive lubricants reference book, SAE, (2004).
8. R. A. Kishore Nadkarni, Spectroscopic analysis of petroleum products and lubricants, ASTM, (2011).
9. ASTM International, Elemental analysis of fuels and lubricants: recent advances and future prospects, ASTM, (2005).
10. M. P. Granchi, J. A. Biggerstaff, L. J. Milliard and P. Grey, Use of a robot and flow injection for automated sample preparation and analysis of used oils by ICP emission spectrometry, Spectrochimica Acta, Part B, Vol. 42, Nos. 1 and 2, 169-180, (1987).
11. D. Li, J. Sedman, D. L. García-González, and F. R. van de Voort, Automated acid content determination in lubricants by FTIR spectroscopy as an alternative to acid number determination, Journal of ASTM International, Vol. 6, No. 6, 1-12, (2009).
12. F. R van de Voort, J. Sedman, V. Yaylayan, and C. Saint-Laurent, The determination of acid and base number in lubricants by FTIR spectroscopy," Appl. Spectrosc., Vol. 57, 1425–1431, (2003).
13. R. Q. Aucelio, R. M. de Souza, R. C. de Campos, N. Miekeley, C. L. P. da Silveira, The determination of trace metals in lubricating oils by atomic spectroscopy, Spectrochimica Acta, Part B, 62, 952-961, (2007).
14. Y. H. Khraisha, I. M. Shbib, Thermal analysis of shale oil using thermogravimetry and differential scanning gravimetry, Energy Conversion & Manangement, Vol. 43, 229-239, (2002).
15. M. Pouzar, T. Cernohorsky, and A. Krejocova, Detrmination of metals in lubricating oils by X- ray fluorescence spectrometry, Talanta, Vol. 54, 829-835, (2001).
16. P. Yaroshchyk, R. J. S. Morison, D. Body, and B. L. Chadwick, Quantitative determination of wear metals in enginer oils using laser induced breakdown spectroscopy: a comparison between liquid jets and static liquids, Spectrochimica Acta, part B, Vol. 60, 986-992, and (2005).
17. C. M. Gambrill, A. G. Gassmann, W. R. O.'Neill, Spectrographic Analysis of new and used lubricating oils, Analytical Chemistry, Vol. 23, No., 10, 1365-1369, (1951).
18. M. H. Jones, Ferrography applied to diesel engine oil analysis, Wear, Vol. 56, No.1, 93-103, (1979).
19. T. J. Cardwell, R. Colton, N. Lambropoulos and J. C. Traeger, Electrospray mass spectrometry of zinc dithiophosphate derivatives and its application to the analysis of engine oil antiwear additives, Analytica Chimica Acta, Vol. 280, No.2, 239-244, (1993).
20. D. M. Levermore, M. Josowicz, W. S. Rees, Jr., and J. Janata, Headspace Analysis of Engine Oil by Gas Chromatography/Mass Spectrometry, Analytical Chemistry, Vol. 73, No. 6, 1361-1365, (2001).
21. H. Shakoori Langeroodi and A. Semnani, African Journal of Pure and Applied Chemistry, 3: 11, 241-246, (2009).
22. A. Semnani and H. Shakoori Langeroodi, An investigation on the behavior of solvent neutral 500: polyisobutene blends, Petroleum Science and Technology, (accepted).
23. H. Shakoori Langeroodi and A. Semnani, the production of a group (iii) base oil and investigation on its polyisobutene blends, Petroleum Science and Technology, (accepted).
24. L. R. Rudnick, Synthetic, mineral oils and bio-based lubricants, Taylor & Francis (2006).
25. T. Mang, and W. Dresel, Lubricants and lubrication, Second Edition, Wiley VCH, (2007).

Table 1 : Typical properties of fresh oil Speedy SL from Sepahan oil Company

Typical Properties	Test Method	Fresh oil	Used oil
Viscosity@40°c cSt	ASTM D-445	141.6	152.2
Viscosity@100°c cSt	ASTM D-445	16.51	18.4
Viscosity Index	ASTM D-2270	125	118.3
Flash Point	ASTM D-92	222	218
Pour Point	ASTM D-97	-26	-30
Specific Gravity	ASTM D-1298	0.8910	0.9037
Color	ASTM D-1500	2	8
TAN (mg KOH/g)	ASTM D-664	1.52	3.2
TBN (mg KOH/g)	ASTM D-664	12.37	9.75
Water content	ASTM D-6304	0	1%

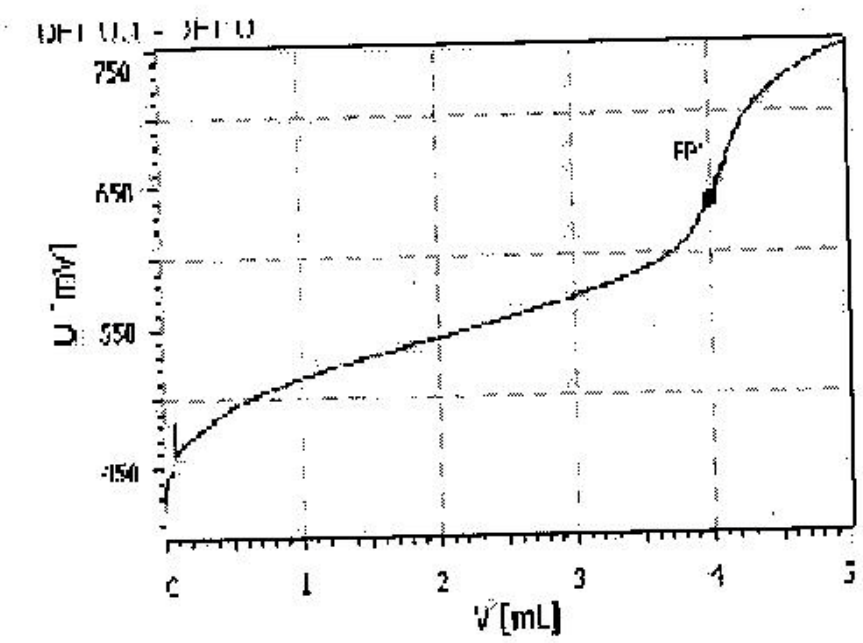


Figure 1 : Titration curve due to TBN determination of fresh oil.

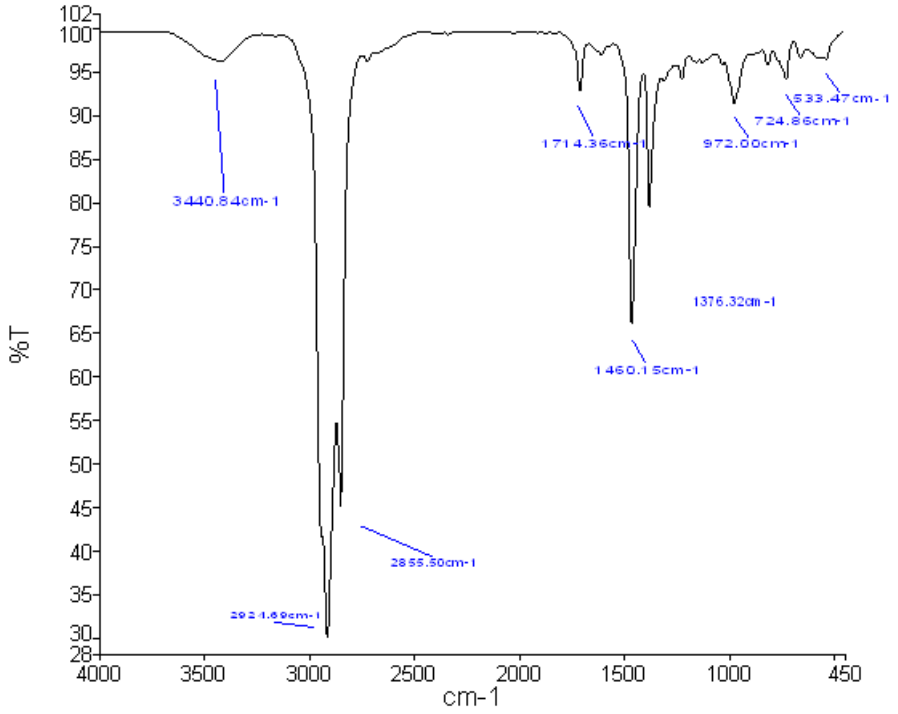


Fig. 2 : IR spectrum of used oil

Table 2 : Concentration of different elements in fresh and used oils

No.	Element	Fresh oil	Used oil	No.	Element	Fresh oil	Used oil
1	Sulfur	1108.0	938.0	13	Nickel	1.1	2.0
2	Zinc	784.0	160.0	14	Sodium	0.5	1.1
3	Phosphorous	811.2	318.8	15	Manganese	0.0	25.3
4	Magnesium	228.9	185.9	16	Iron	0.0	12.8
5	Silicon	61.4	45.5	17	Copper	0.0	2.8
6	Calcium	56.7	16.1	18	Tin	0.0	1.6
7	Barium	29.4	22.8	19	Titanium	0.0	1.6
8	Bohr	6.7	2.9	20	Vanadium	0.0	1.5
9	Molybdenum	6.5	8.9	21	Lead	0.0	0.6
10	Aluminum	5.0	10.6	22	Cadmium	0.0	0.5
11	Silver	1.7	2.3	23	Antimony	0.0	0.4
12	Chromium	1.1	2.2	24	Potassium	0.0	0.0

Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Interaction Between Cryptand 222 And Tetracyanoethylene In Di And Trichlorom Ethane Solutions

By Abolfazl Semnani, Ali Reza Firooz, Leila Kashefi Kheyrabadi, Hamid Shakoori Langeroodi & Safieh Heidarizadeh Rizi

University of Isfahan,Isfahan, I.R. Iran

Abstract - A spectrophotometric study concerning the interaction between cryptand 222 as n-donor and TCNE as π-acceptor has been performed in di and tri chloromethane solutions at temperatures 5, 10, 15, and 20°c. The results of continuous variation and mole ratio methods indicate the formation of 1:1 complexes in both solvents and at all temperatures. The stability constants and the molar absorption coefficients at different temperatures have been calculated from the computer fitting of absorbance- mole ratio data in MATLAB soft ware. The results indicate that Kf values in $CHCl_3$ are more than the corresponding amounts in CH_2Cl_2. In the case of ϵ, the reverse trend is observed. The $\Delta H°$ and $\Delta S°$ values were obtained by Vant Hoff method. The obtained data show that the enthalpy of complex formation in two solvents is favorable. While entropy is favorable in the case of $CHCl_3$ and unfavorable in the case, of CH_2Cl_2. The possible reasons for such observation are discussed. The kinetic results confirm an overall second order reaction which is first order with regard to each reactant. The formation of free ions is rejected by the conductometric measurments.

Keywords : Halomethanes, C222, TCNE, Spectro - photometry, Charge transfer, Thermo-dynamic, Kinetics.

GJSFR-B Classification: FOR Code: 030503

Strictly as per the compliance and regulations of:

Interaction Between Cryptand 222 And Tetracyanoethylene In Di And Trichlorom Ethane Solutions

Abolfazl Semnani[α], Ali Reza Firooz[σ], Leila Kashefi Kheyrabadi[σ], Hamid Shakoori Langeroodi[ρ] & Safieh Heidarizadeh Rizi[α]

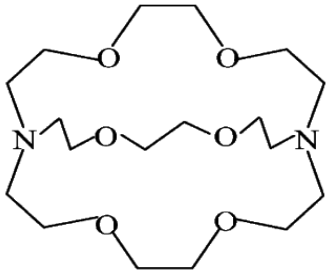
Abstract - A spectrophotometric study concerning the interaction between cryptand 222 as n-donor and TCNE as π-acceptor has been performed in di and tri chloromethane solutions at temperatures 5, 10, 15, and 20°c. The results of continuous variation and mole ratio methods indicate the formation of 1:1 complexes in both solvents and at all temperatures. The stability constants and the molar absorption coefficients at different temperatures have been calculated from the computer fitting of absorbance- mole ratio data in MATLAB soft ware. The results indicate that Kf values in $CHCl_3$ are more than the corresponding amounts in CH_2Cl_2. In the case of €, the reverse trend is observed. The $\Delta H°$ and $\Delta S°$ values were obtained by Vant Hoff method. The obtained data show that the enthalpy of complex formation in two solvents is favorable. While entropy is favorable in the case of $CHCl_3$ and unfavorable in the case, of CH_2Cl_2. The possible reasons for such observation are discussed. The kinetic results confirm an overall second order reaction which is first order with regard to each reactant. The formation of free ions is rejected by the conductometric measurments

Keywords : Halomethanes, C222, TCNE, Spectro - photometry, Charge transfer, Thermodynamic, Kinetics.

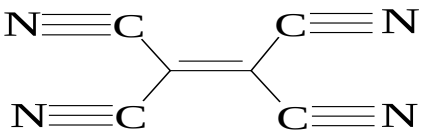
I. Introduction

Since the first synthesis of crown ethers [1] and cryptands [2], there has been an intensive amount of research work on the thermodynamic and kinetics of complexation of these ligands with various cations in a wide variety of solvent systems [3]. Moreover, the molecular complexes of crowns and cryptands have been followed [4]. Interest in molecular complexes is strongly stimulated by their possible applications in different areas such as separation processes, biomimetric receptors, catalytic reactions and conversion of chemical energy to optical or electronic signals. In continue of our interest to molecular complexes of crowns and cryptands [5-10], here we report the results of complexation of cryptand 222 with TCNE in di and trichlolromethane solutions.

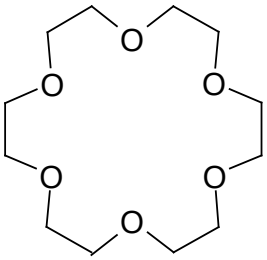
Experimental : The macrocycle C222 and TCNE (both from Merck) were recrystallized from reagent gradre n-hexane and dried over P2O5. Reagent grades of di and trichlorometahne (both from Merck) were used without any further purification.



Cryptand 222 (C222)



Tetracyanoethylene (TCNE)



18-Crown-6

All UV-Vis spectra and absorbance measurements were made with a UV-Vis-NIR spectrophotometer Cary 500 at different temperatures. Conductance measurements were carried out with a conductivity meter 180 from Orion research Company.

II. Results And Discussion

Absorption spectra of $1.0\times 10\text{-}4$ M solution of C222 in trichloromethane in the precence of varying concenteations of TCNE are shown in Fig. 1. Because of similarity, the corresponding spectra due to dichloromethane are not shown. Each spectrum was recorded 20 minutes after preparing the fresh solution. As it can be seen upon addition of C222 to the solution

Author[α] : Department of Chemistry, Faculty of Science, University of Isfahan,Isfahan, 81746-73441, I.R. Iran.
Author[σ] : Department of Chemistry, Faculty of Science, University of Shahrekord,Shahrekord, 115, I.R. Iran.
Author[ρ] : Petrokimia Sepahan Company, Mobarekeh Industrial City, Isfahan, Iran.

of TCNE, a new band is appeared in 350-450 nm region. As, none of the reactants, do not have any absorption in this spectral region. The new band can be attributed to the formation of charge transfer complex between C222 as n-donor and TCNE as π- acceptor [11].

In order to determine the effective site of complexation, the spectrum of 1:1 mixture of 18C6 (i.e. a compound without nitrogen atom) and TCNE was recorded. As, new band was not observed, it can be concluded that the oxygen atoms of C222 do not play an important role and the complexation mainly occur through nitrogen atoms.

The needed time for reaction completeness was determined by monitoring the absorbance of 1:1 mixture of 1.0×10-4 M solution of C222 and TCNE at 400 nm and at different temperatures (Fig. 2). As it can be seen, after 15 minutes, reaction will be terminated. Therefore, in the next experiments, absorbances were measures 20 minutes after mixinof reagents.

The stoichiometry of the complexes at different temperatures was obtained by the absorbance vs. mole ratio [12] and Job methods [13]. Sample plots are shown in Figs. 3 and 4, respectively. Both series of plots clearly confirm 1:1 stoichiometry. Moreover, in both cases upon temperature rising, the curvature of plots is decreased.

Based on spectral, mole ratio and Job evidences it can be concluded that through the reaction between C222 and TCNE, 1:1 charge transfer complex is formed.

$$TCNE + C222 \leftrightarrows TCNE: C222 \quad (1)$$

For the evaluation of the formation constants from absorbance-mole ratio data, a none-linear least squares curve fitting program (curve fitting toolbox in MATLAB) was used [14,15]. The program is based on the iteration adjustment of calculated absorbances to the observed values.

The observed absorbance of complex at its λ_{max} is given by equation (2). The mass balance equations can be written as (3) and (4), and the formation constant of the complex as in (5). Substitution of (3) and (4) in (5) and rearrangement yield (6).

$$Abs. = \epsilon b[DA] \quad (2)$$

$$C_D = [D] + [DA] \quad (3)$$

$$C_A = [A] + [DA] \quad (4)$$

$$K_f = [DA]/[D][A] \quad (5)$$

$$K_f [DA]^2 - (C_A K_f + C_D K_f + 1) [DA] + K_f C_D C_A = 0 \quad (6)$$

With use an approximation value for Kf, the free DA concentration, [DA], were calculated by solution of second order equation. Then, with using from data of DA concentration as x data and data of observed absorbance as y data, the least squares fit technique is used for fitting the data. The output of this fitting is the coefficient of line fit. The coefficient of x values is ϵ (molar absorption coefficient). The obtained coefficient were used for calculation of data of absorbance with using of parabolic fit. To find the least squares error, the sum of squares of differences between the parabolic fit and the actually data must be evaluated. Refinement of parameters (Kf value) was continued until the sum of squares of the residuals between calculated and observed values of the absorbance for all experimental points was minimized.

Sample curve fittings are shown in Fig. 5. The good agreement between the experimental and calculated data confirm the accuracy of the results. The final logKf and ϵ values obtained by MATLAB are given in Table 1. The data indicate that at all temperatures logKf values due to trichloromethane are higher than the corresponding values in dichloromethane. In the case of ϵ, the reverse trend is observed. Greater logKf in trichloromethane means that in this media, the contribution of solvent in entropy, enthalpy or both of them is more favorable than dichloromethane. On the other hand, despite both TCNE and C222 are nonpolar. Their resulting complex is polar and will show diplole-diploe interactions with polar species. Clearly, such interactions will be higher with more polar species. So, it is anticipated that dicholoromethane with dipole moment of 1.5 [16], do have more solute–solvent interactions (with polar charge transfer complex) than that of trichloromethane with dipole moment of 1.15 [16]. Greater dipole-dipole interactions cause that in CH_2Cl_2 orientation of complex particles to be more than of $CHCl_3$. This results in higher absorption cross section [17]. The net effect is the enhancement of ϵ. Therefore, the observation of higher ϵ in CH_2Cl_2 is not unexpected.

The thermodynamic parameters were obtained by the plot of log Kf vs. 1/T (sample plot is shown in Fig. 6) [18]. The obtained values for $\Delta H°$ are -26.3 kJ/mol and -25.4 kJ/mol in di and trichloromethane, respectively. Also, the $\Delta S°$ values were obtained as -16.3 J/mol.°K in dichloromethane and +25.13 J/mol.°K in trichloro-methane.

It is well known that the final stability of complex depends on the sum of entropy and enthalpy changes through the complexation process [18]. On the other hand, the salvation, affects both $\Delta S°$ and $\Delta H°$ values. The effect on $\Delta S°$, relates to positive entropy changes due to desolvation of reactants and negative entropy changes due to solvation of complex. The effect on

$\Delta H°$, relates to enthalpy changes during desolvation of reactants and solvation of complex.

The enhanced enthalpy changes in both solvents indicate that the amount of realized energy through complex formation and complex solvation is higher than consumed energy for desolvation of reactants. In addition, positive $\Delta S°$ in dichloromethane indicates that absolute entropy increase through desolvation of reactants is more than absolute entropy decrease through complex formation and complex solvation. Negative $\Delta S°$ in trichloromethane proves that entropy changes through desolvation of reactants or solvation of complex in recent solvent differs from corresponding values of dichloromethane.

The existence of nitrogen atoms on C222 and TCNE beside the location of three electron-withdrawing groups on carbon atom of trichloromethane, enhances the hydrogen bond formation between solvent and reactants. These bonds are broken through complex formation and some solvent molecules are realized in solvent. The net result is the positive $\Delta S°$. In the case of dichloromethane, the hydrogen bonds are considerably weaker. So, positive effect (through solvent realization) on overall $\Delta S°$ is considerably less than trichloromethane, which causes the observation of overall negative $\Delta S°$ in this solvent.

With the aim of determination of reaction order relative to each of reactants, the absorbance of the various solutions with different TCNE/C222 mole ratios were measured. The measurements were made two minutes after mixing the reactatns. Sample data due to trichloromethane are given in Tables 2 and 3, respectively. As it can be seen, at all temperatures and in both cases, the variation of absorbance is proportional to the variation of TCNE/C222 mole ratio or vice versa. Similar trend was observed in dichloroemethane. Based on the recent data it can be concluded that in both solvents a second order reaction in which the order of TCNE and C222 are 1 is followed.

The conductances as a function of C222/TCNE or TCNE/C222 in both solvents were measured. Considerable change was not observed. So it can be concluded that the adducts of TCNE and C222 in both solvents are nonionic.

III. Conclusions

Based on the obtained results it can be concluded that:

1. In both solvents 1:1 complexes are formed.
2. The stability of complexes in CHCl3 are higher than CH2Cl2.
3. The ϵ of complexes in CH2Cl2 are greater than CHCl3.
4. In both solvents, the $\Delta H°$ of complex formation are negative.
5. Because of hydrogen bonding between the solvent and reactants, the $\Delta S°$ $CHCl_3$ is positive.
6. At all temperatures, the reaction order relative to both of reactants is 1:1
7. The resulting adducts are nonionic.

References Références Referencias

1. C. J. Pedersen, J. Am. Chem. Soc., 89, 7017 (1967).
2. B. Dietrich, I. M. Lehn, and J. P. Sauvage, Tetrahedron Lett., 2885 (1969).
3. R. M. Izatt, J. S. Braclshaw, S. A. Nielson, J. D. Lamb, J. J. Christensen, and D. Sen, Chem. Rev. 85, 271 (1985).
4. R. M. Izatt, J. S. Bradshaw, K. Pawlak, R. L. Bruening & B. J. Tarbet, Chem. Rev. 92, 1261 (1992).
5. A. Semnani & M. Shamsipur, Spectrochim. Acta, 49A, 411 (1993).
6. A. Semnani & M. Shamsipur, J. Chem. Soc., Dalton Trans, 22, 15 (1996).
7. A. Semnani, H. R. Pouretedal, M. H. Keshavarz & A. R. Firooz, Polish J. Chem. 80, 2055 (2006).
8. A. Semnani, A. R. Firooz, H. R. Pouretedal, and M. H. Keshavarz, Chemistry, Vol. 19, No. 4, 1 (2009)
9. M. Javsadian, A. R. Firooz, A. Semnani, H. R. Pouretedal, and M. H. Keshavarz, Bull. Chem. Soc. Ethiop., Vol. 22, 2, 287 (2008).
10. A. Semnani, A. R. Firooz, M. H. Keshavarz and M. Oftadeh, Chemistry, Vol. 19, No. 3, 80 (2010)
11. R. Foster, "Organic Charge Transfer Complexes", Academic Press, London nd New York (1969).
12. M. T. Beck, I. Nagypal, "Chemistry of Complex Equilibria", John Wiley & Sons; NewYork (1990).
13. P. Job, Ann Chim. 9, 113 (1928).
14. P. Gans, "Data Fitting in the Chemical Sciences by the Method of Least Squares", Sohn Wiley & Sons; England (1992).
15. M. Quhn, J. Guckenheimer, B. R. Land, R. Harrs, A. S. Warrick, Nurophysiology, 94, 2883 (2005).
16. CRC hand book
17. R. S. Mulliken, "Molecular Complexes ", Wiley-Interscience, NewYork (1990).
18. C. E. Mortimer, "Chemistry", 7th ed., Wadsworth Publishing Company: NewYork (1986).

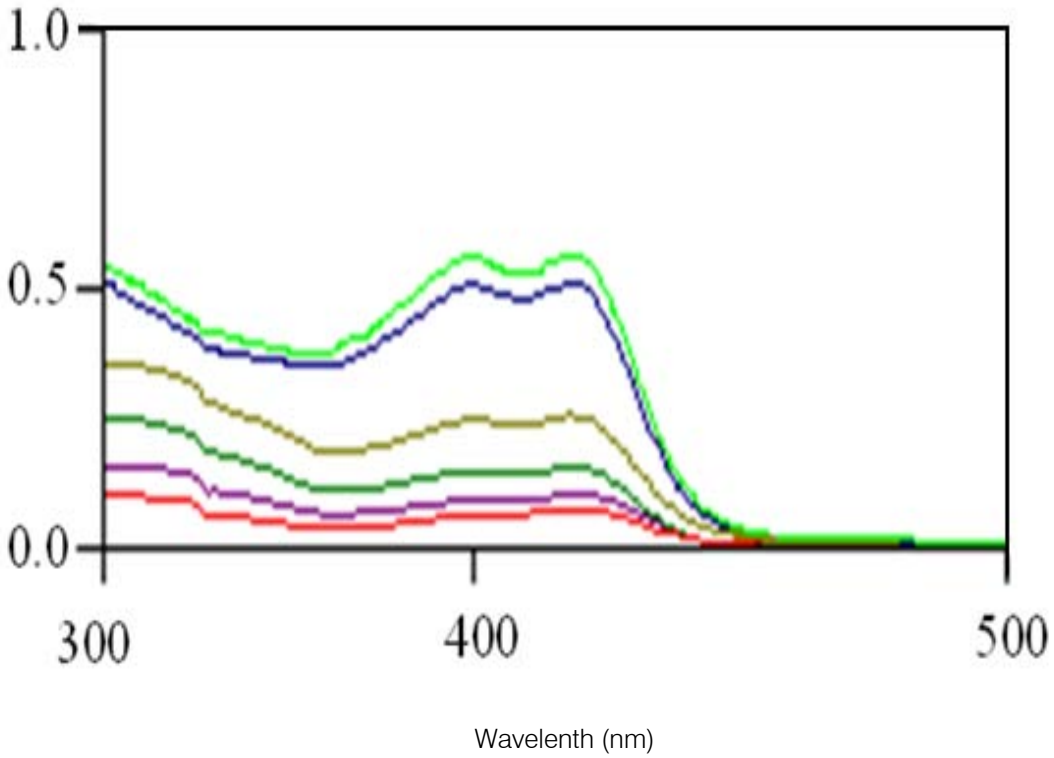


Fig.1 : Absorption spectra of 1.0×10-4 M C222 in the presence of varying concentration of TCNE at 20 0C. The ratio of TCNE to C222 from bottom to top are: 0.15, 0.25, 0.50, 1.00, 1.50, 2.5.

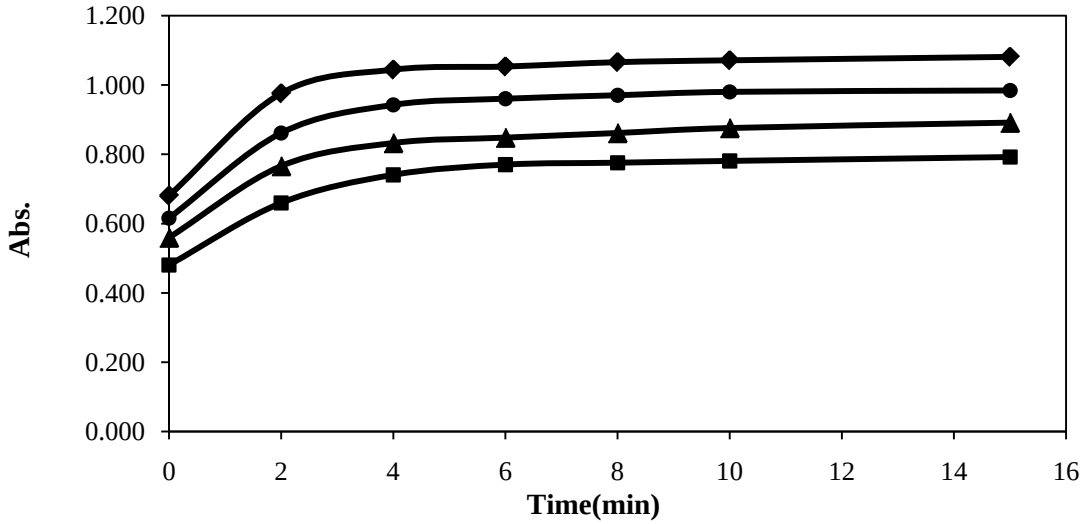


Fig. 2 : Plots of absorbance vs. time for 1.0× 10-4 M C222 in trichloromethane in different temperatures. From bottom to top: 5, 10, 15, and 20°c.

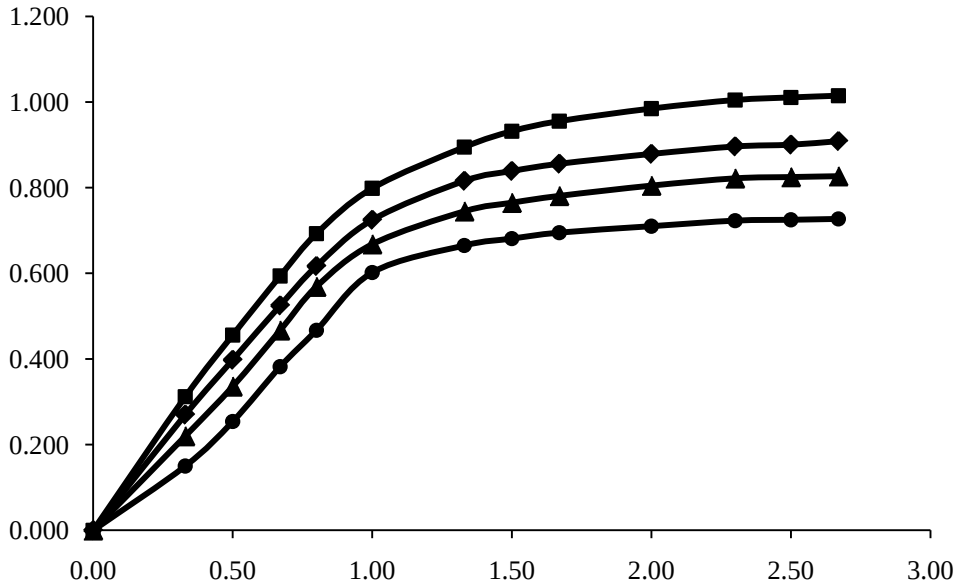


Fig.3 : Absorbance vs. mole ratio plots for 2.0×10-4 M C222 in dichloromethane solution at different temperatures. (■) 5 0C, (▲) 10 0C, (●) 150C and (♦) 20 0C.

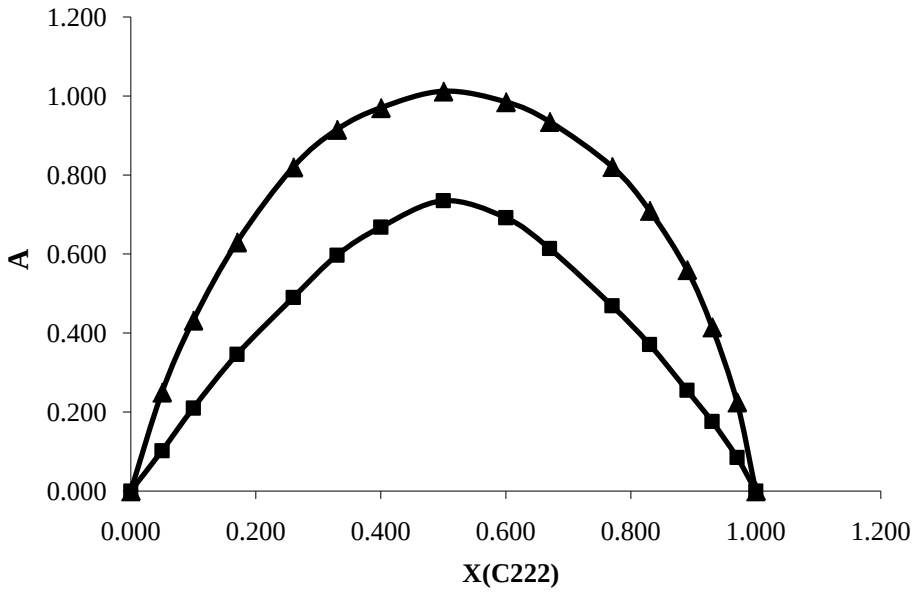


Fig.4 : Job plots at different temperatures in dichloromethane. The concentration of stock solutions and the final volume of each solution are 2.5×10-4 M and 3ml, respectively. (■) 5 0C, and (●) 20 0C.

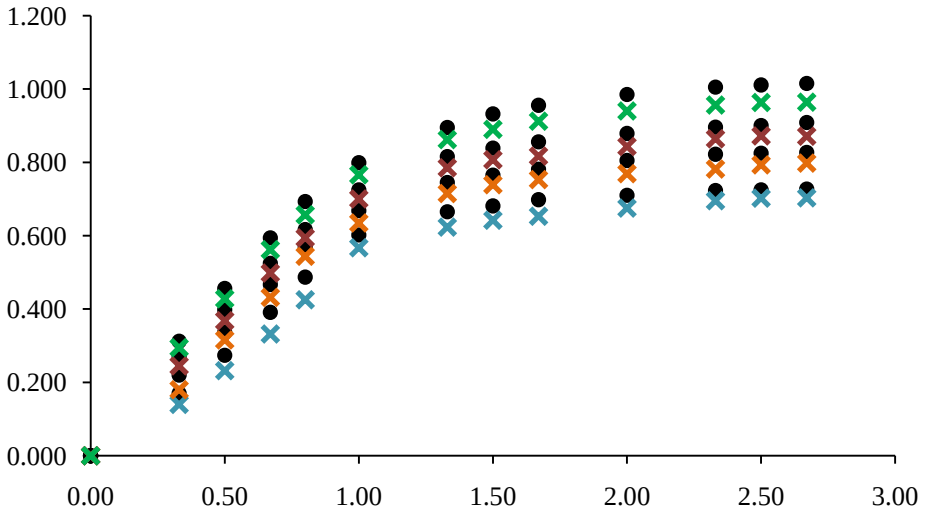


Fig.5 : Computer fitting of absorbance vs. mole ratio data indichloromethane at different temperatures; (●) experimental points and (×) calculated points.

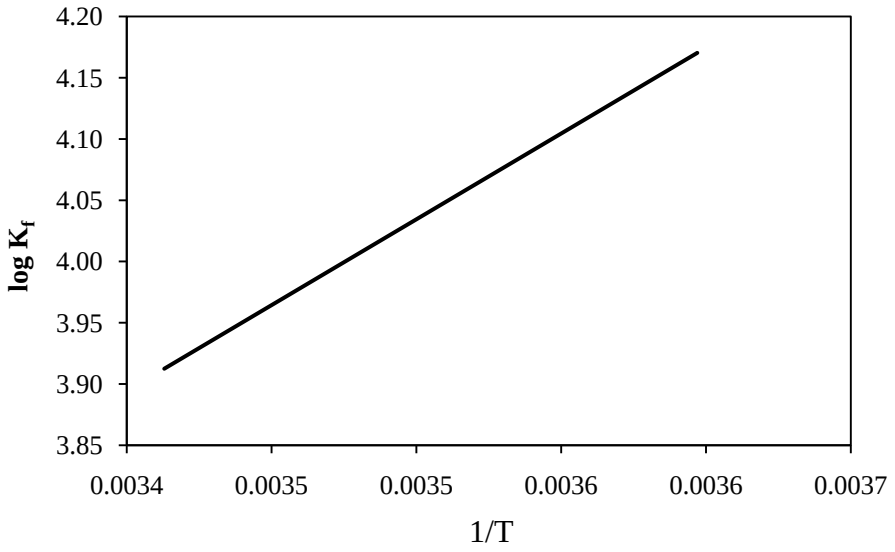


Fig.6 : The plot of log Kf vs. 1/T in dichloromethane solution.

Table 1 : Final log Kf and Є values at different temperatures in CH_2Cl_2 and $CHCl_3$

T	278	283	288	293
Log K_f in CH_2Cl_2	4.17±0.02	4.08±0.02	4.01±0.01	3.91±0.01
Log K_f in $CHCl_3$	6.08±0.04	5.99±0.01	5.91±0.02	5.84±0.01
Є in CH_2Cl_2	7947±238	8549±256	9088±273	1186±336
Є in CHCl3	3552±107	3927±117	4576±138	4912±147

Table 2 : Data due to determination of reaction order relative to TCNE in trichloromethane solution

TCNE/C222	Absorbance at 5°c	Absorbance at 10°c	Absorbance at 15°c	Absorbance at 20°c
0.33	0.095	0.143	0.211	0.287
0.67	0.191	0.228	0.422	0.577
1.00	0.290	0.432	0.639	0.867
1.33	0.383	0.575	0.850	1.142

Table 3 : Data due to determination of reaction order relative to C222 in trichloromethane solution

C222/TCNE	Absorbance at 5°c	Absorbance at 10°c	Absorbance at 15°c	Absorbance at 20°c
0.33	0.098	0.151	0.211	0.295
0.67	0.195	0.302	0.442	0.640
1.00	0.293	0.461	0.668	0.878
1.33	0.391	0.611	0.892	1.181

This page is intentionally left blank

Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Synthesis of Biologically Important Pyrrole Derivatives in Any ^{13}C and ^{15}N Isotope Enriched Form

By Prativa B. S. Dawadi & Johan Lugtenburg
Leiden University, Leiden

Abstract - Recently the synthesis of **[3-^{13}C]-**, **[4-^{13}C]-**, and **[11-^{13}C]-** porphobilinogen, **[^{15}N,^{13}C4]-1H** - pyrrole-**2,3,5 -** tricar - boxylic acid, [1-^{15}N]-3-cyano-4-methyl-1H-pyrrole and **[2-^{13}C]-** and **[3-^{13}C]-**cyano-**4-**methyl-**3-**pyrrolin-**2-**one have been published. Incorporation of **^{13}C** and **^{15}N** in these systems at any position and combination of positions has become accessible. Also mild alkylations of active methylene compounds with **α**-halo carbonyl compounds open up many 3-pyrrolin-2- ones and pyrrole systems based on stable isotope building blocks that have been published. This gives the access to a whole new library of stable isotope enriched pyrroles in any stable isotope enriched form. This is also the case for biliverdin **IXα** which after enzymatic treatment has been converted into **(2R)-**phytochromobilin that reacts with its apoprotein to form intact active phytochrome.

Keywords : [1-^{15}N]-3-Cyano-4-methyl-1H-pyrrole, [3-^{13}C], [4-^{13}C]-, and [11-^{13}C]-porphobilinogen, [^{15}N, ^{13}C4,] -1H-pyrrole-2,3,5-tricarboxylic acid and biliverdin IXα.

GJSFR-B Classification: FOR Code: 030503, 040203,

SYNTHESIS OF BIOLOGICALLY IMPORTANT PYRROLE DERIVATIVES IN ANY 13C AND 15N ISOTOPE ENRICHED FORM

Strictly as per the compliance and regulations of:

Synthesis of Biologically Important Pyrrole Derivatives in Any ^{13}C and ^{15}N Isotope Enriched Form

Prativa B. S. Dawadi[α] & Johan Lugtenburg[σ]

Abstract - Recently the synthesis of **[3-^{13}C]-, [4-^{13}C]-, and [11-^{13}C]-porphobilinogen, [^{15}N,$^{13}C_4$]-1H-pyrrole-2,3,5-tricarboxylic acid, [1-^{15}N]-3-cyano-4-methyl-1H-pyrrole and [2-^{13}C]- and [3-^{13}C]-cyano-4-methyl-3-pyrrolin-2-one** have been published. Incorporation of ^{13}C and ^{15}N in these systems at any position and combination of positions has become accessible.

Also mild alkylations of active methylene compounds with α-halo carbonyl compounds open up many 3-pyrrolin-2-ones and pyrrole systems based on stable isotope building blocks that have been published. This gives the access to a whole new library of stable isotope enriched pyrroles in any stable isotope enriched form. This is also the case for biliverdin **IXα** which after enzymatic treatment has been converted into **(2R)**-phytochromobilin that reacts with its apoprotein to form intact active phytochrome.

Keywords :[1-^{15}N]-3-Cyano-4-methyl-1H-pyrrole, [3-^{13}C], [4-^{13}C]-, and [11-^{13}C]-porphobilinogen, [^{15}N,$^{13}C_4$]-1H-pyrrole-2,3,5-tricarboxylic acid and biliverdin IXα.

I. Introduction

Pyrroles and their derivatives are one of the most important classes of heterocyclic compounds.[1] They exhibit extensive biological and pharmacological properties.[2] Many pyrrole derivatives have shown interesting biological properties such as antibacterial[3], antiinflammatory[4], antioxidant[5], antitumor, antifungal[6] and immune suppressant activities.[7] Highly functionalized pyrroles are subunits of heme, chlorophyll, bile pigments, vitamin B12 and pyrrole alkaloids isolated from marine source.[8] Atrovastatin (Lipitor) is a drug for lowering cholesterol.[9]

Access to stable isotope enriched systems (^{2}H, ^{13}C and ^{15}N) allows the metabolic conversions of these systems to be followed with mass spectroscopic techniques when they have at least three stable isotopes.[10] ^{13}C-NMR Techniques have been used to study the conversion of [5-^{13}C]-aminolevulinic acid into porphobilinogen in vivo in living Rhodobacter sphaerhoides cells.11

Similarly, the conversion of [2-^{13}C]- and [11-^{13}C]-porphobilinogen in the body into uroporphyrinogen III and coproporphyrinogen III has been investigated.[12] Very recently the ^{13}C photo-CIDNAP MAS NMR spectra of membrane fractions of Heliobacillus mobilis that was grown on media containing [4-^{13}C]-aminolevulinic acid have been obtained.[13]

Besides NMR spectroscopy, vibrational techniques such as resonance raman spectroscopy have been applied in heme protein research.[14] In this case some of the vibrations coupled to an electronic transition of the chromophore showed enhanced inelastic scattering up to 10^6 fold.

Access to pyrroles enriched on each position and any combination of positions with stable isotopes such as ^{2}H, ^{13}C and ^{15}N is essential to study the metabolism of important pyrrole derivatives using non-invasive isotope sensitive techniques.The chromophores of heme proteins and photosynthetic reaction centres have been prepared with stable isotope enriched pyrrole building blocks.

Recently, we have published a review paper about the stable isotope enriched systems in heme and (bacterio)chlorophyll protein systems that were known at that time.[15] In the meantime a number of important stable isotope enriched pyrrole systems have been published together with a new method to prepare pyrroles and stable isotope enriched building blocks that allow access to a whole new range of stable isotope enriched pyrroles.

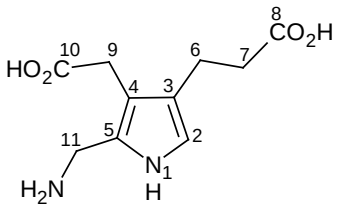
In this paper we focus on those new possibilities that allow access to biliverdin **IXα** which can be converted into (2R)-phytochromobilin, the chromophore of phytochrome via one enzymatic conversion.[16] We mainly focus on ^{13}C and ^{15}N enriched building blocks leading to the labels at all atoms in the molecular skeleton of the pyrroles and tetrapyrrole systems. We have not focused on ^{2}H systems because ^{2}H occupies the peripheral positions on the molecular system and is more prone to isotope loss and scrambling during the synthetic process. However, the schemes for ^{13}C incorporation can easily be adjusted to ^{2}H incorporation as well.

II. Synthesis And Discussion

a) *Synthesis Of [3-^{13}C]-, [4-^{13}C]- And [11-^{13}C]-Porphobilinogen 1.*

Enzymatic incorporation of [11-^{13}C]- and [2,11-$^{13}C_2$]-porphobilinogen 1 (fig. 1) into uroporphyrinogen I and III has been reported.[17,18]

Author α σ : Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA, Leiden. E-mail : p.b.s.dawadi@gmail.com



1

1a: [4-^{13}C]

1b: [3-^{13}C]

1c: [11-^{13}C]

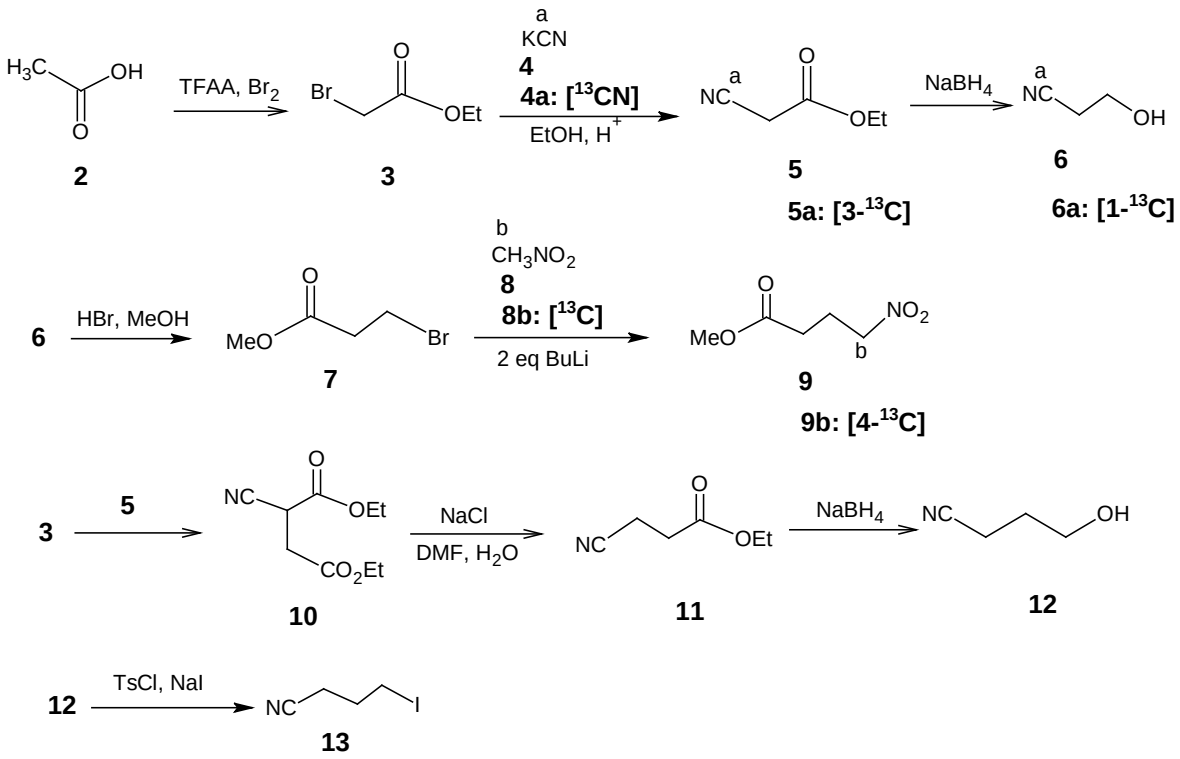
Figure 1 : Structure and numbering of porphobilinogen 1 and its highly enriched isotopomers 1a, 1b and 1c.

Porphobilinogen 1 is a biosynthetic precursor of tetrapyrrole chromophores in heme proteins, photosynthetic antennae proteins, photosynthetic reaction centres and phytochromes. The synthetic access to ^{13}C and ^{15}N enriched porphobilinogen will allow access to enrich stable isotopes in the above mentioned systems at any possible position. With ^{13}C and ^{15}N isotope incorporation in the chromophores of these biologically important proteins can be investigated with noninvasive isotope sensitive techniques.

In figure 1 the structure and numbering of porphobilinogen 1 is depicted. The synthesis of [3-^{13}C]-, [4-^{13}C]- and [11-^{13}C]-porphobilinogen 1a, 1b and 1c via a scheme that allows access to any stable isotopomer and isotopologue has been reported.[19]

Acetic acid 2 is treated with 1 eq of bromine in the presence of trifluoroacetic anhydride to afford a high yield of the 2-bromoacetic acid which is esterified with ethanol into ethyl bromoacetate 3. The bromine is easily substituted for the cyano group with KCN 4 to give ethyl cyanoacetate 5. The ester function is reduced with $NaBH_4$ to give an alcohol function in 3-hydroxypropionitrile 6. Treatment of 6 with aqueous HBr and subsequent esterification afforded methyl 3-bromopropionate 7 in high yield. The S_N2 reaction of reagent 7 with nitromethane 8 in the presence of 2 eq BuLi to obtain methyl 4-nitrobutanoate 9 is somewhat difficult.

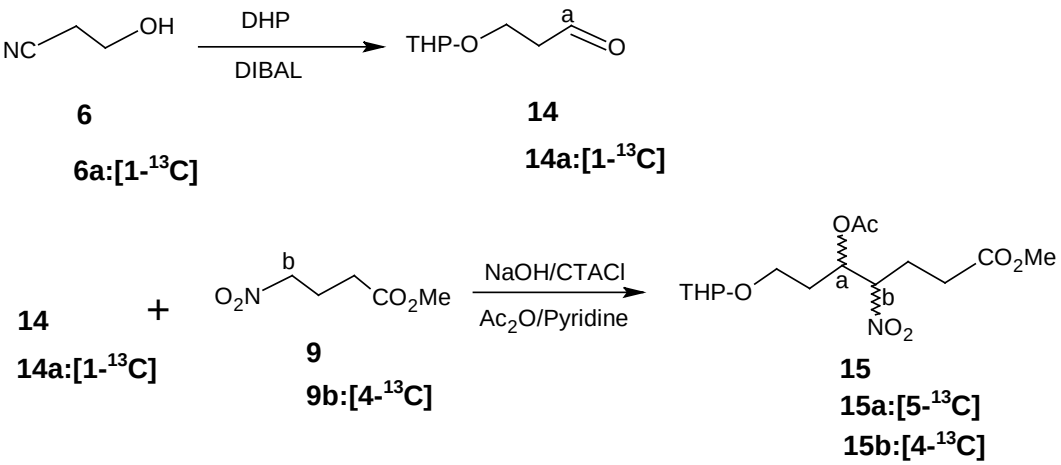
An alternative method to obtain the product 9 is to treat reagents 3 and 5 in the presence of NaOEt to afford diethyl 2-cyanopentanedioate 10.[20] Selective removal of one of the ester functions in NaCl, DMSO, H_2O gave ethyl 3-cyanopropionate 11. Subsequent reduction of the ester function with NaBH4 afforded 4-hydroxybutyronitrile 12 which is further converted into 4-iodobutyronitrile 13. S_N2 substitution of the iodo function with $NaNO_2$ and subsequent conversion of the nitrile function into ethyl carboxylate is expected to give ethyl 4-nitrobutanoate 9 without problem. In porphobilinogen 1 (fig. 1) the carbon atoms 3, 6, 7 and 8 are derived from the compound 9 and carbon atoms 4, 9 and 10 are derived from 3-hydroxypropionitrile 6.



Scheme 1. The synthesis of ethyl [4-^{13}C]-4-nitrobutyrate 9b. The starting compound acetic acid 2 is commercially available in the [1-^{13}C]-, [2-^{13}C]- and [1, 2-$^{13}C_2$] isotopomeric forms.

3-Hydroxypropionitrile 6 is first protected with dihydropyrane via acid catalyzed reaction to afford 3-(tetrahydropyran-2'-yloxy)-propionitrile (scheme 2).[19] DIBAL-H reduction of the nitrile afforded the required 3-hydroxypropanal derivative 14. Henry reaction between the nitro ester 9 and the aldehyde 14 in the presence of a phase transition catalyst and acetylation afforded the nitro derivative 15.

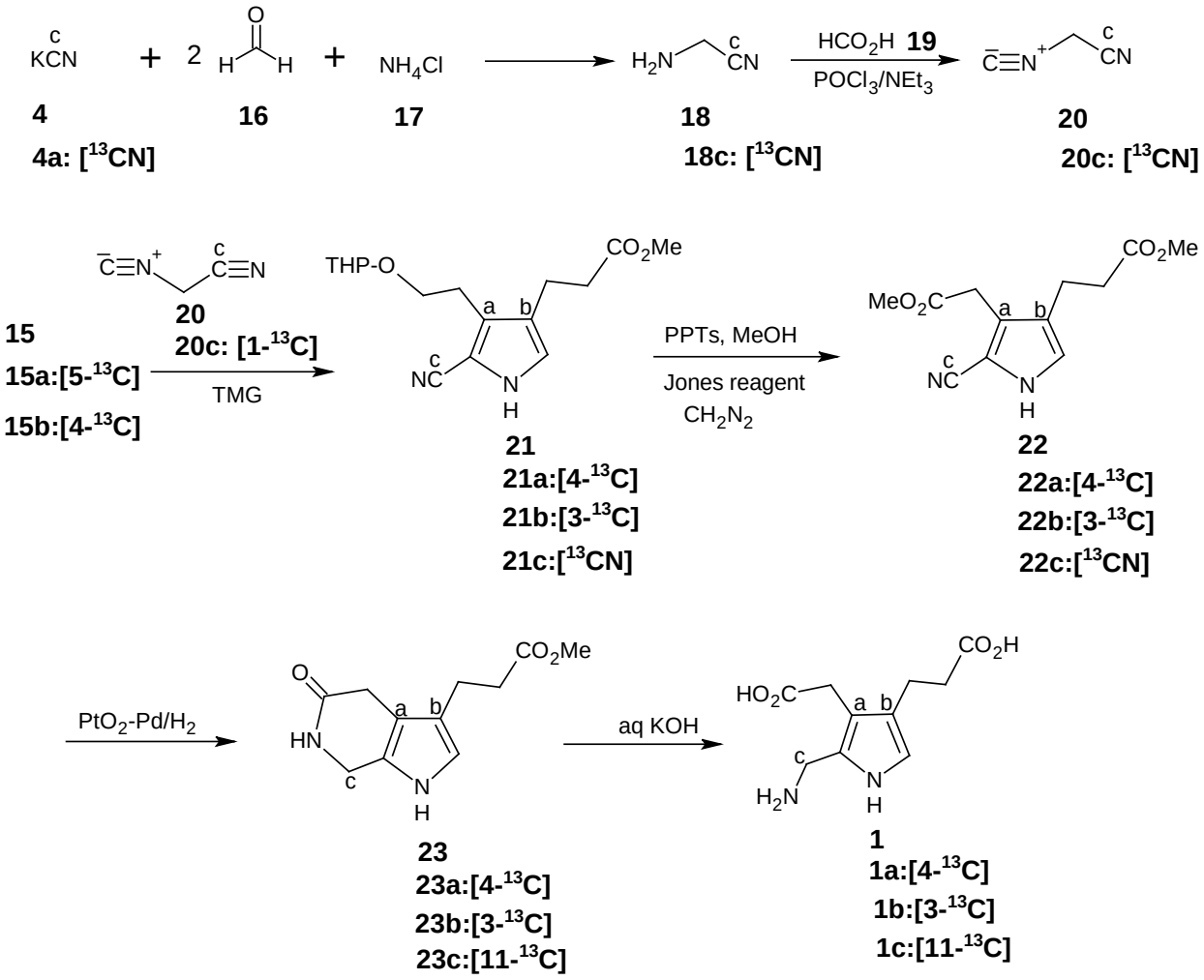
Isocyanoacetonitrile 20 is the building block that provides carbon atoms 2, 5 and 11 and two nitrogen atoms of porphobilinogen 1. It is shown that in scheme 3 a Strecker reaction of KCN 4, formaldehyde 16 and NH_4Cl 17 leads to 2-aminoacetonitrile 18. This molecule reacted with formic acid 19 in acetic anhydride to give the formyl derivative of 2-aminoacetonitrile that upon treatment with $POCl_3$ and triethylamine afforded 2-isocyanoacetonitrile 20. The base (tetramethylguanidine) induced the reaction between compounds 15 and 20 to give 5-cyano-3-(methoxycarbonylethyl)-4 (tetrahydro-pyran-2'-yl-ethyl)-pyrrole 21. The tetrahydropyranyl protective group is removed by acid to obtain the primary alcohol. The alcohol function is converted into the carboxylic acid by Jones oxidation and this acid is subsequently converted into the methyl ester 22. The nitrile and methyl ester functions are catalytically reduced to give an amide function in product 23. Base treatment converted the ester and amide functions into carboxylic acid and a methylene amine function in porphobilinogen 1. The conversion of products 15 and 20 into the pyrrole 21 is a so-called Barton-Zard reacton.[21]



Scheme 2. 3-Hydroxypropionitrile 6 is converted into methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2'-yloxy)-heptanoate 15, 15a and 15b.

The synthetic routes shown in schemes 1-3 have been developed for the synthesis of [3-^{13}C]-, [4-^{13}C]- and [11-^{13}C]-porphobilinogen 1. It is clear that based on commercially available ^{13}C and ^{15}N isotope enriched starting materials any carbon and nitrogen with stable isotope enrichment are accessible in porphobilinogen 1. Schemes 1 and 3 can be modified in such a way that a whole series of vicinal acetoxy and nitro derivatives can be made in the required stable isotope enriched form.

Tertiary butyl isocyanoacetate has been used to prepare pyrroles with methyl carboxylate groups on the 2-position.[22] This building block is accessible in any stable isotope enriched form via reactions analogous to those described in scheme 3. Tosylmethyl isocyanide in all possible stable isotopically labelled forms has been used to prepare pyrroles and other heterocyclic systems via building blocks that can be easily prepared in stable isotope enriched form.[23]



Scheme 3. Preparation of 2-aminoacetonitrile 18 and its conversion into isocyanoacetonitrile 20. Synthesis of [4-^{13}C]-porphobilinogen 1a, [3-^{13}C]-porphobilinogen 1b and [11-^{13}C]-porphobilinogen 1c via base catalyzed condensation of methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2'-yloxy)-heptanoate 15 and isocyanoacetonitrile 20.

b) Synthesis Of [1-^{15}N]- Formyl-4-Methyl-1H-Pyrrole 24a

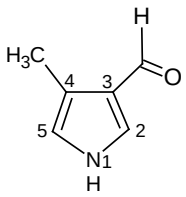
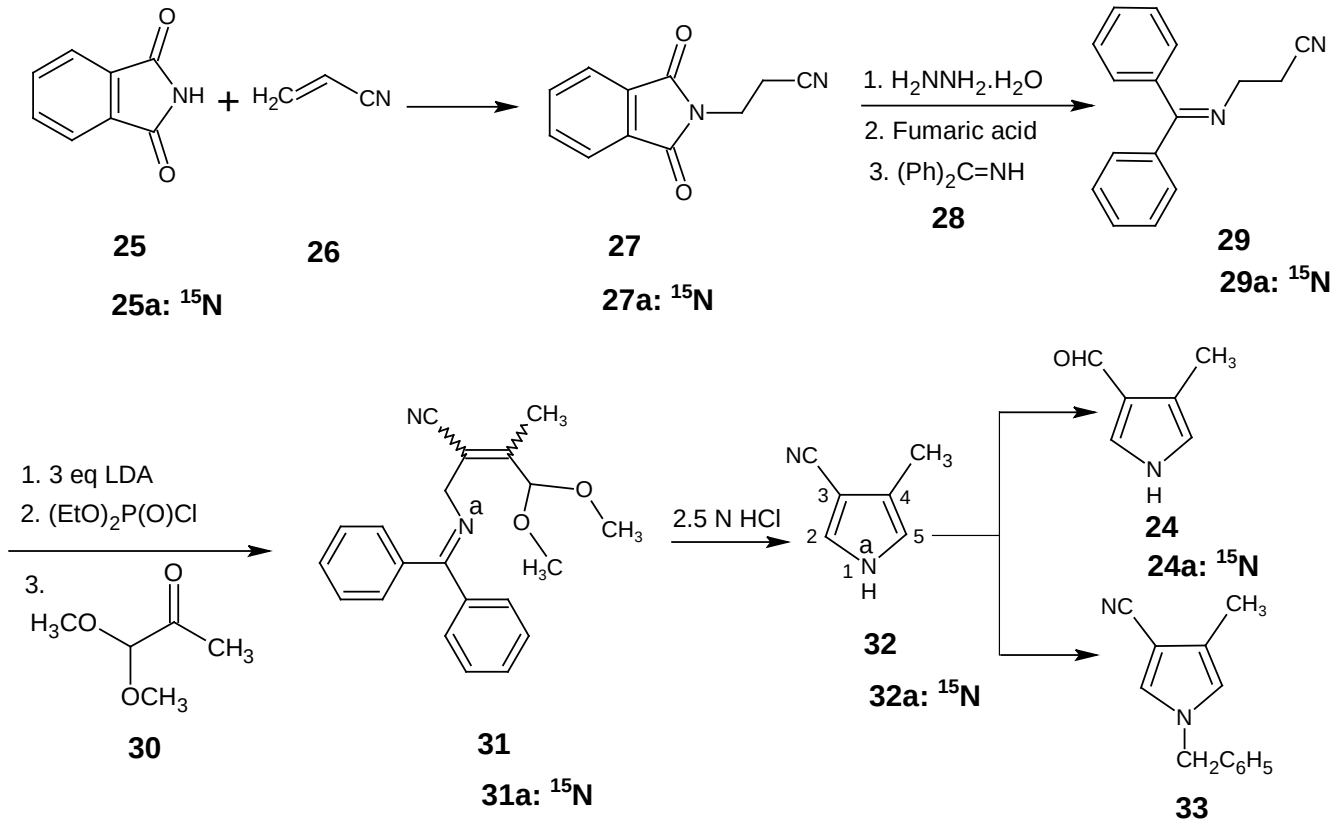


Figure 2. Structure and numbering of [1-^{15}N]-3-formyl-4-methyl-1H-pyrrole 24a.

This pyrrole is a building block for the biologically important tetrapyrrole systems. In Scheme 4 it is indicated that phthalimide 25 treated with acrylonitrile 26 to form β-phthalimidopropionitrile 27. ^{15}N-Phthalimide 25a is commercially available. All possible isotopologues of acrylonitrile are accessible via isotopically enriched 3-hydroxypropionitrile 6 (scheme 1).[24]



Scheme 4. Reactions to prepare [1-^{15}N]-3-formyl-4-methyl-1H-pyrrole 24a starting from[^{15}N]-phthalimide 25a and acrylonitrile 26.

The product 3-[(diphenylmethylene) amino] propionitrile 29 is prepared by first treating β-phthalimidopropionitrile 27 with hydrazine hydrate, then treating the mixture with fumaric acid to obtain the fumaric acid salt of 3-aminopropionitrile followed by reaction with benzophenone imine 28. Product 29 is treated with 3 eq LDA at low temperature and subsequently diethyl chlorophosphate is added followed by 1,1-dimethoxyacetone 30. After the Wittig-Horner reaction compound 31 is isolated as a mixture of E-and Z-forms. In a solution of 2.5 N HCl the acetal and the nitrogen protection groups are removed and the free amino group and aldehyde group reacted to give a high yield of 3-cyano-4-methyl-1H-pyrrole 32. DIBAL-H reduction of the nitrile function in the product 32 afforded 3-formyl-4-methyl-1H-pyrrole 24. Repeating this reaction with commercially available [^{15}N]-phthalimide 25a afforded [1-^{15}N]-3-formyl-4-methyl-1H-pyrrole 24a.

As expected the NH group in the product 32 can be easily alkylated under basic condition. In this case benzyl bromide has been used to obtain N-benzyl-3-cyano-4-methylpyrrole 33 in high yield.

1,1-Dimethoxyacetone 30 or its homologous in any isotope enriched form is easily accessible via a Pummerer reaction of 1-phenyl sulfoxyl acetone.[25] Also many homologous of acrylonitrile are easily accessible. This means synthetic routes shown in scheme 4 will give access to many isotopically labelled pyrroles directly via subsequent functional group transformations.

c) Synthesis Of [1-^{15}N, 2, 2', 3, 3'-^{13}C4]-Pyrrole-2,3,5-Tricarboxylic Acid 34a.

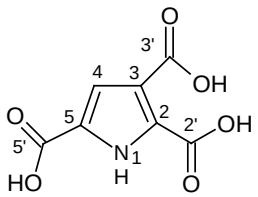
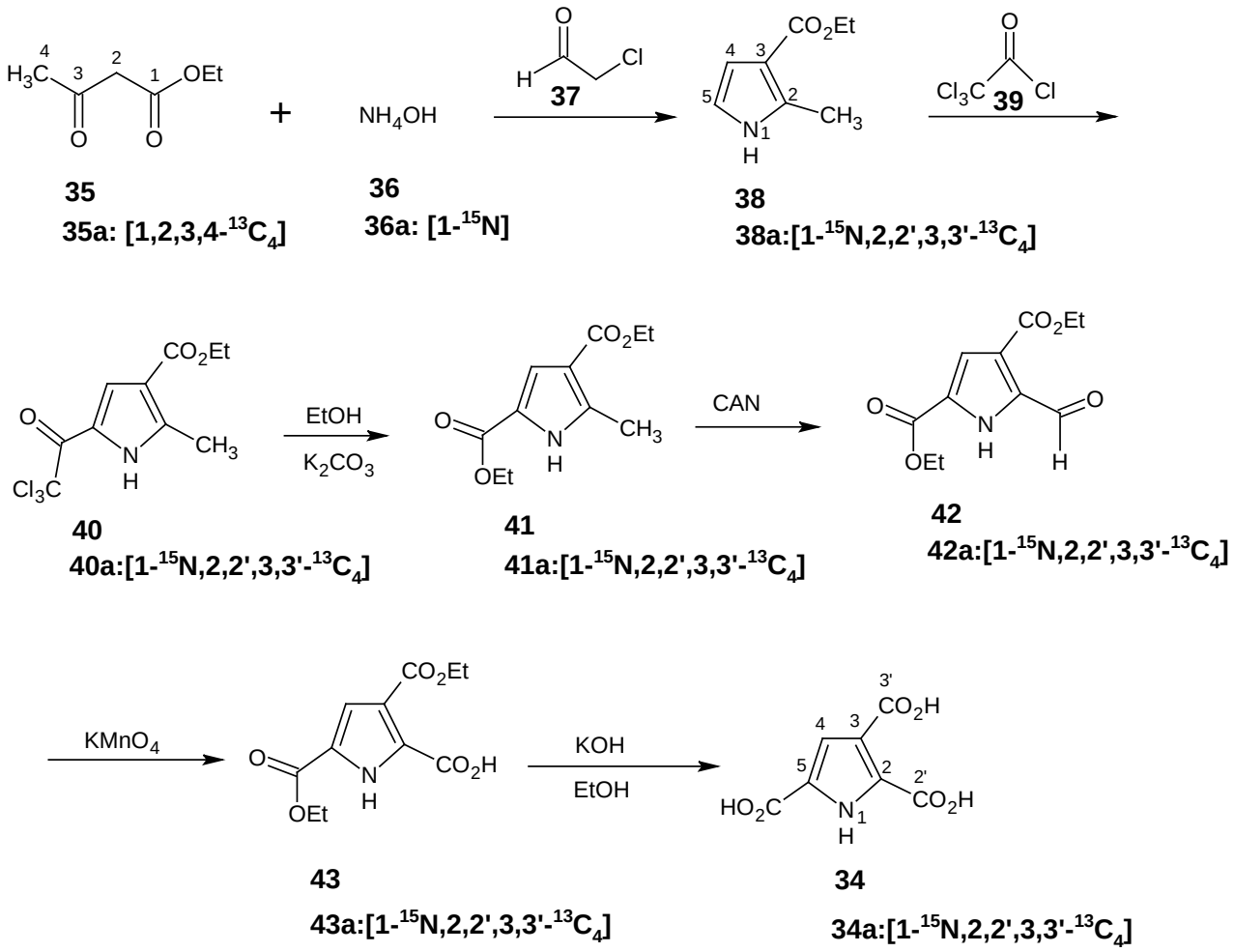


Figure 3. Structure and numbering of [1-^{15}N, 2, 2', 3, 3'-^{13}C4]-pyrrole-2,3,5-tricarboxylic acid 34a.

The synthesis of the product 34a has been reported.[10] Commercially available [^{13}C4]-ethyl acetoacetate 35a is treated with commercially available [^{15}N]-ammonia in water in the presence of 2-chloroacetaldehyde 37. [1-^{15}N, 2,2',3,3'-^{13}C4]-2-Methyl-3-carbethoxypyrrole 38a is obtained via Hantzsch pyrrole synthesis in 35% yield which is in competition with a Feist-Benary reaction leading after HCl treatment to [2,2',3,3'-$^{13}C_4$]-2-methyl-3-carbethoxyfuran. In the Hantzsch pyrrole synthesis the enamine of the 3-keto carboxylate attacks the aldehyde function of the 2-chloro keto molecule. After ring closure and dehydration the pyrrole system is obtained. In the Fiest-Benary reaction the initial reaction is the attack of the anion of the active methylene derivative on the aldehyde function of chloroacetaldehyde and subsequent ring closure.[25]

Product 38a is treated with trichloroacetyl chloride 39 to give a quantitative yield of trichloromethyl keto compound 2-methyl-3-carbethoxy pyrrole 40a. Treatment with ethanol in the presence of potassium carbonate afforded the diester 41a, upon cerium ammonium nitrate oxidation the 2-methyl group is converted into an aldehyde function (compound 42a).

Treatment with potassium permanganate oxidized the aldehyde function into a carboxylic acid function 43a. A final base induced saponification afforded [1-^{15}N, 2,2',3,3'-^{13}C4]-pyrrole-2,3,5-tricarboxylic acid 34a.



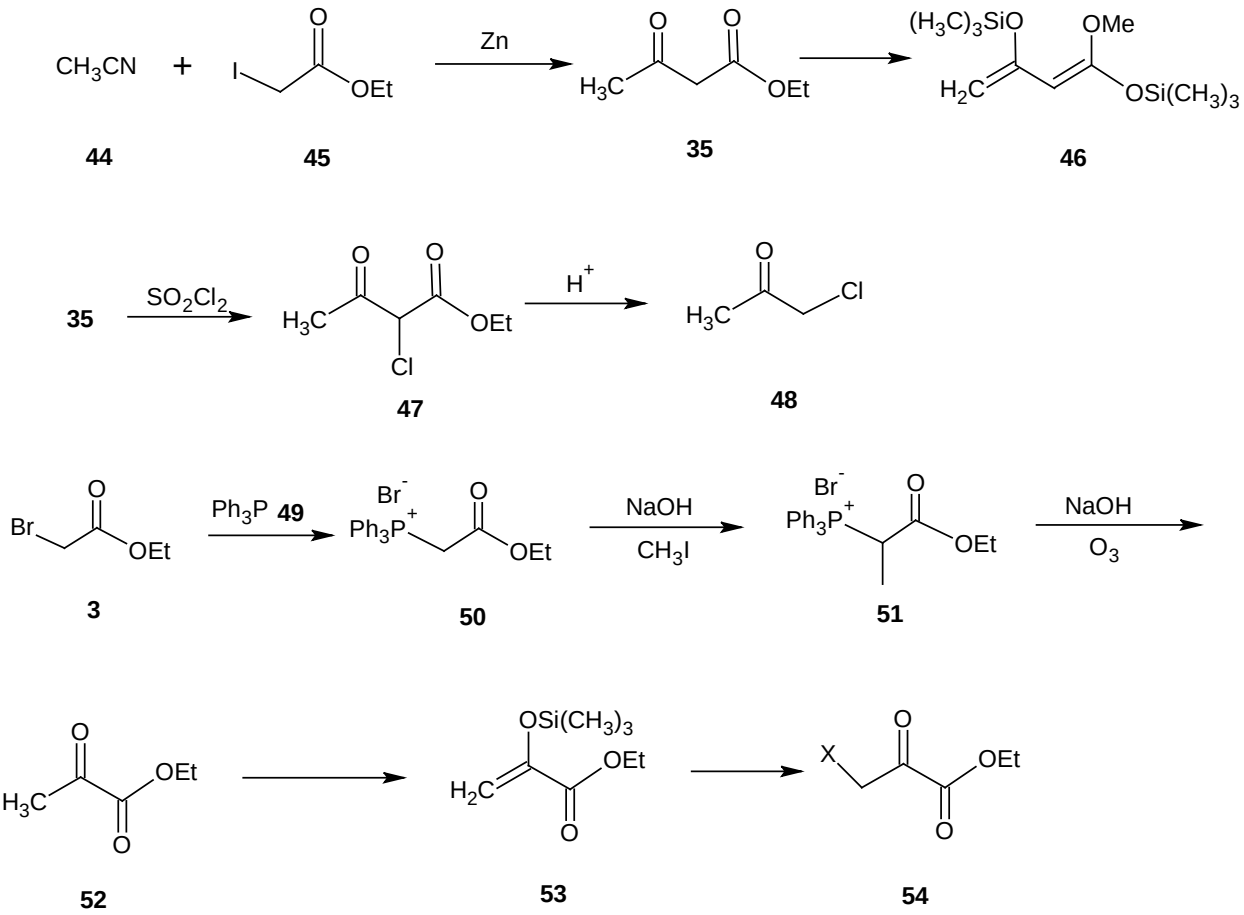
Scheme 5. Synthesis of [1-^{15}N, 2,2',3,3'-^{13}C$_4$]-pyrrole-2,3,5-tricarboxylic acid 34a.

Pyrroles 38a, 40a, 41a, 42a and 43a in scheme 5 have isotope incorporation in ^{15}N on position 1 and ^{13}C$_4$ incorporation on positions 2, 2', 3 and 3'. Via scheme 5 many pyrrole systems can be enriched besides the positions 2, 2', 3 and 3'.

α-Halogenated aldehydes such as 2-chloroacetaldehydes are accessible. Aldehydes that can be easily obtained from stable isotope enriched nitrile esters and alcohol etc.[27]

A general method to prepare the corresponding α-chloroderivatives has been reported.[28] Many α-amino acids are commercially available in stable isotope enriched form. Recently, an efficient method to convert them into 2-chloroaldehydes has been reported.[29] Trichloroacetic acid is commercially available in ^{13}C-enriched form; it can be easily converted into the corresponding chloride.[30]

The Blaise reaction of acetonitrile 44 with zinc enolate of ethyl iodoacetate 45 has been reported.[31] Acetonitrile 44 is commercially available in all possible isotopomers. Zinc derivative of 45 is accessible via ethyl 2-bromoacetate 3 (scheme 1). Many other nitriles and α-bromoesters are accessible in all possible stable isotope enriched forms. Trimethyl silylation of 35 gives the bis(trimethylsilyloxy)butadiene derivatives 46. Treatment with 1 mol of bromine afforded methyl 4-bromo-3-ketobutyrate.[32] This means that besides ethyl acetoacetate 35 many 3-ketoesters are accessible.



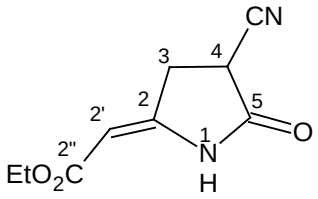
Scheme 6. Preparation of ethyl acetoacetate 35 and ethyl 2-chloro-3-oxobutyrate 47 in any isotopomeric form using acetonitrile 44 and ethyl iodoacetate 45.

Ethyl acetoacetate 35 can be easily monochlorinated by treatment with sulphuryl chloride. Subsequent acid catalyzed hydrolysis and carbon dioxide elimination results in 1-chloroacetone 48. Similarly, many monochloroketones will be accessible in any stable isotope labelled form.

Ethyl bromoacetate 3 treated with triphenyl-phosphine 49 to form triphenyl phosphonium salt 50. After addition of 1eq NaOH and subsequent treatment with CH_3I, the propionate phosphonium salt 51 is formed. Further treatment with base and ozonolysis ethyl pyruvate 52 is formed.[33]

Ethyl pyruvate 52 can be converted in the corresponding trimethyl silyl ether 53.[34] Halogenation of the product 53 afforded the 3-halogenopyruvate 54. Using the reactions discussed in scheme 5 together with the building blocks given in scheme 6 it is clear that a very extended range of pyrroles in all possible stable isotopomeric forms are now accessible.

d) Synthesis Of Ethyl (2Z)-(4-Cyano-5-Oxopyrrolidin-2-Ylidene)Ethanoate 55.



55

Figure 4. Structure and numbering of ethyl (2Z)-(4-cyano-5-oxopyrrolidin-2-ylidene)ethanoate 55.

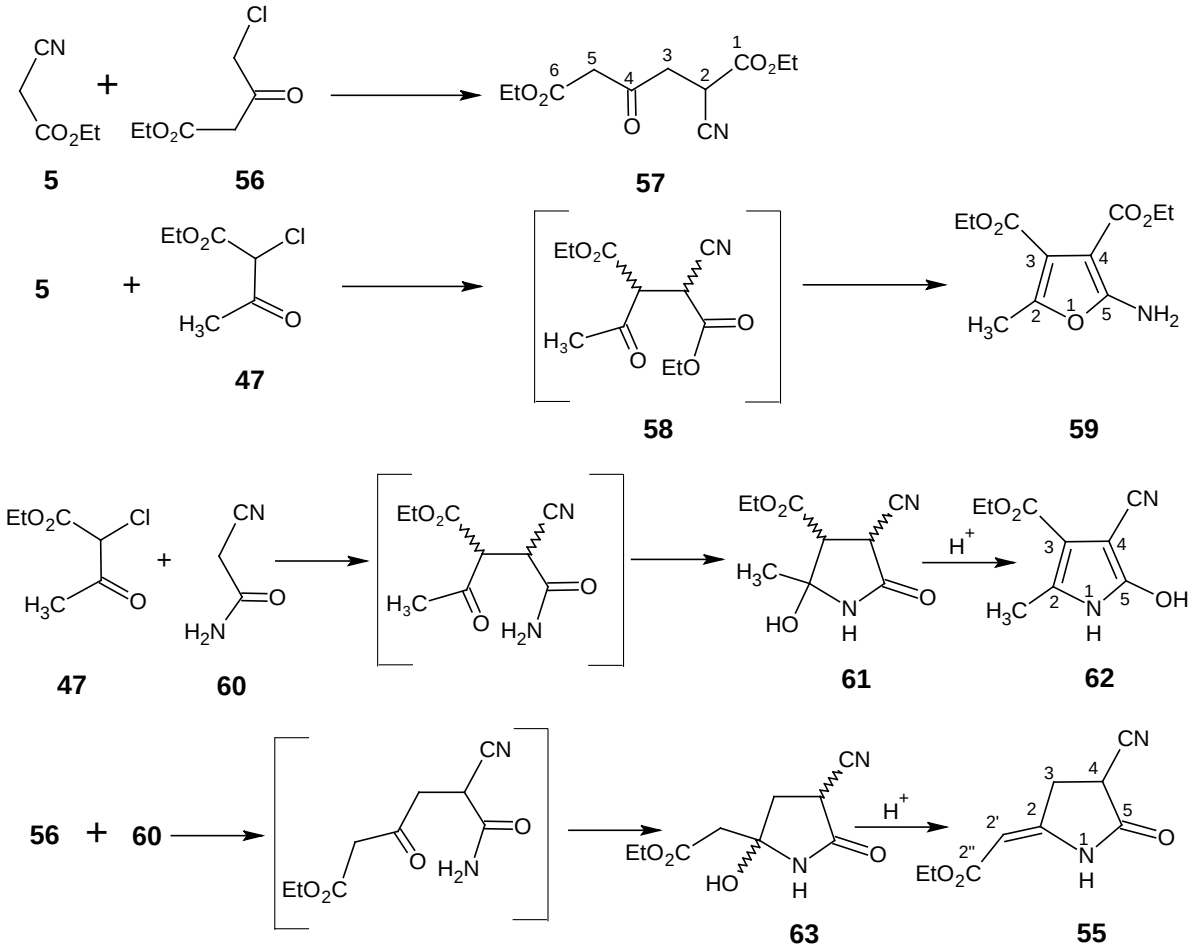
Ethyl cyanoacetate 5 is treated with ethyl 4-chloro-3-oxobutyrate 56 (prepared by chlorination of 46 in scheme 6) in the presence of 1 eq of triethylamine in refluxing toluene (scheme 7).[35] Diethyl 2-cyano-4-oxohexanedioate 57 is the single product in high yield. This product is the result of an S_N2 reaction of the anion of the active methylene compound without competing Feist-Benary reaction in the Hantzsch pyrrole synthesis because the nonnucleophilic base triethylamine cannot give the Hantzsch pyrrole system.

A similar reaction between ethyl cyanoacetate 5 and ethyl 2-chloro-3-oxobutyrate 47 afforded 2-amino-3,4-dicarbethoxy-5-methylfuran 59 in high yield (scheme 7). This molecule has been described in the literature.[36,37] In this case the S_N2 reaction of the anion of ethyl cyanoacetate 5 must have been the first step followed by a base catalyzed cyclization to the furan derivative 59.

It is to be expected that triethylamine induced alkylations of active methylene derivatives with aldehyde or keto functions in both the chloride reagent and the active methylene compound will give 1,4-dicarbonyl systems in Paal-Knorr pyrrole, Paal-Knorr furan and Paal-Knorr thiophene syntheses in high yield.[38]

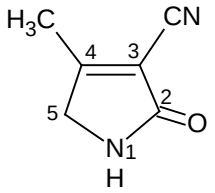
2-Cyanoacetamide 60 is expected to react with ethyl 2-chloro-3-oxobutyrate 47 using triethylamine as a base to give the initial S_N2 reaction on the active methylene carbon without reaction on the amide function due to its higher pKa value.

The carbonyl group and the amide group cyclised to give compound 61. Acid catalyzed dehydration afforded 2-methyl-3-ethoxycarbonyl-4-cyano-5-hydroxypyrrole 62.[35] Similarly, ethyl 4-chloro-3-oxoacetate 56 and 2-cyanoacetamide 60 afforded a high yield of the cyclic derivative 63. Product 55 is obtained via acid catalyzed dehydration of product 63. These 2-oxypyrrole derivatives have important pharmaceutical and biological properties.



Scheme 7. Preparation of ethyl (2Z)-(4-cyano-5-oxopyrrolidin-2-ylidene)ethanoate 55.

e) Synthesis Of [2-^{13}C]-3-Cyano-4-Methyl-3-Pyrrolin-2-One 64a And [3-^{13}C]-3-Cyano-4-Methyl-3-Pyrrolin-2-One 64b.



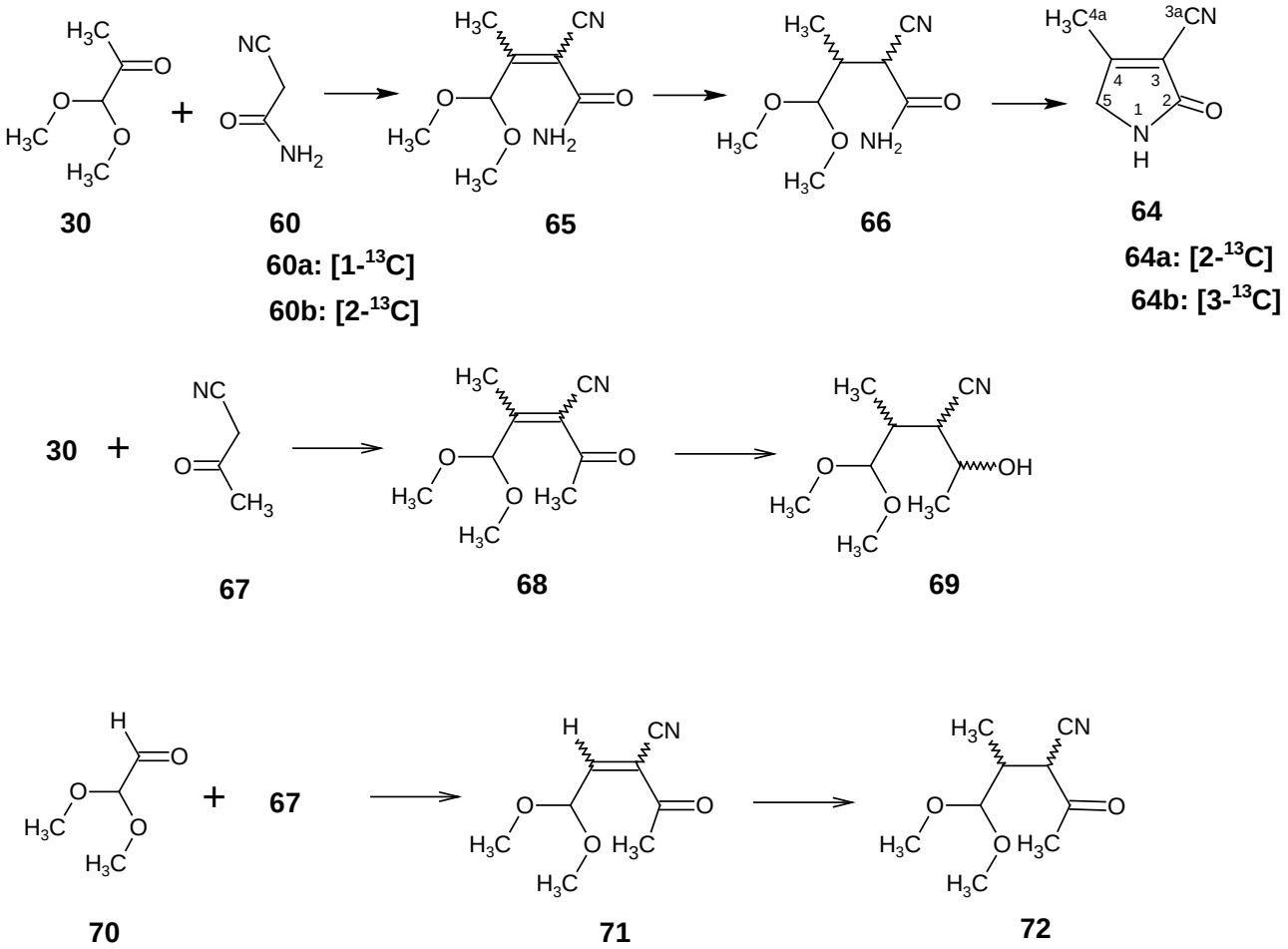
64

64a: [2-^{13}C]

64b: [3-^{13}C]

Figure 5. Structure and numbering of [2-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64a and [3-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64b.

Even earlier than the above discussed alkylation of active methylene reagents, the Knoevenagel reaction between active methylene compounds, ketones and aldehydes to form [2-^{13}C]- and [3-^{13}C]-3-cyano-4-methyl-3-pyrrolin-3-one 64a and 64b, respectively (fig. 5) has been reported.[39] In scheme 8 it is depicted that 1,1-dimethoxyacetome 30 and 2-cyanoacetamide 60 in refluxing toluene in the presence of ammonium acetate and acetic acid afforded a high yield of a mixture of (E)- and (Z)-2-cyano-3-methyl-4,4-dimethoxybut-2-enamide 65. Due to the presence of two electron withdrawing groups in the molecule the acetal function is relatively acid stable. The double bond can easily be reduced by sodium borohydride in ethanol to form the enantiomeric mixtures of 2-cyano-3-methyl-4,4-dimethoxybutanamide 66.



30 **60** **65** **66** **64**

60a: [1-^{13}C]

60b: [2-^{13}C]

64a: [2-^{13}C]

64b: [3-^{13}C]

30 **67** **68** **69**

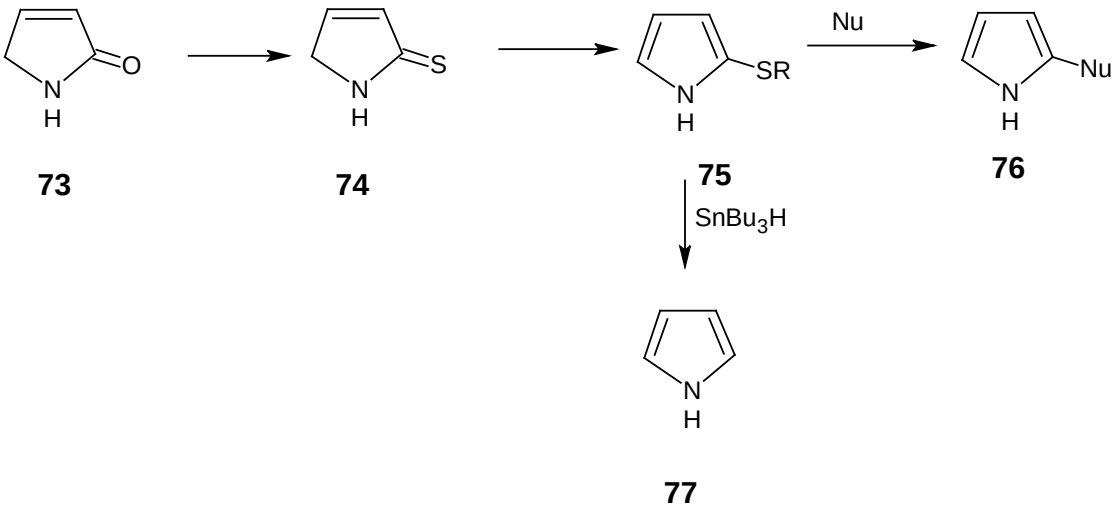
70 **67** **71** **72**

Scheme 8. The preparation of [2-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64a and [3-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64b from 1,1-dimethoxyacetone 30 and [1-^{13}C]-2-cyanoacetamide 60a and [2-^{13}C]-2-cyanoacetamide 60b, respectively.

Upon mild acid treatment of (E)- and (Z)- 2-cyano-3-methyl-4,4-dimethoxybutanamide 66 afforded 3-cyano-4-methyl-3-pyrrolin-2-one 64 in a high yield. Using [1-^{13}C]-2-cyanoacetamide 60a and [2-^{13}C]-2-cyanoacetamide 60b, [2-^{13}C]-64a and [3-^{13}C]-64b have been prepared, respectively. In order to get to 3-vinyl-4-methyl-3-pyrrolin-2-one 89 for the ring D of biliverdin IXα and phytochromobilin (vide infra), 1,1-dimethoxyacetone 30 is treated with 1-cyanoacetone 67 under Knoevenagel conditions to give an excellent yield of 5,5-dimethoxy-4-methyl-3-cyanopentan-2-one 68 (scheme 8). The reduction of the 3,4 double bond and keto function occurred simultaneously to give an enantiomeric mixture of 5,5-dimethoxy-4-methyl-3-cyanopentan-2-ol 69. Pinner reaction of 69 in aqueous media to convert the nitrile function into the amide function and subsequent ring closure to get 3-(1'-hydroxyethyl)-4-methyl-3-pyrrolin-2-one could not be realized.

As an alternative the Knoevenagel condensation between 1,1-dimethoxyacetaldehyde 70 and 1-cyanoacetone 67 afforded a high yield of 5,5-dimethoxy-3-cyanopent-3-ene-2-one 71. Treatment of 71 with methyl magnesium iodide and cuprous cyanide gave in a high yield of the 1,4-addition product 5,5-dimethoxy-4-methyl-3-cyanopentan-2-one 72. Conversion of product 72 to the required 3-pyrrolin-2-one was not successful. However, 3-pyrrolin-2-ones are now accessible via alkylation reactions of active methylene compounds with amide functions.

Compound 72 (a protected 1,4-dicarbonyl compound) can easily be converted in the corresponding pyrrole, thiophene and furan systems.[25] The scope of the Knoevenagel reaction has been extended.[40] At present various 3-pyrrolin-2-ones are easily available. This is a new approach to pyrrole synthesis. These systems react with Lawesson's reagent to give the corresponding thioamide system 74.[25]



73 **74** **75** **76** **77**

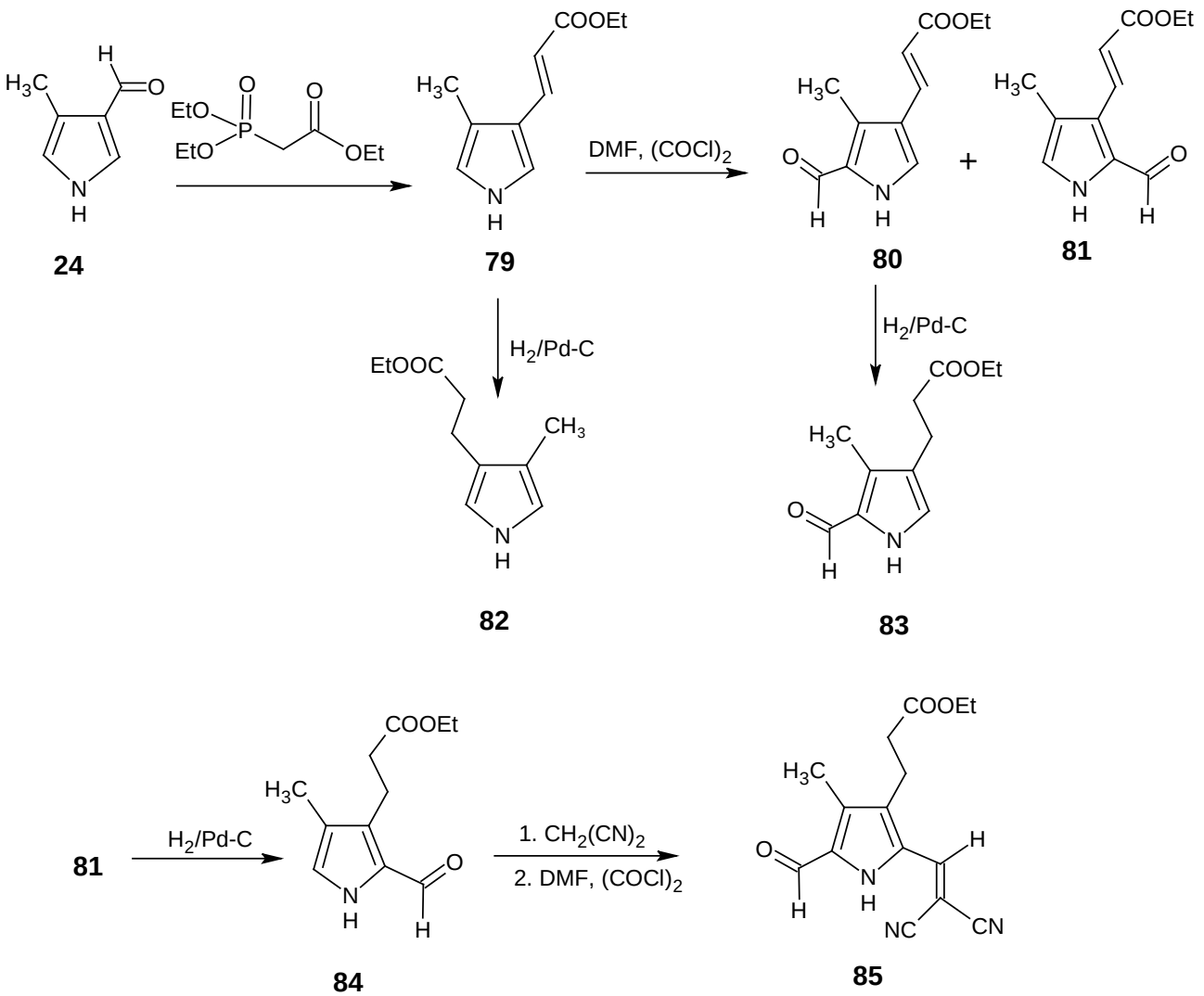
Scheme 9. Conversion of 3-pyrrolin-2-one 73 into 2-substituted pyrrole 76 and pyrrole 77.

The thioamide treated with various alkylhalo - genides to form the 2-thioalkyl substituted pyrroles. The 2-thioalky is easily substituted for many other 2-substituents via various nucleophiles.[41] The thioalkyl group has been substituted for hydrogen via radical reaction with tributyltin hydride to give the pyrrole without a substituent on position 2.[42]

f) Chemoenzymatic Synthesis Of (2R)-Phytochromobilin 80, The Chromophore Of Phytochromes

Biliverdin IXα 78 can be converted into (2R)-phytochromobilin[16] 91 that spontaneously reacts with the apoprotein of phytochromes to form fully active phytochrome (fig. 6).[43,44]

3-Formyl-4-methyl-1H-pyrrole 24 (scheme 4) which can be obtained in any stable isotope enriched form forms the primary building block of both rings B and C of biliverdin IXα 78.

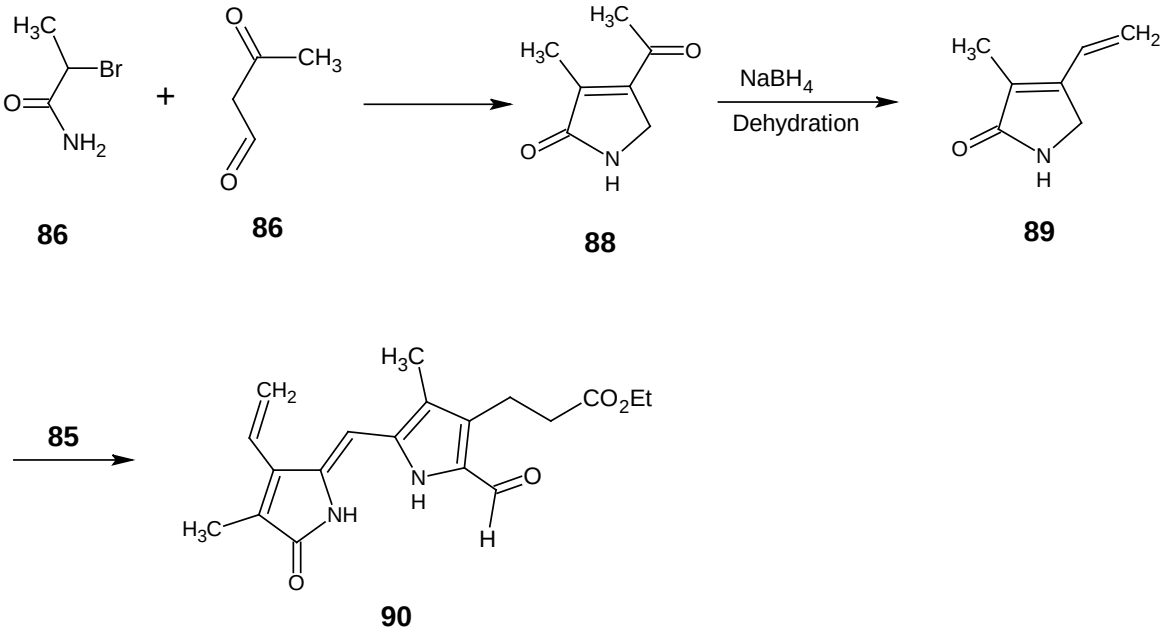


Scheme 10. Conversion of 3-formyl-4-methyl-1H-pyrrole 24 into the necessary building blocks of phytochromobilin 91.

In scheme 10 a synthetic scheme is shown that converts 3-formyl-4-methyl-1H-pyrrole 24 into the necessary building blocks of phytochromobilin 91.

Triethyl phosphonoacetate is obtained from the reaction of ethyl bromoacetate 3 and triethylphosphite. Triethyl phosphonoacetate is treated with 3-formyl-4-methyl-1H-pyrrole 24 (scheme 4) to obtain ethyl 3-(4-methyl-1H-pyrrol-3-yl)acrylate 79.[15] Vilsmeier formylation of product 79 afforded a mixture of the two pyrrole aldehydes which can easily be separated into ethyl 3-(5-formy-4-methyl-1H-pyrrol-3-yl)acrylate 80 and ethyl 3-(2-formyl-4-methyl-1H-pyrrol-3-yl)acrylate 81. Catalytic reductions of the double bond in pyrrole aldehydes 80 and 81 led to 3-[2'-(ethoxycarbonyl)ethyl]-4-methyl-1H-pyrrole-5-aldehyde 83 and 3-[2'-(ethoxycarbonyl)ethyl]-4-methyl-1H-pyrrole-2-aldehyde 84, respectively. Via the Knoevenagel reaction the 2-formyl group in the product 84 is protected as a dicyanovinyl group. A subsequent Vilsmeier formylation afforded in ethyl 3-[2-(2,2-dicyanoethenyl)-5-formyl-4-methyl-1H-pyrrol-3yl] propanoate 85.

In scheme 11 it is indicated that the pyrromethenone building block containing rings A and B is accessible in any stable isotope enriched form.



Scheme 11. Synthesis of pyrromethenone 90 (ring A and ring B) of the phytochrome 91.

2-Bromopropionamide 86 is condensed with 3-oxobutanal 87. Subsequent dehydration of the product gave product 88.[35] Reduction of the carbonyl function and subsequent dehydration results in 3-vinyl-4-methyl-3-pyrrolin-2-one 89. The condensation of product 89 with product 85 and subsequent deprotection of the aldehyde function afforded the product 90.[45]

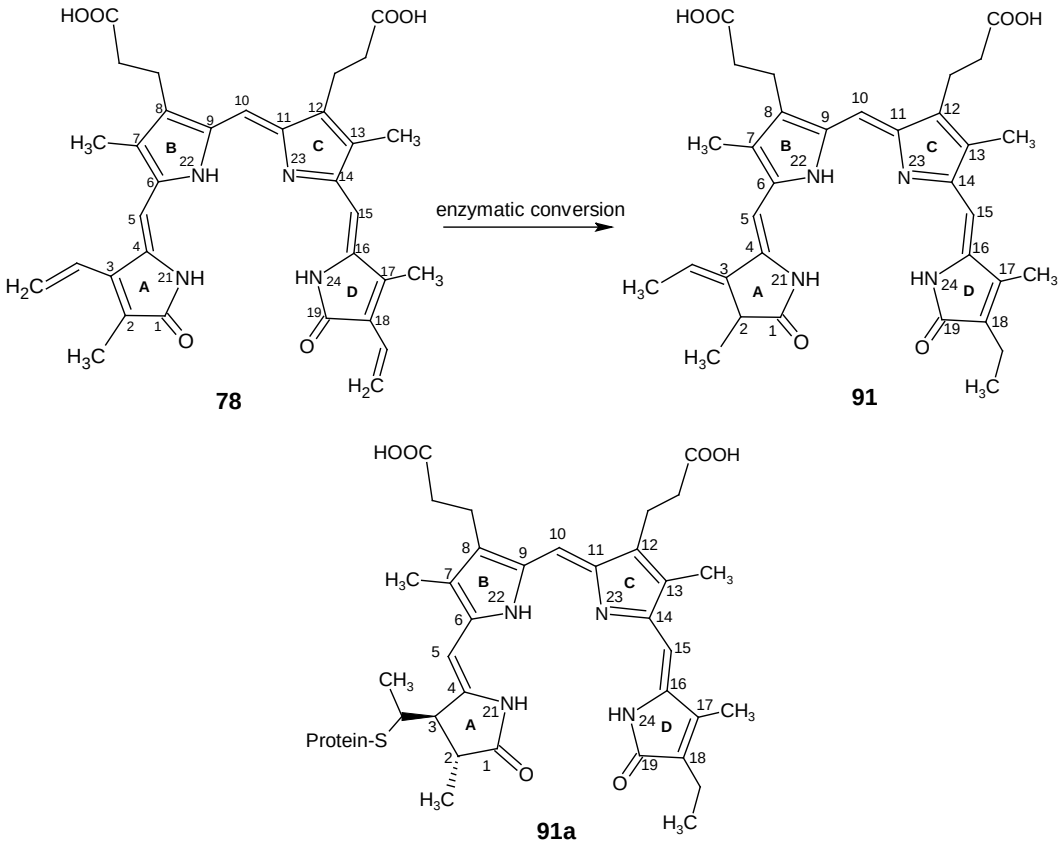
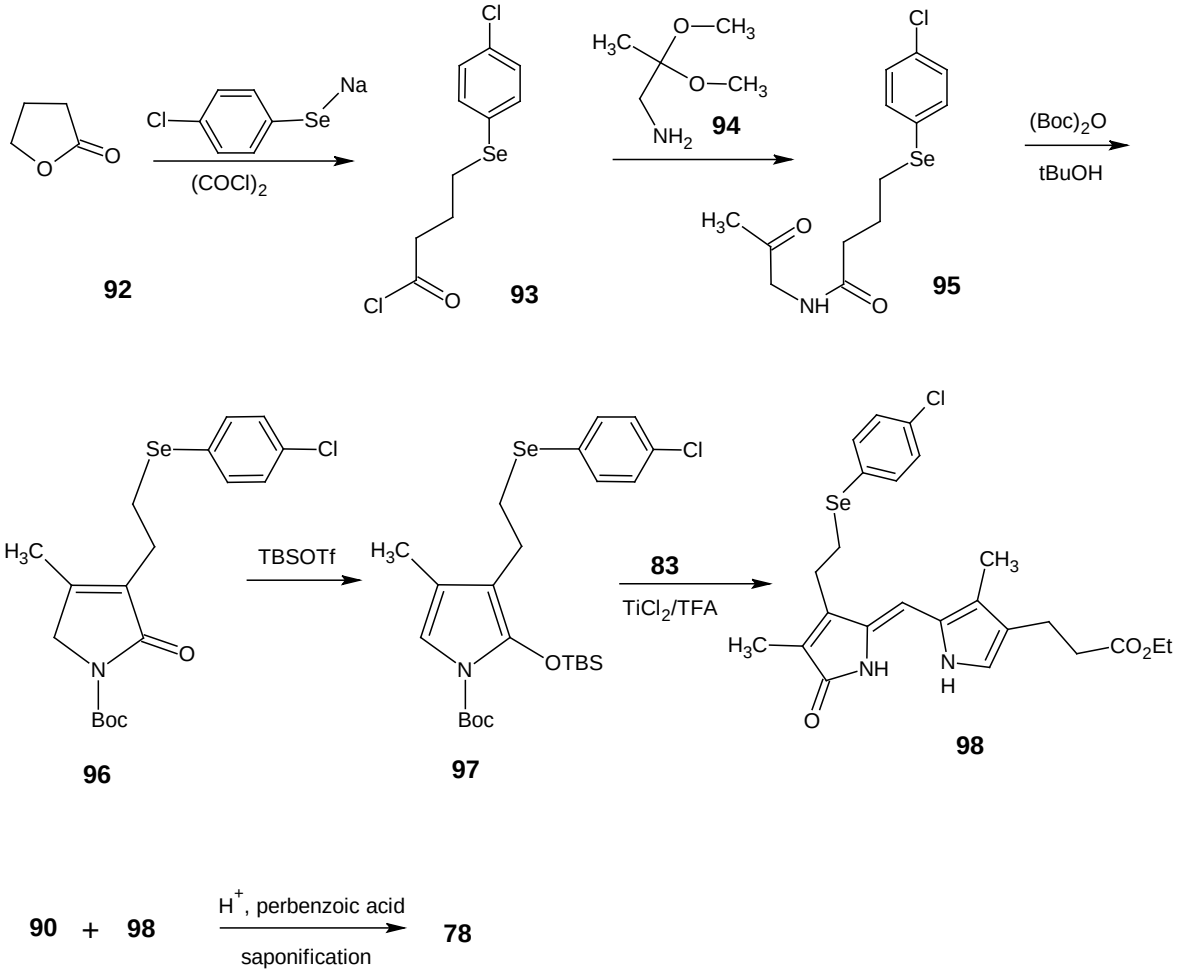


Figure 6. Structure and numbering of biliverdin IXα 78 and (2R)-phytochromobilin 91. Structure 91a represents the linkage of ring A to the protein of the phytochrome.

In scheme 12 it is indicated that the C and D building blocks are accessible in any stable isotope enriched form. Butyrolactone 92 treated with the sodium salt of parachlorobenzene selenol to give the 4-selenophenyl butyrate that treated with oxalyl chloride to form the chloride product 81.[46]

Butyrolactone 92 is accessible in any stable isotope labelled form via a Pinner reaction of 4-hydroxybutyronitrile 12 (scheme 1). Product 93 treated with 2,2-methoxypropylamine 94 to give product 95 after deacetalization. The protected 1-aminoacetone 94 is easily accessible via nitrosation of ethyl acetoacetate 35 (scheme 5).[47]

After introduction of BOC protection of the amide base induced ring closure to the 3-pyrrolin-2-one with tert-butyl alcoholate is effected.[46] Treatment of product 96 with tributyl silyl triflate results in the double protected pyrrole 97. This building block condensed with the pyrrole aldehyde 82 under formation of the pyrromethenone 98.[46] A final acid condensation afforded in the tetrapyrrole system which after oxidative desalination gives the vinyl group in the D ring giving the dimethyl ester of biliverdin IXα. A mild saponification afforded in biliverdin IXα 78.



Scheme 12. Synthesis of biliverdin IXα 78.

III. Conclusion

Nowadays there is a strong synthetic effort in the pyrrole field. Many new synthetic reactions and new pyrroles are worked out and reported. Many of the building blocks that are used in these processes can be made accessible in various stable isotope enriched form. In the near future whole new libraries of stable isotope enriched pyrroles will become available.

IV. Acknowledgment

This paper is written with great indebtedness to investigators who have been involved in pyrrole synthesis and the preparation of ^{13}C and ^{15}N enriched building blocks. We dedicate this paper to future investigators who will use the now accessible isotopomers to unravel the role of medically, pharmaceutically and biologically important pyrrole systems without perturbation at the atomic level.

References Références Referencias

1. Black, D. StC. '1H-Pyrroles', in Science of synthesis, hetarenes and related ring systems, Thieme Verlag, Stuttgart. 2001, 9, 441 – 552.
2. Bellur, E.; Freifeld, I.; Langer, P. Tetrahedron Lett. 2005, 47, 2151-2154.
3. Daidone, G.; Maggio, B.; Schillaci, D. Pharmazie 1990, 45, 441-442.
4. A. Kimura, T.; Kawara, A.; Nakao, A.; Ushiyama, S.; Shimozato, T.; Suzuki, K. PCT Int Appl. CODEN:PIXXD2 WO 2000001688 A1, 200001132000, p 173.

   B. Kaiser, D. G.; Glenn, E. M. J. Pharm. Sci. 1972, 61, 1908-1911.
5. Demir, A. S.; Akhmedov, I. M.; Sesenoglu, O. Tetrahedron 2002, 58, 9793-9799.
6. Meshram, H. M.; Prasad B. R. V.; Kumar, D. A. Tetrahedron Lett. 2010, 51, 3477–3480.
7. Davis, F. A.; Bowen, K.; Xu, H.; Velvadapu, V.; Ballard, C. Tetrahedron 2008, 64, 4174-4182.
8. Reisser, M.; Maas, G. J. Org. Chem. 2004, 69, 4913-4924.
9. Mathew, P.; Asokan, C. V. Tetrahedron 2006, 62, 1708-1716.
10. Skaddan, M. B. J. Labelled Comp. and Radiopharm. 2010, 53, 73–77.
11. Scott, A. I.; Burton, G.; Fagerness, P. E. J. Chem. Soc., Chem Commun. 1979, 199–202.
12. Battersby, A. R.; Hunt, E.; McDonald, E.; Paine III, J. B.; Saunders, J. J. Chem. Soc., Perkin Trans. 1 1976, 1008–1018.
13. Roy, E.; Rohmer, T.; Gast, P.; Jeschke, G.; Alia, A.; Matysik, J. Biochemistry 2008, 47, 4629–4635.
14. Siebert, F.; Hildebrandt, P. Vibrational Spectroscopy in Life Science; Wiley-VCH: Weinheim, 2008.
15. Dawadi, P. B. S.; Lugtenburg, J. Targets in Heterocyclic Systems, 2008, 12, 1-30. Eds. O. A. Attanasi and D. Spinelli.
16. Andel III, F.; Murphy, J. T.; Haas, J. A.; McDowell, M. T.; van der Hoef, I.; Lugtenburg, J.; Lagarias, J. Biochemistry, 2000, 39, 2667–2676.
17. Buldain, G.; Valasinas, A. J. Labelled Comp. and Radiopharm. 1980, 19, 1–5.
18. Burton, G.; Fagerness, P. E.; Hosozawa, S.; Jordan, P. M.; Ian Scott, A. J. Chem. Soc., Chem Comm. 1979, 202-204.
19. Dawadi, P. B. S.; Schulten, E. A. M.; Lugtenburg, J. J. Labelled Compd. Radiopharm. 2009, 52, 341-349.
20. Raap, J.; Wolthuis, W. N. E.; Hehenkamp, J. J. J.; Lugtenburg, J. Amino Acids 1995, 8, 171-186.
21. Barton, D. H. R.; Zard, S. J. Chem. Soc.,Chem. Commun. 1985, 1098–1100.
22. Cappon, J. J.; Witters, K. D.; Baart, J.; Verdegem, P. J. E.; Hoek, A. C.; Luiten, R. J. H.; Raap, J.; Lugtenburg, J. Recl. Trav. Chim. Pays-Bas 1994, 113, 318–328.
23. Van Leusen, A. M. Lect. Heterocyclic Chem. 1980, 5, S111-122.
24. Dawadi, P. B. S.; Lugtenburg, J. Eur. J. Org. Chem. 2008, 2288–2292.
25. Li, J. J. Name Reactions Third Expanded Edition Springer-Verlag Berlin 2000.
26. Bravo, P.; Resnati, G. J. Chem. SOC., Chem. Commun. 1988, 218-219.
27. Mundy, B.P.; Ellerd, M.G.; Favaloro, F. J. Name Reactions and Reagents in Organic Synthesis: 2nd edition, John Wiley & Sons: Hoboken, NJ, 2005.
28. Halland, N.; Braunton, A.; Bachmann, S.; Marigo, M.; Jorgensen, K. A. J. Am. Chem. Soc., 2004, 126, 4790-4791.
29. Dekeukeleire, S.; D'hooghe, M.; T rnroos, K. W.; De Kimpe, N. J. Org. Chem. 2010, 75, 5934-5990.
30. Boullais, C.; Breton, J.; Nabedryk, E.; Mioskowski, C. Tetrahedron 1997, 53, 2505-2512.
31. Creemers, A. F. L.; Lugtenburg, J. J. Am. Chem. Soc. 2002, 124, 6324–6334.
32. Chan, T. H.; Brownbridge, P. J. Chem. Soc., Chem. Commun., 1979, 578-579.
33. Siebum, A. H. G.; Woo, W. S.; Lugtenburg, J. Eur. J. Org. Chem. 2003, 4664-4678.
34. Krebs, A.; Bolm, C. Tetrahedron 2011, 67, 4055 - 4060.
35. Dawadi, P. B. S.; Lugtenburg, J. Tetrahedron Lett. 2011, 52, 2508-2510.
36. Bakavoli, M.; Feizyzadeh, B.; Rahimizadeh, M. Tetrahedron Lett. 2006, 47, 8965-8968.
37. Hu, Y. G.; Li, G. H.; Ding, M. W. Arkivoc. 2008, xiii, 151–158.
38. Kurti, L.; Czako, B. Strategic applications of the named reactions, Elsvier Academic Press, 2005.
39. Dawadi, P. B. S.; Lugtenburg, J. Eur. J. Org. Chem. 2007, 1294–1300.
40. Dawadi, P. B. S.; Lugtenburg, J. Synth. Comm. 2010, 40, 2539-2546.
41. Khalifa, A. F.; Ismail, A. N.; Elghandour, H. H. A.; Zohdi, F. H. Tetrahedron, 1991, 47, 8243-8250.
42. Antonio, Y.; Cruz, M. E. D. L.; Maddox, M. L.; Muchowski, J. M. Can. J. Chem . 1994, 72, 15-22.
43. Rohmer, T.; Lang, C.; Bongards, C.; Gupta, K. B.; Neugebauer, J.; Hughes, J.; Gärtner, W.; Matysik, J. J. Am. Chem. Soc. 2010, 132, 4431-4437.
44. Makhynya, Y.; Hussain, Z.; Bauschlicher, T.; Schwinte, P.; Siebert, F.; Gaertner, W.; Eur. J. Org. Chem. 2007, 1287-1293.
45. Plieninger, H.; Hentschel, K.-H.; Kohle, R.-D. Liebigs Ann. Chem., 1974, 1522-1530.
46. Jacobi, P.; Pippin, D. Org. Lett. 2000, 65, 827–830.
47. Kato, T.; Sato, M.; Yoshida, T. Chem. Pharm. Bull., 1971, 19, 292-296.

Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Effect of Blanching, Ripening and Other Treatments on the Production Characteristics of Pectinolytic Enzymes from Banana Peels By Aspergillus Niger

By Ogunlade & Ayodele Oluwayemisi
Ekiti State University, Ado Ekiti

Abstract - Three different strains of Apergillus niger isolated from decayed banana peels in Ibadan metropolis, Nigeria depolymerized citrus pectin. The best strain having pectinolytic activity as indicated by the diameter of clear hydrolyzed zone on the medium plates containing commercial citrus pectin as the sole carbon source was selected among the three strains having the largest zone. This isolate was able to produce polygalacturonase and pectin layse enzymes using banana peels (agrowastes) as the sole carbon source. When Solid state fermentation (SSF) and Submerged fermentation (SMF) were carried out with the banana peels as the substrate using the Aspergillus niger with the largest zone, SSF yielded higher level of pectinolytic activity than the SMF. Different treatments of the banana peels used as substrate were carried out by blanching the substrate with cold sodium chloride, treating the banana peels with wood ashes and also allowing the unripe banana to go through ripening stages. Higher yield of pectinases production was obtained when the banana peels were treated compared with when they were not treated at all. Therefore pretreatment of banana peels increases pectinases production.

Keywords : Aspergillus niger, Pectinolytic Activity, Banana peel, Fermentation, Pectinase.

GJSFR-B Classification: FOR Code: 820214, 070602, 030406

Strictly as per the compliance and regulations of:

Effect of Blanching, Ripening and Other Treatments on the Production Characteristics of Pectinolytic Enzymes from Banana Peels by Aspergillus Niger

Ogunlade & Ayodele Oluwayemisi

Absract - Three different strains of *Apergillus niger* isolated from decayed banana peels in Ibadan metropolis, Nigeria depolymerized citrus pectin. The best strain having pectinolytic activity as indicated by the diameter of clear hydrolyzed zone on the medium plates containing commercial citrus pectin as the sole carbon source was selected among the three strains having the largest zone. This isolate was able to produce polygalacturonase and pectin layse enzymes using banana peels (agrowastes) as the sole carbon source. When Solid state fermentation (SSF) and Submerged fermentation (SMF) were carried out with the banana peels as the substrate using the *Aspergillus niger* with the largest zone, SSF yielded higher level of pectinolytic activity than the SMF. Different treatments of the banana peels used as substrate were carried out by blanching the substrate with cold sodium chloride, treating the banana peels with wood ashes and also allowing the unripe banana to go through ripening stages. Higher yield of pectinases production was obtained when the banana peels were treated compared with when they were not treated at all. Therefore pretreatment of banana peels increases pectinases production.

Keywords : Aspergillus niger, Pectinolytic Activity, Banana peel, Fermentation, Pectinase.

I. Introduction

Enzymes are proteins that catalyze (*i.e.*, increase or decrease the rates of) chemical reactions. (Smith *et al.,* (1997), (Grisham *et al.,* 1999). In enzymatic reactions, the molecules at the beginning of the process are called substrates, and they are converted into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Pectinases are of great significance with tremendous potential to offer to industry (Dayanand *et al.*, 2003.). They are one of the upcoming enzymes of the commercial sector, especially the juice and food industry (kashyap *et al.*, 2001) and in the paper and pulp industry (Beg, Viikari *et al.*, 2001). Pectinolytic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyze the pectic substances, present mostly in plants. Pectinolytic enzymes are widely distributed in higher plants and microorganisms (Whitaker *et al.*, 1990). They are of prime importance for plants as they help in cell wall extension (Ward *et al.*, 1989) and softening of some plant tissues during maturation and storage (Sakai, 1992, Aguilar *et al.*, 1990).

Pectinases are group of enzymes that attack pectin and depolymerise it by hydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 1998). These enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network (Thakur *et al.*, 1997). Pectinases account for 10% of the global industrial enzymes produced (Stutzenberger, 1992).

These pectinases have wide applications in fruit juice industry and wine industry. In fruit juice industry, it is used for clarification, where reduction in viscosity is caused which ultimately leads to formation of clear juice. They increase the yield of juices by enzymatic liquefaction of pulps; these pectinases also helps in formation of pulpy products by macerating the organized tissue into suspension of intact cells. In wine industry pectinases are mainly used for decreasing astringency by solubilizing anthocyanins without leaching out procyadin polyphenols, and pectinases also increase pigmentation by extracting more anthocyanins (Tucker and Woods, 1991).

A group of pectinolytic enzymes known as pectinases hydrolyses pectin. Pectinases are complex hydrolytic enzymes that function as esterases and depolymerases. They include pectinmethyl esterases which catalyse the hydrolysis of methylated carboxylic ester groups in pectin into pectic acid and methanol, pectin lyase which cleave α(1.4)-glycosidic linkages by

Author : Ekiti State University, Ado Ekiti.

transelimination resulting into galacturonide with a double bond between C-4 and C-5 at the non reducing end ant polygalacturonase which hydrolyse the α(1,4)-glycosidic linkages in homo galacturonans (Call *et al.*, 1985;Delgado *et al.*, 1992; Soares *et al.*, 1999).

Solid state fermentation (SSF) and Submerged fermentation (SmF) are important fermentation methods employed for the production of microbial enzymes (Kavitha *et al.*, 2000; Martin *et al.*, 2000). Microbial growth and product formation usually occur at or near the surface of solid substrate particles with low moisture content; hence SSF appear to be advantageous for microbial enzyme production. The advantages of SSF over the SmF process include higher yield of products (Pandey, 1994), generation of less effluent and requirement of simpler equipment (Bennett, 1998). Reports are very few on the comparison of SmF and SSF for the production of pectinases. The present study involves screening of the *Aspergillus niger* isolated from decayed banana peels for pectinolytic activity using banana peels as fermentation substrate and determining the effect of different treatments of banana peels by blanching it in cold NaCl, treating it with wood ashes and allowing it to undergo ripening stages fot pectinolytic enzymes production.

II. Materials And Method

a) Sample Collection

Banana peels were collected from Ibadan metropolis and were transported to the University of Ibadan Postgraduate Laboratory where they were used as analysis.

b) Isolation And Identification Of Fungal Isolate

Decaying banana peels were collected from fruit selling points within the University of Ibadan and then transported to the laboratory for isolation.

10g of the fungi infected region of the banana peels were weighed into 90ml of sterile distilled water and was shaken properly to obtain the stock solution. Dilutions of 10^{-4} was made and pour plating of 10^{-2} and 10^{-3} was done using sterile Potato Dextrose Agar(PDA) which was sterilized by autoclaving at 121^{0}C for 15mins.Streptomycin was added to the PDA to prevent bacterial contamination. The above dilutions were then plated in duplicates. The inoculated plates were incubated at 25±2^{0}C (room temperature) for 5-7days. Pure cultures were obtained by repeated subculturing on PDA plates and maintained on PDA slants. The isolates were examined and identified in the department of Botany and Microbiology University of Ibadan based on cultural and morphological characteristics of the organism. The microscopic structures of the isolates were studied using microscope. Compendium of soil fungi was also consulted.

c) Screening Of Fungal Isolates For Pectinolytic Activity

The isolates were screened for pectinases producing ability by inoculating them in a sterile medium containing 1% citrus pectin, 0.14% $(NH4)_2SO4$, 0.20% K_2HPO4, 0.02% $MgSO4.7H_20$, 0.1% Nutrient solution (5Mg/L $FeSO4.7H_2O$, 1.6Mg/L MnSO4, 1.4mg/L $ZnSO4.7H_20$, 2.0mg/L $CoCl_2$), 3% agar PH 5.0(Martin *et al.*, 2004).

The medium was sterilized and distributed aseptically on petri dishes. The plates were then inoculated with the organism and incubated for 3-5 days. After incubation plates were stained with iodine solution. Clear zones were formed around the pectinase producing isolates.

d) Quantitative Estimation Of Pectinolytic Activity Of Screened Isolates

Quantitative estimation of pectinolytic activity was done on submerged and solid state fermentation.

e) Submerged Fermentation

The liquid medium containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01% with 1% pure pectin and 1% dry banana peels as the sole carbon sources and these were added separately to the basal medium. The PH value was adjusted to 5.5 before sterilization at 121^{0}C for 15mins. The pectinolytic isolates identified was used for inoculation using a flamed and cooled cork borer two disc of fungal hyphae from leading edge of actively growing colonies was cut on petri plates with a flamed and cooled needle disc were then transferred to the fermentation medium in sterile flasks and the Erlenmeyer flasks were then plugged properly and incubated for 12 days at room temperature (25±2^{0}C). Aliquots were withdrawn every day 3, 6, 9, and 12 using whatman No.1 filter paper for carrying out polygalacturonase and pectin lyase assays.

f) Preparation Of Substrates For Solid State Fermentation

Fresh banana peels were dried, ground and sieve to obtain smaller substrate particle which provides larger surface area for microbial attack. (Pandey *et al.,* 2002).

g) Production Of Pectinolytic Enzyme By Solid State Fermentation

Solid state fermentation was carried out using a medium containing 15g of ground dried banana peels and 10ml of the mineral salt solution of 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al.*, 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. The flasks were then

incubated at room temperature (25±2^{0}C). For 12days 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. The obtained fiterates were used for conducting polygalacturonase and pectin lyase assays.

III. Effect of Different Treatments On Pectinase Production

a) Effect Of Blanching On Pectinase Production

Banana peels were dipped inside 5% NaCl and removed at different time intervals of 5mins, 10mins, 15mins, and 20mins. 15g of the substrate was weighed and 10ml of mineral solution containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al*., 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. Part of this treated peels were also dried and allowed to be subjected to the fermentation also. The flasks were then incubated at room temperature (25±2^{0}C) for 12days. 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. Filtrate was then used as crude enzyme for assay.

b) Effect Of Ripening On Pectinase Production

Banana fingers were monitored for ripening and peels were removed at different days intervals 0, 2, 4,and 6 and both fresh and dry peels were then subjected to SSF by weighing 15g of the substrate and 10ml of mineral solution containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al*., 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. The flasks were then incubated at room temperature (25±2^{0}C). For 12days 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. The filtrates were later used for assays.

c) Effect Of Ashes On Pectinase Production

Banana peels were dipped inside ashes and removed at different time intervals of 5mins, 10mins, 15mins, and 20mins.They were then subjected to SSF by weighing 15g of the substrate and 10ml of mineral solution containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al*., 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. The flasks were then incubated at room temperature (25±2^{0}C). For 12days 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. The filtrates were later used for assays.

d) Polygalacturonase Assay

Polygalacturonase (PG) activity was determined by measuring the release of reducing groups from citrus pectin using the 3,5 dinitrosalicylic acid (DNS) reagent.(Miller, 1959).

The reaction mixture containing 2ml of 1% citrus pectin in 0.2M phosphate citrate buffer P^H (5.5) and 0.5ml of crude enzyme solution was incubated at 40^0 C for 10mins.(Somogyi *et al*., 1945), a modified method. After this 3ml of DNS reagent was added and boiled in water bath for 15mins. After cooling, colour absorbance was read at 540 nm using a spectrophotometer.

e) Pectin Lyase Assay

Pectin lyase activity was determined by measuring the increase in absorbance at 235nm of substrate solution (0.8ml of 1% citrus pectin in 0.2M tris Hcl buffer PH 8.5 hydrolysed by 0.2ml enzyme solution at 40^{0}C (Martin *et al*., 2004).

IV. Results

The present study was carried out in order to determine the effect of blanching, ripening, and other treatments on the production characteristics of pectinolytic enzymes from banana peels by *Aspergillus niger.* It is well reported that *Aspergillus niger* have pectinolytic activity, the isolates was screened for the production of the pectinolytic enzymes.

Figure 1 shows the production of polygalacturonase enzymes by *Aspergillus niger* in both solid state fermentation (SSF) and submerged fermentation (SMF). The fermentation was carried out for 12days and assay of the aliquots was conducted on day 3, 6, 9, 12. For (SSF) the highest yield of polygalacturonase was produced on day 3 of the fermentation and the value ranged from 4.9676-7.5544U/ml. However in SMF the polygalacturonase produced ranged from 4.6265-5.3118U/ml and the highest yield of production was observed on day 9 with 5.3118U/ml. Generally from this study SSF was higher than SMF.

Figure 2 shows the production of pectin lyase enzymes by *Aspergillus niger* in both solid state fermentation (SSF) and submerged fermentation (SMF). For SSF the highest yield of production was observed on day 3 with 22.3214U/ml, while the lowest was seen on day 12 with 19.0178U/ml. For SMF the highest yield of pectin lyase production was observed on day 12 having 18.8393U/ml.

V. Production of Polygalacturonase By Aspergillus Niger

A) Effect Of Blanching On Banana Peels

The polygalacturonase production by *Aspergillus niger* using blanched banana peels (fresh) as substrate in solid state fermentation is shown in figure 3. The polygalacturonase produced ranged from 13.8235-18.5294U/ml in which banana peels blanched for 20mins at day 12 had the highest yield of production while the lowest yield of production was observed at the same 20mins on day 3. However the same quantity of enzyme was produced at 10mins day 12, 15mins day 9 and 20mins day 9 having 17.3529U/ml in all the three.

b) Effect Of Ashing On Banana Peels

The polygalacturonase production by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation is shown in figure 4. The highest yield of production was 19.8529U/ml observed on day 3 at 15mins while the lowest yield was 13.6765U/ml observed on day 12 at 10mins.However the same quantity of enzyme was produced at 5mins day 3 and 15mins day 9 having 14.7059U/ml of polygalactturonase production.

c) Effect Of Ripening On Banana Peels.

Figure 5 shows the polygalacturonase production by *Aspergillus niger* using banana peels ripened at different days as substrate in solid state fermentation. During the ripening of these banana, the peels were removed at different days intervals as unripe, moderately ripe, ripe and extremely ripe and when SSF were carried out, changes in their polygalacturonase production ranged from 13.9706-29.8529U/ml. The highest yield of production was observed on day 9 when the banana was extremely ripe while the lowest was observed on day 12 when the banana was ripe.

VI. Production of Pectin Lyase By Aspergillus Niger

a) Effect Of Blanching On Banana Peels

The production of pectin lyase by *Aspergillus niger* using blanched banana peels (fresh) as substrate in solid state fermentation is shown in figure 6. The pectin lyase produced ranged from 13.3928-21.9643U/ml in which banana peels blanched for 15mins at day 6 had the highest yield of production while the lowest yield of production was observed at 10mins on day 12. However the same quantity of enzyme was produced at 15mins day 3 and 5mins day 6 having 17.8125U/ml.

b) Effect Of Ashing On Banana Peels

The pectin lyase production by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation is shown in figure 7. The highest yield of production were observed on day 3 at 5mins and the same day 3 at 15mins with 20.1339U/ml while the lowest yield was 17.0536U/ml observed on that same day 3 at 10mins. However the same quantity of enzyme was produced at 5mins day 9 and 10mins day 9 also having 18.75U/ml of pectin lyase production.

c) Effect Of Ripening On Banana Peels.

Figure 8 shows the pectin lyase production by *Aspergillus niger* using banana peels ripened at different days as substrate in solid state fermentation. During the ripening of these banana, the peels were removed at different days intervals as unripe, moderately ripe, ripe and extremely ripe and when SSF were carried out, changes in their pectin lyase production ranged from 17.7232-20.5804U/ml. The highest yield of production was observed on day 3 when the banana was ripe while the lowest was observed on the same day 3 when the banana was unripe. The same quantity of pectin lyase was produced on day 6 with unripe banana and day 3 with extremely ripe banana with both having 18.3929U/ml.

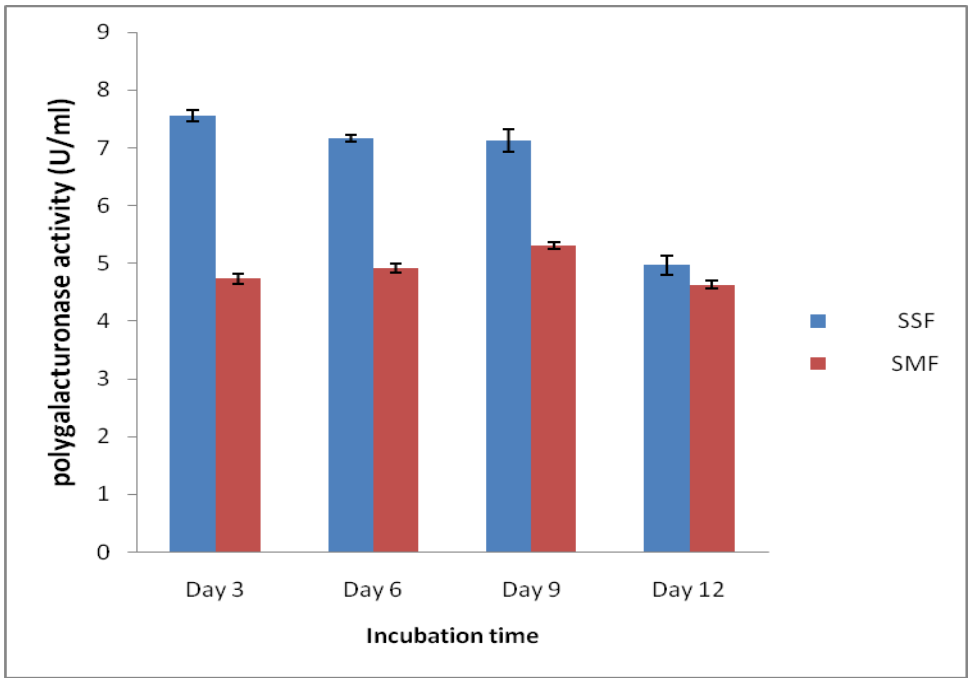


Fig 1 : Production of polygalacturonase by *Aspergillus niger* in solid state fermentation (SSF) and Submerged fermentation (SMF)

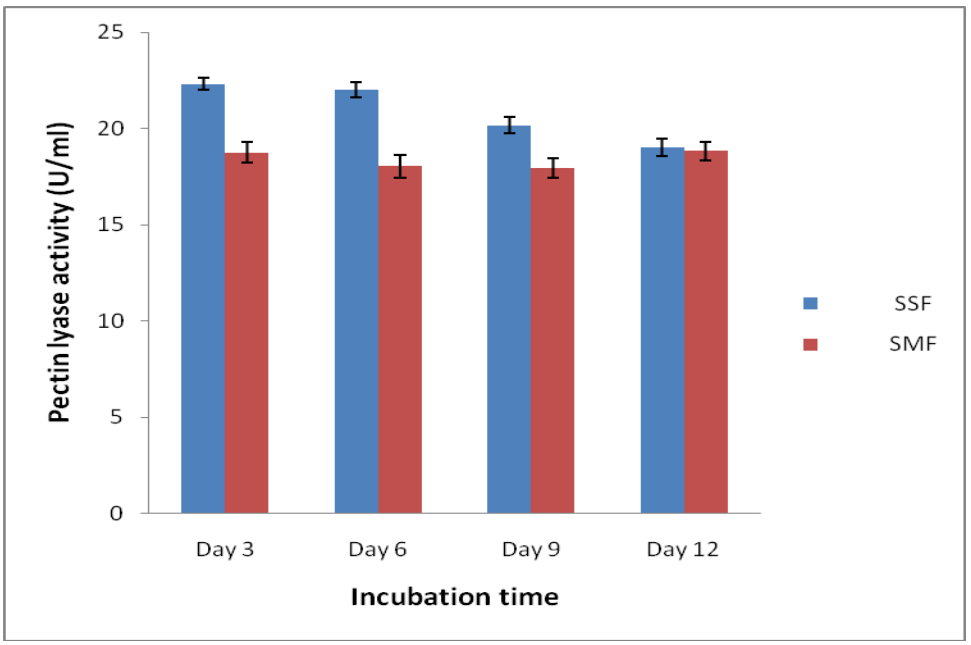


Fig 2 : Production of Pectin lyase by *Aspergillus niger* in solid state fermentation (SSF) and Submerged fermentation (SMF)

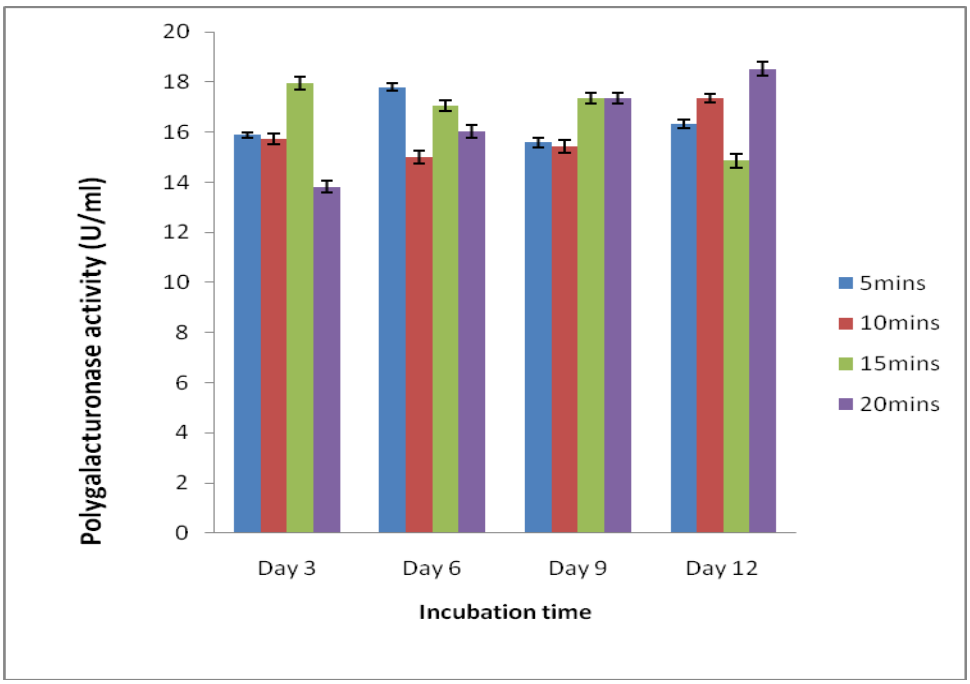


Fig 3 : Production of by polygalacturonase by *Aspergillus niger* using blanched banana peels as substrate in solid state fermentation (fresh).

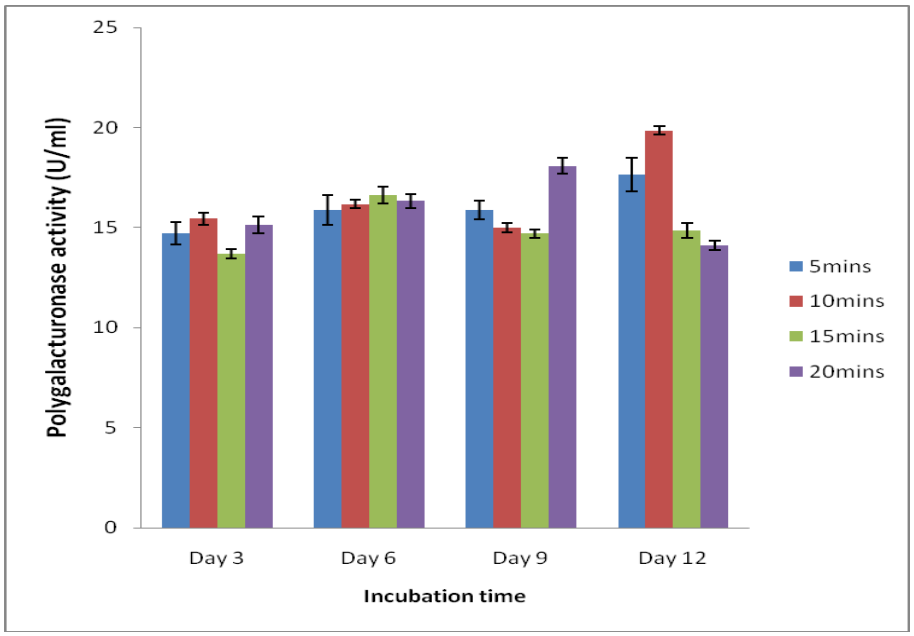


Fig. 4 : Production of polygalacturonase by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation

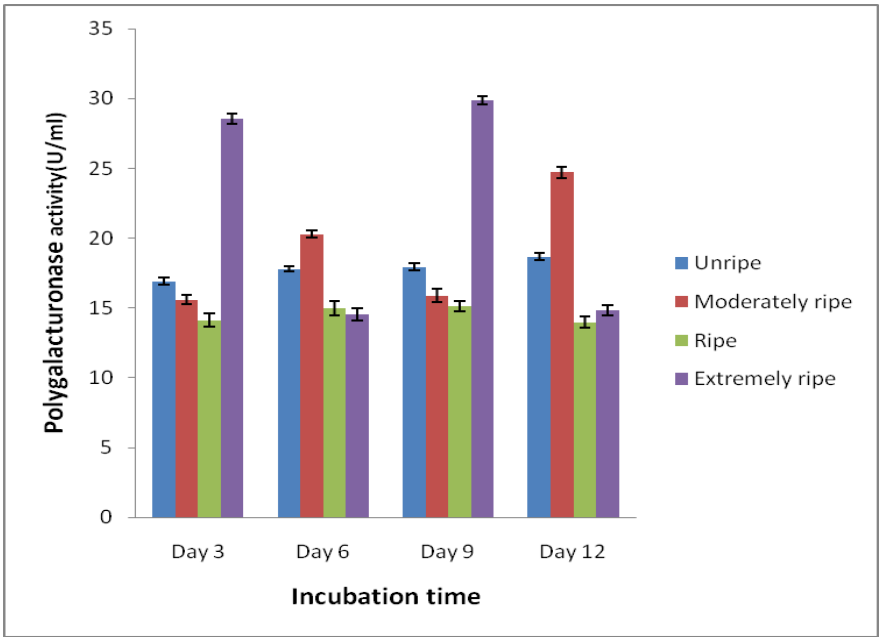


Fig. 5 : Production of polygalacturonase by *Aspergillus niger* using ripening banana peels as substrate in solid state fermentation (fresh)

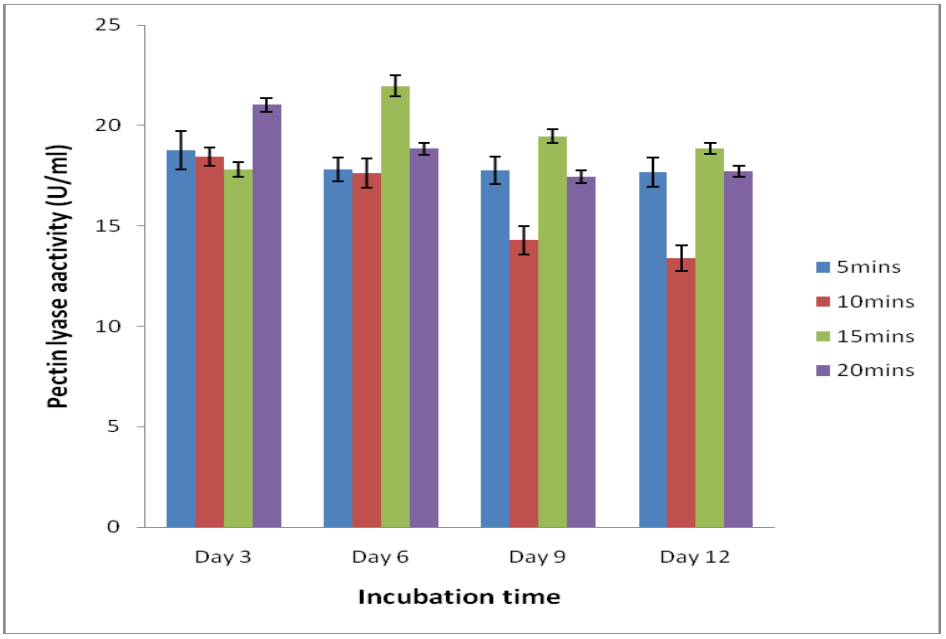


Fig. 6 : Production of Pectin lyase by *Aspergillus niger* using blanched banana peels as substrate in solid state fermentation (fresh)

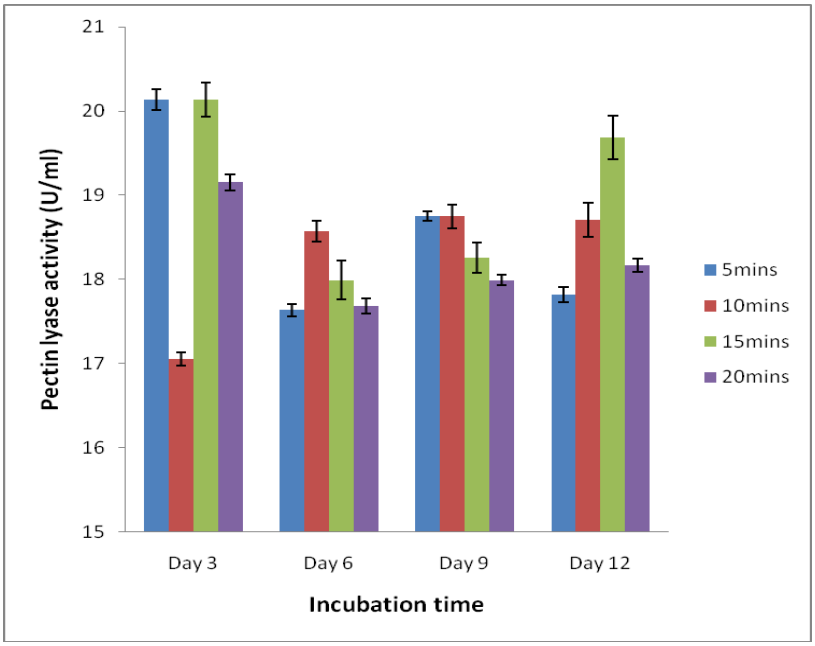


Fig.7 : Production of Pectin lyase by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation

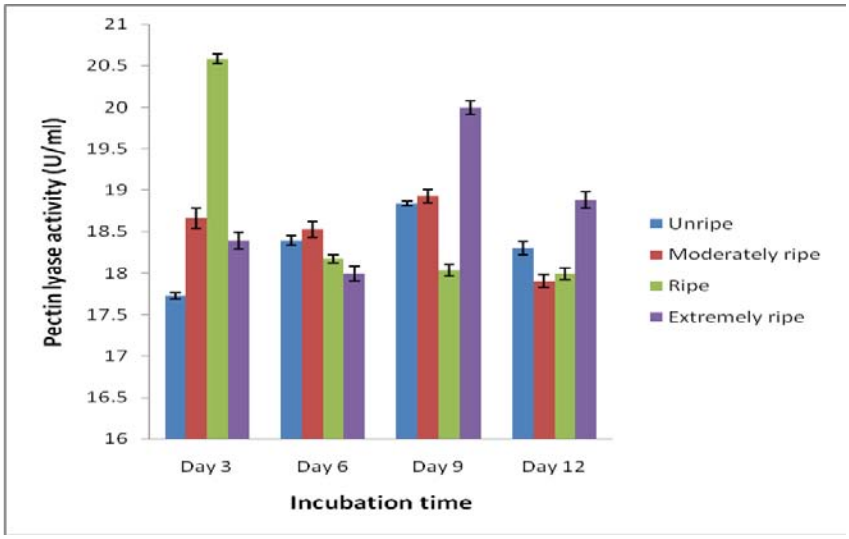


Fig.8 : Production of Pectin lyase by *Aspergillus niger* using ripening banana peels as substrate in solid state fermentation (fresh)

VII. Disscussion And Conclusion

Three different Aspergillus niger were isolated from banana peels in Ibadan metropolis as strain A-C. The best pectinolytic activity based on screening method was given by strain B. The present study shows that the strain of *A. niger* produce pectinases which hydrolyze pectins. *Aspergillus niger* isolated was reported to produce cellulases (Chinedu *et al*., 2008 a; Nwodo-Chinedu *et al*., 2007a) and xylanases (Chinedu *et al*., 2008b; Okafor *et al*., 2007a, b). Thus, this fungus produce the full complement of enzymes required for the hydrolysis of pectinolytic biomass. This explains why the fungi thrive on waste plant matter and are capable of utilizing such waste materials as carbon sources in their culture media (Nwodo-Chinedu *et al*., 2007 b). Pectinase production from Solid state fermentation (SSF) culture of this organism was significantly higher than that obtained by submerged fermentation (SmF). The higher level of pectinase activity by SSF is observed in polygalacturonase and pectin lyase. Several workers proposed the use of SSF for pectinase production, using different solid agricultural and agro-industrial residues as substrates such as wheat bran banana peels and soy bran (Castilho *et al*., 1999, 2000; Singh *et al*., 1999). The present result clearly supports the use of SSF over SmF for pectinases production by filamentous fungi.

Lignocellulosic waste of Banana plant left over otherwise for natural degradation in field was effectively used as component in the medium for the production of enzymes (Baig *et al*., 2003). Subsequently these enzymes produced on the medium containing banana agro waste can be further implicated in the saccharification of the same agro waste. Many researchers have studied the effect of agrowaste pre-treatment by alkali or steam (Okeke and Obi, 1994; Kirk and Farrel, 1987; Durand *et al*., 1984; Waldron and Eveleigh, 1986; Ekhlund *et al*., 1990)

In conclusion, *Aspergillus niger* isolated from banana peels from Ibadan metropolis, Nigeria is a pectinolytic fungi. Banana peels had been identified as a suitable low-cost substrate for pectinase production by the strains of *A. niger* . Higher levels of pectinase activity were obtained by SSF compared to SmF. The use of banana peels for pectinase production will not only reduce the production costs of the enzyme but also help decrease pollution-load due to the agro-industrial waste. Banana peels offer a good medium for the production of pectinase and *Aspergillus niger* can be used for large scale production of pectinase using banana peels and when they were subjected to different treatments pectinase production was higher than when the peels were not treated at all.

References Références Referencias

1. Baig MMV, Mane VP, More DR, Shinde LP, Baig MIA (2003). Utilization of Agricultural Waste of Banana: Production of Cellulases by Soil fungi, *Journal of Environmental Biology* 24:173 -176.
2. Beg QK, Kapoor M, Tiwari RP, Hoondal GS. (2001) Bleach-boosting of eucalyptus kraft pulp using combination of xylanase and pectinase from *Streptomyces sp*. QG-11-3. *Resource Bulletin*. Panjab University; 57:71–8
3. Bennett JW (1998). Mycotechnology: The role of fungi in biotechnology. *Journal of Biotechnology*., **66**: 101-107.
4. Call HP, Walter J, Emeis CC (1985). Maceration activity of an endopolygalacturonase from Candida macedoniensis. *Journal of Food Biochemistry*, **9**: 325-348.
5. Castilho LR, Alves TLM, Medronho RA (2000). Production and extraction of pectinases obtained by solid-state fermentation of agroindustrial residues with *Aspergillus niger*. Bioresources Technology. **71:** 45–50.
6. Ceci, L. and Loranzo. J. (1998). Determination of enzymatic activities of commercial pectinases for the clarification of apple juice. *Food Chemistry*. **61**, 237-241
7. Chinedu SN, Nwinyi OC, Okochi VI (2008). a. Growth and cellulose activity of wild- type *Aspergillus niger* ANL301 in different carbon sources. *Canada Journal of pure and Applied Science* **2**(2): 357-362.
8. Chinedu SN, Okafor UA, Emezue TN, Okochi VI (2008b). Xylanase production of Aspergillus niger and Penicillium chrysogenum from ammonia pretreated cellulosic waste. *Resource Journal of Microbiology*, 3(4) 243-256
9. Dayanand A, Patil SR.(2003) In: Detection of potential fungal isolates for the production of pectinase from deseeded dried sunflower head.
10. Delgado L, Blanca A.T, Huitron C, Aguilar G. (1992). Pectin lyase from Aspergillus sp.CH- Y-1043. *Applied Microbiology and Biotechnology*.,**39**:515-519
11. Durand H, Soucaille P, Tiraby G (1984). Comparative study of cellulases and hemicellulases from four fungi: mesophiles *Trichoderma reesei* and *Penicillium sp*. And thermophiles *Thielavia terrestris* and *Sporotrichum cellulophilum*. *Enzyme Microbiology and Technology*. **6**: 175 -180
12. Ekhlund R, Galbe M, Zachi G (1990). Optimization of temperature and enzyme concentration in the enzymatic saccharification of steam pre-treated willow. *Enzyme Microbiology and Technology* **12**: 225-228.
13. Grisham, Charles M.; Reginald H. Garrett (1999).

Biochemistry. Philadelphia: Saunders College Pub. pp. 426–7. ISBN 0-03-022318-0.
14. Kashyap DR, Vohra PK, Chopra S, Tewari R.(2001). Applications of pectinases in commercial sector: a review. *Bioresources Technology*; **77:**215–27.
15. Kavitha R, Umesh-Kumar S (2001). Genetic improvement of Aspergillus carbonarius for pectinase overproduction duringsolid state growth, *Biotechnology and Bioengineering*., **67:** 121–125.
16. Kirk TK, Farrel FL (1987). Enzymatic combustion: the microbial degradation of lignin. *Annalytical and Revolutional. Microbiology*. **41**: 465-505.
17. Martin N, De Souza SR, Da Silva R, Gomes E (2004). Pectinase production by fungal strains in solid-state fermentation using agroindustrial bioproduct, *Brazillian Archeology Biology and Technology*., 47: 813–819.
18. Martins E.S, Silva R and Gomes E. (2000). Solid state production of thermostable pectinases from thermophilic *Thermoascus aurantiacus*. *Process Biochemistry*.,**37**:949-954
19. Miller GL (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*. **31**:426-429.
20. Nwodo-Chinedu S, Okochi VI, Omidiji O, Omowaye OO, Adeniji BR, Olukoju D, Chidozie F. (2007b). Potentials of cellulosic wastes in media formulation. *African Journal of Biotechnology*, **6(**3): 243-246.
21. Nwodo-Chinedu S, Okochi VI, Smith HA, Okafor UA, OnyegemeOkerenta BM, Omidiji O (2007 a). Effect of carbon sources on cellulase (EC 3. 2. 1. 4) production by wild-type *Penicillium chrysogenum* PCL501. *African Journal of Biochemical Resources*, **1**(1): 6-10.
22. Okafor UA, Emezue TN, Okochi VI, Onyegeme-Okerenta BM, NwodoChinedu S (2007a).Xylanase production by *Penicillium chrysogenum* PCL501 fermented on cellulosic wastes. *African Journal of Biochemical Resources*, **1**(4): 48-53.
23. Okeke BC, Obi SKC (1994). Lignocellulose and sugar compositions of some agro waste materials. *Bioresource Technology*. **50**: 222-227.
24. Pandey A (1994). Solid-State Fermentation: An overview. In: Solid State Fermentation, A. Pandey (Ed.), Wiley Eastern Ltd.New Delhi, India, pp. 3–10.
25. Pandey, A.; Selvakumar, P.; Soccoi, C.R. and Nigam Poonam. (2002). Solid State Fermentation for the Production of Industrial enzymes. http://tejas.serc.iisc.ernet.in/~c urrsci/july10/articles23.html
26. Singh SA, Plattner H, Diekmann H (1999). Exopolygalacturonate lyase from a thermophilic *Bacillus sp*. *Enzymology of Microbial Technology*, 25: 420–425.
27. Smith AL (Ed) (1997). Oxford dictionary of biochemistry and molecular biology. Oxford [Oxfordshire]: Oxford University Press. ISBN 0-19-854768-4
28. Soares MMCN, Silva R, Gomes E (1999). Screening of Bacterial Strains for Pectinolytic Activity Characterization of the pgase Produced by *Bacillus species*. *Revised. Microbiology* **30**: 229-303.
29. Somogyi, M. 1945. A new reagent for the determination of sugars *Journal of Biology and Chemistry* 160, 195- 19.
30. Stutzenberger F. 1992. Pectinase Production. Encyclopedia of Microbiology (Lederberg J.editor in-chief), Academy press, New York.3: 327-337.
31. Thakur, B. R.; Singh, R. K and Handa, A.K. (1997). Chemistry and uses of pectin. Crit *Revised Food Science and Nutrition*. 37:47.
32. Tucker, G. A. and Woods, L. F. J. (1991).Enzymes in production of Beverages and Fruit juices. Enzymes in Food Processing, Blackie, New York., 201-203.
33. Waldron CR, Eveleigh DE (1986) Saccharification of cellulosics by *Microbispora bispora*. *Journal of Microbiology and Biotechnology* **24:** 489-492.
34. Ward OP, Moo-Young M.(1989).Enzymatic degradation of cell wall and related plantpolysaccharides. CRC *Critical Revolution Biotechnology*, **8**: 237–74.
35. Whitaker JR.(1990). Microbial pectinolytic enzymes. In: Fogarty WM, Kelly CT, editors.*Microbial enzymes and biotechnology*. 2nd edition. London: Elsevier Science Ltd.; p. 133–76.

Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Protective role of sphaeranthus amaranthoides on hepatic marker enzymes

By L.Swarna latha & Dr.P.Neelakanta Reddy
Sathyabama University, Jeppiaar nagar, Chennai, India

Abstract - The ethanolic extract of Sphaeranthus amaranthoides at an oral dosage 500mg/kg body weight exhibited a significant protection against D-galactosamine induced liver damage. Sphaeranthus amaranthoides showed hepatoprotective activity by reducing a D-galactosamine induced alteration in biochemical changes, that was evident by examination of the levels of hepatic marker enzymes. The plant extract may interfere with free radical formation or it may exert antioxidant activity which may result in hepatoprotective action.

Keywords : *hepatic marker enzymes; sphaeranhtus amaranthodies; ethanol; D-galactosamine; hepato- toxicity; hepatoprotective activity; antioxidant activity.*

GJSFR-B Classification: *FOR Code: 060107,030406*

Strictly as per the compliance and regulations of:

Protective role of sphaeranthus amaranthoides on hepatic marker enzymes

L.Swarna latha [α] & Dr.P.Neelakanta Reddy [σ]

Abstract - The ethanolic extract of *Sphaeranthus amaranthoides* at an oral dosage 500mg/kg body weight exhibited a significant protection against D-galactosamine induced liver damage. *Sphaeranthus amaranthoides* showed hepatoprotective activity by reducing a D-galactosamine induced alteration in biochemical changes, that was evident by examination of the levels of hepatic marker enzymes. The plant extract may interfere with free radical formation or it may exert antioxidant activity which may result in hepatoprotective action.

Keywords : hepatic marker enzymes; sphaeranhtus amaranthodies; ethanol; D-galactosamine; hepato-toxicity; hepatoprotective activity; antioxidant activity.

I. Introduction

Liver, a vital organ of the body plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. The liver has a paramount importance in the maintenance and regulation of the homeostasis of the body. Liver can also detoxify the xenobiotics and antibiotics(Ahsan et al., 2009). Any hepatic damage leads to the distortion of these metabolic functions. Unfortunately, the conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes have serious side effects on the other organs. Herbal drugs or their extracts are prescribed for treatment of liver diseases widely, though their biological active compounds are unknown (Gupta et al., 2005). Herbal drugs speed up the natural healing process of liver. Thus growing interest on the herbal plants continues due to their effectiveness and safety in treatment of liver diseases.

The plant sphaeranthus amaranthoides (asteraceae) is called as sivakaranthai in Tamil. It is weed in paddy feild. This plant reported to possess a variety of medicinal properties(swarnalatha et al 2009). Sphaeranthus amaranthoides posses the antimicrobial activity, anti dioarrheal activity(swarnalatha et al 2009). *Sphaeranthus amaranthoides* also showed wound healing acativity (swarnalatha et al 2009).

Author [α] : Department of Biotechnology, Sathyabama University, Jeppiaar nagar, Chennai 600119, India. Corresponding author. Tel +91-44-24503145(o)/09841419450.
E-mail : lokiswarna@gmail.com
Author [σ] : Department of Bioorganic laboratory, CLRI, Adyar, Chennai, India. E-mail : neelakantareddyp@gmail.com

Anticancerous activity of sphaeranthus amaranthoides was reported from the whole plant (swarna latha et al 2011). The phytochemical analysis of ethanol extracts of the plant revealed the presence of flavonoides, phenolics, tannins, steroids and glycosides(swarna latha et al 2009). The current pharmcological study determines the efficacy of hepatoprotective activity of *Sphaeranthus amaranthoides* againest D-galactosamine induced hepatotoxicity. Hepatitis induced by D-galactosamine (D-galn) have been reported to show many metabolic and morphological changes in the liver of experimental animals which is similar to the viral hepatitis. The mode of action of is attributed to peroxidation of endogenous lipid and loss of plasma lipid membrane integrity(Ananham *et al* 1998, Kucera *et al* 2006).

II. Materials and Methods

a) Plant material

The plant *Sphaeranthus amaranthoides* was collected from the Sengottai, Tirunelveli, Tamil Nadu, India. The plant material was identified and authenticated by Mr. V.Chelladurai, Retired Research officer-botany, Central Council For Research In Ayurveda and Sidha (C.C.R.A.S). Govt. of India, Tirunelveli. The Collected plant material was free from diseases and also free from contamination of other plants.

b) Preparation of Plant extract

About 1 Kg of powdered material was taken in a clean, flat bottomed glass container and Soaked in petroleum ether to remove the pigments. Then the plant material is transferred into 2.75lt of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper no.42. The filtrate (ethanol extract) obtained was evaporated using rotary evaporator under reduced pressure. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of ethanol. The extract was transferred to a closed container for further use and protection. The extract obtained was 14 %(w/w) of dry powder. This extract is given to the rats by mixing in a Tween 80.

c) Experimental animals

The experimental animals were divided into four groups of six animals each. Group 1 served as control, group II animals were intraperitoneally injected with D-galn (500mg/kg, dissolved in saline for 1day) for induction of hepatitis as described by Deaciue *et. al*., (1993). Group III animals were pretreated with plant extract alone for 21 days(500mg/kg). Group IV animals were orally pretreated with plant extract(500mg/kg per day, for 21 days, dissolved in Tween 80) and then intraperitoneally injected with D-galn (500mg/kg per day) for one day.

d) D-galactosamine induced hepatotoxicity

D-galactosamine was obtained from SRL laboratories. Animals of the test groups were given the plants extracts in Tween 80 prior to the administration of D-galactosmine for 21 days. The control group received normal diet alone The biochemical parameters were determined 24 h after the D-galactosamine challenge or administration

e) Assessment of liver function

Rats of all groups were anaesthetetized by diethyl ether, 24h after the administration of hepatotoxin. The blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and analyzed for various biochemical parameters:

Alanine aminotransferatse, aspartate amino-transferase and acidific transferase were assayed by the method of (king J 1965) he enzyme activity was expressed as μ moles ofpyruvate liberated per min/mg protein. ALP was assayed by the method of (Reitman S and Frankel SA, 1957). The enzyme activity is expresed as IU/l for plasma and μmoles of phenol liberated per min/mg protein for tissue LDH was assayed according to method of Rosalki and Rau (32), and its activity is expressed as IU/l for plasma and μmoles of pyruvate liberated per min/mg protein for tissue. The assay of GGT was carried out by the method of (Malloy E and Evelyn K, 1987)

f) Statistical analysis

Results of the biochemical estimations are reported as mean S.E.M.Total variation, present in a setof data was estimated by one-way analysis of variance (ANOVA), Student's t-test was used for determining significance (Woolson,1987).

III. Results and Discussion

Intraperitoneal administration of D-galacto-samine led to significant increse the serum enzymes level by 2-3 fold as compared to the normal control group ($p<0.001$). The results for hepatoprotective marker enzymes are shwon in the table. Pretreatment of the rats with ethnolic extract of *sphaeranthus amaranthoides* at 500mg/kg body weight induced significant decrease in the serum enzymes levle($P<0.001$),($P<0.01$).when compared with D-GalN treated rats.

The liver marker enzymems (ALT, AST, ACP, ALP, GGT and LDH) are cytoplasmic in nature upon injury these enzymes enter in to the circulatory system due to the altered permeability of membrane (Zimmerman and Seeff, 1970). D-GalN is a well-established hepatotoxicant that induces a diffuse type of liver injury morphologically and functionally closely resembling human viral hepatitis (Decker and Keppler 1972). GalN causes the hepatic injury with spotty hepatocyte necrosis and marked elevation portal and parenchymal infiltration (Keppler and Decker, 1969). Galactosamine also causes depletion of uridine diphosphate (UDP) by increase the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration(Decker et al 1973, Elmofty et al 1975). This results in the disruption of the plasma membrane causing leakage of the enzymes from cell, (Naik and Panda, 2007) which leads to elevation in serum levels of ALT, AST, ACP, ALP, GGT and LDH is consistent with a number of earlier reports (Muntae et al 2000, Wills and Asha 2006 and Zhou et al 2008). The increase in plasma LDH level indicates the necrosis of hepatocytes (Quintero et al 2002). Further intense galactosamination of membrane structure is thought to be responsible for loss of activity of ionic pumps. The impairment in the calcium pump with consequent increase in the intracellular calcium is considered to be responsible for cell death(Tsai et al., 1997).

The hepatocellular damage leads to the raise in the ALT which is followed by AST raise in the serum (Rao et al., 1989). It has been shown that pretreatment with plant extract exerts its protective action against D-GalN induced liver injury by impairment of D-GalN mediated lipid peroxidation, either through decreased production of free radical derivatives or due to the antioxidant activity of the protective agent itself (Naik and Panda, 2007). In the present study the structural integrity of the hepatocellular membrane was preserved by *sphaeranthus amaranthoides* as evidenced by the decrease in the markder enzyme lelves when compared with the D-GalN treated rats.. This concludes that the crude ethanolic extracts of *sphaeranthus amaranthoides* showed a potent protective effect on D-galN induced acute liver toxicity.

References Références Referencias

1. Ahsan MR, Islam KM, Bulbul IJ (2009). Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur. J. Sci. Res. 37(2): 302-310.
2. Ananham R, Devi KP, Devaki T, Govindaraju P. (1998) Preventive effect of *Picrochiza kuroa* on D-galactosamineinduced hepatitis in rats. J. Clin. Biochem. Nutr. (25): 87-95.
3. Castro,J.A.,deFerreyra,G.C.,deCastro,C.R.,Sesame, H.,deFenos,O.M.,Gillette,J.R.,1974.Preventionofcarb ontetrachlorideinducednecrosisbyinhibitorsofdrugm etabolism.Furtherstudiesonthemetabolismoftheiracti on.BiochemicalPhar-macology23,295–302.
4. Decker K, Keppler D, Pausch J. 1973. The Regulation of Phyrimidine Nucleotide Level and its Role in Experimental Hepatitis. Adv Enz Reg 11:205-230.
5. Decker.D. and Keppler.D. (1972) Prog. Liver. Dis. 4, 183 : Galactosamin induced liver injury.
6. El-Mofty SK, Scrutton MC, Serroni A, Nicolini C, Farber JL. 1975. Early Reversible Plasma Membrane Injury in Galactosamine-Induced Liver Cell Death. Am J Pathol 79:579-596.
7. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. 2005. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. J Ethnopharmacol 99(1):75-81.
8. Handa, S.S., Sharma, A., Chakarborti, K.K., 1986. Natural products and plants as liver protecting drugs. Fitoterapia 57 (5), 307–351.
9. Keppler,D. and Decker,K. (1969) Eur. J. Biochem. 10, 219-225 : Studies on the mechanism of galactosamine hepatitis : accumulation of galactosamine-1- phosphate and its inhibition of UDP-glucose pyrophosphorylase.
10. King J. The dehydrogenase of oxidoreductase-lactate dehydrogenase. In King JC (eds.), Practical Clinical Enzymology, Van D Nostrand Company, London, 1965, pp 83-93.
11. Kucera O, Lotkova H, Kand'ar R, Hezova R, Muzakora V, Cervinkova Z. (2006) The model of D-galactosamine-induced injury of rat hepatocytes in primary culture. Acta. Medica (Hrad ec kralova) (49): 59-65.
12. L.swarna latha and P.Neelakanta Reddy(2011) Evaluation of Anticancer and Antibacterial Activiy of ShpaeranthusAmaranthoides. CiiT International Journal of Biometrics and Bioinformatics.
13. L.Swarna latha and P.Neelakanta Reddy, Indian Journal of Science and Technology vol.2 no.3 (2009) pp 45-48.
14. L.swarna latha, P.Neelakanta Reddy and Kanchan amarnathoides(2009) PROTECTIVE ROLE OF SPHAERANTHUS AMARANTHOIDES EXTRACT ON DERMAL WOUNDS IN WISTAR RATS International Journal on Applied Bioengineering, Vol.3, No.1, (2009) pp52-55.
15. L.swarna latha, P.Neelakanta ReddyProtective Role of S. Amaranthoides on Drug Metabolizing Microsomal Enzymes in b-D-Galn Induced Hepatitic Rats Journal of Pharmacy Research 2011,4(7), pp2143-2145
16. Malloy E and Evelyn K. The determination of bilirubin with the photoelectric colorimeter. J. Bio. Chem. 199: 481-485 (1987).
17. Muntane, J., F.J. Rodríguez, O. Segado, A. Quintero, J.M. Lozano, E. Siendones, C.A. Pedraza, M. Delgado, F. OValle, R. Garcia, J.L. Montero, M. De La Mata and G. Mino, 2000. TNF-alpha dependent production of inducible nitric oxide is involved in PGE (1) protection against acute liver injury. Gut, 47: 553-562.
18. Naik, S.R. and V.S. Panda, 2007. Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int., 27: 393-399.
19. Quintero, A., C.A. Pedraza, E. Siendones, A.M. Kamal ElSaid, A. Colell, C. García-Ruiz, J.L. Montero, M. De la Mata, J.C. Fernandez-Checa, G. Mino and J. Muntane, 2002. PGE1 protection against apoptosis induced by D-galactosamine is not related to the modulation of intracellular free radical production in primary culture of rat hepatocytes. Free Radic Res., 36: 345-355.
20. Rao GM, Morghmom LO, Kabur MN, Ben Mohamed BM, Ashibani K. 1989. Serum gluatmic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in diabetes mellitus. Indian J Med Sci 43:118-121.
21. Reitman S and Frankel SA. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathology. 28: 56-63 (1957).
22. Rosalki SB and Rau D. Serum gamma-glutamyl transpeptidase activity in alcoholism. Clinica. Chimica. Acta. 39: 41-47 (1972).
23. Tsai CC, Kao CT, Hsu CT, Lin CC, and Lin JG. Evaluation of four prescriptions of traditional chinese medicine: Syh-Mo-Yiin, Guizhi-Fuling-Wan, Shieh-Qing-Wan and Syh-Nih-Sann on experimental acute liver damage in rats. J. Ethnopharm. 55: 213-222 (1997).
24. Wills, P.J. and V.V. Asha, 2006. Protective effect of Lygodium flexuosum (L.) Sw. Lygodiaceae) against D-galactosamine induced liver injury in rats. J Ethnopharmacol., 108: 116-123.
25. Yasuda,H.,Izugami,N.,Shimadar,O.,KobaYakawa,Y., Nakanishi,M.,1980.The protective effect of tinoride against carbontetrachloride hepatotoxicity. Toxi-cology and Applied Pharmacology 52,42074213.

26. Zhou, Y., C.M. Park, C.W. Cho and Y.S. Song, 2008. Protective effect of pinitol against D-galactosamine-induced hepatotoxicity in rats fed on a high-fat diet. Biosci Biotechnol Biochem., 72: 1657-1666.

Effect of *S.amarathoides* on the levels of hepatic marker enzymes

Parameters	Group I Control	Group II D-GalN intoxicated	Group II *S.amaranthoides* treated	Group IV D-GalN+ *S.amaranthoides* treated
ALT	114.2 ± 12.9	274.4 ± 26.9***	115.9 ± 12.31^{NS}	138.2 ± 12.9***
AST	86.9 ± 9.1	189.9 ± 19.3***	86.8 ± 9.10^{NS}	95.4 ± 9.23***
ACP	14.9 ± 2.3	36.5 ± 2.9***	15.1 ± 1.09^{NS}	16.6 ± 2.31***
ALP	110.5 ± 9.9	226.2 ± 22.7***	110.4 ± 9.17^{NS}	117.8 ± 9.91***
GGT	6.1 ± 0.29	11.20 ± 0.78***	6.21 ± 0.48^{NS}	7.27 ± 0.49***
LDH	220.0 ± 22.1	378.8 ± 36.7***	222.1 ± 18.9^{NS}	227.4 ± 24.9

Values are expressed as mean ± SD (six animals in each group).
As compared with respective controls (comparisons are made between Group II and Group I; Group III and Group I, Group IV and Group II) statistical analysis by *students t-test* ***P<0.001, ^{NS}–Non significant.
Units : IU/l

Global Journals Inc. (US) Guidelines Handbook 2012

www.GlobalJournals.org

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (FARSS)

- 'FARSS' title will be awarded to the person after approval of Editor-in-Chief and Editorial Board. The title 'FARSS" can be added to name in the following manner. eg. Dr. John E. Hall, Ph.D., FARSS or William Walldroff Ph. D., M.S., FARSS
- Being FARSS is a respectful honor. It authenticates your research activities. After becoming FARSS, you can use 'FARSS' title as you use your degree in suffix of your name. This will definitely will enhance and add up your name. You can use it on your Career Counseling Materials/CV/Resume/Visiting Card/Name Plate etc.
- 60% Discount will be provided to FARSS members for publishing research papers in Global Journals Inc., if our Editorial Board and Peer Reviewers accept the paper. For the life time, if you are author/co-author of any paper bill sent to you will automatically be discounted one by 60%
- FARSS will be given a renowned, secure, free professional email address with 100 GB of space eg.johnhall@globaljournals.org. You will be facilitated with Webmail, SpamAssassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
- FARSS member is eligible to become paid peer reviewer at Global Journals Inc. to earn up to 15% of realized author charges taken from author of respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account or to your PayPal account.
- Eg. If we had taken 420 USD from author, we can send 63 USD to your account.
- FARSS member can apply for free approval, grading and certification of some of their Educational and Institutional Degrees from Global Journals Inc. (US) and Open Association of Research,Society U.S.A.
- After you are FARSS. You can send us scanned copy of all of your documents. We will verify, grade and certify them within a month. It will be based on your academic records, quality of research papers published by you, and 50 more criteria. This is beneficial for your job interviews as recruiting organization need not just rely on you for authenticity and your unknown qualities, you would have authentic ranks of all of your documents. Our scale is unique worldwide.
- FARSS member can proceed to get benefits of free research podcasting in Global Research Radio with their research documents, slides and online movies.
- After your publication anywhere in the world, you can upload you research paper with your recorded voice or you can use our professional RJs to record your paper their voice. We can also stream your conference videos and display your slides online.
- FARSS will be eligible for free application of Standardization of their Researches by Open Scientific Standards. Standardization is next step and level after publishing in a journal. A team of research and professional will work with you to take your research to its next level, which is worldwide open standardization.

- FARSS is eligible to earn from their researches: While publishing his paper with Global Journals Inc. (US), FARSS can decide whether he/she would like to publish his/her research in closed manner. When readers will buy that individual research paper for reading, 80% of its earning by Global Journals Inc. (US) will be transferred to FARSS member's bank account after certain threshold balance. There is no time limit for collection. FARSS member can decide its price and we can help in decision.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (MARSS)

- 'MARSS' title will be awarded to the person after approval of Editor-in-Chief and Editorial Board. The title 'MARSS" can be added to name in the following manner. eg. Dr. John E. Hall, Ph.D., MARSS or William Walldroff Ph. D., M.S., MARSS
- Being MARSS is a respectful honor. It authenticates your research activities. After becoming MARSS, you can use 'MARSS' title as you use your degree in suffix of your name. This will definitely will enhance and add up your name. You can use it on your Career Counseling Materials/CV/Resume/Visiting Card/Name Plate etc.
- 40% Discount will be provided to MARSS members for publishing research papers in Global Journals Inc., if our Editorial Board and Peer Reviewers accept the paper. For the life time, if you are author/co-author of any paper bill sent to you will automatically be discounted one by 60%
- MARSS will be given a renowned, secure, free professional email address with 30 GB of space eg.johnhall@globaljournals.org. You will be facilitated with Webmail, SpamAssassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
- MARSS member is eligible to become paid peer reviewer at Global Journals Inc. to earn up to 10% of realized author charges taken from author of respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account or to your PayPal account.
- MARSS member can apply for free approval, grading and certification of some of their Educational and Institutional Degrees from Global Journals Inc. (US) and Open Association of Research,Society U.S.A.
- MARSS is eligible to earn from their researches: While publishing his paper with Global Journals Inc. (US), MARSS can decide whether he/she would like to publish his/her research in closed manner. When readers will buy that individual research paper for reading, 40% of its earning by Global Journals Inc. (US) will be transferred to MARSS member's bank account after certain threshold balance. There is no time limit for collection. MARSS member can decide its price and we can help in decision.

AUXILIARY MEMBERSHIPS

ANNUAL MEMBER

- Annual Member will be authorized to receive e-Journal GJSFR for one year (subscription for one year).
- The member will be allotted free 1 GB Web-space along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted free 500 MB email space.

PAPER PUBLICATION

- The members can publish paper once. The paper will be sent to two-peer reviewer. The paper will be published after the acceptance of peer reviewers and Editorial Board.

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the 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.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(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, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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

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

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive 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) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

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

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin 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 research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, 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. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. 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: It is the rule of the Global Journals Inc. (US) for authors 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.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. 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.

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

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. **Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. 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 to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After 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 to 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 in 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 criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of 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 the 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 evade

- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose 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 sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic

principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, 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 the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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

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 Inc. (US).

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

INDEX

T

V

Global Journal of Science Frontier Research

Visit us on the Web at www.GlobalJournals.org | www.JournalofScience.org
or email us at helpdesk@globaljournals.org

ISSN 9755896