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VALIDATED RP –UPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND ZIDOVUDINE IN PHARMACEUTICAL FORMULATIONS

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Abstract

A validated, sensitive, reliable and rapid UPLC method has been developed for the quantitative simultaneous determination of Lamivudine and Zidovudine in combined pharmaceutical dosage form. The ultra-performance liquid chromatography was performed on an Acquity UPLC BEH (C18, 50 x 2.1 mm, 1.7 µm) column with UV detection at 270 nm. Mobile phases A, B and C were made of ammonium acetate buffer, methanol and tetrahydrofuran in the ratio 40:40:20 (w/v:v/v:v/v) respectively; injected at a flow rate of 0.5 mL/min. The method is characterized by relatively shorter retention times; 0.65 min and 1.42 min for Lamivudine and Zidovudine, respectively.The linear dynamic range was 0.5-3.0 µg/mL and 0.5- 5.0 µg/mL for LAM and ZID, respectively. Percentage recoveries for LAM and ZID were 100.03 and 99.72 %, respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise and robust for the simultaneous determination of LAM and ZID in pharmaceutical preparation and it can be used for the quality control of formulation products.

Key words: *Lamivudine, Zidovudine, UPLC, pharmaceutical formulations.*

Introduction

Lamivudine, is chemically 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (Fig.1) is a potent is a potentnucleoside analog reverse transcriptase inhibitor (nRTI). It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It needs to be phosphorylated to its triphosphate form before it is active. 3TC-triphosphate also inhibits cellular DNA polymerase. Zidovudine, is chemically known as 1-[(2*R*,4*S*,5*S*)-4-Azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione [1-2] (Fig.2) is a nucleoside analog reverse-transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the

treatment of HIV/AIDS infection. The simultaneous determination of two or more active components in different pharmaceutical preparations, without previous chemical separation, is a common analytical problem [3]. Official monographs, such as The Brazilian Pharmacopoeia and The United States Pharmacopoeia [4-5] describe the determination of AZT and 3TC separately, either as raw material or in different pharmaceutical preparations. No official monograph has been found that describes how this association in tablets can be determined [6-9].

At the present, chromatographic techniques like HLPC, UPLC, GLC and HPTLC are the techniques commonly employed in the pharmaceutical industry to monitor the quality of the end products and the processes, especially when the product has an association of active compounds. These methods are based upon the measurement of specific and nonspecific physical properties of the substances.

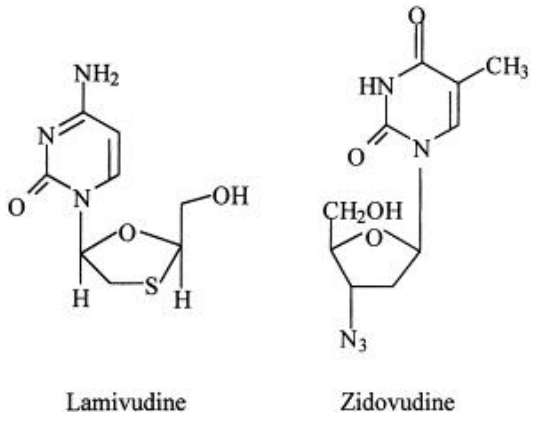

Fig. 1: Structure of Lamivudine and Zidovudine

The literature survey reveals that several HPLC methods [10-16] were reported for the individual estimation of LAM and ZID and few methods for its simultaneous determination [17-19] of the ingredients in combined dosage forms. But there is no simple and sensitive method for the analysis of LAM and ZID. Hence, it is necessary to develop a rapid, accurate and validated RP-UPLC method for the determination of LAM and ZID in tablet dosage form.

In the present research study, attempts were made to develop a rapid, economical, precise, and accurate method for the simultaneous estimation of the LAM and ZID in combined dosage form. A good separation of the analytes of this combination was achieved by using a mobile phase containing ammonium acetate buffer, methanol and terahydrofuran in the ratio 40:40:20 (w/v:v/v:v/v), respectively.The proposed method is rapid, less

expensive, and is successfully applied for the simultaneous determination of LAM and ZID in combined-dosage form (tablets) available in the commercial market. It can be used for the quality control of formulation products.

Experimental

Reagents and standards

Lamivudine and Zidovudine samples were received as gifted samples and were used as received. Standard Zidovudine and Lamivudine were procured from Strides Arcolab Ltd., Bombay, India and from Cadila, Ahmadabad. The Pharmaceutical formulations of LAM and ZID were purchased from local market. HPLC grade Methanol, Merck grade Ammonium acetate and Milli-Q water were used for the assay.

Instrumentation and UPLC conditions

Waters Acquity UPLC column with BEH C18, (50 x 2.1 mm, 1.7 µm) and Empower 2.0 version software was used for data acquisition. The separation was achieved at ambient temperature (30±5 °C) on the column using the mobile phase at a flow rate of 0.5 mL/min. The variable wavelength ultraviolet spectrophotometric detector was set at 270 nm and the injection volume of 1 µL was used for the assay.

Preparation of mobile phase

Mobile phase A: Ammonium acetate buffer of pH 5.5 (prepared by dissolving 7.7 g of ammonium acetate in 100 ml of water and the pH was adjusted to 5.5 by adding ~5.0 ml of glacial acetic acid)

Mobile phase B: Methanol (HPLC grade methanol was used for the assay)

Mobile phase C: Tetrahydrofuran

The mobile phase used was prepared by mixing mobile phase A, mobile phase B and mobile phase C in the ratio, 40:40:20 (A:B:C). The same mobile phase was also used as a diluent for the sample preparations.

Preparation of standard solution

Stock standard solutions of 100 µg/mL of LAM and ZID were prepared separately by using the mobile phase prepared above. All solutions were freshly prepared on the day of analysis.

Procedures

Calibration graph

Working standards of 0.5 to 5.0 µg/mL of LAM and 0.5 to 3.0 µg/mL ZIDwas prepared by taking aliquots from the stock standard solution (100 µg/mL) and diluted to get required concentration for calibration plot. 5.0 µl aliquot of each solution was injected automatically on to the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of the studied drugs.

Preparation of tablet dosage form

Five tablets (each contained 150 mg of LAM and 300 mg of ZID) were accurately weighed and finely powdered. A quantity of the powder containing weight equivalent to 10 mg each of LAM and ZID was transferred to a 100 mL volumetric flask and diluents (75 mL) was added followed by ultra-sonication for 10 min. The solution was then diluted to volume with the same solvent and filtered. 5 µl of the assay preparation was injected and chromatographed.

Results and discussion

Method development

For the preliminary analysis different columns in combination with different solvent systems were tried out. Finally, Waters Acquity UPLC column with BEH (C18, 50 x 2.1 mm, 1.7 µm) was used. The column temperature was set at 30±5 ºC. A series of working standard solutions prepared was injected in duplicate on to the column and was monitored by UV detection at 270 nm. A mobile phase consisting of ammonium acetate buffer adjusted to pH-5.5 with glacial acetic acid, methanol and tetrahydrofuran in the ratio 40:40:20 (w/v/v) were selected after several preliminary experiments. At a flow rate of 0.5 mL/min the retention time was 0.65 and 1.42 min for LAM and ZID, respectively (Fig. 2).

Method validation

The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines [20].

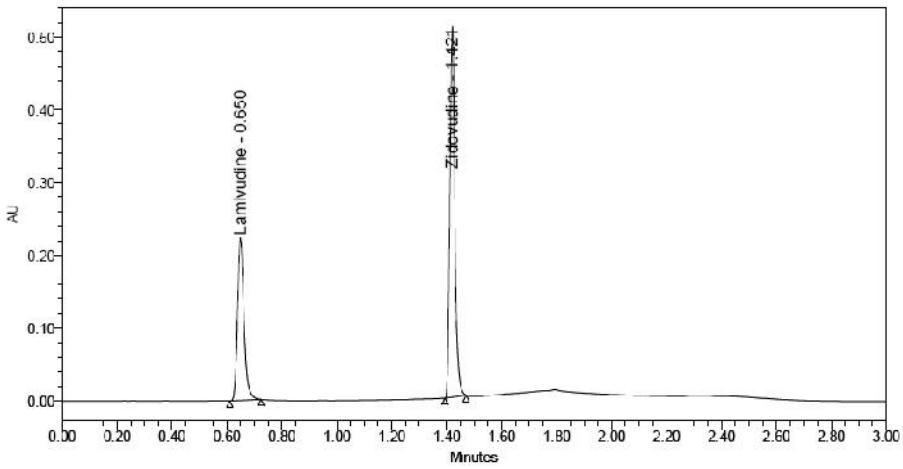

Fig. 2.UPLC Chromatogram of Lamivudine and Zidovudine (1 µg/mL)

Linearity

The linearity of an analytical method indicates its ability to obtain the response directly proportional to the concentration of the analyte in the sample within a definite range. The standard curves in the range of 0.5-5.0 and 0.5-3.0 µg/mL for LAM and ZID were prepared on 3 consecutive days. Each set of standards was injected into the column from lowest to highest concentration.

Accuracy

The accuracy experiment was performed by recovery study at three levels 80%, 100%, and 120% of concentration. The recovery was found between 98 to 101.5% and the results are presented in Table 1.

Precision

The method precision was assessed using multiple preparations of a single sample. Five different concentration of the working standard solution was analyzed in triplicate on the same day. The method precision data is also shown in Table 1. The relative standard deviation values obtained for the peak areas of LAM and ZID was not more than 1.0%.

Table 1. Intra-day accuracy and precision results

LAM taken, µg/mL	LAM found*, µg/mL	RE %	RSD[a] %	RSD[b] %	ZID taken, µg/mL	ZID found*, µg/mL	RE %	RSD[a] %	RSD[b] %
1.0	0.98	0.24	0.30	0.41	0.5	0.52	0.25	0.33	0.45
2.0	1.94	0.61	0.71	0.73	1.0	0.98	0.68	0.78	0.77
3.0	2.93	0.70	0.92	0.93	1.5	1.53	0.79	0.87	0.99

RE: Relative error; RSD: Relative standard deviation.

*Mean value of five determinations; a Based on peak area; b Based on retention time.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established by evaluating the minimum level at which the analyte could be readily detected and quantified accurately. The LOD and LOQ for each component are 0.3 µg/mL (LAM) and 0.22 µg/mL (ZID), respectively. Signal to noise ratio is more than 3 for LOD and more than 10 for LOQ.

Robustness

By introducing small but deliberate changes in the mobile phase composition (±5.0%), detection and flow rate (± 0.3 –0.5 mL/min) robustness of the described method was studied. The % RSD for peak area response was less than 1.0% for retention time which demonstrated that the RP-HPLC method developed was robust (Table 2).

Table 2. Robustness data of the proposed method

Parameter	Proposed	Variation	LAM	ZID
			% RSD	
pH of buffer	5.5	3.56	0.844	0.862
		3.49	0.776	0.738
Wavelength (nm)	270	272	0.688	0.780
		268	0.721	0.691
Mobile phase	40:40:20	40:38:22	0.783	0.563
		38:38:22	0.765	0.977
Flow rate (ml/min)	0.5 ml/min	0.6	0.839	0.680
		0.4	0.782	0.874

*%RSD –Percent Relative Standard Deviation (Six determination)

Application of the method for the analysis of commercial formulations

The developed UPLC method was applied to the simultaneous determination of the studied drugs in tablets containing LAM and ZID (150 mg Lam and 300 mg ZID per tablet) which are available in the local market using the procedure described earlier. Evaluation was performed using the calibration curve method since no significance difference between the slopes of the calibration curves for standards and tablet extracts was observed and with high recovery values and no additional peaks, in the chromatogram indicate that the proposed procedure is free from interference of the commonly used excipients in the formulation.

Recovery

The accuracy and validity of the proposed method was further ascertained by performing recovery experiments. Pre-analyzed tablet powder was spiked with pure LAM and ZID at three different levels and the total was found by the proposed method. Each determination was repeated three times. The recovery of pure drug added was quantitative (Table 3) and revealed that co- formulated substances did not interfere in the determination

Table 3. Results of Analysis of formulation and recovery study by the proposed method.

Drug	Amount mg/tablet		% Label claim	% Recovery
	Label claim	Amount found ± SD		
LAM	150	148.89 ± 0.08	99.99	99.26
ZID	300	289.10 ± 0.15	100.05	96.37

*Average of six determinations, SD- Standard deviation

Conclusion

The RP-UPLC method developed in the present assay for the simultaneous determination of LAM and ZID in bulk form or in tablet dosage form was successfully validated as per the current ICH guidelines. The method was found specific, linear, precise, accurate, and robust. The total run time required for the method is only 3 mins for eluting both Lamivudine and Zidovudine. Statistical analysis proves that the method is suitable for the analysis of LAM and ZID in bulk drug or in pharmaceutical formulations without any interference from the excipients. Hence, this method could be recommended to the industry for quality control of drug content in pharmaceutical preparations.

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SYNTHESIS OF THE INTERMEDIATE 2-(BROMOACETYL)-3*H*-BENZO [*F*] CHROMEN-3-ONE

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Abstract

In the present study the 2-(Acetyl)-3*H*-Benzo[*f*] Chromen-3-one was synthesized through Knovenogel condensation reaction. 2-(Acetyl)-3*H*-Benzo[*f*] Chromen-3-one was brominated using bromine in chloroform in the reflux temperature. The synthesized compound has been characterized using NMR, MASS, and IR spectroscopy.

Keywords: *2-(Acetyl)-3H-Benzo[f] Chromen-3-one, Bromine/Chloroform, 2-Naphthol, Mass spectra*

Introduction

Coumarone (2H-1-benzopyran-2-one) and coumarin derivatives are natural compounds [1] and are important chemicals in the perfume, cosmetic and pharmaceutical industrial production [2]. Knoevenagel reaction a century old reaction is one of the most common Synthetic methods to produce coumarins. Coumarin derivatives exhibited useful and diverse activity in pharmaceuticals, fragrances, agrochemicals, insecticides and polymer science have become the most extensively investigated and commercially significant group of organic fluorescent materials in recent years [3-6]. Coumarins played vital role in electro photographic and electroluminescent devices and laser dyes. Several 3-substituted 7-hydroxycoumarins rank among the most efficient photostable laser dyes emitting in blue green region of the visible spectrum. The lasing range covered by coumarin dyes is appreciably extended when the 3-substituent is a heterocyclic moiety [7,8]. Therefore it is relevant to design and synthesize coumarins bearing different heterocycles at the 3-position with the aim to obtain a new photostable laser dyes having rigid structures that are tunable

over a wide wavelength range within the visible spectrum. In recent years, the use of coumarins as fluorescent labels for a variety of compounds has been reported [9, 10]. Their benzo counterparts, namely benzocoumarins, have been less studied. Akira Taka date *et al*., studied fluorescence properties of coumarins and benzocoumarin [11]. Coumarins posses important photochemical and photo physical properties [12,13] leading to numerous industrial applications [14, 15]. Some of the derivatives are also served as fluorescence brighteners [16], Since fluorescence is highly sensitive to physicochemical environments, a variety of such organic fluorescent compounds have been widely used in many scientific fields, for example, as analytical tools such as fluorescent labeling reagents [17], fluorescence probes [18], fluorescence sensors [19], and laser dyes. Several 7- hydroxyl coumarins having substituents at 3rd position rank among the most efficient photostable laser dyes, emitting in the blue green region in the visible spectrum..In 1999, the world production of fluorescent brighteners amounted to 40000 tons of active substances [20]. The lasing range covered by coumarin dyes has been appreciably extended when the substituents at 3rd position is a heterocyclic moiety [21,22]. Therefore, the synthesis and study of electronic properties of coumarins bearing different heterocycles at 3rd position has gained much attention in order to obtain highly sensitive fluorescence coumarin brighteners. Recently we have reported the synthesis and fluorescent properties of 2-(5-alkyl-1,3,4-oxadiazol-2-yl)-3*H*-benzo[*f*]chromen-3-ones as strong blue fluorescent compounds [12]. In connection to this we report in this paper, the syntheses and the fluorescence properties of various 2-{5-[2-arylethenyl]-1,3,4-oxadiazol-2-yl}- 3*H*-benzo[*f*]chromen-3-ones **(5a-e)** as candidate of novel fluorophores emitting in green region, which could be among the most sensitive and practically useful fluorescent brighteners. This work was encouraged by our previous investigation on new routes and new synthesis of various heterocyclic compounds [23-32].

Materials and Methods

The melting point of the synthesized compounds were determined in open capillary using LABHOSP melting point apparatus and recorded without correction. Progress of the reaction and the purity of the compounds were checked using precoated silica gel TLC plates (60 GF, 254 MERCK) and a mixture of petroleum ether and ethyl acetate (1:1) as a mobile phase. The IR spectra of the synthesized compounds were recorded on SHIMADZU FTIR 8400 spectrometer by KBr pellet technique. The ^{1}HNMR spectra of the synthesized compounds were taken using BRUKER SPECTROSPIN-400MHz spectrometer using

CD_3OD/CD_3COCD_3 as solvent and TMS as internal standard. The Mass spectra the chemical shift data's were expressed as δ ppm.

Synthesis of 3-Acetyl benzocoumarin

A mixture of 2-hydroxy-1-naphthaldehyde (0.029mol, 5g), an equivalent amount of ethyl acetoacetate (0.0029mol, 3.77g,), and catalytic amount of piperidine in ethanol (1.0ml) was refluxed for 30 minutes on water bath. After the reaction was complete, the reaction mixture was cooled to room temperature and poured into 100g crushed ice with stirring. The precipitate obtained was then filtered, washed with water, dried and recrystallized by using ethanol to get pure compound (**Scheme – 1)**.

3- Acetyl benzocoumarin

Solid; (94%); m.p.117^{0}C; IR (KBr) ν (cm^-1): 1765 (C=O pyrone), 1750 (C=O Acetyl): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):) 2,7 (S, 3H, CH_3), 7.4 (m, 6H, ArH), 9.3 (s, 1H, CH).

Synthesis of substituted 2-(bromoacetyl)-3*H*-benzo[*f*]chromen-3-one

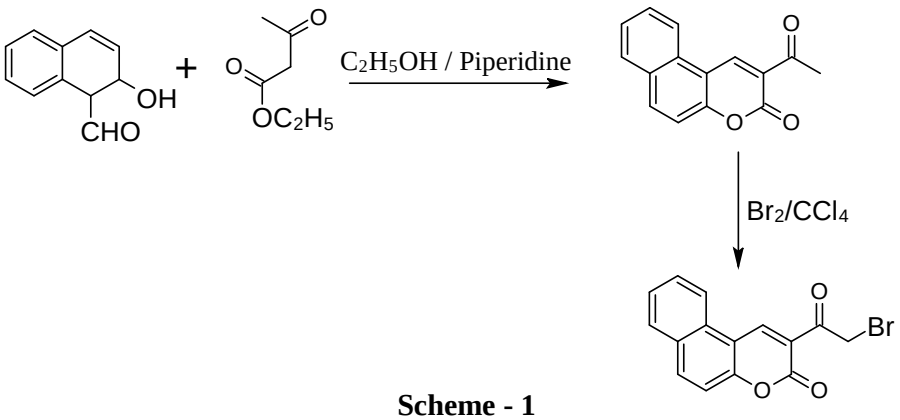
The compound 3-Acetyl benzocoumarin (0.0042, 1g) in chloroform was cooled, added an equivalent amount of bromine in chloroform, stirred for half an hour. Then it was refluxed for 3-4 hours on water bath. After the reaction was completed, the reaction mixture was cooled to room temperature and poured into 100g crushed ice with stirring. The precipitate obtained was then filtered, washed with water, dried and recrystallised by using ethanol to get pure compound (**Scheme-1)**.

2-(bromoacetyl)-3*H*-benzo[*f*]chromen-3-one

Solid; (75%); m.p.158^{0}C; IR (KBr) ν (cm^-1): 1765 (C=O pyrone), 1810 (C=O Acetyl): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):) 4.8 (S, 2H, CH_2), 7.8 (m, 6H, ArH), 9.4 (s, 1H, CH): UPLCMS: **317**

Results and Discussion:

The yields of the synthesized compounds 3- Acetyl benzocoumarin, 2-(bromoacetyl)-3H-benzo[f]chromen-3-one were found to be 94% and 75% respectively. The Mass, IR and ^{1}HNMR Spectra of these compounds clearly indicates that the assigned structures are in good agreement with them. The molecular mass of the target compound 2-(bromoacetyl)-3*H*-benzo[*f*]chromen-3-one is exactly matches at 370.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C2H5OH / Piperidine
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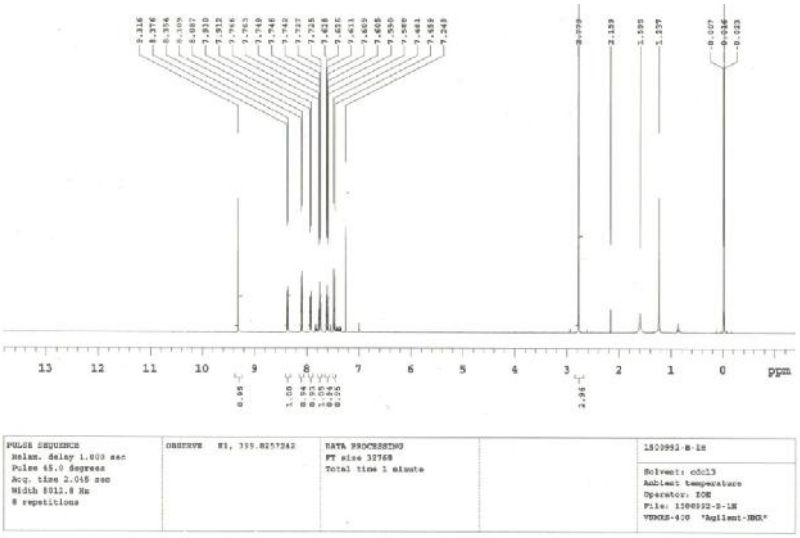
Scheme - 1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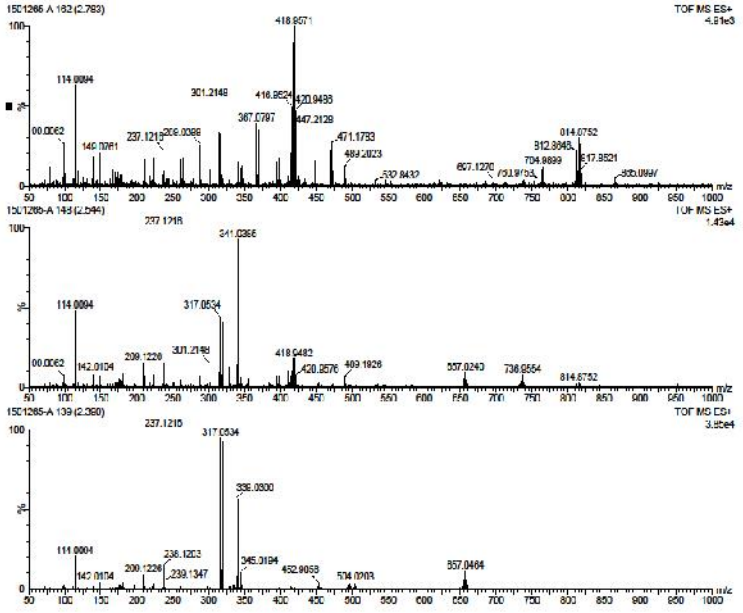
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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF NEW SCAFFOLD OF SCHIFF BASE DERIVATIVES

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Abstract

The Schiff base ligands *N*′[1],*N*[3]-bis[(E)-4-(dimethylamino) benzylidene] benzene-1,3-dicarbohydrazide and *N*′-{(*Z*)-[4-(dimethyl-amino)phenyl]methylidene}-2-oxo-2*H*-chromene-3-carbohydrazide have been synthesized and charaterized by elemental analysis, FTIR, UV-visible and mass spectral studies. The newly synthesized ligands were screened for their biological activity against bacterial species, *gram* positive bacteria (Staphylococcus aureus) and *gram* negative bacteria (Escherichia coli). Both the Schiff base ligands showed significant activity against both *gram* positive and *gram* negative bacteria at higher concentration (**100 µg**) and lower concentration (**50 µg**).

Key Words: *Schiff base, UV-Visible, Antibacterial activity.*

Introduction

Schiff bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications in many fields, e.g., biological, inorganic and analytical chemistry [1,2]. Applications of many new analytical devices require the presence of organic reagents as essential compounds of the measuring system. Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties [3,4]. Schiff bases play important roles in coordination chemistry as they easily form stable complexes with most transition metal ions [5,6]. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds.

Azomethine group (–C=N–) containing compounds typically known as Schiff bases have been synthesized by the condensation of primary amines with active carbonyls. Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial, antifungal and antitumor activity. They have been studied extensively as a class of ligands and are known to coordinate with metal ions through the azomethine nitrogen atom [7]. Schiff's base is a functional group or type of chemical compound containing a carbon nitrogen double bond with the nitrogen atom connected to an aryl group or an alkyl group but not hydrogen [8]. Schiff's bases can be synthesized from an aromatic amine and a carbonyl compound in a nucleophilic addition to a hemiaminal followed elimination of water to the imine [9].

Schiff-base macrocycles have been of great importance in macrocyclic and supramolecular chemistry. In coordination chemistry the functionally substituted Schiff bases bearing additional donor groups represent the most important class of hetero polydentate ligands capable of forming mono-bi, and polynuclear complexes with transition and non-transition metals. They were among the first artificial metal macrocyclic complexes to be synthesized. Interest in exploring metal ion complexes with hydrazone Schiff-base ligands has been continuously increasing owing to the recognition of the role played by these structures in metalloproteins. During the last two decades, considerable efforts have been made for developing metal-free methods for furnishing macrocycles starting from various dicarbonyl compounds and diamines in addition to standard metal-templated protocols.

Schiff base ligands are capable for supporting metals in a variety of oxidation states. However, the metal or the ligand may become oxidized [10]. Generally Schiff bases are prepared under acid or base catalysis or with heat. Schiff bases of aliphatic aldehydes are unstable and readily polymerizable while those of aromatic aldehydes, having an effective conjugation system, are more stable. The common Schiff bases are crystalline, these are feebly basic but at least some form insoluble salts with strong acids. Schiff bases are readily hydrolyzed by aqueous acid to give back the amine and aldehyde. They can be reduced and may be used in the preparation of secondary amine [11]. The majority of Schiff bases usually act as multidentate N-N and N-O donors with the formation of mono-or polynuclear complexes [12].

The literature survey clearly shows that the study of Schiff base ligand systems is linked with many of the key advances made in inorganic chemistry. They played a seminal role in the development of modern coordination chemistry.

Hydrazones or aroylhydrazones have been widely used as ligands in the preparation of a variety of transition metal complexes. The hydrazone ligands and their complexes are biologically active and their biological activities may be due to the presence of an "azomethine" linkage. The acyl/aroylhydrazone or coumarin moiety may co-ordinate to the metal through the keto or enol forms. The complexing ability of the coumarin hydrazone ligand increases through the enol form [R-C(O) NH-N=C<R-C(OH)N-N=C<] as the π-conjugation or π - character of the R-group increases [13].

Hydrazide-hydrazones compounds are not only intermediates but they are also very effective organic compounds in their own right. When they are used as intermediates, coupling products can be synthesized using the active hydrogen component of –CONHN=CH- azomethine group [14]. Hydrazides and hydrazones are of wide interest because of their diverse biological and clinical applications. This created interest in researchers who have synthesized variety of hydrazide derivatives and screened them for their various biological activities. In the present study, we made an attempt to collect biological properties of hydrazide and hydrazone derivatives reported in the new millennium [15].

Interest in the study of hydrazones has been growing because of their antimicrobial, antituberculosis, and antitumour activity. Hydrazones derived from condensation of isonicotinic acid hydrazide with pyridine aldehydes have been found to show better antitubercular activity than INH .The remarkable biological activity of acid hydrazides $R-CO-NH-NH_2$, their corresponding aroylhydrazones $R-CO-NH-N=CH-R$, and the dependence of their mode of chelation with transition metal ions present in the living system have been of significant importance in the past [16].

On the other hand, interest in coumarin chemistry has flourished for many years; largely as a result of the wide spread use of coumarin derivatives. Apart from the medicinal, biological and pharmacological applications coumarins are also used as sweeteners, fixatives of perfumes, additives in food and cosmetics, odor stabilizers in tobacco and an odor masker in paints and rubber [17].

Derivatives of coumarin are known to possess significant antifungal as well as antibacterial properties, and there are a number of commercially available coumarin-based antibiotics such as Novobiocin, Clorobiocin and Coumermycin A1. Many of the coumarins present in plants, and also their synthetic analogues, have been reported to be good antifungal

and antibacterial agents. Preliminary structure–activity relationship studies have shown that the presences of hydroxyl or carboxylic groups on the coumarin nucleus are necessary for antimicrobial activity. Coumarin-derived Schiff bases are well known compounds and several reports have been written about their applications as dye and fluorescent agents. Iminocoumarins have also been shown to exhibit anti-inflammatory, antibacterial and antifungal activities [18].

Although Schiff bases containing coumarin moiety have been used in a variety of enzymes, little attention has been given to their metal binding properties and the structural features of their complexes [19].

Coumarin derivatives are known for their physiological, photodynamic, anticoagulant, bacteriostatic, and antitumor activity. A large number of structurally novel coumarin derivatives have ultimately been reported to show substantial cytotoxic and anti-HIV activity in vitro and in vivo systems. Coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase. Several authors have reported on the use of Coumarin containing hydrazones for the treatment of some human carcinomas. The Coumarin derivatives have been the focus for designing of new cytotoxic agents. It is well known that many investigations have proved that binding of a drug to a metal enhances its activity and in some cases the complex possesses even more healing properties than the parent drug. Metal ions in coordination with coumarin derivatives at different positions have attracted the researchers for chelation [20].

Enlightened by the above mentioned facts and in continuation of our research work, [21-24] towards the synthesis of potentially bioactive Schiff base ligands by simple and practical approach, herein we report a rapid and efficient method for the synthesis of novel linear and bis-aroyl Schiff base ligands *via* simple condensation method afford good yield. The synthesized compounds exhibited significant antibacterial activity [25].

Materials and Methods

Materials and Physical measurements

All reagents and solvents required were of AR grade, purchased commercially. All the solvents were purified by distillation and N,N-dimethyl benzaldehyde, Diethyl malonate, Piperidine and DMF were purchased from Himedia.

The melting points are determined by open capillary methods and are uncorrected. The UV‑Visible spectra are recorded on a Shimadzu model impact 1650 UV‑Visible double beam spectrometer. The FT-IR spectra are recorded on a Shimadzu model impact 8400S FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution), ^{1}HNMR, on a Bruker 400 MHz and mass spectra are recorded on LCMS Shimadzu, Japan 800 MHz spectrometer. Elemental analyses are done on Vario EL.CHNOS elemental analyzer.

Synthesis

Synthesis of benzene-1,3-dicarbohydrazide

Calculated amount of isophthalic acid was dissolved in absolute ethanol and was placed in a freezing mixture with constant stirring. Then it was treated with conc. H_2SO_4 drop-wise for about half an hour. After the addition the reaction mixture was refluxed for about 8-10 hours at 80-90^{o}C. After completion of the reaction monitored by TLC, the mixture was poured in to ice cold water, neutralized and then it was extracted with ethyl acetate to well achieve the conversion. The resulting ester solution was refluxed with hydrazine hydrate in absolute ethanol to get the final product benzene-1,3-dicarbohydrazide.

Synthesis of N'^{1},N^{3}-bis[(E)-4-(dimethylamino) benzylidene] benzene-1,3-dicarbohydrazide (L_1)

0.002 mol of N,N-dimethyl benzaldehyde was dissolved in ethanol (15 mL) and treated drop-wise with an aqueous solution of benzene-1,3-dicarbohydrazide (0.001 mol) in presence of catalytic amount of glacial acetic acid and then the reaction mixture was stirred for about six hour at 90-100 ^{0}C. After completion of the reaction monitored by TLC, the resulting solution was cooled and poured into ice cold water with constant stirring. The obtained product was filtered and washed with water for three to four times, dried and recrystallized using DMF and H_2O. The resulting compound was a pale yellow solid. The yield was 86 % and m. p. = 176-178 °C. Anal. (%) Calc. for [$C_{26}H_{28}N_6O_2$]: C, 68.40; H, 6.18; N, 18.41. Found: C, 68.42; H, 6.17; N, 18.39. LC-MS (m/z) 457 [$C_{26}H_{28}N_6O_2$ + H]$^{+}$. IR (cm^{-1}): 3505.1, 3421.1 (-NH), 3077.8 (Ar -CH), 5807.4 (Ali-CH), 1650.9 (C=O), 1600 (C=N), 1519.6 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 7.4-8.4 ppm (m, Ar-ring),12.10 ppm (s,2H,-NH), 9.9-10.22 ppm (s,2H,-CHN), 1.30 ppm (s,6H,-CH_3). UV–Vis in DMF [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 358 (5820).

Synthesis of *N*'-{(*Z*)-[4-(dimethylamino)phenyl]methylidene}-2-oxo-2*H*-chromene-3-carbohydrazide-(L_2)

N,N^1-dimethylbenzaldehyde (0.146g, 0.001 mol) was dissolved in dimethyl formamide (20 mL) and treated drop-wise with 2-oxo-2H-chromene-3-carbohydrazide (0.2g, 0.001 mol) and then the reaction mixture was stirred for about six hour at 90-100 °C. After completion of the reaction monitored by TLC, the resulting solution was cooled and poured into ice cold water with constant stirring. The obtained product was filtered and washed with water for three to four times, dried and recrystallized using Ethanol. The resulting compound was a light brown solid with 81% yield and m.p. = 206-208 °C. Anal. (%) Calc. for [$C_{19}H_{17}N_3O_3$]: C, 68.05; H, 5.11; N, 12.53. Found: C, 68.03; H, 5.10; N, 12.55. LC-MS (m/z) 335 [$C_{19}H_{17}N_3O_3$ + H]$^+$. IR (cm^{-1}): 3435.2 (-NH in amide), 2926.7 (Ali-CH), 1620.1 (C=O in amide), 1572.1 (C=N), 1526.1 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.54-10 ppm (s, 1H, CHN), 4.53-4.9 ppm (S, 1H, -NH in amide), 8.4-8.73 ppm (m, 4H, Ar-ring), 8.22-8.5 ppm (m, 4H, Ar-ring), 0.92-0.94 ppm (s, 6H, -CH_3). UV–Vis in DMF [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 358 (7800), 330 (5820).

Antibacterial activity

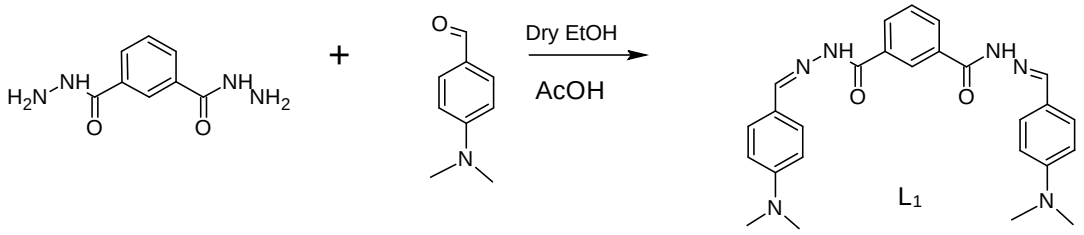
The synthesized Schiff base ligands were screened for their antibacterial activity by nutrient agar diffusion method in dimethyl sulphoxide (DMSO) solvent against Gram-positive (S. aureus) and Gram-negative (E. coli) bacterial species [26]. The stock solution (1 mg mL^{-1}) of the test chemical was prepared by dissolving 10 mg of the test compound in 10 mL of DMSO. The stock solution was suitably diluted with sterilized distilled water to get dilution of 100, 50 and 25 µg mL^{-1}. Control for each dilution was prepared by diluting 10 mL of solvent instead of stock solution with sterilized distilled water.

The bacteria were subcultured in agar medium. The petridishes were incubated for 24 h at 37 °C. Standard antibacterial drug, Ciprofloxacin was also screened under similar conditions for comparison. The petridishes were incubated for 48 h at 37 °C. Sterile disks were used in the agar media. The wells were dug in the agar media using a sterile metallic borer. Activity was determined by measuring the diameter of the zone showing complete inhibition. Growth inhibition was compared with the standard drugs. In order to clarify any effect of DMSO on the biological screening, separate studies were carried out with solutions alone of DMSO and they showed no activity against any microbial strains. Compounds showing promising antibacterial activity were selected for minimum inhibitory concentration (MIC) studies [27].

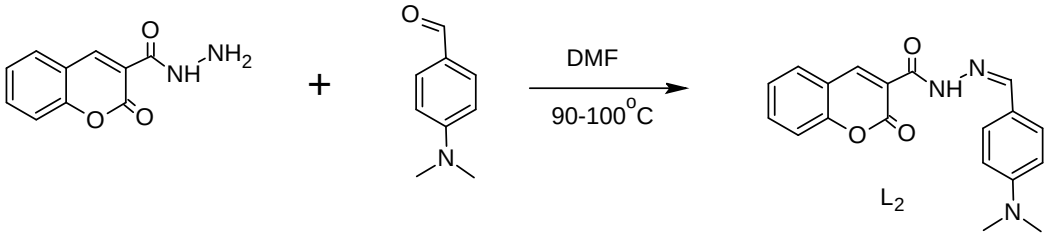
Results and Discussion

Due to the vital importance of the symmetrical bis-aroyl schiff ligands containing heterocyclic groups such as N,O etc, we have synthesized *N*'1,*N*^{3}-bis[(E)-4-(dimethylamino)benzylidene]benzene-1,3-dicarbohydrazide (L_1) and *N*'-{(*Z*)-[4-(dimethylamino)phenyl]methylidene}-2-oxo-2*H*-chromene-3-carbohydrazide (L_2) derivatives. The strategy adopted for the synthesis of new Schiff base ligand system involves a successive building up of the (L_1) and (L_2) on the synthesized benzene-1,3-dicarbohydrazide and 2-oxo-2H-chromene-3-carbohydrazide with carbonyl compound such as 4-(dimethylamino) benzaldehyde. The path followed (L_1) were of synthesized using two reaction path ways.

In first step the isophthalic acid ester when reacts with hydrazine hydrate in absolute alcohol, the resulting product was filtered and recrystallized **(1a),** in second step the resultant hydrazide compounds reacts with different carbonyl compounds in presence of acetic acid as a catalyst using ethanol solvent. The product was isolated from ice cold water. The obtained precipitate was removed by filtration and the usual work-up gave the desired products in almost quantitative yields. We have observed that addition of acetic acid conditions gives better yields.



Scheme-1: Synthesis of N'^{1},N^3-bis[(E)-4-(dimethylamino)benzylidene] benzene-1,3-dicarbohydrazide (L_1)



Scheme-2: Synthesis of *N*'-{(*Z*)-[4-(dimethylamino)phenyl]methylidene}-2-oxo-2*H*-chromene-3-carbohydrazide (L_2).

Table-1: Physical and analytical data of **L_1** and **L_2.**

Compd.	Color	Yield (%)	m. p ºC	Cryst. solvent	Mol. Formula (Mol. wt.)
L_1	Pale yellow	86	180-182	DMF	$C_{26}H_{28}N_6O_2$ (456)
L_2	Pale brown	81	206-208	Ethanol	$C_{19}H_{17}O_3N_3$ (335)

The newly synthesized compounds were characterized by IR, ^{1}HNMR, Mass and UV-visible spectral studies. Our mechanistic investigation gives the proof for ligands **L_1** and **L_2**.

IR Studies

The IR spectra of the compound **1a** showed a sharp peak in the region of 3240-3450 cm^{-1} was corresponding to NH-NH_2 stretching vibration in benzene-1,3-dicarbohydrazide, The IR spectra of the compound **L_1** showed a broad peak in the region 3505.1, 3421.1 for –NH stretching. Two broad peaks observed in the region of 3077.8 cm^{-1} and a weak band observed at 5807.4 cm^{-1} respectively is attributed to aromatic and aliphatic –CH stretching vibrations. Two sharp peaks present in the region 1650.9 and 1600 cm^{-1} confirms the presence of C=O and C=N functionality. Similarly, the IR spectrum of L_2 showed a broad peak in the region 3435.2 cm^{-1} for –NH stretching amide group. A broad peak observed in the region of 2926.7 cm^{-1} is attributed to aromatic and aliphatic –CH stretching vibrations. Two sharp peaks present in the region 1620.1 and 1572.1 cm^{-1} confirms the presence of C=O and C=N functionality. This structure was further confirmed by ^{1}H NMR spectra.

^{1}H NMR Studies

The ^{1}HNMR spectra of L_1 show two singlets at 12.10 ppm and 9.9 ppm is corresponding to –NH protons and CHN protons respectively. The resonate multiplets in the region of 7.4-8.4 ppm and a sharp singlet observed at 1.30 ppm is attributed to aromatic and a –CH_3 protons present in the compound. On the other hand, ligand L_2 shows two sharp singlets at 4.9 and 9.54 ppm corresponding to –NH and –CHN protons respectively. This structure was further confirmed by mass spectra.

Mass Studies

The molecular ion peak observed at m/z = 457 (M+H) and at m/z = 335 (M+H) for L_1 and L_2 is corresponding to their molecular weight confirms the formation of both the ligands. The corresponding spectra's are given below.

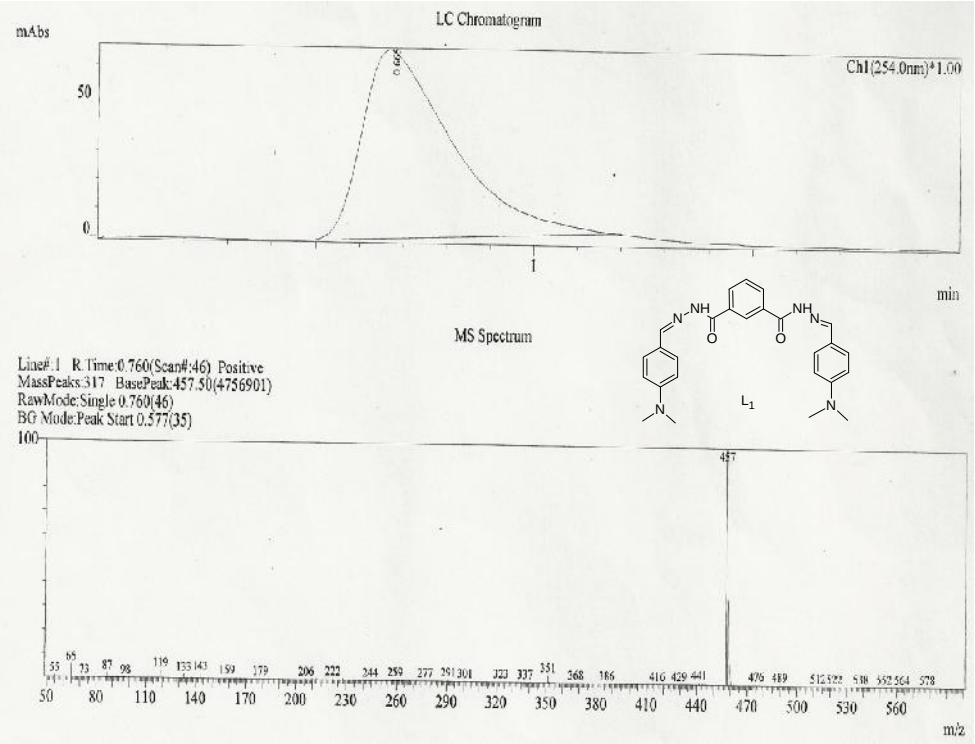


Fig. 1: LC-Mass spectrum of N'[1],N[3]-bis[(E)-4-(dimethylamino)benzylidene] benzene-1,3-dicarbohydrazide (L_1).

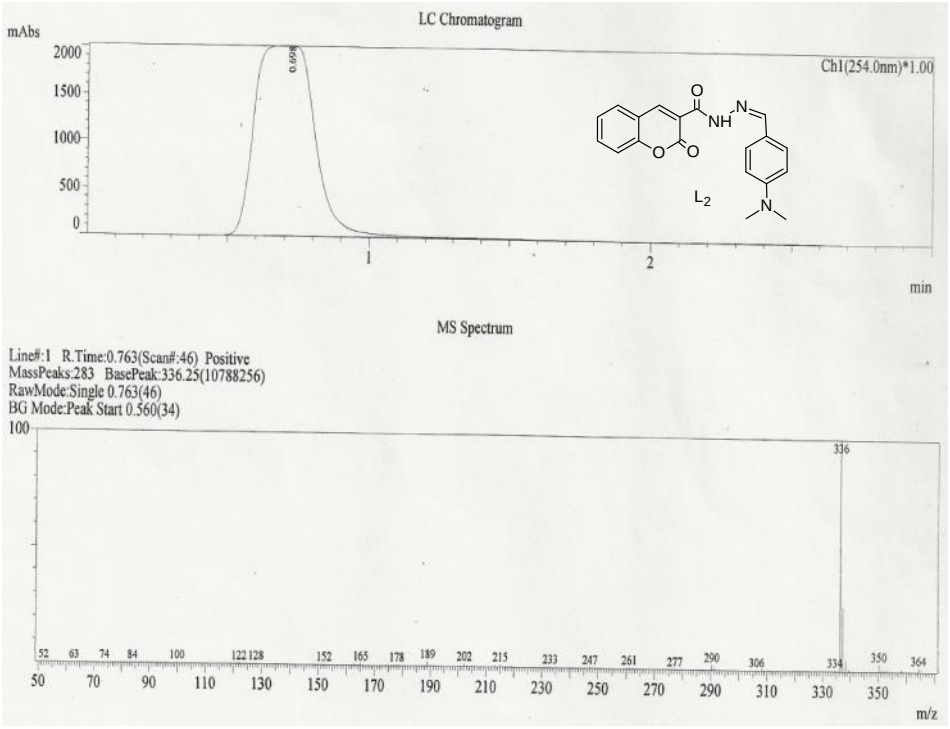


Fig. 2: LC-Mass spectra of N'-{(Z)-[4-(dimethylamino)phenyl]methylidene}-2-oxo-2H-chromene-3 carbohydrazide (L_2).

The Electronic Spectra

The electronic spectra of the newly synthesized Schiff base ligands were recorded in DMF solution in the range 200-800 nm regions (Figures 3 & 4) and the obtained protocol was summarized in **table 1**. The absorption maxima of all the compounds exhibited was due to π-π* transitions.

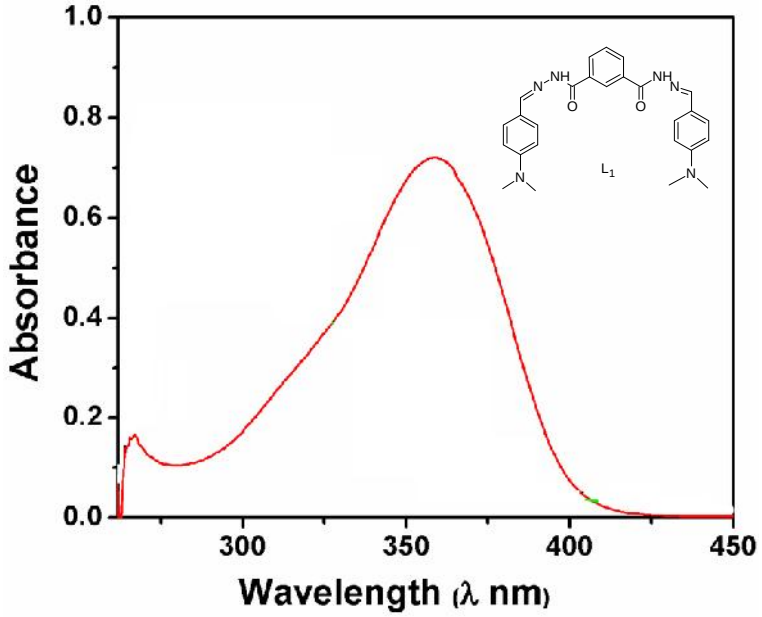


Fig. 3: UV-Visible spectrum of N'^{1},N^{3}-bis[(E)-4-(dimethylamino)benzylidene] benzene-1,3-dicarbohydrazide **(L_1)** in DMF.

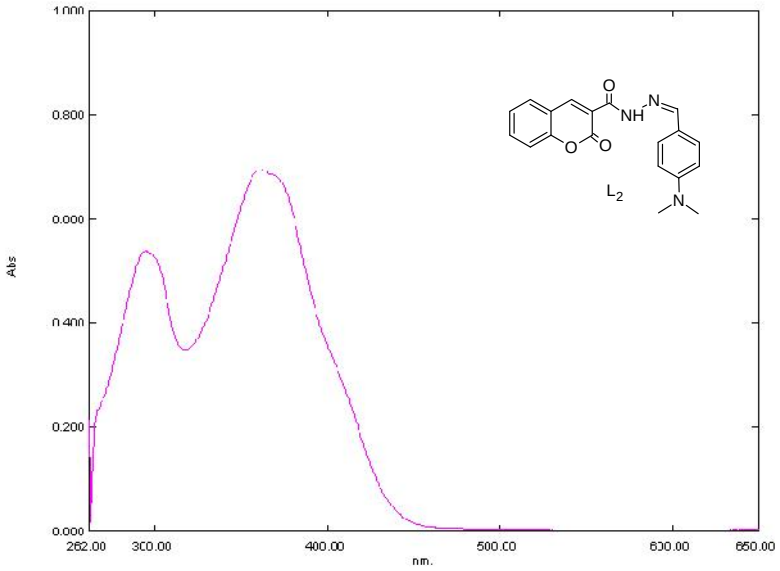


Fig. 4: UV-Visible spectra N'-{(Z)-[4-(dimethylamino)phenyl]methylidene}-2-oxo-2H-chromene-3-carbohydrazide **(L_2)** in DMF.

Table-2: Summarized UV-Visible spectral data of $\mathbf{L_1}$ and $\mathbf{L_2}$ in DMF.

Compounds	Solvent	λ_{max} in nm	Absorbance (A)	Log ε
$\mathbf{L_1}$	DMF	358	0.717	3.58
$\mathbf{L_2}$	DMF	362, 295	0.72, 0.57	3.62

Evaluation of Antibacterial activity

The *in-vitro* antibacterial activity was carried out against 24-48 hrs in cultured media using two bacteria ***Staphylococcus aureus*** and ***Escherichia coli*** by cup-let method. The compounds have been tested for the antibacterial activity against 1 and 2. Nutrient agar and potato dextrose agars were used for the preparation of cultural media. The compounds were tested at the concentration of 100 µg/mL in DMSO. The compounds were tested at two different concentrations ie, 50 µg/mL and 100 µg/mL, the compounds shows very good MIC against the *gram* positive and *gram* negative bacteria at 100 µg/mL than that of the 50 µg/mL in DMSO solution. Inhibition was recorded by measuring the diameter of inhibition zone at 24-48 hrs. Each experiment was carried out at 28 $^{\circ}$C thrice and the average reading was recorded.

Table-4: Evaluation of antibacterial activity of L_1 and L_2 performed at **100 µg/mL.**

Compounds	*Staphylococcus aureus*	*Escherichia coli*
$\mathbf{L_1}$	1.7 cm	1.1 cm
$\mathbf{L_2}$	1.7 cm	1.5 cm
Ciprofloxacin	**2.6 cm**	**2.6 cm**

Determination of minimum inhibitory concentrations (MICs)

Table-5: Evaluation of antibacterial activity of (L_1) and (L_2) performed at **50 µg/mL.**

Compounds	Staphylococcus aureus	Escherichia coli
L_1	1.3 cm	0.9 cm
L_2	1.3 cm	1.1 cm
Ciprofloxacin	**2.6 cm**	**2.6 cm**

The established compounds give significant activity towards the tested bacteria and obtained protocol was summarized in Table 4 and 5. The compound $\mathbf{L_1}$ and $\mathbf{L_2}$ were exhibited more significant activity towards the tested bacteria. The significant activity of given synthesized compounds due to presence of azomethine nitrogen atoms.

Conclusion

The synthetic route adopted for the synthesis of N^1,N^3-bis[(E)-4-(dimethylamino) benzylidene]benzene-1,3-dicarbohydrazide (**L_1**) and *N*'-{(*Z*)-[4-(dimethyl-amino)phenyl]methylidene}-2-oxo-2*H*-chromene-3-carbohydrazide (**L_2**) was very simple and gives good yield. The efficiency and simple methodology based on utility of acid catalyzed condensation reaction. The efficiency and fastness of the reaction employed was explained by fact that the energy required for the conversion is attributed to the role of acetic acid as a catalyst. The activation energy required for the conversion very less .The visible spectroscopy of the compounds gives bathochromic shift (red shift) due to high conjugation present in the given compound. The antibacterial activity of the compound having azomethine groups (**L_1**) exhibited predominant activity compared to other ligand (**L_2**). And also, both the synthesized Schiff base ligands shows significant activity against both *gram* positive and *gram* negative bacteria at higher concentration (**100 µg**) and lower concentration (**50 µg**).

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VINYL POLYMERIZATION OF ACRYLONITRILE INITIATED BY Ce(IV)-ISOBUTYRIC ACID REDOX SYSTEM: A KINETIC STUDY

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Abstract

The Polymerization of Acrylonitrile (AN) initiated by Ce(IV)- Isobutyric acid redox system has been studied in aqueous sulphuric acid medium under nitrogen atmosphere in the temperature range of 40 to 70 ^{o}C. The rate of polymerization is proportional to $[AN]^{1.0}$ $[Isobutyric]^{0.75}$ and $[Ce(IV)]^{0.33}$ and the rate of ceric ion disappearance is proportional to [Isobutyric], [Ce(IV)]. The effects of organic solvents, surfactants, ionic strength, dielectric constant and temperature have been investigated. A kinetic scheme has been proposed and various +rates, activation energy and other thermodynamic parameters were evaluated.

Keywords: *Vinyl Polymerization, AN. Ce (IV), Isobutyric redox.*

Introduction

Redox polymerization has advantages of no induction period or a very short induction time, low activation energy, production of high molecular weight polymers with high yields and high purity, easy control of the polymerization reaction, at low temperature reduction of the side reactions can be achieved and the direct experimental evidence of the transient radical inter mediates [1]. In redox systems, oxidant forms initially a complex by reacting simply organic molecules; which then decomposes unimolecularly to produce free radicals that initiate polymerization [2].

Numerous redox pairs containing organic & Inorganic components as polymerization initiator have been used successively. Commonly used oxidants include peroxides. Persulfates, permanganates etc, are the salts of transition metals. These oxidants form potential redox systems with various reducing agents like Alcohols, aldehydes, amines,

amides, ketones, acids, thiolsetc for the aqueous polymerization of vinyl monomers [3]. Ce(IV) ion has been used for the oxidation of many organic compounds, in the form of Ce(IV) ammonium sulfate, Ce(IV)-Sulfate, Ce(IV) ammonium nitrate and ceric perchlorate [4]. The oxidation of alcohol by Ce(IV) is believed to proceed by disproportionation of coordination of complexes. According to the complex formation, unimolecular disproportionation of complex yields cerrous ion, a proton and a free radical on the alcohol substrate [5-7].

This paper describes the results of the investigation of the redox polymerization of AN with Isobutyric acid, initiated by Ce (IV)/H_2SO_4 redox system. The aim is to evaluate the effects of Ce(IV) / isobutryic acid, acrylonitrile (AN), H_2SO_4 concentration, temperature on the rate of polymerization and yield. In addition of organic solvents and surfactants the rate of polymerization were studied. The PAN characterization such as molecular weight, density, Refractive index (R.I), and FTIR spectral analysis is also studied. The energy of activation and other thermo dynamic parameters were computed.

Experimental

Materials

Acrylonitrile (S.d. fine Chem. India) was purified by the method of *Bamford et al* [8]. The reagents ceric ammonium Sulfate, Sulphuric acid and Isobutyric acid (s.d. fine Chem.) was also of Analar grade. Water was used doubly distilled for the preparation of reagents and solutions throughout the experiment. Reaction mixtures were deaerated by passing pure nitrogen through a column of Fieser's solution and distilled water.

Methods

All the polymerization experiments were carried out in Pyrex glass vessels. The reaction vessels were covered completely with rubber gasket. The requisite amounts of Isobutyric acid ($2.0x\ 10^{-2}$ mol/dm^3), Acrylonitrile ($8.05x10^{-2}$mol/dm^3) and H_2O (to keep the volume constant) were placed in the reaction glass vessels. The system was thermostated at 50 ^oC and degassed by flushing with free nitrogen. An appropriate amount of Ce(IV)($4.0x10^{-3}$mol/dm^3) in aqueous H_2SO_4 was ($2.0x10^{-3}$mol/dm^3) then added to the reaction mixture under nitrogen atmosphere and the polymerization was attained for 75 minutes. The reaction product was precipitated, filtered and dried to constant weight at 35^oC.

Characterization of Polyacrylonitrile Product

The Molecular weight (Mv) of the purified sample of polyacrylonitrile product was determined by viscometry. A 1% Solution of the polymer in DMF was filtered through a fritted glass filter and placed in Ubbelhode type suspended level dilution viscometer. The intrinsic viscosity [η] for the reaction mixture was determined, and the Mv value was calculated using the following Mark-Houvink equation given by Stock Mayer and Cleland [9] $[\eta]=(3.335x10^{-4}x\ Mv^{0.72}$ at 30°C for polyacrylonitrile. The molecular weight of the polymer product under standard conditions was found to be $5.68x10^{5}$. It is increases with increase in monomer concentration and decreases with increase in Ce(IV), Isobutyric acid and temperature. This effect is attributed to the fact that increase in [Ce(IV)] [Isobutyric acid], & temperature provides more chances for premature termination of growing chain radicals reducing the degree of polymerization. [10,11].The density of the product polyacrylonitrile was determined using density bottle (25 mL) and R.I (1.245 & 1.432) by Abbe's refractometer respectively. Finally, the FTIR Spectra of the PAN were taken on a Shimadzu FTIR 8101A China instrument in pressed KBr Pellets. The formation of PAN in this system was endured by FTIR analysis.

Stoichiometry and product analysis

Reaction mixture of various constituents of [Ce(IV)], [IBA], [AN], and $[H_2SO_4]$ were equilibrated at 50° C for 24h.The reaction mixture showed that one mole of [Ce (IV)] was consumed per mole [IBA] according to equations are as follows:

$$Ce(SO_4)_2 + HSO_4^- \longleftrightarrow Ce(SO_4)_3^{2-} + H^+ \quad (1)$$

$$Ce(SO_4)_2 + C_4H_7O_2^- \longleftrightarrow Ce(SO_4)_2 - C_4H_6O_2^{2-} + H^+ \quad (2)$$

$$Ce(SO_4)_2 + C_4H_6O_2^{2-} \longleftrightarrow Ce(SO_4)_2 + {}^{\bullet}C_4H_6O_2^{2-} \quad (3)$$

$$Ce(SO_4)_2 + C_4H_6O_2^{2-} + {}^{\bullet}C_4H_6O_2^{2} \longrightarrow Ce(SO_4)_2 + C_4H_6O_2^{-} + (CH_3)_2CO + CO_2 + 2C \quad (4)$$

The product polyacrylonitrile was identified by IR spectra. The FTIR spectra of the polyacrylonitrile were taken on a Shimadzu China, Model FTIR 1801A instrument in pressed KBr pellets. The formation of PAN in this system was endorsed by FTIR analysis at University of Mysore.

The FTIR (Scan fig 6) of the prepared polymer revealed the manifestation of peaks at (C-H Stretching), 2940 cm^{-1} (C≡N Stretching) 2243.5 $cm^{-1}$and 1454.4 cm^{-1} (C-H bending) which matched with the reported FTIR spectra for polyacrylonitrile [12].

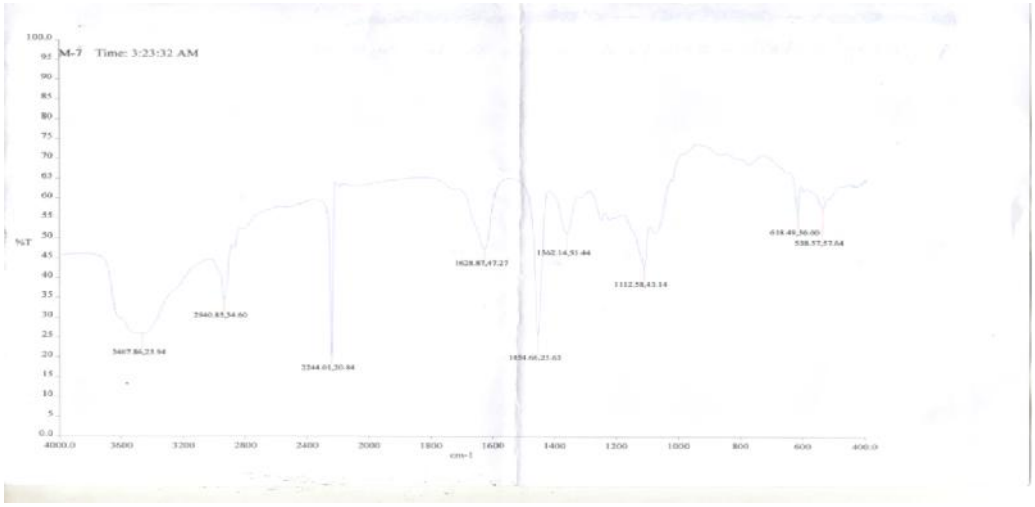


Fig 6: FTIR Spectra for polyacrylonitrile from Isobutyric acid and Ce(IV)

Results and Discussion

The polymerization of acrylonitrile initiated by Ce(IV)-Isobutyric acid redox system takes places under stoichiometric conditions at a measurable rate at 50^{o}C. Although no induction period was observed under deaerated conditions. The steady state attained at 75 minuts.The rate of Ce(IV) disappearance (-Rm) was found to be independent of acrylonitrile concentration for [Ce(IV)]= $4.0x10^{-3}$ mol/dm^3, a plot of (-Rm) versus [Ce (IV)] was Linear and passed through origin. A plot of – 1/Rm V/s [1/Isobutyric] was Linear with an intercept on the rate axis indicating Line weaver–Burk [14] kinetics for complex formation as shown in Fig. 1.

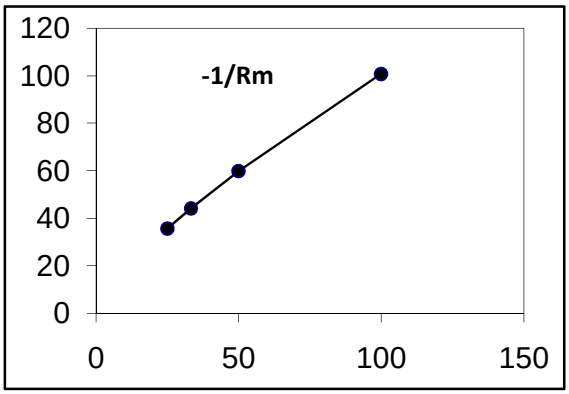


Fig. 1: Inverse plot of Cerium (IV) disappearance versus Isobutyric acid
[Ce(IV)] =$4.0x10^{-3}$ mol/dm^3,
[Isobutyric] = $2.0x10^{-2}$ mol/dm^3,
[AN] = $8.05x10^{-2}$mol/dm^3),
[H_2SO_4] = $2.0x10^{-3}$ mol/dm^3, Temperature = 323^0 K

Effect of Monomer on rate of Polymerization

The rate of polymerization (Rp) increased with increasing monomer concentration ($4.02x10^{-2}$–$20.12x10^{-2}$mol/dm^3). The plot of log Rp v/s log[M] was linear and passed through origin as shown in Fig. 2. Hence the order with respect to [M] was Unity (1.20).

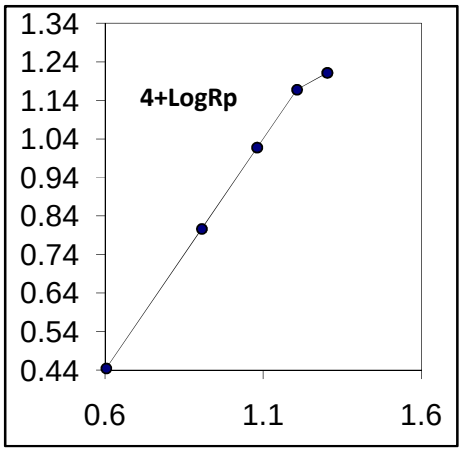


Fig. 2: Effect of [Monomer] on the rate of polymerization

[Ce (IV)]=($4.0x10^{-3}$ mol/dm^3), [Isobutyric] =$2.0x10^{-2}$mol/dm^3
[Monomer] =$4.02x10^{-2}$ ----$20.12x10^{-2}$mol/dm^3, [H_2SO_4] =$2.0x10^{-3}$mol/dm^3
Temperature =323^0K

Effect of [Ce(IV)] on rate of polymerization

The rate was also directly proportional to the Ce (IV). This increase of Rp with increase of [Ce (IV)] rules out mutual termination and points into the possibility of a linear mode of termination as a major process.

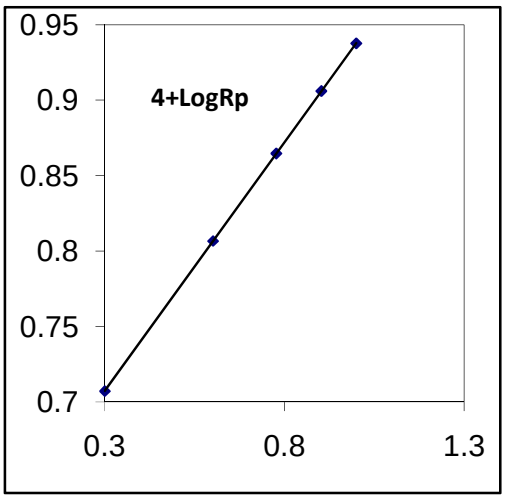


Fig. 3: Effect of [Ce(IV)] on the rate of polymerization

[Ce (IV)]=($2.0x10^{-3}$ ---$12.0x10^{-3}$ mol/dm^3), [Isobutyric] =$2.0x10^{-2}$ mol/dm^3
[Monomer] =$8.05x10^{-2}$mol/dm^3, [H_2SO_4] =$2.0x10^{-3}$mol/dm^3
Temperature =323 ^{0}K

Plots of 1/Rp v/s [Ce (IV)] were Linear up to [Ce (IV)] = 2.0 -$12.0x10^{-3}$ mol/dm^3. The departure from Linearity of the plots at higher temperature and very low [Ce(IV)] could be ascribed to change in the relative proportions of mutual and primary radical terminations. The order was found to be fractional (0.33) order of the [Ce (IV)] for standard condition as observed in Fig.3.

Effect of Isobutyric acid on rate of polymerization

The rate of polymerization also increased with increasing of Isobutyric acid concentration and reacts optimum level, later it decreases gradually as shown in table 1.

Table1. Effect of Variation of [Isobutyric], [Ce (IV)], [H_2SO_4] [Monomer] and Temperature

Ce (IV) x 10^3 mole/dm^3	**[Isobutyric] x 10^2 mole/dm^3**	**[AN]x10^2 mole/dm^3**	**[H_2SO_4]x10^3 mol/dm^3**	**Temp K**	**Rp x 10^4 mole/dm^3**
2.0	2.0	8.05	2.0	323	5.096
4.0	2.0	8.05	2.0	323	6.407
6.0	2.0	8.05	2.0	323	7.323
8.0	2.0	8.05	2.0	323	8.052
10.0	2.0	8.05	2.0	323	8.667
12.0	2.0	8.05	2.0	323	8.609
4.0	1.0	8.05	2.0	323	3.809
4.0	2.0	8.05	2.0	323	6.407
4.0	3.0	8.05	2.0	323	8.683
4.0	4.0	8.05	2.0	323	10.77
4.0	5.0	8.05	2.0	323	9.98
4.0	2.0	8.05	2.0	323	9.00
4.0	2.0	4.02	2.0	323	2.785
4.0	2.0	8.05	2.0	323	6.406
4.0	2.0	12.07	2.0	323	10.418
4.0	2.0	16.10	2.0	323	14.72
4.0	2.0	20.12	2.0	323	16.271
4.0	2.0	8.05	2.0	328	6.407
4.0	2.0	8.05	4.0	323	3.802
4.0	2.0	8.05	6.0	323	2.754
4.0	2.0	8.05	8.0	323	2.238
4.0	2.0	8.05	10.0	323	1.905
4.0	2.0	8.05	2.0	313	4.701
4.0	2.0	8.05	2.0	323	6.406
4.0	2.0	8.05	2.0	333	8.102
4.0	2.0	8.05	2.0	343	10.001
4.0	2.0	8.05	2.0	353	13.201

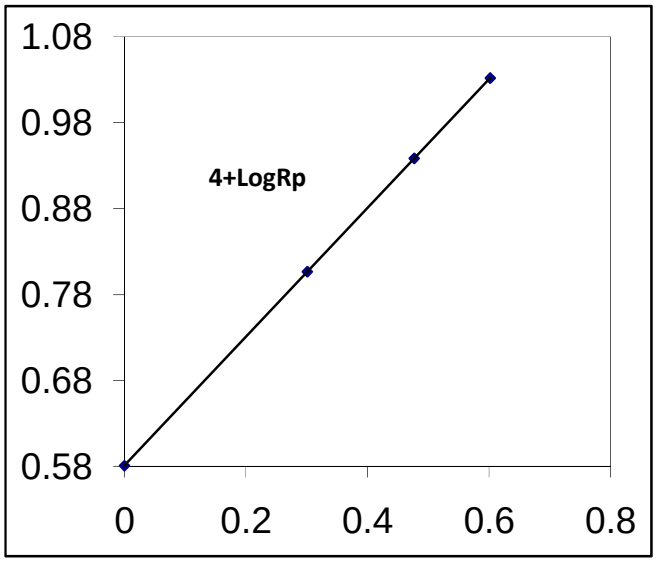


Fig. 4: Effect of [Isobutyric acid] on the rate of polymerization

[Ce (IV)]=(4.0×10^{-3} mol/dm^3), [Isobutyric] =1.0×10^{-2} –6.0×10^{-2} mol/dm
[Monomer] =8.05×10^{-2}mol/dm^3, [H_2SO_4] =2.0×10^{-3}mol/dm^3
Temperature =323^0K

Then the order of the reaction with respect to Isobutyric acid concentration was found to be of fractional order (0.75) from the plot of log Rp v/s log [Isobutyric] in the concentration range of 1.0×10^{-2} to 6.0×10^{-2}mol/dm^3 as shown in figure 4.

Effect of temperature on rate of polymerization

The rate of polymerization and the percentage conversion increases steadily with rising temperature. At a higher temperature, the maximum conversion and Rp was decreases. The activation energy. (Ea = 22.36 KJ/mol) is calculated from the Arrehenius plot of log Rp v/s 1/T in the temperature range 30-70^{o}C. The other thermodynamic parameters calculated from the Erying plots are:

$\Delta H^{\neq}$= 19.67 KJ/mol, $\Delta G^{\neq}$= 99.12 KJ/mol and $\Delta S^{\neq}$= -245.90 JK mol^{-1}

The overall activation energy for this system was Ea = 22.36 KJ/mol.

Effect of [H_2SO_4] on rate of polymerization

The kinetic measurements were performed in H_2SO_4 solution of different ion concentration. The rate of polymerization decreases with an increased in [H_2SO_4], indicates that the hydrolyzed species Ce(IV) is more reactive than the unhydrolysed species and also that the unprotonated form of Isobutyric acid is more reactive than the protonated form. The

order with respect to [H^+] was found to be inverse fractional (-0.75) order from the plot of log Rp against log [H^+]. As shown in Fig. 5.

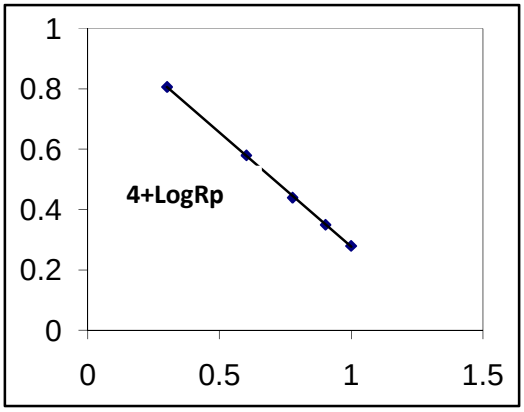


Fig. 5: Effect of [H_2SO_4] on the rate of polymerization

[Ce (IV)]=(4.0×10^{-3} mol/dm^3), [Isobutyric] =2.0×10^{-2}mol/dm^3

[Monomer] =8.05×10^{-2} ,[H_2SO_4] =2.0×10^{-3} −10.0×10^{-3}mol/dm^3, Temperature =323^0K.

Effect of Organic Solvents on the rate of polymerization

These solvents due to the decrease in the area of shielding of a strong hydration layer in the aqueous medium, resulting in the termination of the radical end of the growing chain of due to the increase the regulated rate of production of primary radicals, caused by the solvents which renders the termination rate to be relating fast as compared to the growth of the polymer chains as shown by *Schulz et al* [15]. The water – miscible organic solvents such as methanol and DMF both are depressed the rate of polymerization in this system.

Effect of Surfactants on the rate of polymerization

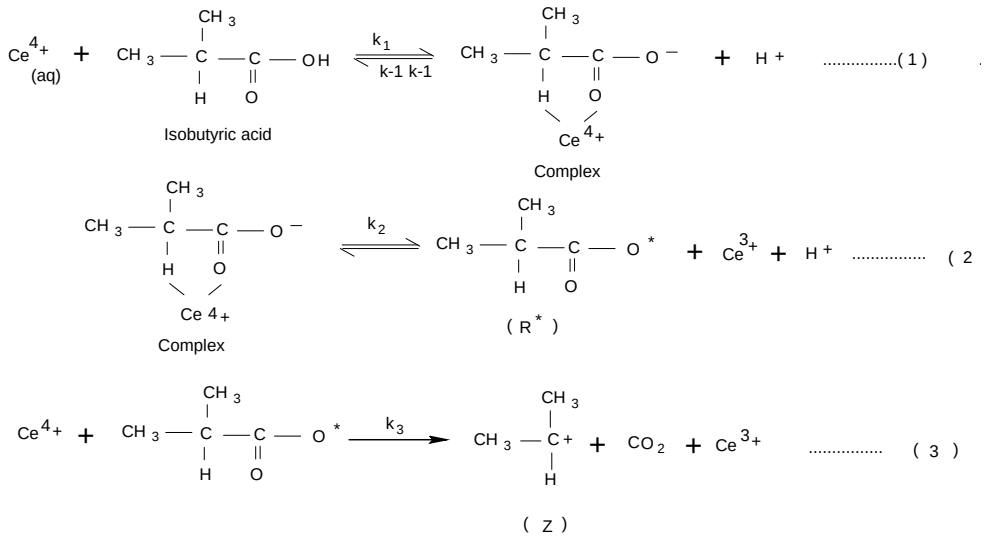
Addition of anionic surfactant, Sodium lauryl sulphate, increases the rate of polymerization (Rp) above and below the CMC value. The cationic surfactants Cetyltrimethyl ammonium bromide decreases the Rp above and below the CMC value. But in contrast, the non-ionic surfactants Triton x-100 has no effect on the rate. The reason may be due to the hydrophobic interactions, and electrostatic attractions which are mainly responsible for the enhancement or the inhibition of the rate of polymerization [16-20].

The reaction mixture which was initially homogeneous, then becomes heterogeneous as soon as the polymerization started due to the formation of an insoluble product, control experiments involving the individual addition of Ce(IV) or Isobutyric acid to the monomer

solution did not induce polymerization suggesting that the free radicals formed *in situ* by the Ce (IV)-Isobutyric acid system were responsible for polymerization. Based on the above results the following Mechanistic scheme is proposed:

Mechanism and Rate law

The polymerization of acrylonitrile in the presence of ceric ion and Isobutyric acid in aqueous sulphuric acid medium shows characteristic features of heterogeneous polymerization, as the polymer is insoluble in the aqueous phase. From the proportionalities obtained between the measurable parameters and the variables a reaction scheme-1 is suggested involving initiation by organic free radicals produced by the interaction of Ce(IV) with Isobutyric acid and termination by ceric ions termination of vinyl polymerization by metal ions well recognized



Scheme–1

$$\text{Isobutyricacid} + Ce(IV) \xrightarrow{K} \text{Complex}$$

$$\text{Complex} \xrightarrow{k_d} R^{\bullet} + Ce^{3+}$$

Initiation:

k_i $\quad M + R^{\bullet} \longrightarrow RM^{\bullet}$

Propagation:

k_p $\quad RM^{\bullet} + M \longrightarrow RMM^{\bullet}$

$\quad RM^{\bullet}x\text{-}1 + M \longrightarrow RM^{\bullet}x$

Termination:

k_t $\quad RM^{\bullet}x + RM^{\bullet}y \longrightarrow$ Polymer

Applying steady state approximation

$$-d/dt[R^{\bullet}]=K_d\,[complex]-k_i\,[M]\,[R^{\bullet}]=0$$

$$K = [complex]/\,[Isobutyric]\,[Ce\ (IV)]$$

$$[Complex]= K\,[Ce\ (IV)][Isobutyric]$$

$$K_dK[Ce(IV)][Isobutyric]- k_i\,[M][R^{\bullet}]=0$$

$$[R^{\bullet}]=k_dK[Ce(IV)][Isobutyric]/k_i[M]$$

$$k_i\,[R^{\bullet}][M] =k_t\,[RM^{\bullet}n]\,[RM^{\bullet}n]=k_t[RM^{\bullet}n]^2$$

$$[RM^{\bullet}n]^2 = \left(\, k_i/k_t \,\right) \quad [R^{\bullet}][M]$$

$$[RM^{\bullet}n]= \left(k_i/k_t\right)^{1/2}\ [R^{\bullet}]^{1/2}[M]^{1/2}$$

$$k_p=[RM^{\bullet}n][M]$$

$$R_p=\left(k_pk_i/k_t^{\,½}\right)[R^{\bullet}]^{1/2}\,[M]^{1/2}\,[M]$$

$$R_p=k_p[RM^{\bullet}n][M]$$

$$R_p=\left(k_pk_i/k_t\right)½ \qquad \left(k_dK/k_t\right)½\left\{\,[Ce(IV)[Isobu]\,/[M\,]\right\}½\,[M]^{\,3/2}$$

$$R_p= \left(k_pk_dK/k_t\right)\ ½\ \ [Ce\ (IV)]^{\,1/2}\,[Isobutyric]^{\,1/2}\,[M]^{\,1/2}/[M]^{\,1/2}$$

$$R_p=\left(k_pk_dK\,/k_t\right)½\,[Ce(IV)]^{1/2}\,[Isobutyric]^{1/2}\,[M]$$

Thus, the dependence of Rp on [M], [Ce (IV)] and [Isobutyric] all of which are observed, are consistent with the experimental results. The low energy of activation (Ea = 22.36 kJ/mol) is an indication of the high reactivity of the initiator and provides direct experimental evidence of the existence of transient radical intermediates generated in redox system. It also enables the identification of these radicals as end groups of the polymer. Further work on the kinetics of polymerization various vinyl monomers initiated by the

reaction of cerium (IV) and other transition metal ions with suitable reductant is in progress in our laboratory.

Conclusion

Redox initiated free-radical polymerization of Acrylonitrile with Ce(IV)- Isobutyric acid using H_2SO_4 medium was investigated. The effects of Isobutyric acid, Ce(IV), AN H_2SO_4, and temperature on the rate of polymerization (Rp) and yield were clarified or calculated. Acrylonitrile conversion as well as the polymerization rate (Rp) increased on increasing Ce(IV), Isobutyric & temperature and then decrease gradually thereafter. This behavior is the result of both effects in activation of the active initiator species which brings about premature termination due to the higher concentration of isobutyric acid. Acrylonitrile viscosity and rate of polymerization initially increases then gradually decreases depending on the Ce(IV) concentration. This behavior is the result of simultaneous effect of Ce (IV) ion concentration on the rate and the oxidative termination of the primary radicals. The effect of the temperature on the polymer yield and polymerization rate of acrylonitrile was investigated over the 30-70 ^{o}C range. Monomer convention and Rp increased on increasing temperature then decreased at higher temperatures. This behavior is very likely due to side reactions occurring as higher temperatures. Some of water- miscible organic solvents and surfactants on the rate of polymerization were investigated and activation parameters were computed from Arrhenius plots. The other thermodynamic parameters also calculated.

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CRYSTAL STRUCTURE OF 4-(4-CHLOROPHENYL)-4-HYDROXYPIPERIDINIUM SUCCINATE HYDRATE

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Abstract

The title compound, $C_{13}H_{15}ClNO_4$, characterized by single crystal X-ray diffraction method. This crystallizes in monoclinic crystal system with cell parameters a = 15.282 (2) Å, b = 10.065 (1) Å, c = 18.496 (3) Å, space group $C2/c$, β = 103.250 (8)^0, Z = 8, and volume = 2769.2 (8) Å^3. The piperidine ring adopts a chair conformation. In the crystal, the intermolecular hydrogen bonds of the type N---H…O and intramolecular hydrogen bond C---H…O is observed.

Key words: *4-Hydroxypiperidinium, Crystal Structure, Succinate, Hydrogen bonds.*

Introduction

The 4-(4-chlorophenl)-4hdroxypiperidinium is one of the intermediate material used in the synthesis of pharmacological compounds. The compounds such as haloperidol are used for the treatment of psycohotic illness, extreme agitation and Tourette's syndrome. One of the derivative loperamide is used in diagnosing diarrhea [1]. A review on the synthesis and biological activity of uncondensed cyclic derivatives of piperidine is reported [2]. The crystal structures of 1,2,2,4,6,6-hexamethyl-4-piperidinol [3], Structure of piperazinium succinate**,** [4], three isomers of (±)-1,2,3-trimethyl-4-phenyl-4-piperidinol [5] and 1-(4-nitrophenyl)-4-piperidinol [6] and 4-[(E)-(2,4-difluorophenyl) (hydroxyimino)methyl]piperidinium picrate [7], 3,4-diaminopyridinium hydrogen succinate [8] , 4-(4-chlorophenyl)piperidin-4-ol [9], 2-amino-5-bromopyridinium hydrogen succinate [10], 4-(4-chlorophenyl)-4-hydroxypiperidinium benzoate [11], 2-Ethyl-6-methylpyridin-3-ol and its salt bis(2-ethyl-3-

hydroxy-6-methylpyridinium) succinate [12] and structure and properties of domperidone and its succinate salt [13] have been reported. In view of the importance of the title compound, we report the crystal structure of 4-(4-chlorophenl)-4hdroxypiperidinium Succinate hydrate.

Experimental

Synthesis and crystallization of the title compound

The title was synthesized as follows, 4-(4-chlorophenyl)-piperidin-4-ol (2.2 g, 0.01mol) and succinic acid (1.18 g, 0.01mol) were dissolved in 20 ml of ethanol resulting the title compound (Fig. 1). The mixture was stirred for 10 minutes in room temperature. Then the solution was kept aside for 3 days at room temperature. Yellow crystals were obtained (m.p: 395–398 K) by slow evaporation of ethanol solution.

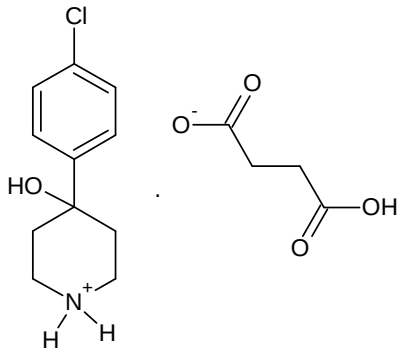


Fig. 1- Chemical Structure of the title Compound

Crystal Structure determination

A good single crystal of the title compound with dimension 0.23 × 0.23 × 0.22 mm was chosen for X-ray diffraction study. Data collection and cell refinement were carried out using Bruker X8 Proteum diffractometer with Mo *Kα* radiation. The absorption correction was applied using multi-scan technique for data collection (SADABS; Bruker, 2013). The lattice parameters were determined by the least-squares methods on the basis of all reflections with F2 > 2σ (F2). The structure was solved by the direct methods using SHELXS-97 [14]. All the non-hydrogen atoms were revealed in the Fourier map itself. Full-matrix least squares refinement using SHELXL-97 [14] with isotropic temperature factors for all the atoms was done. Refinement of non-hydrogen atoms with anisotropic parameters was started at this stage. The hydrogen atoms were placed at chemically acceptable positions and were allowed to ride on their parent atoms. About 175 parameters were refined with 2293 unique reflections which saturated the residuals to R1 = 0.0839 and wR2 = 0.2490. The details of the

crystal data and refinement are given in **Table 1**. All the figures (*ORTEP*, packing and hydrogen bonding) were plotted using *MERCURY* [15].

Results and Discussion

In the title compound (Fig. 2), the piperidine ring adopts chair conformation and the groups (hydroxy and NH) occupy the axial position. The torsional angles for the groups (hydroxy and NH) are O2-C4-C14-C15 = 63.93 (2)^0, O2-C4-C7-C9 = -61.99(1)^0, C7-C9-N3-H3A = 66.42 (1)^0, C14-C15N3-H3A = 64.68 (3)^0 which are comparable to the values of reported 4-(4-Chlorophenyl)piperidin-4-ol molecule.

In the crystal structure (Fig. 3), the molecules are linked through N—H···O, O—H…O and short contacts of the type Cl···O. The N3---H3A···O8 and N3---H3B…O12 hydrogen bonds have distance of 2.675 (2) Å [angle = 161.86 (1) ^{0}] and 2.775 (2) Å [angle = 167.84 (1) ^{0}]. The O2---H2…O8 hydrogen bond exists with a distance of 2.663 (1) Å [angle = 149.32 (2) ^{0}]. In addition, the short contact between Cl1 and hydrate molecule O1 is observed with a distance of 3.16 (1) Å.

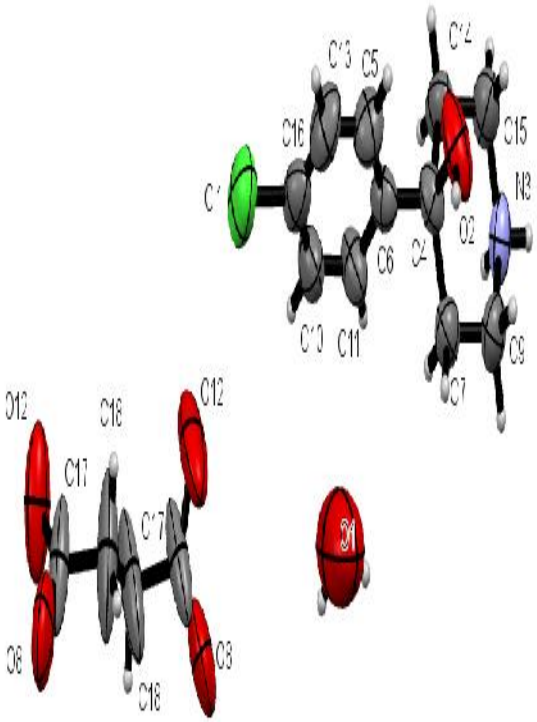


Fig. 2- *ORTEP* diagram of the title compound with 50% probability ellipsoids.

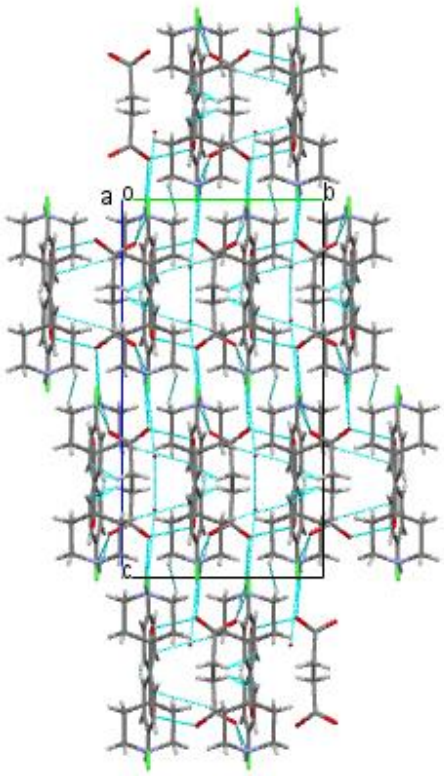
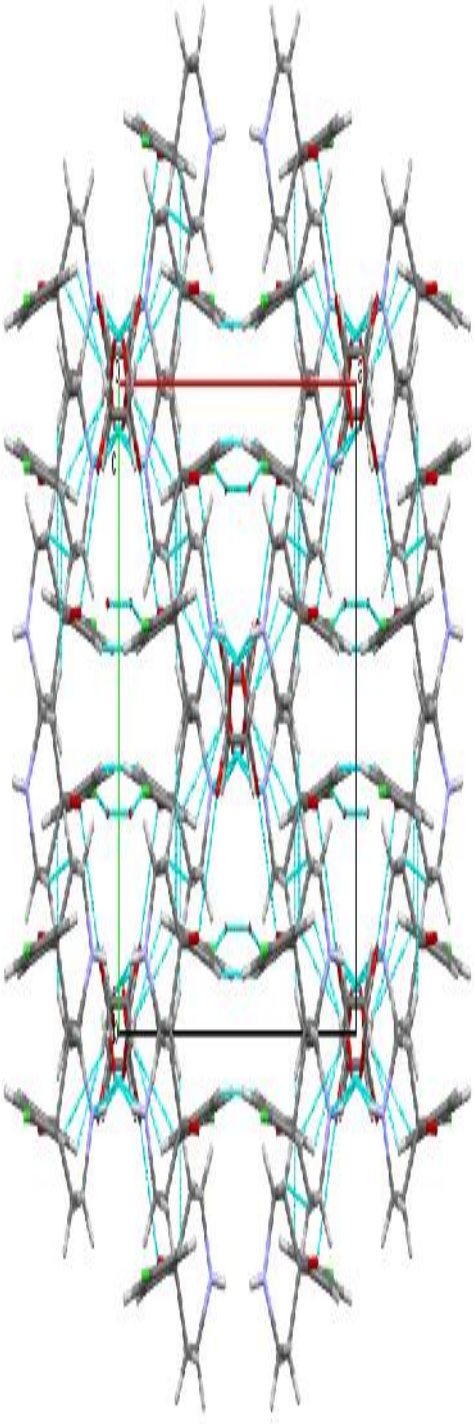


Fig. 3 (a) -Packing of molecules along *a*-axis



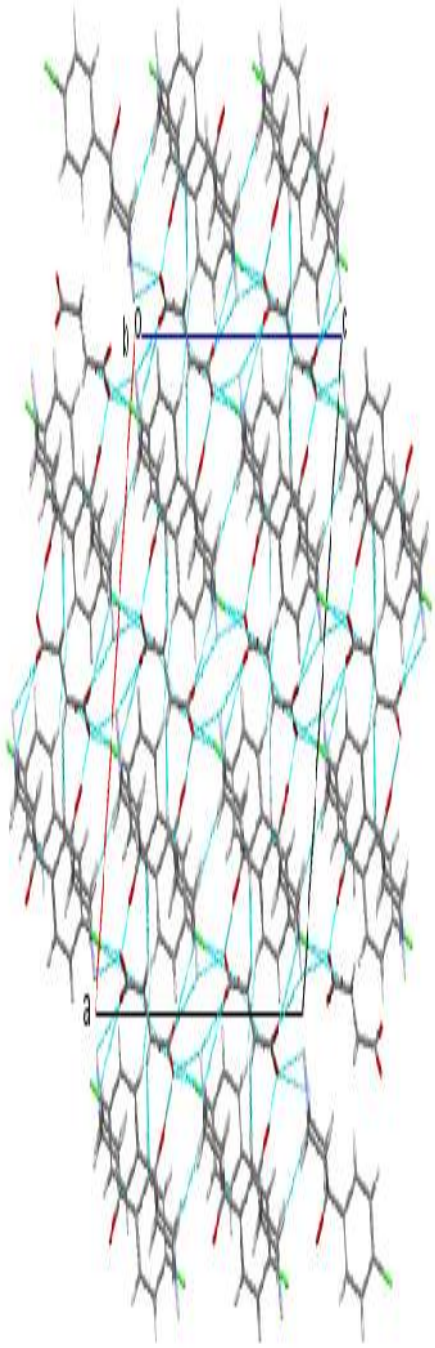


Fig.3(b)-Packing of molecules along *b*-axis; **Fig.3(c) -** Packing of molecules along *c*-axis.

4-(4-chlorophenl)-4-hdroxypiperidinium Succinate hydrate

Crystal data

Empirical formula	$C_{13}H_{16}O_4NCl$
Formula weight	288.75
Temperature	296.15K
Crystal system	monoclinic
Space group	C2/c
a=15.282(2) Å	Z = 8
b=10.0649(16) Å	F(000) =1230.8
c=18.496(3) Å	Dx = 1.3851Mg m^{-3}
α=90°	μ= 2.545mm^{-1}
β=103.250(8)°	Cu Kα radiation, λ = 1.54178
γ=90 °	θ= 9.82 to 129.18°
Crystal size	0.23 × 0.23 × 0.22 mm^3
Prism, Colourless	

Data collection

Reflections collected 10894	
Independent reflections	2293 [R_{int} = 0.0414, R_{sigma} = 0.0305]
Radiation source:	
Detector resolution:	
Absorption correction:	
Index ranges	-13 ≤ h ≤ 17, -11 ≤ k ≤ 11, -21 ≤ l ≤ 13

Refinement

Data/restraints/parameters	2293/0/175
Goodness-of-fit on F^2	1.085
Final R indexes [I>=2σ (I)]	R_1 = 0.0839, wR_2 = 0.2490
Final R indexes [all data]	R_1 = 0.1064, wR_2 = 0.2774
Largest diff. peak/hole	Δρmax = 0.58 e $Å^{-3}$ Δρmin = −0.58 e $Å^{-3}$

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($Å^2$)

Atoms	x	y	z	Uiso*/Ueq
H1a	200(170)	6400(300)	2780(80)	351(6)
H1b	240(120)	6300(400)	3530(150)	351(6)
H2	3600(30)	7830(30)	1430(40)	152(2)
O2	3342(2)	8544(5)	1344(2)	101.3(14)
H3a	909(2)	8780(4)	-94.4(19)	85.5(13)
H3b	642(2)	8880(4)	610.0(19)	85.5(13)
N3	1116(2)	8775(4)	402.1(19)	71.2(11)
C4	2613(3)	8598(4)	1705(2)	61.5(10)
C5	3846(3)	8903(5)	2870(3)	80.9(14)
H5	4239(3)	9083(5)	2566(3)	97.0(16)
C6	2959(3)	8617(4)	2547(2)	60.5(10)
H7a	2294(3)	6618(4)	1583(2)	81.3(13)
H7b	1500(3)	7478(4)	1735(2)	81.3(13)
C7	1971(3)	7447(4)	1462(2)	67.7(11)
C9	1547(3)	7482(5)	633(2)	74.8(13)
H9a	1100(3)	6782(5)	512(2)	89.8(15)
H9b	2006(3)	7314(5)	359(2)	89.8(15)
C10	2707(4)	8461(5)	3782(3)	80.0(13)
H10	2313(4)	8331(5)	4091(3)	95.9(16)
C11	2396(3)	8407(5)	3025(2)	74.3(13)
H11	1791(3)	8225(5)	2829(2)	89.2(15)
C13	4165(4)	8931(5)	3629(3)	90.6(16)
H13	4769(4)	9102(5)	3833(3)	108.7(19)
C14	2136(3)	9900(4)	1448(3)	76.9(13)
H14a	1660(3)	10040(4)	1708(3)	92.3(16)
H14b	2559(3)	10628(4)	1573(3)	92.3(16)
C15	1740(4)	9897(5)	619(3)	82.3(14)
H15a	2222(4)	9835(5)	358(3)	98.8(17)
H15b	1423(4)	10726(5)	477(3)	98.8(17)
C16	3591(4)	8705(4)	4080(3)	78.0(14)
O1	498(7)	6629(11)	3214(7)	234(4)
O8	4135(4)	3701(5)	6077(3)	121.4(17)
O12	4810(3)	5623(7)	6159(2)	149(2)
C17	4540(3)	4613(11)	6441(3)	116(3)
C18	4649(4)	4536(12)	7266(3)	170(4)
H18a	4337(4)	3733(12)	7353(3)	205(5)
H18b	4302(4)	5270(12)	7394(3)	205(5)

Atomic displacement parameters ($Å^2$)

Atoms	U11	U22	U33	U12	U13	U23
Cl1	138.8(14)	103.5(12)	77.8(9)	2.0(8)	-17.6(8)	-2.8(7)
O2	68(2)	156(4)	89(2)	19(2)	38.1(18)	43(2)
N3	72(2)	87(3)	55(2)	12.8(18)	16.4(16)	-12.8(17)
C4	58(2)	63(2)	67(2)	5.6(18)	23.6(18)	10.9(18)
C5	65(3)	83(3)	94(4)	-5(2)	18(2)	-6(3)
C6	59(2)	51(2)	71(3)	2.4(16)	12.4(19)	9.3(17)
C7	84(3)	56(2)	69(3)	2(2)	28(2)	-3.2(18)
C9	93(3)	66(3)	72(3)	-1(2)	32(2)	-17(2)
C10	89(3)	79(3)	69(3)	-9(2)	13(2)	15(2)
C11	65(3)	89(3)	67(3)	-7(2)	12(2)	19(2)
C13	70(3)	89(4)	101(4)	0(2)	-5(3)	-16(3)
C14	98(3)	51(2)	71(3)	0(2)	-4(2)	5.2(19)
C15	104(4)	63(3)	72(3)	8(2)	2(2)	5(2)
C16	90(3)	53(2)	81(3)	3(2)	0(3)	4(2)
O1	246(9)	213(9)	216(10)	-67(7)	-3(7)	38(6)
O8	133(4)	171(5)	70(3)	14(3)	43(3)	46(3)
O12	111(3)	257(6)	78(3)	-76(4)	23(2)	32(3)
C18	96(4)	362(14)	48(3)	-28(6)	5(2)	52(5)
C17	48(3)	245(9)	54(3)	-2(4)	11(2)	56(4)

Geometric parameters (Å,°)

C11 – C16	1.742(5)	O2 – C4	1.425(5)
N3 – C9	1.477(6)	N3 – C15	1.474(6)
C4 – C6	1.527(6)	C4 – C7	1.518(6)
C4 – C14	1.521(6)	C5 – C6	1.380(6)
C5– C13	1.377(8)	C6– C11	1.384(6)
C7– C9	1.522(6)	C10– C11	1.372(7)
C10– C16	1.359(7)	C13– C16	1.361(8)
C14– C15	1.514(6)	O8 – C17	1.220(9)
O12– C17	1.255(9)	C18– C18	1.215(12)
C17– C18	1.498(8)		
C15−N3−C9	112.2(4)	C7−C9−N3	111.7(3)
C6−C4−O2	110.7(3)	C16−C10−C11	120.1(5)
C7−C4−O2	110.7(4)	C10−C11−C6	121.6(4)
C7− C4− C6	111.1(3)	C16−C13−C5	119.7(5)
C14−C4−O2	104.9(4)	C15−C14−C4	111.5(4)
C14−C4−C6	109.7(3)	C14−C15−N3	111.1(4)
C14−C4−C7	109.5(4)	C10−C16−C11	119.7(4)
C13−C5−C6	121.9(5)	C13−C16−C11	120.2(4)
C5− C6− C4	121.3(4)	C13−C16−C10	120.1(5)
C11−C6−C4	121.9(4)	O12− C17− O8	123.7(5)
C11−C6−C5	116.7(4)	C18−C17−O8	116.5(7)

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REPRODUCTIVE TRAITS, STOMATAL FREQUENCY AND KARYOTYPIC STUDIES OF THREE VARIETIES OF MULBERRY (*MORUS SPP.)*

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Abstract

Regarding reproductive traits, stomatal frequency and karyotypic studies of three mulberry varieties namely DD, S_{799} and S_{1635} were selected. Height, internodal distance, colour of leaves, stem, stomatal frequency, length inflorescence and number of flowers / inflorescence and karyotypic studies were studied. Mulberry varieties studied exhibited considerable variations in height, internodal distance, leaf texture, stomatal frequency, length and breadth. Height and stomatal frequency was higher in diploids when compared to triploid variety. Triploid showed increased stomatal size, reduction in height, number of branches and internodal distance followed by diploid varieties. DD and S_{799} are found to be diploids with somatic chromosome number of 2n=28. While variety S_{1635} revealed triploid chromosome number of 2n=42. Somatic chromosomes measured 1.83 μm to 3.61 μm in length. Karyotypes of these taxa are symmetric.

Key words*: Mulberry (Morus spp.), diploids, triploid, mitosis, karyotype analysis*.

Introduction

Mulberry (*Morus spp*.) is a multipurpose, predominantly dioecious, heterozygous and out breeding tree. The foliage of the plant is used mainly as a sole food of silkworm (*Bombyxmori* L.). The information in the field of cytology, genetics and plant breeding is inadequate to seek the answer for some of the problems faced by the classical taxonomists. Hence, reproductive characters, stomatal studies and cytogenetical data of different ploidy levels of mulberry are essential for taxonomists to classify different species and varieties to assist plant breeders to evolve promising genotypes for commercial exploitation.

Modern taxonomy is a synthetic entity which tries to align the related groups in an order, drawing information from various branches such as morphology, reproductive

characters, cytogenetics etc., for better utilization of genetic resources. Most of the cultivated varieties of mulberry are diploid with 2n=28 chromosomes, a few are polyploids [8,13]. Micro-morphology and reproductive traits of different ploidy level of the mulberry varieties were studied by [15] and are considered diploid parents are superior to triploids and tetraploids. Stomatal frequency and karyotypic studies have been reported by [14]. Karyotypes of these taxa are symmetric, only metacentric and submetacentric chromosomes are found in the somatic complement. In the present study focused on the reproductive characters, stomatal studies and karyotypic studies of three different mulberry varieties.

Materials and methods

Root tips are collected from potted plants of DD, S_{799} and S_{1635} between 9.30 to 10.30 a.m. and pre-treated in saturated solution of 0.002 M 8-hydroxyquinoline at 10^0 C for 3 hours and then fixed in 1:3 glacial acetic acid: alcohol. Root tips were transferred to 45% acetic acid for 15 min., stained with mixture of 2% aceto-orcein: 1N HCl (9:1) for seven minutes and squash preparations were made in 45% of acetic acid. Photomicrographs and drawings were made on the same day of preparation. For each variety numbers of preparations were made to ascertain the chromosome number and their morphology. Ideograms were drawn using suitable scale. Karyotype classification was made according to the literature [10].

Stomatal frequency

Stomatal frequency was determined by nail-polish impression method. Stomatal frequency was calculated using the formula and expressed as number of stomata/mm^2 [1, 11].

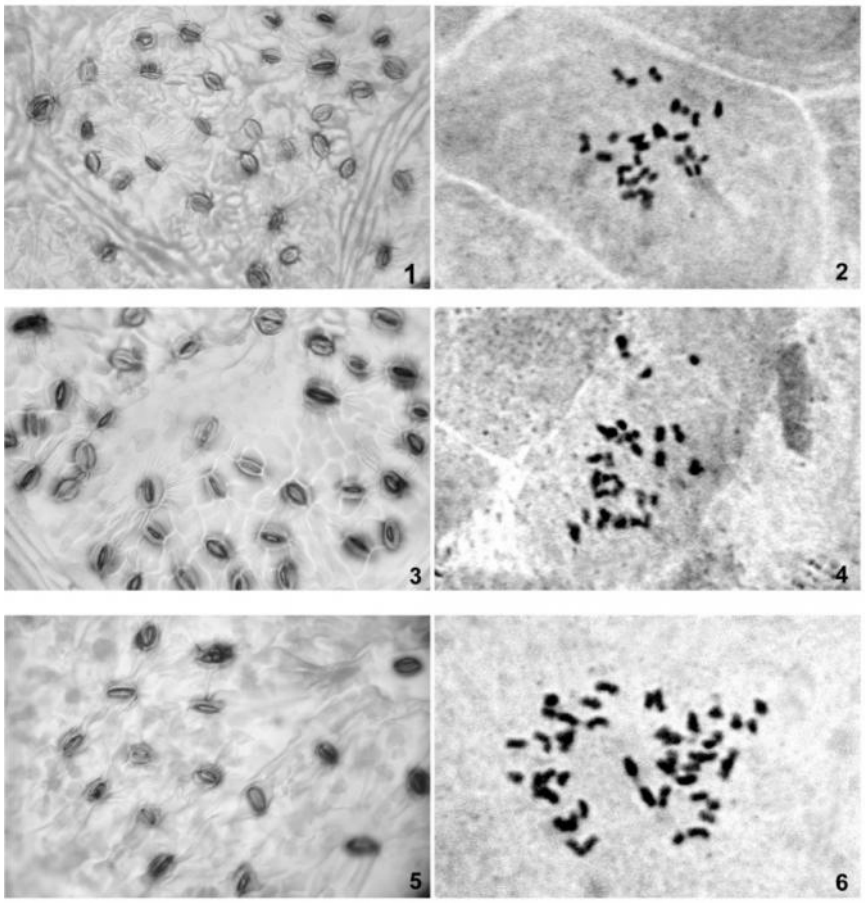
$$\text{Stomatal frequency} = \frac{\text{Number of Stomata}}{\text{Area of microscopic field}} \times mm^2$$

Results and Discussion

Comparative account of reproductive characters, stomatal frequency, somatic chromosome number, ploidy level, range of chromosome length, karyotype formula, arm ratio and haploid chromatin length are presented in Table 1.

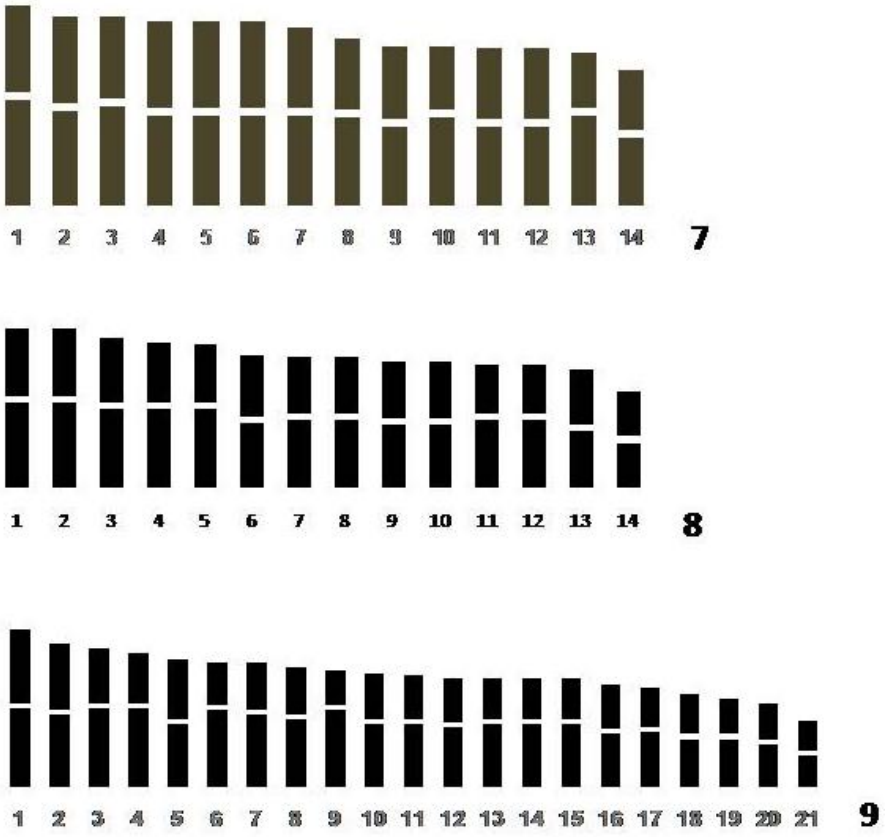
Table 1 Reproductive traits and karyotype of three mulberry varieties

Characters	DD (2n)	S_{799} (2n)	S_{1635} (3n)
No. of stomata /unit area (mm^2)	266.66	196.24	166.66
Length of inflorescence (cm)	2.9	3.0	3.4
Diameter of inflorescence (cm)	1.1	1.0	1.4
No. of flowers/ inflorescence	23	26	32
Length of flower (cm)	0.61	0.65	0.70
Pollen stain ability (%)	98.24%	96.28%	93.42%
2n chromosome number	28	28	42
Ploidy level	Diploid	Diploid	Triploid
Karyotype formula	$24B^m+2B^{sm}+2C^m$	$22B^m+4B^{sm}+2C^m$	$16B^m+24B^{sm}+2C^m$
Range of chromosome length (µm)	1.90-3.61	1.96-2.86	1.80-3.19
Arm ratio (µm)	0.70-1.00	0.62-0.97	0.60-1.00
Haploid chromatin length (µm)	36.36	34.11	49.87



Figures:1-6.

1 & 2, Stomatal frequency and somatic chromosomes (2n=28) of variety DD.
3 & 4, Stomatal frequency and somatic chromosomes (2n=28) of variety S_{799}.
5 & 6, Stomatal frequency and somatic chromosomes (2n=42) of variety S_{1635}.



Figures: 7, 8 & 9, Ideogram of varieties DD, S_{799} and S_{1635} respectively.

Variety DD

Scientists working at Karnataka State Sericulture Research & Development Institute (KSSR&DI), perfected, this variety through clonal selection. This variety yields 40,000 to 42,000 Kgs of leaves / ha / year. The stomatal frequency was found to be 266.66 / mm^2 (Fig.1). Chromosomes are very small (1.90 to 3.61 μm) in size. This taxon revealed diploid chromosome number of 2n=28 (Fig.2) with 22 medium chromosomes with median primary constriction, 4 medium chromosomes with sub median primary constriction and 2 short chromosomes with median region primary constriction. The karyotype formula of this taxon is $2n=28=22B^{m}+4B^{sm}+2C^{m}$ (Fig. 7). The karyotype is symmetrical with an arm ratio ranging from 0.70-1.00.The total chromatin length of haploid complement was 36.36μm.

Variety S_{799}

This variety cultivated as perennial bush and best suited for irrigated condition. Under ideal agro-climatic conditions this genotype yields 58 tonnes of leaf yield / ha / year. The

stomatal frequency was found to be 196.24 / mm^2 (Fig. 3). Chromosomes are small (1.96 to 2.86 μm) in size. This taxon also revealed diploid chromosomes number of 2n=28 (Fig. 4) with 18 medium chromosomes with median primary constriction, 8 medium chromosomes with sub median primary constriction, 2 short chromosomes with median region primary constriction. Only metacentric and sub metacentric chromosomes are found in the somatic complement. The karyotype formula of this taxon is $2n=28=18B^{m}+8B^{sm}+2C^{m}$ (Fig.8). The karyotype is symmetrical with an arm ratio ranging from 0.62 to 0.97. The total chromatin length of haploid complement was 34.11μm.

Variety S_{1635}

It is evolved through polyploidy breeding and selection. The stomatal frequency was found to be 166.66 / mm^2 (Fig. 5). Chromosomes are very small (1.83 to 3.19 μm) in size. This taxon revealed triploid chromosome number 2n=42 (Fig. 6) with 16 medium chromosomes with median primary constriction, 24 medium chromosomes with sub-median primary constriction and 2 chromosomes with median region primary constriction. Only metacentric and sub metacentric chromosomes are found in the somatic complement. The karyotype formula of this taxon is $2n=42=16B^{m}+24B^{sm}+2C^{m}$ (Fig. 9). The karyotype is symmetrical with an arm ratio ranging from 0.60 to 1.00. The total chromatin length of haploid complement was 49.87μm.

To classify existing mulberry populations objectively as taxa of different magnitude is rather difficult owing to the continuous variations as a result of outbreeding. To evolve dependable system of classification, a study of all the three types of relationship viz., Phylogenetic, Phenotypic and Geotropic is imperative as stated by [4]. Determination of biological species in cultivated plants the observational basis are full description of chromosome number and karyotype analysis and evidence of natural hybridization [2].

In the present investigation DD, S_{799} and S_{1635} are morphologically distinct and some similarities in their leaves with identical leaf margin and dissimilarities in their leaf texture, height, internodal distance, stem colour, inflorescence, pollen stainability and stomatal frequency were recorded. Cytologically DD and S_{799} showed 2n=28 and S_{1635} 2n=42 chromosomes respectively. These different chromosome numbers has reflected on their micro-morphology and reproductive data of diploids and triploid and varieties of different ploidy level of same mulberry varieties are scanty.

Stomatal frequency and size are considered as two important parameters in characterization of mulberry genotypes. These two characters are having positive correlation with drought resistance. The observed small size and lesser frequency of stomata in triploids than diploid varieties. Stomatal frequency and size decreases with increase in ploidy level have established by [12]. The observed genotypic level differences in stomatal frequency are in agreement with various other reports of [7, 6, 16]. The present findings also clearly shows frequency of stomata per unit area is significantly less in triploid compared to diploid. Moisture retention capacity will be higher in those mulberry varieties possessing smaller and lower stomatal frequency [3].

Mulberry varieties included in the present work exhibited variations in chromosome numbers, ploidy level and karyomorphology. Basic chromosome number of the genus *Morus* L., as x=14 for majority of the species have been reported by [5, 9]. In the present study, three mulberry varieties belong to *Morusalba*. Out of three varieties studied two are diploids with 2n=28 and one variety showed 2n=42 chromosomes. In general, chromosomes are smaller with a close range of length variation. Confirming the earlier reports, cytologically they showed similar karyotype with only two types of chromosomes, equal chromatin length and also length range. These different chromosome numbers has reflected on their morphology. Previous and present reports on chromosome number and cytology showed the presence of 2n=28 chromosomes in most of the cultivars in this *Genus*.

These reproductive characters, chromosome numbers and karyotypic studies can be made use of while selecting the parents for evolving progeny of different ploidy level both by hybridization and colchicine treatment. In addition, the information will be of much use in establishing a phylogenetic relationship and evolution of mulberry and will also help in selecting mother plants for hybridization based on chromosome number.

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STUDIES ON PALS AND DIELECTRIC CONSTANT OF NOVEL COMPOSITES R^{3+} DOPED ALUMINOPHOSPHATE ZEOLITES

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Abstract

Novel composites R^{3+} (R^{3+}: Eu: Gd: Nd) doped Aluminophosphate Zeolites have been synthesized under hydrothermal conditions in the presence of structure directing agents such as organic amines. The addition of R^{3+} ions into the aluminophosphate zeolites framework not only enhances the stability of the framework but also enlarges the pore diameter and frequency dependence of electrical properties like conductivity, dielectric constant, and capacitance are observed in the range of 10 kHz to 100 kHz. The dielectric properties of these materials have been studied with a view to modify the properties of aluminophosphate zeolite systems for practical applications. Positron annihilation lifetime (PAL) measurements, Brunauer–Emmett–Teller (BET) surface area measurement.

Key Words: *Aluminophosphate zeolite, Positron annihilation lifetime, Dielectric constant, Conductivity, Frequency.*

Introduction

Zeolites and microporous materials form an important family of solids, which are known for their application as ion exchange and gas separation materials. In addition, zeolites are becoming more popular owing to a new range of applications in technology [1]. Their applications are often intimately connected with the structure and composition. The characteristic open structure of zeoliets results in high surface area in them. Different

additives are usually added to Aluminophosphate Zeolites in order to modify and improve its electrical properties. Inorganic additives such as transition metal salts have considerable effect on the electrical properties of Aluminophosphate Zeolites. Such properties like electrical conductivity σ, dielectric constant ε' and capacitance were estimated with respect to frequency at room temperature. Positron annihilation lifetime spectroscopy is a well known non-destructive nuclear technique to investigate the defect distribution in solids [2]. Positrons diffusing through matter may be captured in special trapping sites such as vacancies, voids, dislocations, etc. depending on the sample characteristics. Positron annihilation study is less explored in the area of zeolites.

Experimental methods

Following steps are adopted in the synthesis of micro porous aluminophasphate zeolites.

a) Aluminophasphate gel were prepared first by neutralizing the pseudobohemit (AlOOH-98.35 mmol) homogenously in water (50 mL) was added sphoric acid (H_3PO_4-175 mmol) was added with rigorous stirring. This is known as reactive gel.
b) The gel obtained was aged for 3h over a hot water bath.
c) Organic amine was added to the reactive gel, which acts as a structure directing agent. This is referred to as precursor gel.
d) This precursor gel is aged for 3h over a hot water bath.
e) At this stage, 9 mmol of rare earths such $Eu(NO_3)_3.6H_2O$, $Gd(NO_3)_3.5H_2O$, $Nd(NO_3)_3.6H_2O$ were added.
f) The lastly precursor gel was charged into 30 mL capacity Teflon steel autoclaved and kept nearly 60 % full in the Teflon liner.

The hydrothermal runs were carried out for a period of 60 h at 150^oC in at furnace. The runs were arrested by quenching the autoclaves in the cold water bath. The run products were carefully recovered and washed thoroughly using double distilled water and ultrasonicated to remove adhered organic amines and excess R^{3+} elements. Then the run products were dried in a dust proof environment at 40^oC. The PAL spectroscopic studies were carried out using PAL spectrometer with the 10 micro Ci^{22}Na source sandwiched between two identical pellets of the zeolite sample. The dielectric properties like dielectric constant, capacitance and electrical conductivity (σ) of the samples were

recorded using the LCR Bridge in the frequency range 10k Hz to 100 KHz. By knowing the geometry of the sample calculates the dielectric constant with the help of the formula.

$$\varepsilon = \frac{C\,d}{A\,\varepsilon_0}$$

Where,

C = Capacitance

A = Area of applied silver paste

d = Thickness of the sample

ε_0 = Absolute permittivity

Results and discussion

PALS measurement

Positron annihilation lifetime spectra were recorded using Positron Annihilation Lifetime Spectrometer (PALS). The Positron Lifetime Spectrometer consists of a fast–fast coincidence system with BaF_2 scintillators coupled to photo multiplier tubes type XP2020/Q with quartz window as detectors. Two PMT were used as start channel and stop channel with the energy selections of 300–550 KeV and 700–1320 KeV respectively. The operating voltage was set to +2100 V. To eliminate the effect of time walk, the PMT pulses were processed through constant fraction differential discriminator (CFDD), one in each channel. The pulses from CFDD are connected to time to amplitude converter (TAC), which helps to calibrate the spectrometer and to shift the spectrum to the desired region in the multichannel analyser. The output pulses from TAC whose amplitudes are proportional to the time gap between emissions of the 1.276 MeV start gamma-ray and the subsequent 0.511 MeV annihilation stop gamma-ray pulses were recorded in a PC based multichannel analyser with a time resolution (FWHM) of 230 ps. For each positron annihilation spectrum, nearly 10^5 coincidence counts were recorded. The BET surface area analysis measurements were carried out for AlPO4 zeolites using the nitrogen gas adsorption. Average specific surface area from the 6 adsorption cycles for each sample was calculated using the Micromeritics software. The results of the specific surface area measurements for R^{3+}:$AlPO_4$ zeolite is given in Table 1.

Table 1: **BET surface area data for R^{3+}:$AlPO_4$ zeolites.**

Sample	$AlPO_4$ $m^2 g^{-1}$	Eu:$Alpo_4$ $m^2 g^{-1}$	Gd:$AlPO_4$ $m^2 g^{-1}$	Nd:$AlPO_4$ $m^2 g^{-1}$
BET Surface area	54.05	30.23	28.67	21.13

Capacitance

The variation of capacitance, determined at 10 kHz to 100 kHz, versus the $AlPO_4$ doped with different rare earth metals is shown in Figure 1. It can be seen from the figure that the value of capacitance decreases continuously with increasing frequency. All the doped samples have resulted in higher capacitance if compared to undoped sample. However, the capacitance is found to decrease with the increasing of the different range of frequency. The dielectric constant has been observed to decrease with increasing frequency but the dispersion in all the cases showed an unusual dielectric behavior similar to that observed. The decrease of dielectric constant with increase of frequency as observed in the different dopent of rare earth metals is a normal dielectric behaviour. This normal dielectric behaviour was also observed by several other investigators [3-4].

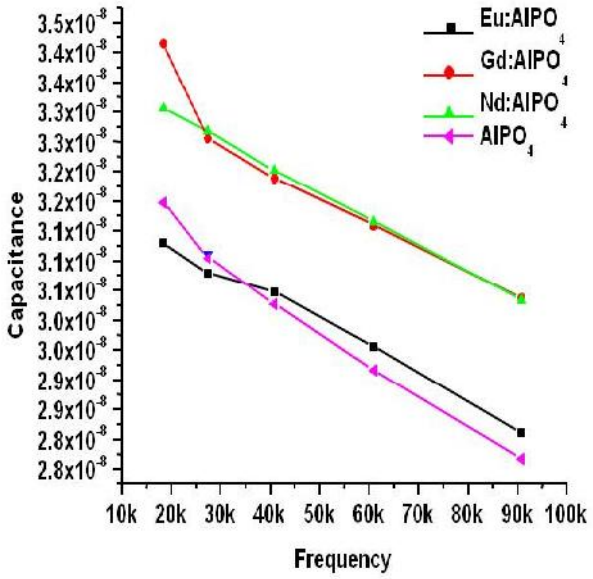

Figure 1

The dielectric behaviour in metals can be explained on the basis of the assumption that the mechanism of dielectric polarization is similar to that of conduction. Many scientists established a strong correlation between the conduction mechanism and dielectric constant of metals. It can be seen from the figure that the dispersion in dielectric constant is analogous to Maxwell–Wagner interfacial polarization in agreement with Koop's phenomenological theory [5].

Conclusion

In our samples, R^{3+} doped $ALPO_4$ have been successfully synthesized using closed hydrothermal method and effect of sintering temperature on the electrical and dielectrically properties have been studied. Positron annihilation lifetime spectroscopy data show that microvoid content increases as the surface area decreases. The electrical property of $AlPO_4$ keeps changing by the addition of different rare earth materials. The Dielectric constant decreases, where as the conductivity increases with respect to frequency at room temperature. We notice that the lattice parameter for all samples seems to be independent of the type of doped rare earth ions. This means that the rare earth ions occupy either the Zeolite positions or go to the grain boundaries.

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ANTHELMINTIC ACTIVITY OF SUBSTITUTED 1H-BENZIMIDAZOLE-2-THIOL MANNICH BASES BEARING N-METHYL PIPERAZINE

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Abstract

The present study involves the reaction of o-phenylenediamine with carbon disulphide in an alkali to give 1H-Benzimidazole-2-thiol (1). This on reaction with 2-chloro-N-substituted-phenylacetamids results in compound 2a-2e, these on further treatment with formaldehyde and N-methyl piperazine (NMP) in presence of Conc. hydrochloric acid yields the title compounds 3a-3e. The structures of the compounds were confirmed by physical data (m.p, R_f) and spectral studies (IR, ^{1}H NMR). Indian earthworm *Pheritimaposthuma* was used to study the possible anthelmintic potentials of the compounds against standard drug piperazine citrate.

Key Words: *Anthelmintic activity, 1H-Benzimidazole-2-thiol, N-methyl piperazine, Mannich Reaction.*

Introduction

Mannich bases are the β-aminoketone/s carrying compounds and are prepared via mannich reaction [1, 2]. It is the condensation of the compounds containing an active hydrogen atom with an amine and formaldehyde [3]. A detailed literature studies reveal that drugs such as ethacrynic acid, rollitetracyclins, fluoxetine, are the amino alkyl ether bearing mannich bases [4] and are currently used in medicine worldwide in the treatment of various ailments effectively. Recently mannich bases have received therapeutic importance due to their wide range of

biological activities viz., anticonvulsant [5], anticancer [6], analgesic [7], anti-tubercular [8], antimalarial [9], local anesthetic [10], antipsychotic [11], antimicrobial [12], antiviral [13], anti-inflammatory [14] etc., however there is a scarcity of documents on the anthelmintic activity of mannich bases in the literature. Infections due to worms are increasing worldwide and need to be treated [15]. Though piperazine and related compounds were found to possess anthelmintic activity [16] benzimidazoleheterocycles such as albendazole, mebendazole, parabendazole, oxfendazole, fenbendazole, thiabendazole, Flubendazole [17] etc., were therapeutically used in the treatment of helminthiasis and these are associated with the side effects headache, dizziness, abdominal discomfort, diarrhea, drowsiness, rash, hallucinations, crystalluria, and leukopenia [18] etc., Hence there is a need for the search of an anthelmintic drug devoid of side effects. In view of the above facts and in continuation of our work on mannich bases [19], about five derivatives of 1H-Imidazole-2-thiol 3a-3e were synthesized from mannich reaction by incorporating N-methyl piperazine.

Materials and Methods:

The chemicals used in the present study were of commercial grade without further purification. The melting points were determined in an open capillary tube using Secor India melting point apparatus and were uncorrected. Purity of the synthesized compounds were checked by TLC, characterized by physical data (R_f, m.p) and spectral studies (IR, ^{1}H NMR). The IR spectra were recorded on Shimadzu 8400 spectrophotometer by KBr pellet technique and the ^{1}H NMR spectra was taken on a Bruker AMX 400 MHz NMR spectrometer.

General Procedure:

Step-1: Synthesis of 1H-Benzimidazole-2-thiol (1).

0.1 mol (10.81g) of o-phenylenediamine, 0.1 mol (6.35 g) of powdered potassium hydroxide and 0.1 mol (5.653g, 7 mL) of Carbon disulphide in 100 mL of ethanol and 20 mL water were refluxed for 3 hours in a 250 mL round bottomed flask. 3-4 g of activated animal charcoal was added to the above refluxed mixture and further heated to 10 min., cooled and filtered. The filtrate obtained was then heated to 60-70 ^{0}C on a water bath with 10 mL of acetic acid and 20 mL of water where these were added under constant stirring. The glistering white crystals were formed during this process and allowed to crystallize in a refrigerator. The product

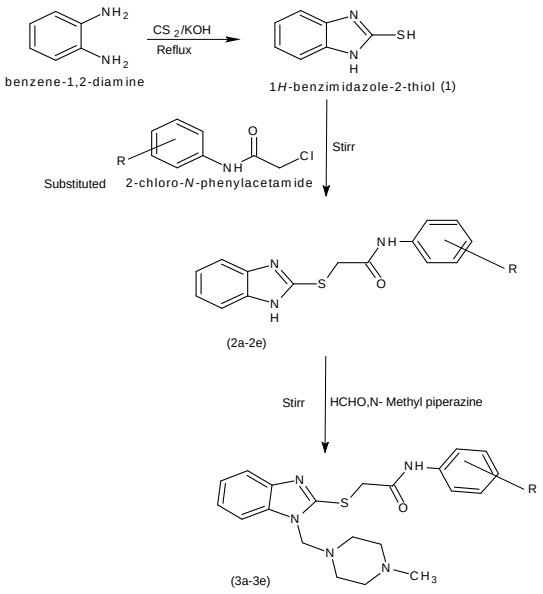
thus obtained was collected and recrystallized from ethanol [20]. % yield: 53; m.p (^{0}C): 303-304; M.F: $C_7H_6N_2S$; R_f: 0.8; IR (KBr, cm^{-1}): 3155 (NH Str), 1506 (C-C Str, Ar), 1259 (CN str, Ar), 2560 (SH str, Ar).

Step-2: Synthesis of compounds 2a-2e.

0.001 mol (0.15 g) of compound 1H-Benzimidazole-2-thiol (1) was dissolved in 10 mL of dimethyl formamide and 4 g (0.04 mol) of anhydrous potassium carbonate taken in a 100 mL round bottomed flask attached with dropping funnel and calcium chloride guard tube. 0.01mol of 2-chloro-N-substituted-phenylacetamids reported in our earlier studies [21] in 10 mL of dimethyl formamide was added separately drop-wise to the above solution with constant stirring at room temperature. The reaction mixture was then continuously stirred for about 24 hours, the solvent was distilled off and the products obtained were collected.

Step-3: Synthesis of compounds 3a-3e.

In a 50 mL round bottom flask, 0.01 mol of compounds 2a-2e obtained from the step-2 in methanol was added 0.01 mol of formaldehyde (0.5ml, 37%) and 0.01 mol (1.09 mL) n-methyl piperazine. The mixture was stirred overnight with 2-3 drops of Conc. Hydrochloric acid and kept at 0-10 ^{0}C for 3 hrs. The precipitate thus obtained were collected and recrystallized from methanol (**scheme1**).



Scheme 1: Synthesis of mannich bases of 1H-Benzimidazole-2-thiol (3a-3e).

Where,

Compound	R
3a	4-NO_2
3b	4-Cl
3c	2-NO_2
3d	2-Cl, 5-NO_2
3e	2-NO_2, 4-OCH_3.

Compound 3a: M.F: $C_{21}H_{24}N_6O_3S$; % yield: 49; m.p (^{0}C): 180-182; R_f: 0.58; IR (KBr, cm^{-1}): 3300 (NH str), 3000 (CH Str, Ar), 2878 (CH Str aliphatic), 1699 (C=O str); ^{1}H NMR (δ ppm): 7.9 (s, 1H, CONH), 7.5-7.0 (m, 8H, Ar-H), 4.9 (s, 2H, CH_2), 3.8 (s, 2H, CH_2CONH), 2.5-2.4 (t, 8H, CH_2 of NMP), 2.2-2.1 (s, 3H, N-CH_3 of NMP).

Compound 3b: M.F: $C_{21}H_{24}N_5SOCl$; % yield: 36; m.p (^{0}C): 169-171; R_f: 0.47; IR (KBr, cm^{-1}): 3510 (NH Str), 3065 (CH Str, Ar), 2953 (CH str, Aliphatic), 1633 (C=O str), 755 (CClStr); ^{1}H NMR (δ ppm): 8.0 (s, 1H, CONH), 7.4-7.2 (m, 8H, Ar-H), 4.8-4.7 (s, 2H, CH_2), 3.8-3.7 (s, 2H, CH_2CONH), 2.5-2.4 (t, 8H, CH_2 of NMP), 2.2 (s, 3H, N-CH_3 of NMP).

Compound 3c: M.F: $C_{21}H_{24}N_6O_3S$; % yield: 56; m.p (^{0}C): 184-185; R_f: 0.52; IR (KBr, cm^{-1}): 3310 (NH Str), 3133 (Str, Ar-H), 2822 (CH str, Aliphatic), 1661 (C=O str); ^{1}H NMR (δ ppm): 8.0-7.9 (s, 1H, CONH), 7.6-7.2 (m, 8H, Ar-H), 4.8 (s, 2H, CH_2), 3.8-3.7 (s, 2H, CH_2CONH), 2.4 (t, 8H, CH_2 of NMP), 2.3-2.2 (s, 3H, N-CH_3 of NMP).

Compound 3d: M.F: $C_{21}H_{23}N_6O_3SCl$; % yield: 39; m.p (^{0}C): 152-154; R_f: 0.4; IR (KBr, cm^{-1}): 3321 (NH Str), 3106 (CH Str, Ar), 2849 (CH str, Aliphatic), 1706 (C=O str), 748 (CClStr); ^{1}H NMR (δ ppm): 8.0 (s, 1H, CONH), 7.7-6.8 (m, 7H, Ar-H), 4.8 (s, 2H, CH_2), 3.8-3.7 (s, 2H, CH_2CONH), 2.5-2.4 (t, 8H, CH_2 of NMP), 2.3-2.2 (s, 3H, N-CH_3 of NMP).

Compound 3e: M.F: $C_{22}H_{26}N_6O_4S$; % yield: 62; m.p (^{0}C): 159-163; R_f: 0.72; IR (KBr, cm^{-1}): 3510 (NH Str), 3065 (CH Str, Ar), 2968 (CH str, Aliphatic), 1663 (C=O str); ^{1}H NMR (δ ppm): 8.0-7.9 (s, 1H, CONH), 7.4-6.5 (m, 7H, Ar-H), 4.9-4.8 (s, 2H, CH_2), 3.8 (s, 2H, CH_2CONH), 3.7-3.6 (s, 3H, Ar-OCH_3), 2.4-2.3 (t, 8H, CH_2 of NMP), 2.2-2.1 (s, 3H, N-CH_3 of NMP).

Anthelmintic activity: Anthelmintic activity was carried out according to the method of Ajaigeoba [22] using Indian earth worm *Pheritimaposthuma*due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings [23,24].

*Pheritimaposthuma*of nearly equal size (6 ± 1 cm) were collected from moist areas and washed with normal saline.

Preparation of sample solutions:

The suspensions of the sample [25] (0.5% w/v, 1.0% w/v and 1.5% w/v) were prepared by triturating the compounds with distilled water and 0.5% w/v ethylene glycol and further transferred to a beaker labeled as 0.5%, 1.0% and 1.5% respectively, stirred for about 30 min at room temperature. The resulting solutions were then used for anthelmintic studies.

Method of testing:

Test Compound	% Concentration	Time in minutes	
		For paralysis	For death
Control (Normal saline)	0.9	-	-
Piperazine citrate (Std)	0.5	10	18
	1.0	07	15
	1.5	04	11
3a	0.5	12	19
	1.0	07	16
	1.5	05	12
3b	0.5	11	19
	1.0	08	16
	1.5	05	13
3c	0.5	12	21
	1.0	09	18
	1.5	07	15
3d	0.5	13	22
	1.0	08	17
	1.5	07	15
3e	0.5	12	23
	1.0	09	19
	1.5	06	16

Nineteen groups of approximately equal-sized (6 ± 1 cm) Indian earthworms *Pheritimaposthama*consisting of six earthworms in each group were placed in Petri dishes (4" size) containing suspensions of specific concentration (0.5%, 1.0%, 1.5%w/v) at room temperature. Each group was treated with one of the following: control (0.5% w/v ethylene

glycol in normal saline), piperazine citrate (0.5%, 1.0%, 1.5% w/v in normal saline) different concentration of compounds (05, 1.0%, 1.5%) respectively. The times taken for complete paralysis and death were recorded using a stopwatch. The mean paralysis time and mean lethal time for each sample was recorded (each reading was taken in triplicate). The time taken by worms to become motionless was noted as paralysis time and to note the death time the earthworms frequently applied with external stimuli by transferring them to a beaker containing hot water in order to check its mortality. The anthelmintic activities of the tested compounds on *Pheritimaposthuma* (earthworm) are indicated in **Table 1.**

Results and Discussions:

The mannich bases of substituted 1H-benzimidazole-2-thiol were prepared, identified by TLC having R_f values in the range of 0.4-0.72 and characterized by IR and ^{1}H NMR. The IR spectra of all the synthesized compounds 3a-3e indicated the presence of aromatic CH str vibrations between 3065-3000 cm^{-1}, aliphatic CH str vibrations in the range 2968-2849 cm^{-1}, NH str at 3510-3300 cm^{-1} and C=O str between 1706-1633 cm^{-1}. The ^{1}H NMR spectra of the compounds 3a-3e exhibited singlet between 7.9-8.0 δ ppm corresponds to NH, multiplets at δ ppm 7.7-6.6 for aromatic protons, at δ 4.9-4.8 ppm as singlet corresponds to methylene bridge between N-methyl piperazine and the nitrogen of benzimidazole, a singlet at δ 3.8-3.7 δ, ppm corresponds to CH_2CONH, triplet between δ, ppm 2.5-2.4 corresponds to methylene bridges in N-methyl piperazine and a singlet at 2.3-3.2 δ, ppm corresponds to a methyl group of N-methyl piperazine. All the above facts confirm the proposed structures. The results of the anthelmintic activity exhibited by title compound on *Pheritimaposthuma*are shown in **Table 1**. Data from this table indicates that compounds 3a, 3b having nitro and chloro group were found to possess markedly higher anthelmintic activity than other compounds compared with standard and hence can be concluded that the presence of electron withdrawing group at 4th position of 2-chloro-N-substituted-phenylacetamids enhances the anthelmintic activity.

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