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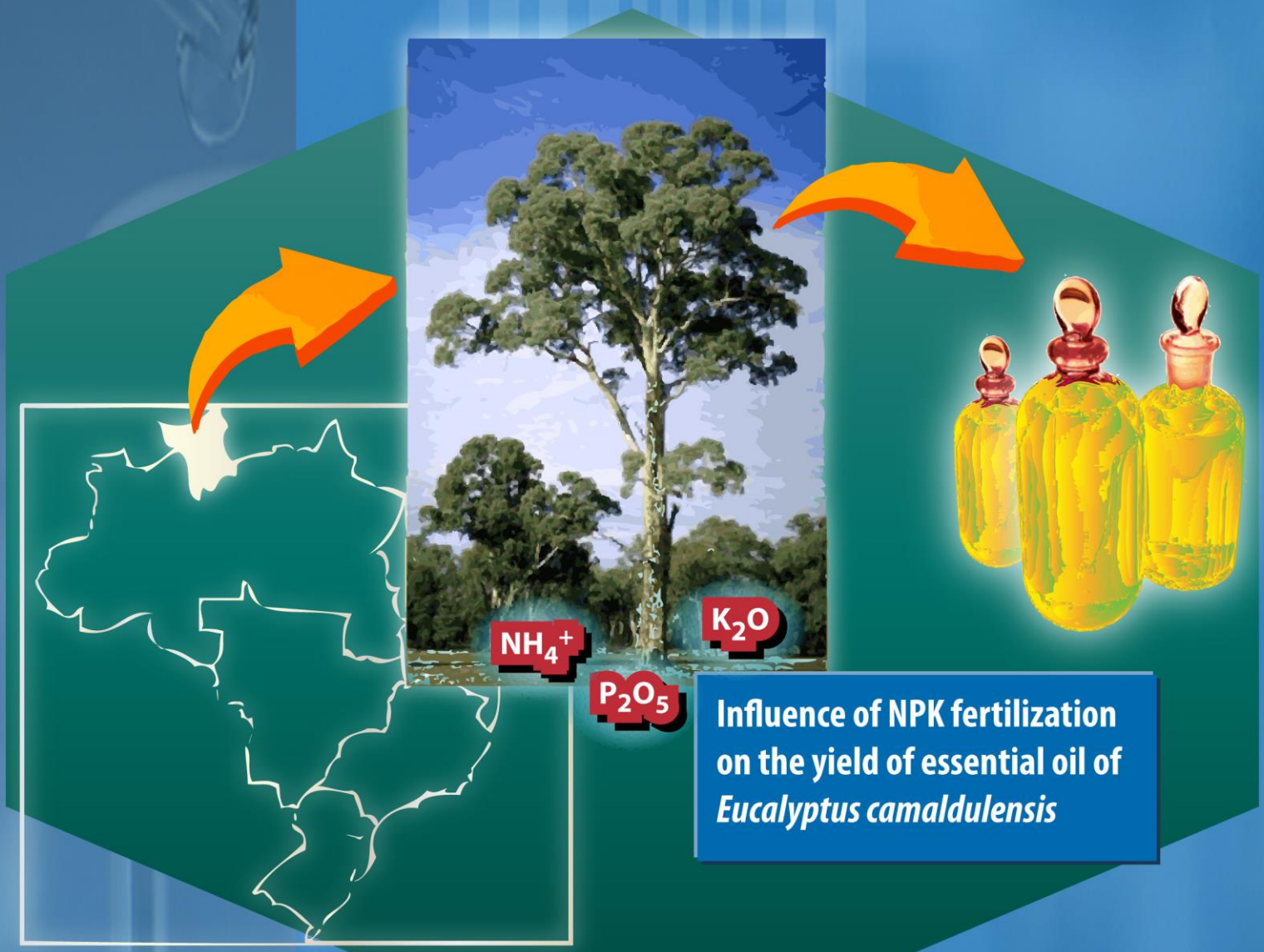

Influence of NPK fertilization on the yield of essential oil of *Eucalyptus camaldulensis*

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Full Paper

Influência da adubação NPK no rendimento do óleo essencial de *Eucalyptus camaldulensis*

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ABSTRACT: *Eucalyptus camaldulensis is a species of eucalyptus with potential for establishment of planted forests in the savannah area of Roraima. Little is known about the appropriate management of E. camaldulensis in the conditions of the savannah of Roraima, and for this reason, an experiment was conducted to evaluate the effects of nitrogen (N), phosphorus (P) and potassium (K) in the production of essential oil of the species. The experiment was conducted in ½ 4^3 fractional factorial design, that evaluated four doses of N (0, 50, 100, 200 kg ha^{-1}), P (0, 30, 60, 120 kg ha^{-1}) and K (0, 50, 100, 200 kg ha^{-1}). One year after planting, the yield of essential oil was determined in fresh leaves, with extraction performed by the method of hydrodistillation in Clevenger type system. The yield of essential oil was changed mainly due to fertilization, increasing from 0.43 to 0.62% as it increased the doses of P. These values were within the range of 0.3 to 2.8% in the literature to yield essential oil in fresh leaves of E. camaldulensis. The highest oil yield was observed with a dose of 120 kg ha^{-1} phosphorus.*

Keywords: *primary macronutrients; no-timber forest products; secondary metabolites; cineole; eucalyptus.*

Introdução

Os vegetais têm duas formas de defesa, estas podem ser físicas e/ou químicas. Essas defesas impedem que inimigos naturais, como as bactérias, fungos, animais e insetos, ataquem ou se aproximem. As defesas físicas dispõem de diversos mecanismos,

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o mais conhecido são os espinhos, e as defesas químicas são os metabólitos secundários. Dentre estes metabólitos estão compostos hidrofóbicos voláteis mais conhecidos como óleos essenciais, os quais podem ser expelidos pela planta para atrair ou repelir insetos polinizadores [1].

Os óleos essenciais são substâncias odoríferas voláteis de mistura complexa de diversas funções orgânicas. São utilizados na indústria de perfumaria e alimentícia, na agricultura e na medicina. Alguns exemplos de espécies produtoras são manjericão, camomila, alfavaca, eucalipto, e outros [2-4].

O gênero *Eucalyptus* pertence à família da Myrtaceae, é nativa da Austrália. A espécie em estudo, *Eucalyptus camaldulensis* Dehn., resiste a situações climáticas extremas, como calor ou frio, bem como deficiências hídricas e nutricionais. Do eucalipto obtém-se a celulose, madeira (lenha, movelaria, postes e cercados) e os óleos contidos em suas folhas possuem uso estético e medicinal, tendo como componente majoritário o cineol [3, 5-8]. O cineol ou eucaliptol é um monoterpeno, o qual é o principal componente encontrado no óleo essencial das folhas de eucalipto, o mesmo é utilizado na medicina tradicional para bronquite, sinusite e resfriados [9-10].

O óleo essencial de eucalipto possui importante controle biológico na agricultura, devido às suas atividades antimicrobianas, acaricida, nematicida, herbicida e repelente de insetos. Sendo assim, pode-se utilizar o óleo essencial de eucalipto como inseticida natural, evitando, assim, uso de agrotóxicos sintéticos. Na medicina é utilizado como antisséptico, antifúngico, antibacteriano, expectorante e aumenta o número de células mortas que causam a leucemia [3, 9, 11-14].

A produção de óleo essencial de eucalipto tem se tornado muito rentável para diversos países, principalmente o Brasil, o qual exportou, no período de 2005-2008, 1.237 toneladas desse óleo. No Brasil os óleos essenciais das espécies *E. globulus*, *E. citriodora* e *E. staigeriana* são os mais utilizados na perfumaria e na medicina [3].

O estabelecimento de florestas plantadas de eucalipto surge como uma alternativa para viabilizar o uso e evitar a degradação de áreas alteradas no cerrado roraimense. A espécie *E. camaldulensis*, por sua rusticidade, apresenta bom potencial para plantios nas condições de clima e solo de Roraima. A espécie em estudo possui bom rendimento de óleo essencial e alto teor de cineol.

Os percentuais de rendimento e composição do óleo volátil variam de acordo com as espécies, fatores climáticos, condições de solo, manejo da cultura, entre outros. Esses percentuais de rendimento e composição do óleo essencial podem aumentar, ou não, devido à concentração da adubação [7, 15-18].

Brant e colaboradores (2010) verificaram que em uma das doses de adubação

com esterco bovino houve aumento significativo de óleo essencial de *Aloysia triphylla*. Enquanto Alsafar e Al-Hassan (2009) apresentaram importante aumento de massa foliar e de óleo essencial da espécie vegetal *Mentha longifolia* L. em tratamento de 75/50 kg N/P_2O_5. Junior e colaboradores (2005) sugerem o tratamento de adubação mista sem calagem de alta concentração, assim fornece alto rendimento de óleo essencial da espécie *Lychonophora ericoides*.

Foram iniciados estudos sobre a influência de doses de macronutrientes primários (NPK) no desenvolvimento inicial do *E. camaldulensis* nas condições de Roraima. Supõe-se que as doses de nutrientes não afetam somente o crescimento e a produção de biomassa da espécie, mas também, a produção de metabólitos secundários. Desta maneira, o presente estudo objetivou avaliar o efeito de doses de NPK no rendimento de óleo essencial do *E. camaldulensis*.

Material e Métodos

Área experimental

O experimento foi realizado em área da Embrapa Roraima, localizada no município de Boa Vista (RR). A localização geográfica da área experimental é 60°43'51"S e 2°45'25,8"N. O clima é Awi, conforme o sistema de classificação de Köppen, com duas estações climáticas bem definidas, uma chuvosa de abril a setembro e outra seca de outubro a março [21]. O solo da área experimental apresentou a seguinte caracterização química na profundidade de 0-20 cm: pH_{H2O}= 4,8; Ca= 0,25 $cmol_c\ dm^{-3}$; Mg= 0,65 $cmol_c\ dm^{-3}$; K= 0,01 $cmol_c\ dm^{-3}$; Al= 0,61 $cmol_c\ dm^{-3}$; H+Al= 2,64 $cmol_c\ dm^{-3}$; CTCt= 3,6 $cmol_c\ dm^{-3}$; CTCe= 1,5 $cmol_c\ dm^{-3}$; V(%)= 25,6 e m(%)= 40.

Para correção do solo da área experimental foi feita calagem utilizando o critério para aumento dos teores de Ca e Mg. Nesse critério, bastante usual dentre as técnicas de recomendação de calagem para espécies perenes, o cálculo da necessidade de calcário é feito com base nos teores iniciais dos cátions no solo, no poder relativo de neutralização do calcário (PRNT) e no fator 20 que normalmente é adotado para o eucalipto [22]. O fornecimento de micronutrientes foi garantido a partir da aplicação de FTE BR12, visando 1 kg ha^{-1} de boro e 4 kg ha^{-1} de zinco. Por ocasião do transplante das mudas no campo no período chuvoso, as doses de P_2O_5 foram aplicadas na sua totalidade no sulco de plantio, enquanto que a adubação NK foi parcelada, visando maior eficiência de utilização dos fertilizantes. A uréia foi a fonte de N utilizada no experimento, enquanto que as fontes de P_2O_5 e K_2O foram o superfosfato triplo e o cloreto de potássio, respectivamente.

Delineamento experimental

O delineamento experimental foi fatorial fracionário ½ 4^3 [23-24]. Metade das

combinações de um fatorial completo foi testada, resultando em 32 tratamentos sem repetições e divididos em dois blocos (Figura 1). Apenas as combinações consideradas biologicamente mais interessantes foram avaliadas (Tabela 1). As parcelas experimentais foram constituídas por 40 plantas com espaçamento de 3 x 2 m. A área útil foi aquela ocupada pelas 18 plantas centrais (Figura 2).

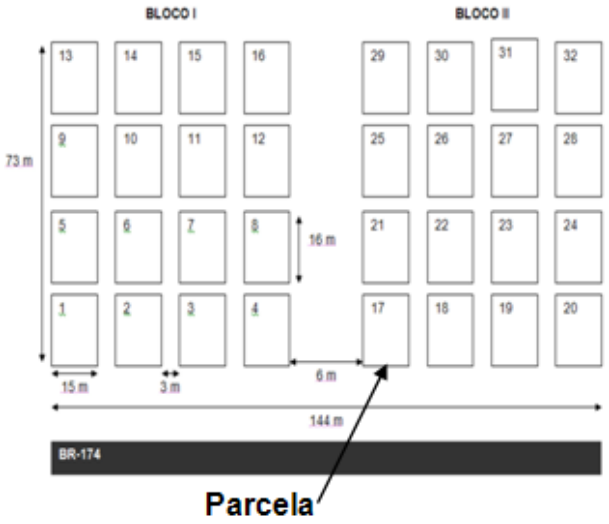

Figura 1. Representação das 32 parcelas distribuídas em dois blocos na área experimental.

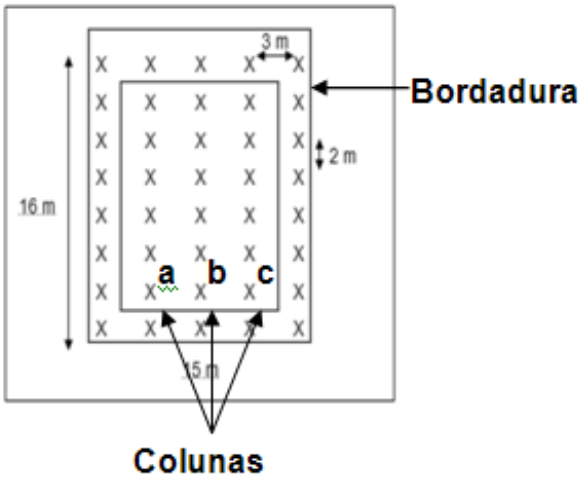

Figura 2. Representação da parcela experimental com 18 árvores na área útil.

Coleta, extração e avaliação do rendimento de óleo essencial

Para avaliar o rendimento de óleo essencial, em dezembro de 2008 foram coletadas folhas de três plantas úteis de cada parcela. A coleta foi realizada no período da manhã, retirando-se somente folhas frescas e de boa aparência das árvores [2].

Para cada amostra, a extração de óleo essencial foi feita em três repetições em

laboratório. Foi empregado o processo de hidrodestilação com sistema tipo *Clevenger*, onde a extração foi realizada até obtenção de volume constante de óleo essencial, ou seja, por período de 4 horas [25-27] e de 3 a 4 vezes ao dia.

Tabela 1. Descrição dos tratamentos e doses totais de nitrogênio (N), fósforo (P) e potássio (K) aplicadas nos tratamentos

Tratamento[1]	N	P	K
Bloco I		**Kg ha^{-1}**	
111	0	0	0
122	0	30	50
133	0	60	100
144	0	120	200
212	50	0	50
221	50	30	0
234	50	60	200
243	50	120	100
313	100	0	100
324	100	30	200
331	100	60	0
342	100	120	50
414	200	0	200
423	200	30	100
432	200	60	50
441	200	120	0
Bloco II		**Kg ha^{-1}**	
114	0	0	200
123	0	30	100
132	0	60	50
141	0	120	0
213	50	0	100
224	50	30	200
231	50	60	0
242	50	120	50
312	100	0	50
321	100	30	0
334	100	60	200
343	100	120	100
411	200	0	0
422	200	30	50
433	200	60	100
444	200	120	200

[1] 1, 2, 3 e 4 representam as doses de nutrientes dentro de cada tratamento

Após a extração, a umidade do óleo essencial foi retirada utilizando sulfato de

sódio anidro. Na seqüência o material foi filtrado, acondicionado em embalagens de vidro âmbar e mantido resfriado [2, 28]. O rendimento de óleo foi avaliado calculando a porcentagem em relação à massa inicial das folhas.

Análise estatística

A análise estatística dos dados foi feita a partir da média aritmética das três repetições do processo laboratorial de extração, de modo que as médias passaram a representar o rendimento de óleo de cada parcela experimental. Os dados foram testados para verificar diferença significativa entre tratamentos usando o teste de análise da variância (ANOVA) em blocos incompletos. Usando o procedimento GLM do SAS [29], foram calculadas funções-resposta do tipo $Y = b_0 + b_1N + b_2N^2 + b_3P + b_4P^2 + b_5K + b_6K^2 + b_7NP + b_8NK + b_9PK$, onde Y é a variável dependente, b_0 a b_9 são os coeficientes de regressão e N, P e K são as doses de NPK aplicadas ao longo do experimento. A variável dependente foi a medida de rendimento de óleo essencial.

Se, por exemplo, respostas foram observadas somente para N, a função-resposta foi simplificada para $Y=b_0 + b_1N + b_2N^2$, calculada pela substituição de valores de P e K iguais à menor dose testada, estimando assim novos coeficientes de regressão de modo que os coeficientes não-significativos não afetaram o novo modelo [30].

Resultados e Discussão

O rendimento de óleo essencial foi afetado pela adubação (Tabela 2). Houve diferença no rendimento de óleo principalmente em função das doses crescentes de P (Figura 3). Com a adubação fosfatada o rendimento de óleo essencial do *E. camaldulensis* passou de 0,45 para 0,62% o que está de acordo com a literatura na qual verifica-se que a faixa de rendimento de óleo em folhas frescas de *E. camaldulensis* é de 0,3 a 2,8% [30-31].

Tabela 2. Função-resposta para rendimento de óleo essencial de *E. camaldulensis* em função da adubação NPK

Parâmetro	Coeficiente	PR > t
N	-0,0006281	0,5870
N^2	0,0000030748	0,5284
P	0,0052652293	0,0114
P^2	-0,0000165365	0,2282
K	-0,0023112973	0,0552
K^2	0,0000078408	0,1170
NP	-0,0000122150	0,0734
NK	0,0000046250	0,2477
PK	-0,0000103252	0,1261
R^2	0,57	
CV (%)	21,78	

Foi constatado que quando o *E. camaldulensis* foi plantado em solos arenosos nas condições climáticas de Anhembi (SP), o rendimento médio de óleo essencial foi

correspondente a 0,32% para diferentes épocas do ano [32]. O máximo rendimento verificado para a espécie foi de 0,41% e esse valor é inferior ao encontrado no presente estudo em que o *E. camaldulensis* foi adubado com diferentes doses de fósforo nas condições edafoclimáticas de Roraima.

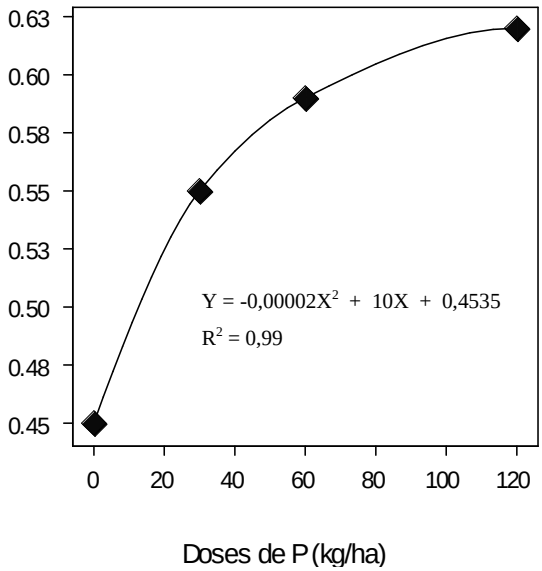

Figura 3. Rendimento de óleo essencial de eucalipto em função das doses de P.

O fósforo é fundamental na composição do trifosfato de adenosina (ATP), o qual armazena e transporta energia do meio externo, permitindo a síntese de substâncias orgânicas e a absorção de nutrientes. Para sintetizar monoterpenos, que constituem 90% dos óleos essenciais, há diversas reações de fosforilação e o ATP é fundamental na doação de energia e do íon fosfato na reação. Conseqüentemente, plantas deficientes em fósforo apresentarão menor taxa de fosforilação, reduzindo sua produção de monoterpenos [33].

Na Tabela 2 é possível verificar que não houve diferença significativa no rendimento de óleo do *E. camaldulensis* em função das doses de nitrogênio (N) e potássio (K). Também não foram observados efeitos quadráticos significativos nos quais o rendimento de óleo essencial poderia reduzir ou aumentar a partir de uma determinada dose de nutrientes. Porém, ao observar o termo linear negativo na Tabela 2 e ao observar as Figuras 4 e 5, constata-se que aumentos nas doses de N e K estão associados à redução no rendimento de óleo essencial do *E. camaldulensis.*

No caso do N esse efeito negativo pode estar relacionado ao maior crescimento vegetativo proporcionado pelo nutriente que causaria diluição na concentração do óleo essencial. A importância do N na formação de biomassa foliar de outra espécie de eucalipto (*E. citriodora)* já foi comprovada em outros estudos [34]. Contudo, pesquisas mostram haver correlação positiva entre fornecimento de N e produção de óleo essencial.

Essa correlação positiva é explicada tanto pela maior produção de biomassa de parte aérea como pela maior quantidade de glândulas que secretam óleo nas folhas [35].

Estudos com outras espécies mostraram que a ausência de N foi a que mais reduziu a produção de óleo essencial em outra espécie vegetal (*Cymbopogon winterianus* Jowitt), juntamente com a omissão de K [36]. Mesmo sem haver diferença estatística significativa, a tendência contrária da curva para N e K observada no presente estudo indica a direção a ser tomada em estudos futuros que foquem, exclusivamente, na relação entre adubação nitrogenada e rendimento de óleo essencial do *E. camaldulensis*.

Interações entre nutrientes também não foram observadas, apesar da interação NP ter apresentado Pr> t mais próximo aos 5% de significância, em comparação com o observado para as interações NK e PK (Tabela 2).

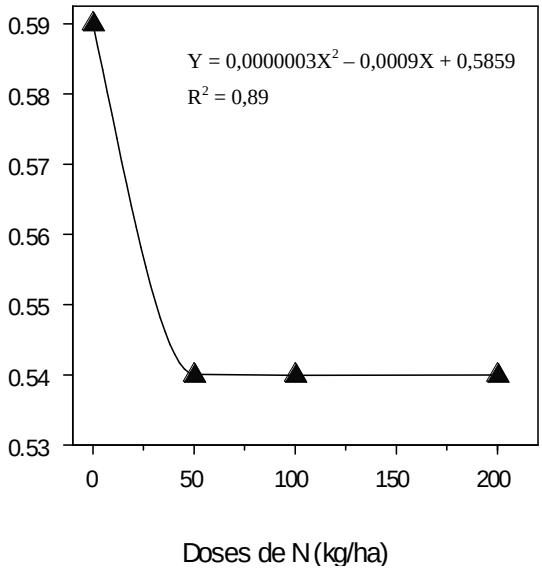

Figura 4. Rendimento de óleo essencial de eucalipto em função das doses de N.

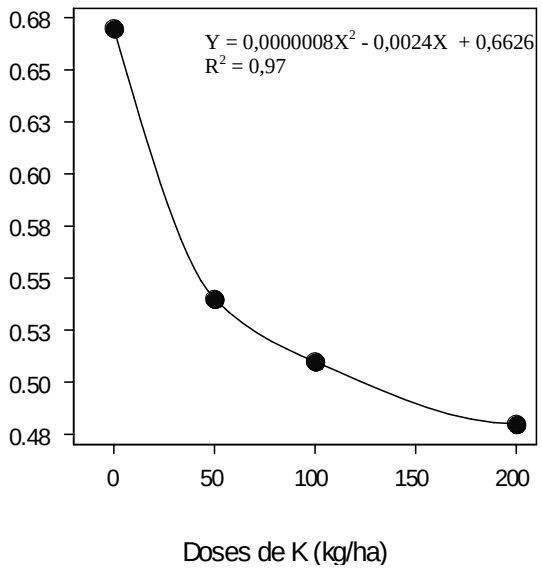

Figura 5. Rendimento de óleo essencial de eucalipto em função das doses de K.

No manejo usualmente praticado por pequenos produtores que utilizam o óleo essencial do eucalipto para obtenção de renda, são feitas colheitas anuais nas quais são retirados cerca de 70% das folhas das copas das árvores [37-38]. Dessa forma, o manejo da adubação com doses de nutrientes que resultem em elevada produção de biomassa de folhas é favorável para aumentar a produção de óleo essencial por hectare, principalmente se associado ao manejo que resulte em maior rendimento de extração.

Conclusão

O rendimento de óleo essencial em folhas frescas de *E. camaldulensis* aumenta com as doses de fósforo aplicadas na adubação de base. O rendimento de óleo essencial obtido para o *E. camaldulensis* nas condições de clima e solo do cerrado roraimense é similar ao obtido para a espécie em outras regiões produtoras do Brasil. A produção de biomassa foliar é fundamental na definição da produção de óleo por hectare do *E. camaldulensis* no cerrado de Roraima.

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Full Paper

2-Hydroxy-4-*n*-butoxy-5-bromoacetophenone thiosemicarbazone as an extractive spectrophotometric reagent for nickel

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ABSTRACT: *2-hydroxy-4-n-butoxy-5-bromoacetophenone thiosemicarbazone (HBBrAT) is spectrophotometric reagent for nickel (II) in chloroform. The metal ion reacts with 2-hydroxy-4-n-butoxy-5-bromoacetophenone thiosemicarbazone (HBBrAT) forming a dark brown coloured complex in the pH range 7.0-11.0. The complex shows maximum absorption at 440 nm. Beer's law is obeyed in the range 2.74-6.86 µg/mL. The molar absorptivity and Sandell's sensitivity are found to be 5229 $Lmol^{-1}cm^{-1}$ and 0.0105 μgcm^{-2}, respectively. The solid complex have been isolated and characterized on the basis of elemental analysis, UV, IR, NMR and Mass spectra. HBBrAT is found to be a selective and strong chelating agent for nickel. The results deduced from Job's method of continuous variation, the mole ratio and the slope ratio method showed that metal: ligand ratio in the complex to be 1:2. The stability constant of the complex found to be 1.92 X 10^7. The free energy change for the complex formation reaction is found to be -10.158 K cal/mole at 32 ^{0}C. The complex is fairly stable for about 24 h and up to 55 ^{o}C.*

Keywords: *bromoacetophenone; thiosemicarbazone; spectrophotometric determination; spectral study*

Introduction

Thiosemicarbazones compound give antifungal and antibacterial activity of different transition metal ions. Thiosemicarbazone are known as analytical reagents [1-5]. Thiosemicarbazones are also found to have biological activity [6]. Further the metal complexes formed with this reagent are of great medicinal value in the treatment of diseases like influenza [7], protozoa [8], small pox [9] and certain kinds of tumor [10].

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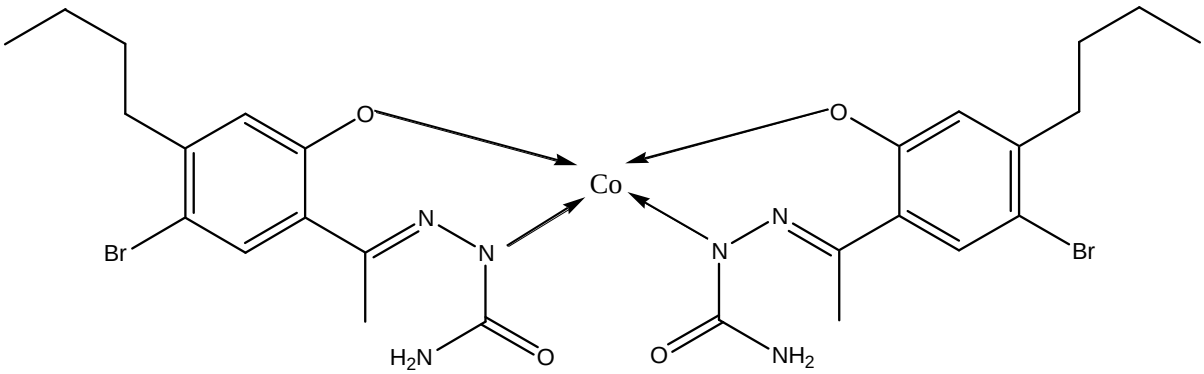
These reagents are known for their antitubercular activity [11]. Metal chelates of these compounds inhibit tumor growth and increase the activity of some drugs [12]. In the treatment of cancer the active species is the metal chelate of thiosemicarbazone [13]. Metal chelates of these reagents are used as pesticides [14] and fungicide [15] in agriculture.

In the present work, we report the use of 2-hydroxy-4-*n*-butoxy-5-bromopropiophenone thiosemicarbazone [16] (HBBrAT) as a spectrophotometric reagent for nickel (II).

Material and Methods

The reagent 2-hydroxy-4-*n*-butoxy-5-bromoacetophenone thiosemicarbazone [M.P. 108-109 ^{o}C] was prepared by simple condensation of 2-hydroxy-4-*n*-butoxy-5-bromoacetophenone with thiosemicarbazide by adopting the standard procedures.

Resacetophenone was prepared from resorcinol, glacial acetic acid and anhydrous zinc chloride according to the method of Robinson and Shah [17]. 2-Hydroxy-4-*n*-butoxyacetophenone (HBA) was prepared by using resacetophenone, *n*-butyl bromide and anhydrous potassium carbonate in acetone [18, 19]. Its 2-hydroxy-4-*n*-butoxy-5-bromoacetophenone was prepared by bromination of HBA. Its thiosemicarbazone was prepared by refluxing its alcoholic solution with thiosemicarbazide for about 4 hour [20]. It was crystallized from ethanol pale yellow crystals were obtained [M.P. 129 ^{0}C]. The molecular weight determination was carried out by Rast's camphor method. The reagent HBBrAT is easily soluble in ethanol, methanol, chloroform, carbon tetrachloride, benzene etc. The structure of Co-HBBrAT is presented below.



Co-HBBrAT

The 0.1 M stock solution of Ni (II) has been prepared by dissolving requisite quantity of nickel sulphate (AR, BDH) in distilled water. The amount of Ni (II) in this solution was determined volumetrically using EDTA.

The elemental analysis of the compound was found to be according the Table 1.

Table 1. Elemental analysis of compound Co-HBBrAT

Element	*Calculated (%)*	*Found (%)*
Carbon	43.25	43.80
Hydrogen	04.91	04.32
Nitrogen	11.61	11.20
Bromine	22.21	22.80
Sulphur	08.91	08.42

Results and Discussion

2-Hydroxy-4-*n*-butoxy-5-bromoacetophenone thiosemicarbazone with nickel gave dark brown colored in basic pH. The absorbance of dark brown colored species at a wavelength corresponding to maximum absorbance i.e. 450 nm remains constant at least one hour. Studies on the effect of pH on the absorbance revealed that the maximum colour was formed in a solution of pH 8. The studies relating to the effect of Ni (II) showed that a linear relationship exists between metal ion concentration and absorbance in the range 14.67–73.36 ppm. Spectrophotometric data of Ni (II)-HBBrPT are as under Table 2.

Table 2. Spectrophotometric data of Ni (II)-HBBrAT

Characteristics	**Results**
Molar absorptivity ($L\ mole^{-1}cm^{-1}$)	5229
Sandell's sensitivity ($\mu g\ cm^{-2}$)	0.0105
Beer's law validity upto (µg/mL)	17.85
Opt. Conc. Range (µg/mL)	2.74-6.86
Stability constant(k)	$1.92X10^{7}$
Standard derivation(S)	0.264
ΔG^{o} (kcal)	-10.158

To determine the stoichiometry of complex, Job's method [21] related data is shown in Figure 1 and mole ratio method [22] related data is shown in Figure 2 are conducted to make these determinations.

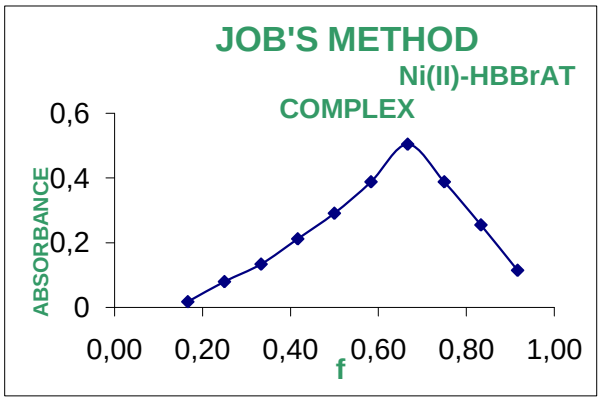


Figure 1. Job's Method.

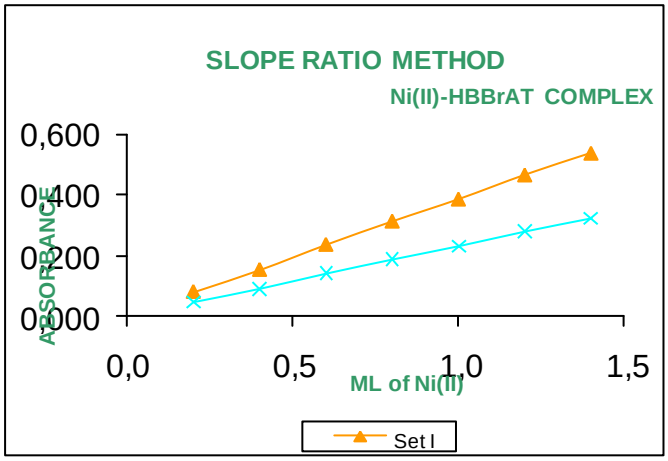


Figure 2. Mole Ratio Method.

It is noticed that Ni (II) forms a stable brown colored 1:2 (metal:ligand) complex with 2-hydroxy-4-*n*-butoxy-5-bromoacetophenone thiosemicarbazone. The stability constant of the complex was found to be 3.54 X10^6 in Table 3.

Table 3. Stability of Ni (II) HBBrAT at 30 ^{0}C

Method Employed	***Em***	***Es***	***a***	***Ks = $\frac{1-a}{4a^3c^2}$***
Mole ratio Method	0.988	0.678	0.31377	3.55 X 10^6
Job's Method	0.784	0.504	0.35714	3.53 X 10^6
Mean Ks				3.54 X 10^6

The newly prepared compound was characterized by elemental analysis as well as spectral analysis, e.g., UV, IR, NMR and CMR method.

Shimadzu 160A UV-visible spectrophotometer (Japan) equipped with 1 cm quartz cell was use in these investigations for making absorbance measurements. A pH meter ELICO L 1-120 (ELICO, Hyderabad) is used to make pH measurements.

IR bands [23, 24] for the ligand and complex are presented in Table 4.

IR spectra of the ligand and complex were recorded on a KBr pellet using Shimadzu – Japan model No. 8400 FTIR.

From the data of NMR spectra of, Ni (II) HBBrAT in Table 5 the ratio of Metal: Ligand is 1:2.

Analytical applications

The nickel-aluminum alloy (0.168 gm.) was dissolved in 1:1 nitric acid by heating on a sand-bath. Excess nitric acid was removed by careful evaporation. The resulting solution was made up to 250 mL. with distilled water in volumetric flask. 1.0 mL of this solution was pipette out in separating funnel. To this solution, 29.0 mL of buffer solution (pH = 8.0) and 10 mL 0.05 M HBBrAT. Solution in chloroform was added. After extraction

the absorbance the organic layer was assured at 500 nm. Against a reagent blank. The results are reported below.

1. Weight of Nickel – aluminum alloy = 0.168 gm.
2. Absorbance of the solution = 0.458 nm
   (Average of three determinations)
3. Nickel found in nickel-aluminum alloy = 50.77%
4. Nickel reported in nickel-aluminum alloy = 50.00%
5. Percent Relative error = 1.54%

Table 4. IR spectra of HBBrAT and Ni-HBBrAT

Vibration mode	***Frequency in cm^{-1}***	
	HBBrAT	**Ni-HBBrAT**
C-H str (asym)	2958	2956
C-H str (aym)	2873	2871
C-H def (asym)	1473	1467
C-H def (aym)	1375	1375
C=C str	1577	1554
C-O-C (sym)	1224	1240
C-O-C (asym)	1037	1022
Ar-OH (intramolecularH-bond)	3647	-
C-Br	557- 619	572,611
C=O	1629	1604
C=S	804	833
C=N	1630	1645
N-H	3253	3035
C-N	1473	1467
C-H	815	833

Table 5. NMR ^{1}H spectra data of Ni-HBBrAT

Signal no.	***Signal position (ppm)***	***Relative no. of ^{1}H***	***Multiplicity***	***Inference***
1	0.91	3H	Triplet	CH_3-CH_2-CH_2-CH_2-O-
2	1.41	2H	Sextet	CH_3-CH_2-CH_2-CH_2-O-
3	1.65	2H	Quintuplet	CH_3-CH_2-CH_2-CH_2-O-
4	2.06	2H	Triplet	CH_3-CH_2-CH_2-CH_2-O-
5	0.93	3H	Singlet	CH_3-
6	2.25	2H	Singlet	$-NH_2$
7	7.7	1H	Singlet	-NH
8	6.51	1H	Singlet	Ar—H
9	7.8	1H	Singlet	Ar—H

Conclusion

Ni (II) forms a 1:2 stable brown colored complex with 2-hydroxy-4-n-butoxy-5-bromoacetophenone thiosemicarbazone. This complex is used for the determination of

nickel in microgram quantities. The stability constant of the complex is 3.54 X10^6. The molar absorptivity and Sandell's sensitivity are 768 $mol^{-1}cm^{-1}$ and 0.0751 $\mu g/cm^{-2}$, respectively. The method has been applied for the analysis of nickel in synthesized mixtures and also in alloys.

Acknowledgments

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Full Paper

Synthesis and biological activities of some 3-chloro-4-(substituted phenyl)-1-{[2-oxo-2-(5-phenyl-1*H*-tetrazol-1-yl) ethyl] amino} azetidin-2-one as potential antibacterial agents

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ABSTRACT: *Some new 3-chloro-4-(substituted phenyl)-1-{[2-oxo-2-(5-phenyl-1Htetrazol1-yl) ethyl] amino} azetidin-2-one have been synthesized from Schiff bases of 5-phenyltetrazole. The Schiff bases were obtained by condensation 2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide with various aromatic aldehydes. Cyclocondensation of Schiff's bases with chloroacetylchloride in the presence of triethylamine results in azetidinone derivatives. The structures of the newly synthesized azatidinones were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds have exhibited significant activity against the bacteria and fungi tested.*

Keywords: ***t****etrazole; azetidinone derivatives; antibacterial activity; antitubercular activity*

Introduction

The emergence and spread of antimicrobial resistance has become one of the most serious public health concerns across the world. Antimicrobial resistance refers to micro-organism that have developed the ability to inactivate, exclude or block the inhibitory or lethal mechanism of the antimicrobial agents [1]. The acid-fast bacillus Mycobacterium tuberculosis is the causative agent of tuberculosis (TB). The tubercle bacillus is a slow growing organism, which does not elicit a sharp and massive reaction from the host. The tubercle bacillus does not produce any substance, which is toxic to the normal host. It acts as an irritating foreign body and tubercle formation can be produced

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by virulent, virulent and nonpathogenic types. The tubercle bacillus is an intracellular parasite, and lives and grows within the host's tissue cells, macrophages and epithelial cells. The recent emergence of outbreaks of multidrug resistant tuberculosis (MDR-TB) is a serious threat to the treatment of the disease. Antitubercular drugs available for the treatments were discovered in the period of 1945-1965 such as isoniazid, rifamycin and ethambutol [2]. No new drugs were synthesized during the last few decades. There are millions of patients suffering from tuberculosis, this tells us about the necessity of searching for and synthesizing new highly active compounds with less side-effects like nausea, skin rashes, vomiting [3].

The search for new anti-tubercular drugs may be done using the Biochemical and Chemical methods [4-5]. Azetidin-2-ones and their derivatives have been extensively investigated, considering the presence of β-lactam ring in their structure just as in the case of highly popular β-lactam antibiotics [6]. Tetrazole derivatives possess very interesting pharmacological and biological properties and are reported to exhibit variety of biological activities like antibacterial [7], antifungal [8, 9], analgesic [10], anti-inflammatory [11, 12], antitubercular activity [13]. Similarly 1,5-substituted tetrazoles have long been known for their pharmaceutical activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic and antithrombotic and antimicrobial properties. Azetidin-2-ones have been reported to possess potent antitubercular, antibacterial [14, 15], antifungal [16-19] activities. By considering the significance of β-lactam ring system as a potential biological agent in treatment of various diseases, in present work a new series of azetidinone derivatives were synthesized from Schiff bases of 5-phenyltetrazole, characterized and their biological activities were investigated.

Material and Methods

General

All the chemicals used were of AR-grade purity. IR spectra (KBr pellets) were recorded on Shimadzu FT-IR model 8010 spectrophotometer. ^{1}H NMR spectra (DMSO-d6) were taken a Varian mercury spectrometer (model YH- 300 FT NMR) using TMS as internal standard and chemical shift are expressed in δ ppm. Analytical thin layer chromatography was performed using E. Merck silica gel G, 0.50 mm plates, (Merck No. 5700). The melting points were determined on an electric melting point apparatus in open capillaries and were uncorrected.

Preparation of ethyl (5-phenyl-1H-tetrazol-1-yl) acetate (1)

A mixture of phenyltetrazole 5 g (0.03 mol) in methanol is prepared, stirred well to dissolve compound **2**.To this add 3.67 mL (0.03 mol) of ethyl chloroacetate drop wise

with continuous stirring to get clear solution. Reflux the reaction mixture on water bath for about 2 hours. A solid residue was obtained by cooling at room temperature. The product was filtered, dried, recrystalised from warm ethanol.

Preparation of 2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide (2)

Ethyl (5-phenyl-1*H*-tetrazol-1-yl) acetate 9 g (0.03 mol) was condensed with 1.95 mL (0.03 mol) 99% hydrazine hydrate. Reflux the reaction mixture on water bath for about 5 hours. The solid residue of acetohydrazide was obtained by cooling. The product was filtered, dried, recrystalised from warm ethanol.

Preparation of Schiff bases (3a-j) [23]

2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide 2 g (0.009 mol) was refluxed with various aromatic aldehydes (0.009 mol) in the presence of sulphuric acid for 6 h. The reaction mixture was then poured into the crushed ice. The resultant solid was washed with distilled water, dried and recrystalised from ethanol.

Preparation of azatidinones(4a-j)

The Schiff bases (0.01 mol) were condensed with chloroacetylchloride (0.02 mol) and triethylamine (0.02 mol) in dry dioxane (50 mL). The mixture was stirred continuously for 20 h. The resulting contents were kept at room temperature for 18 h to complete the reaction. The contents were then poured into the crushed ice. The solid material obtained was filtered, washed with distilled water and recrystallized from warm ethanol.

FT-IR, 1H NMR and 13C NMR of newly synthesized compounds

*3-chloro-4-(phenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl)ethyl] amino} azetidin-2-one (**4a**):* Yield 72%, m.p. 189-191 ^{o}C, IR (KBr) cm^{-1}: 1285 (N–N=N–), 1550(C=N), 1712 (C=0), 2376, 2247 (-NCH2), 1564 (C=C), 2975 (C-H), 3385 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.07 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.46 (s, 2H, -CH2), 7.01-8.45 (m, 10H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.1,167, 153.1, 141, 133, 128.5, 128.4, 128.3, 127.9, 126.9, 123.5, 65.2, 62.5, 50.3. Anal. (%) for $C_{16}H_{14}N_6O$ Calcd. C, 62.70; H, 4.58; N, 27.43. Found: C, 62.58; H, 4.50; N, 27.25.

*3-chloro-4-(2-chlorophenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4b**):* Yield 72%, m.p. 232-234 ^{o}C. IR (KBr) cm^{-1}: 785(C-Cl),1286 (N–N=N–),1551(C=N), 1712 (C=0), 1564 (C=C), 2975 (C-H), 3380 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.07 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.40 (s, 2H, CH2), 7.01-8.45 (m, 9H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 172.9, 167, 153.8, 137.4, 133.5, 130.5, 128.5, 128.4, 128.3, 128.2, 128.1, 127.5, 126.4, 123.2, 65.2, 62.5, 50.30. Anal. (%) for $C_{16}H_{13}ClN_6O$ Calcd. C, 56.37; H, 3.84; N, 24.64. Found: C, 56.30; H,

3.72; N, 24.55.

*3-chloro-4-(4-chlorophenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl] amino} azetidin-2-one (**4c**):* Yield 69%, m.p. 234-236 °C. IR (KBr) cm^{-1}: 785 (C-Cl), 1284 (N–N=N–), 1552 (C=N), 1710 (C=0), 1600 (C=C), 3029 (C-H), 3480 (N-H). ^{1}H NMR (300 MHz) δ ppm: 2.52 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.40 (s, 2H, CH2), 7.60-7.62 (s, 1H, NH), 6.95–8.65 (m, 9H-Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 172.9, 167, 153.8, 141, 133, 130.5, 129.4, 128.5, 128.4, 128.3, 123.5, 65.2, 62.5, 50.3. Anal. (%) for $C_{16}H_{13}ClN_6O$ Calcd. C, 56.37; H, 3.84; N, 24.64. Found: C, 56.30; H, 3.72; N, 24.55.

*3-chloro-4-(4-bromophenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4d**):* Yield 60%, m.p. 243-245 °C. IR (KBr) cm^{-1}: 697 (C-Br), 1284 (N–N=N–), 1553 (C=N), 1695 (C=0), 1576 (C=C), 2939 (C-H), 3404 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.07 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.35 (s, 2H, CH2), 6.98-8.20 (m, 9H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.1, 167, 153.1, 141, 133, 128.5, 128.4, 128.3, 127.9, 126.9, 123.5, 65.2, 62.5, 50.3. Anal. (%) for $C_{16}H_{13}BrN_6O$ Calcd. C, 49.87; H, 3.39; N, 21.81. Found: C, 49.80; H, 3.42; N, 21.70.

*3-chloro-4-(3-nitrophenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4e**):* Yield 72 %, m.p. 199-201 °C. IR (KBr) cm^{-1}: 1282 (N–N=N–), 1550(C=N), 1560 (-NO2), 1682 (C=0), 1574 (C=C), 2979 (C-H), 3411 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.11 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.31 (s, 2H, CH2), 6.82-8.18 (m, 9H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.5, 167, 153.1, 147.2, 141, 130, 129.5, 128.5, 128.3, 127.1, 122.6, 121.9, 65.2, 62.5, 50.3. Anal.(%) for $C_{16}H_{13}N_7O_3$ Calcd. C, 54.65; H, 3.70; N, 27.87. Found: C, 54.58; H, 3.60; N, 27.80.

*3-chloro-4-(4-nitrophenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4f**):* Yield 70 %, m.p. 198-200 °C. IR (KBr) cm^{-1}: 1285 (N–N=N–), 1555(C=N), 1566 (-NO2), 1702 (C=0), 1604 (C=C), 2965 (C-H), 3417 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.14 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.37 (s, 2H, CH2), 6.80-8.13 (m, 9H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.5, 167, 153.1, 148.2, 141, 130.4, 128.5, 128.4, 128.3, 123.6, 122.6, 65.2, 62.5, 50.3. Anal. (%) for $C_{16}H_{13}N_7O_3$ Calcd. C, 54.65; H, 3.70; N, 27.87. Found: C, 54.58; H, 3.60; N, 27.80.

*3-chloro-4-(3-metoxylphenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4g**):* Yield 65 %, m.p. 256-258 °C. IR (KBr) cm^{-1}: 1285 (N–N=N–), 1545 (C=N), 1710 (C=0), 1591 (C=C), 2960 (C-H), 3406 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.07 (s, 1H, CH-Cl), 3.64 (s, 3H, OCH3), 5.30 (s, 2H, CH2), 4.6 (s, 1H, N-CH-Ph), 6.80-8.01 (m, 8H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.1, 167, 153.1, 141, 133.0, 128.5, 128.4, 128.3, 127.9, 126.9, 123.5, 65.2, 62.5, 50.3. Anal. (%) for $C_{17}H_{16}N_6O_2$ Calcd. C, 60.68; H, 4.75; N, 24.92. Found: C, 60.42; H, 4.52; N, 24.78.

*3-chloro-4-(4-metoxylphenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4h**):* Yield 65 %, m.p. 224-226 °C. IR (KBr) cm^{-1} 1165 (-OCH3), 1284 (N–N=N–), 1556 (C=N), 1710 (C=0), 1591 (C=C), 2960 (C-H), 3406 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.07 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.30 (s, 2H, CH2), 3.78 (s, 3H, Ar -0CH3), 6.80-8.01 (m, 8H -Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.1, 167, 159.1, 153, 141, 130.5, 128.5, 128.3, 127.5, 123.2, 114.5, 65.2, 62.5, 55, 50.3. Anal. (%) for $C17H16N6O_2$ Calcd. C, 60.68; H, 4.75; N, 24.92. Found: C, 60.42; H, 4.52; N, 24.78.

*3-chloro-4-(4-methylphenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl]amino} azetidin-2-one (**4i**):* Yield 65%, m.p. 224-226 °C. IR (KBr) cm^{-1}: 1165 (-OCH3), 1284 (N–N=N), 1556 (C=N), 1710 (C=0), 1591 (C=C), 2960 (C-H), 3406 (N-H). ^{1}H NMR (300 MHz) δ ppm: 2.88 (s, 3H, Ar-CH3), 3.07 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.30 (s, 2H, CH2), 3.78 (s, 3H, Ar-0CH3), 6.80-8.01 (m, 8H-Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.1, 167, 153.1, 141, 137.6, 130.5, 129.4, 128.5, 128.3, 126.6, 123.2, 65.2, 62.5, 50.3, 21.1. Anal. (%) for $C_{17}H_{16}N_6O$ Calcd. C, 63.70;H, 5.40; N, 26.20. Found: C, 63.60; H, 5.34; N, 26.27.

*3-chloro-4-(4-dimethylaminophenyl)-1-{[2-oxo-2-(5-phenyl-1h-tetrazol-1-yl) ethyl] amino} azetidin-2-one (**4j**):* Yield 59 %, m.p. 206-208 °C. IR (KBr) cm^{-1}: 1283 (N–N=N), 1548 (C=N), 1700 (C=0), 1598 (C=C), 2968 (C-H), 3155 (-N(CH3)2), 3424 (N-H). ^{1}H NMR (300 MHz) δ ppm: 3.09 (s, 1H, CH-Cl), 4.6 (s, 1H, N-CH-Ph), 5.35 (s, 2H, CH2), 3.45–3.48 (s, 6H, N-(CH3)2), 6.80-8.01 (m, 9H-Ar), 9.27 (s, 1H, NH). ^{13}C NMR (40 MHz, DMSO-d6): δ 173.1, 167, 153.1, 149.5, 141, 130.5, 128.6, 128.4, 128.3, 123.2, 112.1, 65.2, 62.5, 50.3, 40.5. Anal. (%) for $C_{18}H_{22}N_7O$ Calcd. C, 61.84;H, 5.44; N, 28.02. Found: C, 61.80; H, 5.34; N, 27.96.

Biological Evaluation

Antimicrobial activity

The antibacterial activity of the test compounds was tested against *B. subtilis, E. coli. S. aureus and P. aeruginosa* using tryptone Soya agar medium. The antifungal activity of the compounds was tested against *A. niger and C. albicans* using Sabour and dextrose agar medium. The sterilized [20-22] (autoclaved at 120 °C for 30 min) medium (40-50 °C) was inoculated (0.001 mL/mL of medium) with the suspension of microorganism and poured into a petri dish to give a depth of 3-4 mm. The paper impregnated with the test compounds (200 µg/mL in dimethylformamide) was placed on solidified medium. The plates were incubated for 1 h at room temperature and at 37 °C for 24 h and 48 h for antibacterial and antifungal activities respectively. Ampicillin (10 µg/disc) and griseofulvin (10 µg/disc) were used as standard for antibacterial and antifungal activity respectively. The observed values of zone of inhibition are presented in

Table 1.

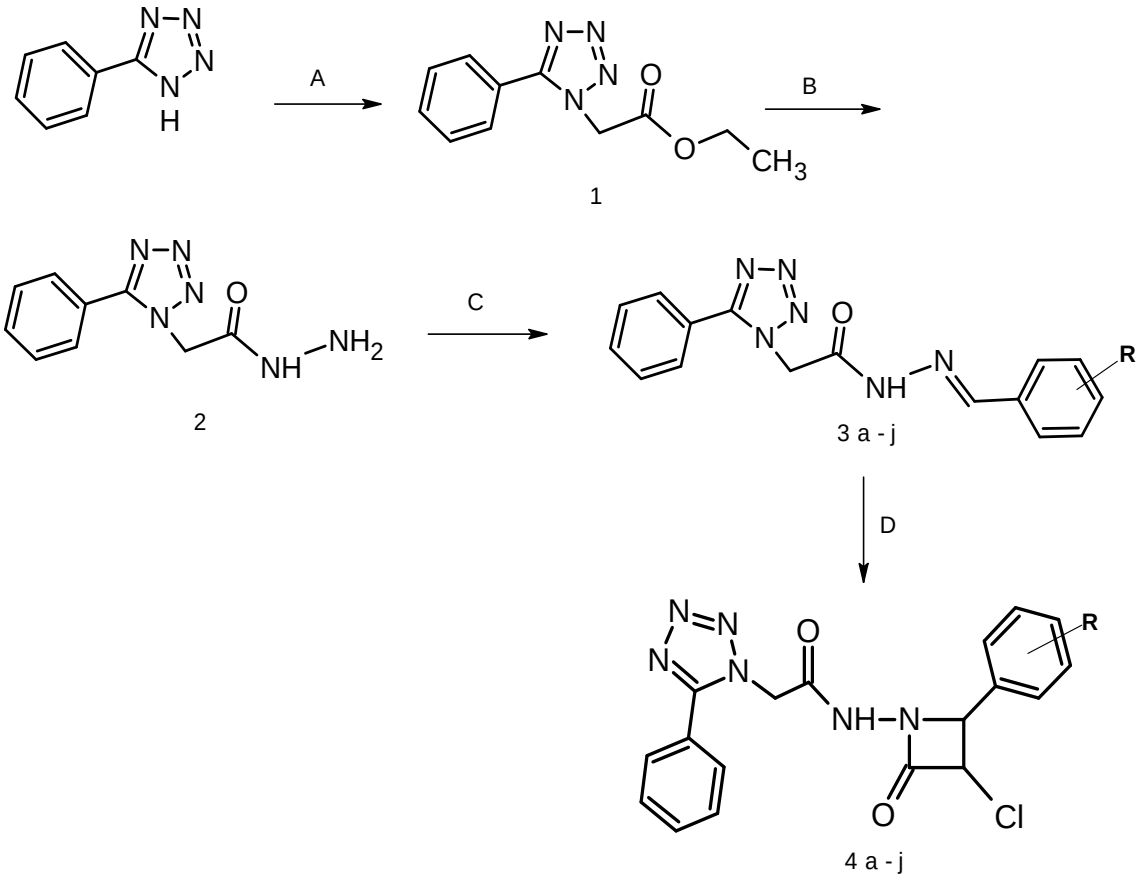

Figure 1. Synthesis protocol for new substituted azatidinone derivatives. Where **A**:$ClCH_2COOC_2H_5$/Methanol;**B**:$NH_2NH_2.H_2O$;**C**:Ar-CHO; **D**:Cl-CH_2CO-Cl/Triethylamine. **4a R = H; 4b R = 2-Cl; 4c R = 4-Cl; 4d R = 4-Br; 4e R = 4-OCH_3; 4f R = 3-NO_2; 4g R=4-NO_2; 4h R=4-CH_3; 4i R=4-OCH_3; 4j R=4-N-$(CH_3)_2$.**

Table 1. Antimicrobial activity of the compounds **4a-j**

Compounds	**Zone of Inhibition in (mm)**					
	S. aureus	***B. subtillis***	***E. Coli***	***P. aeroginosa***	***A. niger***	***C. albicans***
4a	12	14	13	12	09	08
4b	13	15	14	13	14	13
4c	11	13	12	11	10	09
4d	12	14	13	12	11	10
4e	10	12	11	10	13	12
4f	13	15	14	13	10	09
4g	09	11	10	09	12	11
4h	08	10	09	08	11	10
4i	13	15	14	13	09	08
4j	12	14	13	12	10	09
Ampicillin	14	16	15	14	-	-
Griseofulvin	-	-	-	-	15	14
DMF	-	-	-	-	-	-

Antitubercular activity

The procedure followed for anti-TB activity mainly involves the use of Middlebrook 7H-9 broth and standard strain of *M. tuberculosis h37Rv*. The basal medium is prepared according to manufacture's instructions (Hi-Media) and sterilized by autoclaving. 4.5 ml of broth is poured into each one of the sterile bottles. To this, 0.5 mL of ADC supplement is added. This supplement contains catalase, dextrose and bovine serum albumin fraction v. Then a stock solution of the compound is prepared (10 mg/mL). From this appropriate amount of solution is transferred to media bottles to achieve final concentrations of 25, 50, 100 ug/mL. Finally 10 μL suspension of *M. tuberculosis* strain (100000 organisms/mL, adjusted by McFarland's turbidity standard) is transferred to each of the tubes and incubated at 37 °C. Along with this one growth control without compound and drug controls are also set up. The bottles are inspected for growth twice a week for a period of three weeks. The appearance of turbidity is considered as growth and indicates resistance to the compound. The growth is confirmed by making a smear from each bottle and performing a ZN stain. Streptomycin was used as standard drug. The results are presented in Table 2.

Table 2. Antitubercular activity of the compounds **4a-j**

Compounds	Concentration in μg/mL		
	50	**25**	**10**
4a	-	+	++
4b	-	++	++
4c	-	+	++
4d	-	-	-
4e	-	+	++
4f	-	-	++
4g	-	+	++
4h	-	++	++
4i	-	-	++
4j	-	+	++
Streptomycin	-	+	++
Control	++	++	++

(-) – Indicated no growth, (+) indicated growth less than 20 colonies, (++) indicated growth more than 20 colonies.

Results and Discussion

Herein we have described the synthesis, characterization and biological evaluation of 3-chloro-4-(substitutedphenyl)-1-{[2-oxo-2-(5-phenyl-1*h*-tetrazol-1yl) ethyl]amino} azetidin-2-one by cyclocondensation chloroacetylchloride with Schiff bases in the presence of triethylamine (**4a-j**). The Schiff bases were obtained by condensation 2-(5-phenyl-1H-tetrazol-1-yl) acetohydrazide with various aromatic aldehydes. All compounds were obtained in 59-72% yield and analyzed satisfactorily by CHN elemental analysis. All the IR and NMR spectral characteristics of different azatidinones are in good agreement

with proposed structure and are shown in experimental section.

The antibacterial activity of all the synthesized compounds is shown in Table 1. All the compounds exhibited significant antibacterial and antifungal activities. Good antibacterial activity was observed in **4a**, **4b**, **4d**, **4f**, **4i** against *B. Subtilis.* Compounds **4a**, **4b**, **4d**, **4i** showed good activity against *S. aureus.* Compounds **4a**, **4b**, **4d**, **4j** showed significant activity against P. aeruginosa and whereas compounds **4b**, **4f**, **4i** showed noticeable activity against *E. coli.* Compounds **4b**, **4d**, **4e**, **4f** showed marked activity against *A. niger and C. albicans.* Compounds **4d**, **4f** and **4i** showed significant antitubercular activity (25 µg amount) against *M. tuberculosis* in comparison to other.

Conclusion

Conclusively, a variety of azetidinone derivatives of tetrazole have been successfully synthesized in appreciable yields by simple synthetic route and screened in vitro for their antimicrobial activities against both strains of Gram-positive and Gram-negative bacteria and for in vitro antitubercular activity against *M. tuberculosis*. Most of the compounds found to possess good antibacterial activity.

From the above evidence, it is clear that these derivatives can be used to discover bioactive synthetic products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of synthetic origin.

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Full Paper

Ultrasonic velocity and isentropic compressibility of binary fluid mixtures at 298.15 K

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ABSTRACT: *Speed of sound and isentropic compressibility of six polar-nonpolar cyclic liquid binary mixtures has been computed over the whole composition range at 298.15 K with the help of Prigogine-Flory-Patterson theory. Experimental surface tension and experimental density data were utilized in the prediction of sound velocity with the use of Auerbach relation. A comparison has then been carried out as regards the merit and demerits of the employed relations. An attempt has also been made to study the nature and magnitude of molecular interactions involved in the liquid mixture.*

Keywords: *ultrasonic velocity; isentropic compressibility; molecular interactions; Prigogine-Flory-Patterson (PFP) model*

Introduction

Physicochemical behavior and molecular interactions occurring in a variety of liquid mixtures and solutions can be studied with the help of ultrasonic velocity. There has been an increasing interest in the study of molecular interactions and a number of experimental techniques have been used to investigate the same in binary liquid mixtures, since data on sound velocity offers a convenient method for determining certain thermodynamical properties of liquids and liquid mixtures, which are not obtained by other methods. Ultrasonic investigations are important in elucidating internal structure of fluid mixtures involving heat transfer, mass transfer etc. which are applicable in many industrial applications.

Extensive work has been carried out by Pereira et al. [1], Dzida and Ernest [2],

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Al-Kandary et al. [3], Pandey et al. [4] and Pan et al. [5] to investigate liquid state through analysis of ultrasonic propagation parameters and to correlate ultrasonic velocity with other physical and thermodynamic parameters. Substantial amount of work have also been done by Shukla et al. [6-12] successfully for the theoretical evaluation of ultrasonic velocity in binary and multicomponent non polar liquid mixtures by various empirical, semi-empirical and statistical mechanical concepts. Shukla et al. [11-12] have further applied these concepts to polar and ionic liquids. As a part of research concerning the thermochemical studies on new working fluid pair, we present here some useful data on speed of sound and isentropic compressibility for the mixture of trimethylbenzene with tetrahydrofuran, tetrachloromethane and dimethyl sulfoxide. These properties have been determined over the whole composition range. The major objective of the present work is to find out the applicability of Prigogine- Flory- Patterson (PFP) theory in polar-nonpolar cyclic liquid binary mixtures. The necessary parameters for computation have been taken from the work of Pan et al. [5] and CRC hand book of chemistry & physics [13]. To the best of our knowledge, this is the first report in which PFP model has been applied in polar-nonpolar cyclic liquid mixtures.

Theoretical

In dealing with liquid state, Flory et al. [14-16] defined an element (or segment) as an arbitrary chosen portion of the molecules. Considering such segments in a molecule and following Prigogine's treatment of (γ-mer) chain molecules and representing the number of external degree of freedom per segment by 3C, he was able to derive a partition function of the form:

$$Z = Z_{comb}\left[\gamma(v^{1/3} - v^{*1/3})^3\right]^{mc} \exp(-E_0 / kT) \tag{1}$$

where k, N, T and E_0 are, respectively, the Boltzmann constant, number of particles, absolute temperature and excess intermolecular energy. Z_{comb} is combinatorial factor which takes into account the number of ways in which γN elements intersperse among one another. Flory obtained the following expressions for the intermolecular free energy.

$$E_0 = \frac{Nrs\,\eta}{2v} \tag{2}$$

Here η is a constant characterizing the energy of interaction for a pair of neighboring sites, s is the number of intermolecular contact sites per segment and v is the volume per segment. The reduced partition function thus takes the form as:

$$z = z_{comb}(\gamma v^*)^{rNC}(v^{1/3} - 1)^{3rNC} \exp(rNC / \tilde{v}\tilde{T}) \tag{3}$$

the reduced equation of state obtained from the resulting partition function is given by:

$$\frac{\tilde{P}\tilde{v}}{\tilde{T}} = \frac{\tilde{v}^{1/3}}{\tilde{v}^{1/3} - 1} - \frac{1}{\tilde{v}\tilde{T}} \quad (4)$$

Changing from the molecular to molar units per segment for v, v* and η, one gets,

$$\tilde{v} = v/v^* = V/V^* \quad (5)$$

$$\tilde{T} = \frac{T}{T^*} = 2v^* ckT/S_\eta \quad (6)$$

and

$$\tilde{P} = \frac{p}{p^*} = 2pv^*/S_\eta \quad (7)$$

The reduced equation of state at zero pressure becomes,

$$\tilde{T} = \frac{\tilde{v}^{1/3} - 1}{\tilde{v}^{4/3}} \quad (8)$$

$$\tilde{v} = \left[\frac{\alpha T}{3 + 3\alpha T)} + 1\right]^3 \quad (9)$$

Where α is the coefficient of thermal expansion at P = 0. Thus the reduced volume $\tilde{v}$ and reduced temperature $\tilde{T}$ can be obtained from the experimental values of α. The values of $\tilde{v}$, $\tilde{T}$, v* and T* can be computed using eqs (5) and (6). From the reduced equation of state it follows that:

$$P^* = \frac{\alpha}{\beta_T} T\tilde{v}^2 = \gamma T\tilde{v}^2 \quad (10)$$

where $\gamma_P = (\delta P/\delta T)_V$ is the thermal pressure coefficient at P = 0. Characteristic pressure P* is evaluated from this equation for binary liquid mixtures with the component subscript 1 and 2, Flory obtained the following expressions for binary liquid mixtures as:

$$\tilde{T} = \frac{T}{T^*} = \left[\frac{\Psi_1 P_1^* \tilde{T}_1 + \Psi_2 P_2^* \tilde{T}_2}{\Psi_1 P_1^* + \Psi_2 P_2^*}\right]\left[1 - \frac{\Psi_1 \theta P_2 x_{12}}{\Psi_1 P_1^* + \Psi_2 P_2^*}\right] \quad (11)$$

and

$$P^* = \Psi_1 P_1^* + \Psi_2 P_2^* - (\Psi_1 \theta_2 X_{12}) = \frac{T \tilde{v}^{4/3}}{(\tilde{v}^{1/3} - 1)} \left[\frac{\Psi_1 P_1^*}{T_1^*} + \frac{\Psi_2 P_2^*}{T_2^*} \right] \quad (12)$$

where ψ_1 and ψ_2 are the segment fractions and θ_1 and θ_2 are the site fractions given by:

$$\theta_2 = \frac{x_2 v_2 \tilde{v}^{-1}}{(x_1 v_1 \tilde{v}^{-1} + x_2 v_2 \tilde{v}^{-1})} = \left[\frac{s_2 \Psi_2}{s_1 \Psi_1 + s_2 \Psi_2} \right] \quad (13)$$

where x_1 and x_2 are the mole fractions, X_{12} is the interaction parameter and v_1 and v_2 are the molar volumes of the components 1 and 2, respectively. The ration $(s_1/s_2) = (v^*_1/ v^*_2)^{-1/3} = (r_1/ r_2)^{-1/3}$ for spherical molecules. The interaction parameter (X_{12}) can be expressed as:

$$X_{12} = P_1^* \left[1 - \left(\frac{P_2^*}{P_1^*} \right)^{1/2} \left(\frac{v_2^*}{v_1^*} \right)^{1/6} \right]^2 \quad (14)$$

X_{12} is the energy parameter and it is measured of the difference of interaction energy between the unlike pairs and the mean of the like pairs.

The surface tension of binary liquid mixture in terms of Flory's statistical theory can be described by the expression:

$$\sigma = \sigma^* \tilde{\sigma}(\tilde{v}) \quad (15)$$

Where σ^*, $\tilde{\sigma}$ & $\tilde{v}$ are the characteristic and reduced parameters involved in the eqn.

Patterson and Rastogi [17] in their extension of the corresponding state theory dealt with the surface tension by using as a reduction parameter:

$$\sigma^* = k^{1/3} P^{*2/3} T^{*1/3} \quad (16)$$

called the characteristic surface tension of the liquid. Here *k* is Boltzmann constant and P* and T* are the characteristic pressure and temperature, respectively. Starting from the work of Prigogine and Saraga [18] 1952 and Prigogine and Bellmas [19] they derived a reduced surface equation for Van der Waals liquids:

$$\tilde{\sigma}(\tilde{v}) = \left[M \tilde{v}^{-5/3} - \left(\frac{\tilde{v}^{1/3} - 1.0}{\tilde{v}^2} \right) \ln \left(\frac{\tilde{v}^{1/3} - 0.5}{\tilde{v}^{1/3} - 1.0} \right) \right] \quad (17)$$

Where M is the fraction of the nearest neighbor that a molecule loses on moving from the

bulk of the liquid to the surface. Its most suitable value ranges from 0.25 to 0.29.

Although Flory's statistical theory is not directly related with ultrasonic velocity but its evaluation needed the use of well-known and well-tested Auerbach relation which can be successfully used for the evaluation of ultrasonic velocity in binary liquid mixtures represented as:

$$u = \left(\frac{\sigma}{6.3 \times 10^{-4} \rho} \right)^{2/3} \quad (18)$$

where σ and ρ are the surface tension and density of the liquid mixture respectively.

Isentropic compressibility is related to speed of sound and density by the relation:

$$\beta_s = u^{-2}\rho^{-1} \quad (19)$$

Material and Methods

Experimental surface tension and experimental density data have been utilized to evaluate experimental sound velocity with the help of well known and well tested relation of Auerbach for six cyclic binary liquid mixtures over the whole composition range at 298.15K. Parameters of pure components are listed in Table 1 along with the literature values. Values of density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u_{cal} and u_{exp}), percent deviation in speed of sound (%Δu) and isentropic compressibility (β_s) for six binary mixtures; tetrahydrofuran (THF) + 1,2,4-trimethyl benzene (TMB), tetrahydrofuran (THF) + 1,2,3-trimethyl benzene (TMB), tetrachloromethane (TCM) + 1,2,4-trimethyl benzene (TMB), tetrachloromethane (TCM) + 1,3,5-trimethylbenzene, dimethyl sulfoxide (DMSO) + 1,2,4-trimethyl benzene + dimethyl sulfoxide (DMSO) + 1,3,5-trimethyl benzene at 298.5 K are presented in tables 2-7.

All the necessary data for computation were taken from the work of Pan et al. [5]. A close observation of all the tables reveal that experimental and calculated values of speed of sound are very close to each other indicating the success of PFP model in TMB with THF, TCM and DMSO liquid mixtures.

Results and Discussion

Table 1. Parameters of pure components at 298.15 K

Liquid	*ρ^a (exp)*	*ρ^a (lit)*	*$10^4 a/K^{-1}$*	*β_T/TPa^{-1}*	*$V_m/cm^3 mol^{-1}$*	*u/ms^{-1} (exp)*	*u/ms^{-1} (cal)*	*u/ms^{-1} (lit)*
1,2,4-TMB	0.87164	087174	11.168	81.445	137.893	1415.7	1412.3	1421[b]
1,3,5-TMB	0.86103	0.86109	11.320	85.961	139.592	1389.3	1390.8	1397[c]
THF	0.88206	0.88197	11.464	90.440	81.752	1291.2	1283.6	1278[d]
TCM	1.58380	1.58429	11.504	91.704	97.121	921.6	916.6	926[e]
DMSO	1.09554	1.095560	9.8922	50.134	71.316	1480.9	1498.4	1487[d]

a- ref [5], b- ref [20], c- ref [21], d- ref [22], e- ref [7]

Table 2. Values of mole fraction (X_1), density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u-cal, u-exp), %Δu and isentropic compressibility of tetrahydrofuran and 1,2,4-trimethyl benzene

x_1	ρ_m/g.cm^{-3}	V_m/c.c.mol^{-1}	u(cal)/ms^{-1}	u(exp)/ ms^{-1}	(% Δu)	βs/Mpa^{-1}
0.0988	0.8727	132.3	1404.5	1410.1	0.390	0.5809
0.2018	0.8737	126.6	1396.5	1405.3	0.632	0.5869
0.3003	0.8748	121.0	1388.8	1400.4	0.828	0.5927
0.3995	0.8758	115.5	1381.1	1394.4	0.958	0.5986
0.5002	0.8769	109.8	1373.3	1386.8	0.974	0.6047
0.6005	0.8779	104.2	1365.7	1377.9	0.885	0.6107
0.6986	0.8789	98.67	1358.5	1367.8	0.677	0.6165
0.7996	0.8800	93.00	1351.4	1355.9	0.336	0.6223
0.9006	0.8810	87.33	1344.6	1343.7	-0.067	0.6278

Table 3. Values of mole fraction (X_1), density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u-cal, u-exp), %Δu and isentropic compressibility of tetrahydrofuran and 1,3,5-trimethylbenzene

x_1	ρ_m/g.cm^{-3}	V_m/ c.c.mol^{-1}	u(cal)/ ms^{-1}	u(exp)/ ms^{-1}	(% Δu)	βs / Mpa^{-1}
0.0481	0.8620	136.8	1388.0	1381.3	-0.484	0.60217
0.2021	0.8653	127.9	1379.0	1372.6	-0.473	0.60769
0.3002	0.8673	122.2	1373.4	1365.4	-0.587	0.61121
0.4000	0.8694	116.5	1367.8	1358.9	-0.653	0.61475
0.5004	0.8716	110.6	1362.3	1352.8	-0.702	0.61826
0.6010	0.8737	104.8	1356.9	1347.6	-0.687	0.62167
0.7003	0.8758	99.09	1351.8	1342.9	-0.665	0.62488
0.7989	0.8778	93.38	1347.0	1338.8	-0.615	0.62784
0.9000	0.8800	87.54	1342.5	1335.6	-0.515	0.63054

Table 4. Values of mole fraction(X_1), density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u-cal, u-exp), %Δu, isentrtopic compressibility of tetrachloromethane and 1,2,4-trimethyl benzene

x_1	ρ_m/g.cm^{-3}	V_m/ c.c.mol^{-1}	u(cal)/ ms^{-1}	u(exp)/ ms^{-1}	(% Δu)	βs / Mpa^{-1}
0.0496	0.9070	135.9	1371.8	1374.9	0.222	0.58587
0.0996	0.9426	133.8	1333.6	1337.3	0.277	0.59654
0.1986	1.0131	129.8	1264.4	1270.1	0.449	0.61742
0.3001	1.0854	125.7	1201.2	1208.3	0.588	0.63854
0.4006	1.1569	121.6	1145.1	1152.0	0.604	0.65921
0.5987	1.2980	113.5	1049.6	1060.5	1.029	0.69938
0.6986	1.3692	109.4	1007.6	1014.9	0.721	0.71937
0.7985	1.4403	105.3	969.2	973.0	0.394	0.73918
0.9011	1.5134	101.2	932.9	932.1	-0.088	0.75929

Table 5. Values of mole fraction(X_1), density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u-cal, u-exp), %Δu, isentropic compressibility of tetrachloromethane and 1,3,5-trimethyl benzene

x_1	ρ_m/g.cm^{-3}	V_m/ c.c.mol^{-1}	u(cal)/ ms^{-1}	u(exp)/ ms^{-1}	(% Δu)	βs / Mpa^{-1}
0.1009	0.9340	137.5	1311.8	1306.0	-0.445	0.62221
0.2014	1.0066	133.3	1242.8	1235.2	-0.615	0.64319
0.2994	1.0774	129.0	1183.2	1173.6	-0.818	0.66299
0.4007	1.1506	124.7	1128.2	1118.0	-0.912	0.68274
0.4998	1.2223	120.5	1080.1	1071.0	-0.847	0.70131
0.5995	1.2943	116.2	1036.5	1028.8	-0.744	0.71914
0.6988	1.3661	112.0	997.3	991.3	-0.613	0.73592
0.7970	1.4371	107.8	962.4	957.6	-0.494	0.75134
0.9012	1.5124	103.5	929.0	925.1	-0.418	0.76616

Table 6. Values of mole fraction (X_1), Density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u-cal, u-exp), %Δu, isentropic compressibility of dimethylsulfoxide and 1,2,4-trimethylbenzene

x_1	ρ_m/g.cm^{-3}	V_m/ c.c.mol^{-1}	u(cal)/ ms^{-1}	u(exp)/ ms^{-1}	(% Δu)	βs / Mpa^{-1}
0.1007	0.8942	131.2	1259.5	1367.2	7.88	0.70503
0.2007	0.9166	124.5	1264.7	1354.0	6.59	0.68209
0.3007	0.9390	117.9	1270.4	1333.6	4.74	0.65984
0.3995	0.9611	111.3	1276.6	1321.9	3.42	0.63841
0.5011	0.9838	104.5	1283.7	1312.2	2.17	0.61685
0.6012	1.0062	97.9	1291.4	1289.1	-0.18	0.59588
0.6992	1.0282	91.3	1300.0	1271.0	-2.28	0.57552
0.7992	1.0506	84.7	1309.9	1272.4	-2.95	0.55474
0.9013	1.0734	77.9	1321.7	1407.5	6.10	0.53331

Table 7. Values of mole fraction (X_1), density (ρ), molar volume (V_m), theoretical and experimental speed of sound (u-cal, u-exp), %Δu, isentropic compressibility of dimethylsulfoxide and 1,3,5-trimethylbenzene

x_1	ρ_m /g.cm^{-3}	V_m/ c.c.mol^{-1}	u(cal)/ ms^{-1}	u(exp)/ ms^{-1}	(% Δu)	βs / Mpa^{-1}
0.1023	0.8850	132.6	1399.7	1338.4	-4.58	0.57675
0.1987	0.9076	126.0	1408.4	1303.8	-8.02	0.55548
0.3027	0.9320	118.9	1418.1	1274.0	-11.31	0.53355
0.401	0.9551	112.2	1427.7	1275.5	-11.93	0.51371
0.4999	0.9783	105.5	1437.7	1285.3	-11.86	0.49452
0.6003	1.0018	98.6	1448.5	1314.8	-10.17	0.47575
0.6990	1.0250	91.9	1459.7	1350.6	-8.08	0.45791
0.8003	1.0487	85.0	1471.9	1404.3	-4.81	0.44016
0.8998	1.0720	78.2	1484.6	1466.3	-1.25	0.42321

The percent deviations in speed of sound at 298.15 K from figures 1 and 2 shows that they are positive for THF and TCM with 1,2,4-TMB and are negative for THF, TCM and DMSO with 1,3,5-TMB while DMSO with 1,2,4-TMB has both positive and negative values. The maximum positive value of percent deviation in speed of sound follow the order DMSO + 1,2,4-TMB > TCM + 1,2,4-TMB > THF + 1,2,4-TMB and maximum negative values of percent deviation in speed of sound follow the order DMSO + 1,3,5-TMB > TCM + 1,3,5-TMB > THF + 1,3,5-TMB. In both the cases, the trend is similar except for the system DMSO + 1,2,4-TMB where both positive and negative deviations are observed. The possible reason may be that there are no strong intermolecular forces between DMSO and TMB because 1,3,5-TMB is a symmetrical nonpolar molecule. Conversely, stronger forces exists for the systems with 1,2,4-TMB, so the values are positive. Negative values of the systems with 1,3,5-TMB are possibly owing to the greater repulsion due to bulky methyl group as compared to 1,2,4-TMB. These short range repulsive forces are responsible for less compact structure of 1,3,5 TMB. With the increase of concentration, density increases and molar volume decreases due to shrinkage in the volume of DMSO and TCM. Hence, an increase in the speed of sound may be correlated to the structure former of the 1,3,5-TMB. Positive deviations are due to dipolar-dipolar interactions because 1,2,4-TMB is a polar molecule.

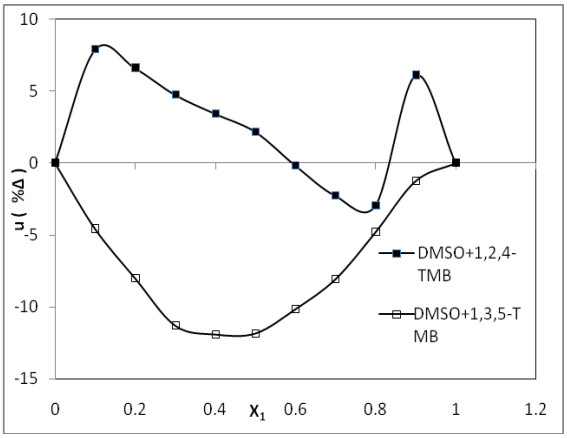

Figure 1. Variation of deviation in speed of sound (%Δu) against mole fraction(X_1) of binary liquid mixture at 298.15 K.

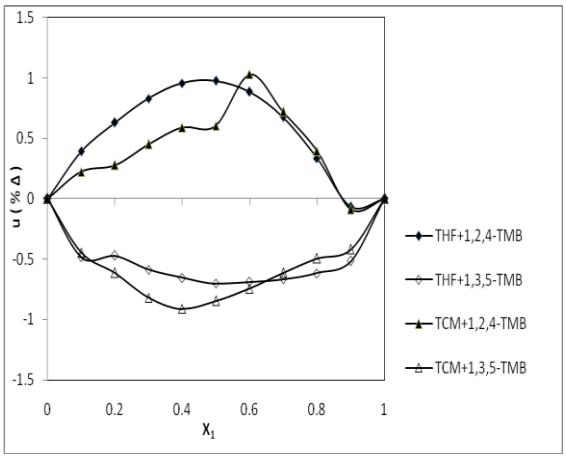

Figure 2. Variation of deviation in speed of sound (%Δu) against mole fraction of binary liquid mixture at 298.15 K.

The lack of smoothness in deviations is due to the interaction between the component molecules. Isentropic compressibility increases regularly with the increase of mole fraction. Increment becomes larger with 1,2,4-TMB. Strong discrepancy is observed in Table 6 where the value of isentropic compressibility decreases. Possibly, it is due to dipolar repulsion which lowers the energy hence regular trend is not observed, while density and speed of sound show regular behavior.

Conclusion

Thus it can be concluded from the preceding discussion that PFP model can be applied successfully to polar-nonpolar cyclic liquid binary mixtures.

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