

Physicochemical studies of N-Acetylcysteine in aqueous ethanol

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Abstract—N-Acetylcysteine (NAC) a sulfhydryl substance is a derivative of amino acid L-cysteine extensively used as medication. NAC is a influential intracellular antioxidant, antitoxin improves immunity. The molecular formula of NAC is $C_5H_9NO_3S$ and molecular mass is 163.195 g/mole. NAC is a powerful scavenger of hypochlorous acid and is capable of reducing hydroxyl radicals and hydrogen peroxide. Ethanol–water mixtures are very important systems exhibiting properties that are of great interest in physics, chemistry and biology. The main streams of this study are comprehending interaction between Water+NAC+Ethanol. Density and viscosity of liquids is important physicochemical properties which affect mass and heat transfer of solutions. NAC had been investigated in ternary system over a concentration range of (1-18) % (w/v) at 298.15 to 323.15 K at 5 K intervals.

Key Words— N-Acetyl-L-cysteine (NAC), Ethanol, Water, Density, Viscosity.

1. INTRODUCTION

Diversity of applying NAC is the source of broad spectrum of used dosage and routes of administrations. Oral administration (tablet or inhalations) can range from 250 to 1800 mg/day and is used mainly in lung diseases [1]. Toxicological data shows that intakes of NAC per day orally could be consumed without causing significant adverse effects [2]. N-acetylcysteine reduced formation of 8-oxoguanine in mice skin protecting melanocytes from UV induced melanoma [3-5]. Most of anti-cancer therapies are based on growth of ROS production in cancer cells leading to their apoptosis [6-10]. The efficiency of vitamin E and NAC as an antioxidant adjuvant therapy was shown in chemotherapy/radiotherapy course during acute lymphoblastic leukemia in 40 children study. Toxicity of chemo- and radiotherapy measured as a diminished level of malondialdehyde, as well as increased level of glutathione peroxidase and decreased occurrence of toxic hepatitis was significantly reduced [11-13]. N-acetylcysteine as a mucoactive, anti-inflammatory and antioxidant agent was expected to have a benefit therapeutic effect in Cystic fibrosis patients. In 4 weeks trial [14-16] NAC was administered orally to 18 Cystic fibrosis patients in a high doses (600-1000 mg) three times daily. Pathophysiology of endometriosis includes chronic inflammation within oxidative stress and pathological angiogenesis [17-18]. As glutathione is an important antioxidant in the lens it has been suggested that increasing GSH level NAC supplementation could be used to reduce cataract risk [19]. Diabetes elevates the risk of cataract formation. This form of cataract can result from sorbitol accumulation in the lens. Pathophysiology of early cataract development and the potential benefit of

supplementation with vitamin B6 and NAC among the diabetic population were studied by Jain *et al.* [20]. High-glucose concentrations can cause the oxidation and modification of proteins in the lens. Vitamin B6 (pyridoxine) and NAC supplementation may be helpful in slowing the oxidation of lens proteins. The study of Liebermann [21] raised the possibility that administration of NAC may reverse early cataracts. Zhang *et al.* [22] evaluated the effect of NAC and glutathione ethyl ester (GSH-EE) eye drops on the progression of diabetic cataract formation in rats. N-Acetylcysteine, used as an ophthalmic drug is promising in the treatment of a range of ophthalmic disorders with oxidative stress component involved in pathogenesis including cataract, glaucoma, dry eye syndrome, vitreous floaters, inflammatory disorders, corneal, retinal and systemic diseases and its ophthalmic complications. Water is worldwide solvent used in every day lifecycle. Ethanol is the organic solvent also comprehensively used in numerous sectors. So investigation of interaction between NAC and ethanol-water mixture could be reasonably curious and applicable.

2. METHODS AND MATERIALS

2.1 Chemicals

N-acetylcysteine was collected from Sigma Aldrich, USA. High performance liquid chromatography (HPLC) graded Ethanol was collected from E-Merk, Germany, and was 99.99% pure. All chemicals and reagents were of analytical grade and were used without further purification.

2.2. METHODS

The densities of the solutions were determined by weighing a definite volume of the solution in a calibrated pycnometer (Glasgow, India) at

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specified temperature. The volumes were obtained by measuring the weight of water at that temperature and using the density of water from literature. The density of solution was determined from the relation.

$$\rho = \frac{W - W_e}{V_0} \dots\dots\dots(1)$$

Where, ρ = density of the solution, w = weight of bottle with solution, w_e = weight of empty bottle, v_0 = volume of bottle.

Viscosity of water, ethanol and several solutions were measured by using the Ostwald U-type viscometer are known (from literature) calibration constant A of the viscometer for different temperature were obtained by using equation,

$$\eta = Apt \dots\dots\dots(2)$$

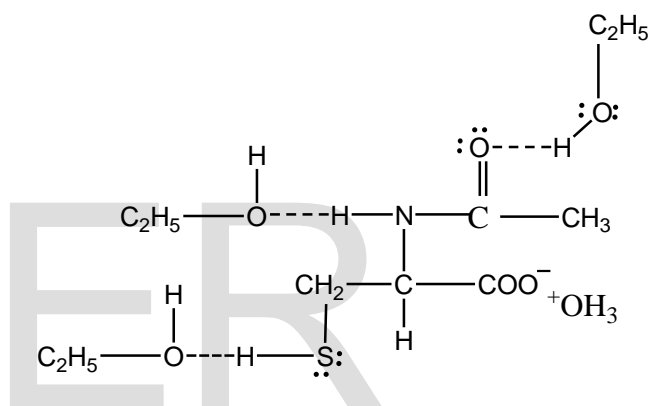
Where, $A = \eta (H_2O) / (\rho (H_2O) \times t(H_2O))$

Like water the flow time of different solutions were determined. Then putting the values of the calibration constant, density and time of flow of the experimental solutions, the viscosities of the solutions were determined by using the equation (2).

3. Results and Discussion

The densities, ρ and viscosities, η of the [4:1][3:2][2:3][1:4] Ethanol–Water–NAC, systems at 298.15K to 318.15 K at 5K interval has been investigated. The density and viscosity values are shown in Table-4 and Table-5. The density values increased with increasing concentration of the NAC in ethanol–water at the entire investigated composition range, 1-18 (w/v) % NAC in mixed solvents. The increase of density values of NAC with concentrations can be attributed to the increase in solvent-solvent, solute-solvent and the solute-solute interactions. Not only ethanol serves as a protic solvent but also aprotic solvent because of moderate dipole moment and long hydrocarbon residues. When ethanol dissolved in water, with increase of the ratio of ethanol in water, the aprotic nature of characteristics increased. The densities decrease significantly with increasing temperature at a constant concentration as in shown Table-4. With the increase of temperature the internal energies of the systems are increased and the solute–solvent, solvent–solvent and solute-solute interaction might be weakened. The consequence is the decrease in density values. All the ternary systems, with the increase of concentration densities are raised due the dipole-dipole, acid-base and hydrogen bond interaction between the NAC–ethanol and NAC–water, NAC–ethanol–water and the steric hindrances of NAC molecules. In case of ternary systems, the highest densities are observed [1:4] ethanol–water– NAC system, probably highest dipole moment as well as interaction are strongly occurring here. On the contrary [4:1] etha-

nol–water–NAC system, probably lowest dipole moment as well as interaction are occur here, comparison in ternary systems. Ternary systems, the intensity of the interaction are increased with the increase of the ratio of water. Detail mechanism of dissolution or solvation of NAC in ethanol–water system is still unknown. Here a possible mechanism has been proposed. We know that hydrophilic NAC molecules are attracted by water molecules. Unlike the charged side chains, polar side chains are overall neutral, but still capable of attracting water to places on their surface. They neither gain nor lose an electron but simply shift the electron towards one of the atoms. Such a polar side chain remains neutral as a whole, but the separation of the "pluses" and "minuses" within it creates two opposite "poles", each capable of attracting water molecules. The molecules of water are closely packed than that of ethanol [23].



Solvation of ethanol, water and NAC molecules

With the increase of dipole-dipole interaction and H–bond formation, results in stronger intermolecular attractions as well as volume of solution are decreased as density is increased. The data of Table-4 are support on this assumption. Ethanol–water systems can have two hydrogen-bonded structures corresponding to the two possible heterodimers where ethanol is a proton acceptor, $CH_3CH_2HO \dots H_2O$, or a proton donor, $CH_3CH_2OH \dots OH_2$. In the water-ethanol heterodimer the $R_{O \dots O}$ and the $R_{O \dots H}$ is 2.843 Å and 1.90 Å and the dipole moment is 1.944 D and in ethanol-water heterodimer the $R_{O \dots O}$ and the $R_{O \dots H}$ is 2.914 Å and 1.951 Å and the dipole moment is 2.494 D and the earlier one is the most stable heterodimer where water plays the role of proton donor [24]. Ethanol and water molecule form OH^- and $C_2H_5OH_2^+$ ions. Carboxylic group of NAC react with OH^- ion and produce water by loss a proton. Thiol group undergoes dipole-dipole interaction with ethanol. The oxygen of carbonyl group pulls the electron towards himself and hold negative charge. Lone pair electron of nitrogen atom fill up the electron defi-

ciency of carbonyl carbon. The structure established H-bond with water. Ternary systems presents are two polar molecules ethanol and water. Due to this polar mixture solvent dipole-dipole interaction are occur highest and density is highest. Ethanol–water mixture solvent dipole moment is 2.494D, Which is highest than water and pure ethanol solvent. And the highest densities are observed [1:4] ethanol + water +NAC system, probably highest dipole moment as well as interaction are stronger. On the contrary [4:1] ethanol +water + NAC system, probably lowest dipole moment as well as interaction are occurred here. The values of η of NAC ethanol–water systems increased with the increase of concentration and decrease considerably with temperature at a constant

temperature shown in Table-5. With the increase of temperature the internal energy of the system is increased and the solute-solvent interaction may be depleted. The increase of η values of NAC with concentration can be attributed to the increase in both solute–solvent, solvent–solvent and solute–solute interactions with concentration. NAC make strong interactions in water–ethanol–NAC ternary systems which is responsible to occur strong cohesive force and change the viscosity a lot. The viscosity maxima follow the order: [1:4] ethanol–water–NAC > [2:3] ethanol–water–NAC > [3:2] ethanol–water–NAC > [4:1] ethanol–water–NAC.

Table-1: Density values, ρ and viscosity values, η of ethanol Literature and experimental values at 298.15 to 318.15 K at 5 K interval.

Temperature(K)	Density (gm. cm^{-3})		Viscosity (mPa.S)	
	Literature Value	Experimental Value	Literature Value	Experimental Value
298.15	0.7858[25]	0.7894	1.0820[26]	1.0889
303.15	0.7813[25]	0.7850	0.9870[26]	0.9895
308.15	0.7761[25]	0.7804	0.9015[27]	0.9073
313.15	0.7718[25]	0.7765	0.8284[28]	0.8332
318.15	0.7651[25]	0.7723	0.7642[27]	0.7718

Table-2: Density values (gm. cm^{-3}), ρ of [4:1], [3:2], [2:3], [1:4] ethanol–water System at 298.15 to 318.15 K at 5 K interval

Name of the system	ρ (gm. cm^{-3})				
	298.15K	303.15K	308.15K	313.15K	318.15K
[4:1] ethanol+ water	0.81044	0.80623	0.80134	0.79834	0.79454
[3:2] ethanol+ water	0.84066	0.83585	0.83140	0.82779	0.82382
[2:3] ethanol+ water	0.87959	0.87561	0.87084	0.86726	0.86368
[1:4] ethanol+ water	0.93217	0.92816	0.92615	0.92334	0.918138

Table-3: Viscosities (mPa.S), η of [4:1], [3:2], [2:3], [1:4] ethanol–water System at 298.15 to 318.15 K at 5 K interval

Name of the system	η (mPa.S)				
	298.15K	303.15K	308.15K	313.15K	318.15K
[4:1] ethanol–water	1.3871	1.2365	1.1201	0.9681	0.8694
[3:2] ethanol– water	1.6251	1.4913	1.3439	1.1966	1.0451
[2:3] ethanol– water	2.1113	1.8235	1.6505	1.4620	1.3132
[1:4] ethanol–water	2.3352	1.9633	1.7407	1.5532	1.3876

Tabl-4: Density (gm.cm^{-3}), ρ of N-Acetylcysteine in [4:1], [3:2], [2:3], [1:4] ethanol–water System at 298.15 to 318.15 K at 5 K interval

Name of the system	Conc. (w/v)%	ρ (gm.cm^{-3})				
		298.15 K	303.15 K	308.15 K	313.15 K	318.15 K
[4:1] ethanol–water– NAC	1	0.81673	0.81249	0.80745	0.80441	0.80057
	5	0.83311	0.82838	0.82313	0.81991	0.81589
	10	0.85527	0.84978	0.84484	0.84021	0.83602
	15	0.87599	0.87070	0.86569	0.86077	0.85573
	18	0.88820	0.88190	0.87649	0.87252	0.86738
[3:2] ethanol–water– NAC	1	0.84657	0.84164	0.83683	0.83299	0.82885
	5	0.86992	0.86398	0.85824	0.85252	0.84842
	10	0.89449	0.88868	0.88355	0.87696	0.87174
	15	0.91402	0.90864	0.90448	0.89986	0.89618
	18	0.92064	0.91628	0.91084	0.90665	0.90312
[2:3] ethanol–water– NAC	1	0.88655	0.88036	0.87487	0.87118	0.86756
	5	0.90365	0.89664	0.88993	0.88556	0.88195
	10	0.92172	0.91466	0.90856	0.90174	0.89727
	15	0.93723	0.93132	0.92619	0.91849	0.91406
	18	0.94345	0.93934	0.93291	0.92480	0.91918
[1:4] ethanol–water– NAC	1	0.93625	0.93212	0.92994	0.92695	0.92169
	5	0.95190	0.94763	0.94492	0.94075	0.93551
	10	0.97040	0.96454	0.96110	0.95734	0.95232
	15	0.98241	0.97667	0.97398	0.96995	0.96469
	18	0.98744	0.98322	0.98102	0.97795	0.97312

Table-5: Viscosities (mPa.S), η of N-Acetylcysteine in [4:1], [3:2], [2:3], [1:4] ethanol–water System at 298.15 to 318.15 K at 5 K interval

Name of the system	Conc./ (w/v)%	η (mPa.S)				
		298.15 K	303.15 K	308.15 K	313.15 K	318.15 K
[4:1] ethanol–water– NAC	1	1.43450	1.24945	1.11495	0.97100	0.85929
	5	1.56606	1.37850	1.23009	1.06302	0.95040
	10	1.75032	1.54491	1.36516	1.18401	1.05773
	15	2.22262	1.91905	1.69701	1.44404	1.26385
	18	2.46917	2.12750	1.86494	1.61420	1.40099
[3:2] ethanol–water– NAC	1	1.70002	1.51721	1.35145	1.21708	1.09077
	5	1.84758	1.63985	1.46012	1.28841	1.12083
	10	2.01977	1.78832	1.58441	1.41778	1.23769
	15	2.20462	1.93895	1.71278	1.51800	1.28965
	18	2.35333	2.05588	1.83274	1.62622	1.41330
[2:3] ethanol–water– NAC	1	2.13742	1.87281	1.66900	1.49653	1.34058
	5	2.27624	1.96998	1.75679	1.56132	1.39216
	10	2.45401	2.1096	1.86536	1.64178	1.46263
	15	2.61736	2.26356	2.00073	1.76974	1.57582
	18	2.76373	2.37607	2.05529	1.81720	1.61258
[1:4] ethanol–water– NAC	1	2.33526	1.96336	1.74076	1.55327	1.38769
	5	2.45535	2.16515	1.92536	1.71107	1.53598
	10	2.63289	2.33621	2.07665	1.83773	1.62400
	15	2.78151	2.46420	2.19083	1.94356	1.72447
	18	2.91042	2.58045	2.27630	2.01197	1.79477

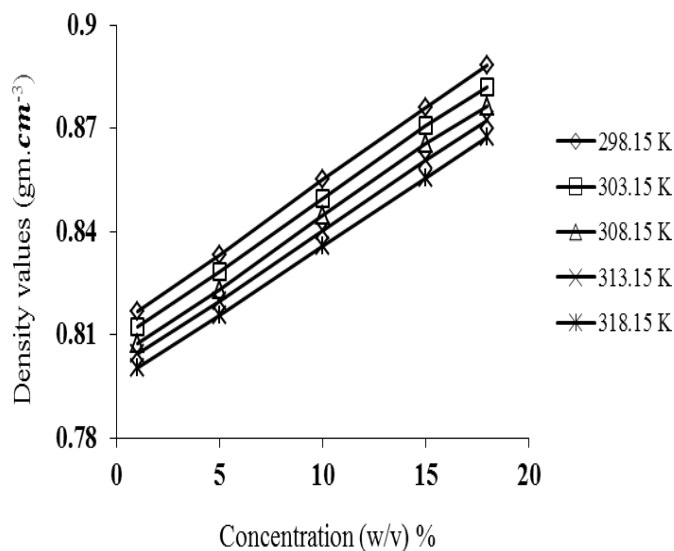


Figure-1: Densities, ρ of N-Acetylcysteine in [4:1] ethanol-water System at 298.15 to 318.15 K at 5 K interval

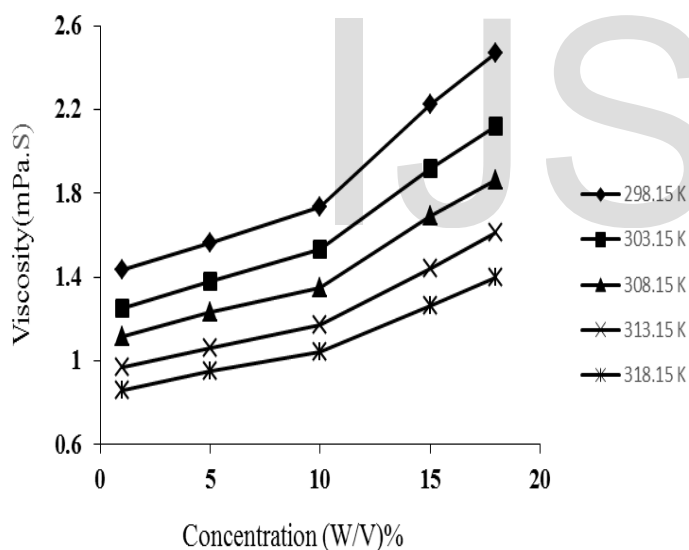


Figure-2: Viscosities, η of N-Acetylcysteine in [4:1] ethanol-water System at 298.15 to 318.15 K at 5 K interval.

4. Conclusion:

The investigation of interaction between NAC and ethanol-water mixture is found that the highest densities are observed [1:4] ethanol + water +NAC system, probably highest dipole moment as well as interaction are stronger. On the contrary [4:1] ethanol +water + NAC system is lowest Densities, probably lowest dipole moment as well as interaction are occurred here. And the largest viscosity is [1:4] ethanol-water-NAC whereas lowest viscosity is [4:1] ethanol-water-NAC.

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