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Determination of physico-chemical properties of Congo red in aqueous solution

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ABSTRACT

Congo red (CR) is a symmetrical azo dye that has been used extensively in histochemistry, as staining agent for amyloid proteins and a host of other pharmacological applications. However, recently is has been found to form aggregates in aqueous solution and, in addition to its π-π packing properties, makes Congo red useful as a supramolecule. Its thermodynamic properties that are pertinent in evaluation and accurate interpretation of its interaction with solvents and other solutes are also very important. These parameters such as partial molar volume, viscosity, density and index of refraction have been determined in this work. The partial molar volume and the refractive index of this compound were determined as 296.86 ± 4.16 cm^3/mol and 1.4118 ± 0.0000, respectively. The rest of its physico-chemical properties were determined as a function of its concentration which ranged from 9.58 x 10^{-3} to 4.69×10^{-2} M.

KEYWORDS

Congo red;
Partial molar volume;
Density;
Viscosity;
Index of refraction;
Supramolecule;
Azo;
Dye.

INTRODUCTION

Physico-chemical properties (partial molar volume, density, viscosity and refractive Index) of a solute are thermodynamic observables of any given solute and are useful in the analyses and prediction of the behavior of solutes solubilized in a solvent[1-9]. Research work in these areas is continuing as more molecules are synthesized/discovered. These properties are used in chemical and biochemical/biological systems study of solute-solute, solute-solvent interactions as well as their isothermal compressibility[9-19]. In this work we determined the physico-chemical properties of Congo red. The chemical structure of Congo red is shown in figure 1.

Congo red is a symmetric, sulfonated azo dye and belongs to the class of protein-binding dyes. The color of the solution of Congo red is pH-dependent. At pH up to 3, this solution is bluish and at pH above 5 the color becomes bright red. As a result it has been used in Chemistry as a pH indicator[20,21]. Congo red is also used as a dyeing agent in textile industry[22-24]. Perhaps,

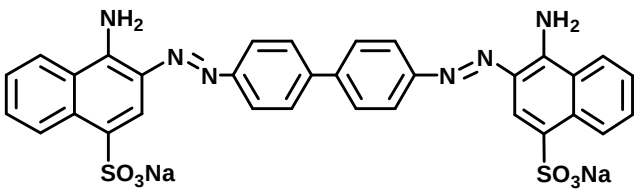

Figure 1 : The chemical structure of Congo red (CR)

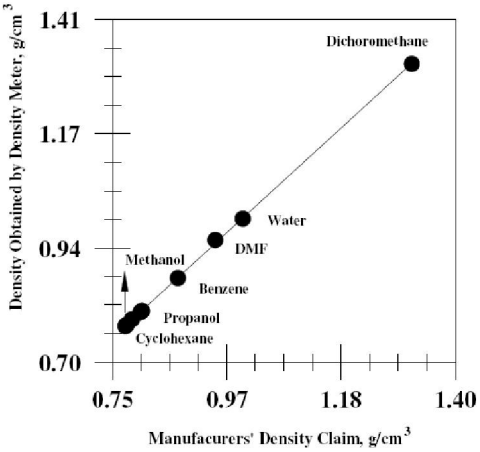

Figure 2 : Comparison of density obtained using dma density meter and that clamed by sovent's manufacturers

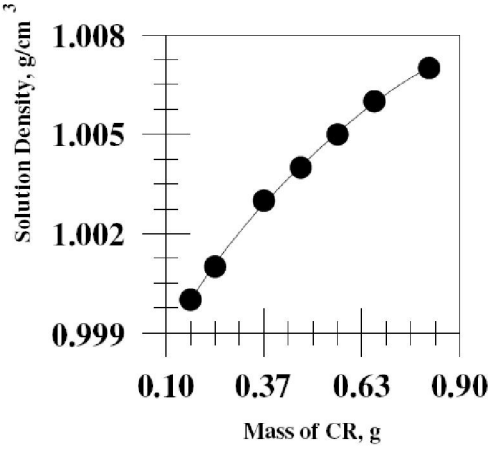

Figure 3 : Solution density versus mass of CR

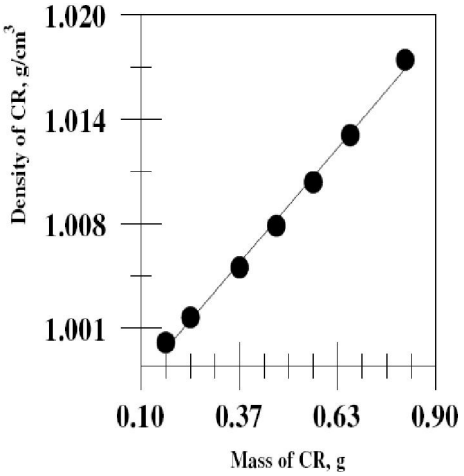

Figure 4 : Calculated density of CR versus mass of CR

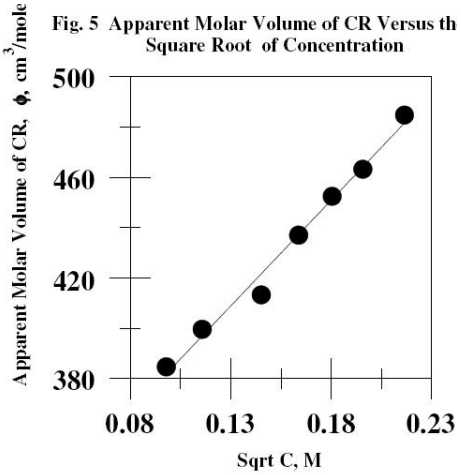

Figure 5 : Apparent molar volume of cr versus the square root of concentration

the greatest use of Congo red is in biology and histochemistry. Under a polarized light Congo red is birefringent[25,26]. It is also known to bind to amyloid proteins and its birefringence under a polarized light is a characteristic[27-31] feature that has made it useful in characterizing its interaction with amyloid formation in Alzheimer patients[32]. More recently it has been found to form aggregates in aqueous solution[33-37], and in addition to its π-π stacking properties, has been found to be useful as a supramolecule[34,38-42]. The thermodynamic properties, particularly its physico-chemical properties, pertinent in evaluation and the interpretation of its interaction with solvents and other solutes are important. However, to the author's knowledge these properties have not been determined. Therefore, we have attempted, in this work, to obtain these properties in aqueous solution.

EXPERIMENTAL

Materials

Congo red (CR) was obtained from Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, USA and was used as received.

Instrumentation

The density of all solutions was determined using the Anton PAAR Digital Density Meter, model DMA 35 obtained from Anton PAAR K.G., A-8054 Graz, Austria, Europe and the viscosities of the various solutions were obtained using the Brookfield Viscometer, model DV-II+ supplied by Brookfield Engineering Laboratories. Inc., Middleboro, Massachusetts, USA. The refractive indices of the solutions were obtained

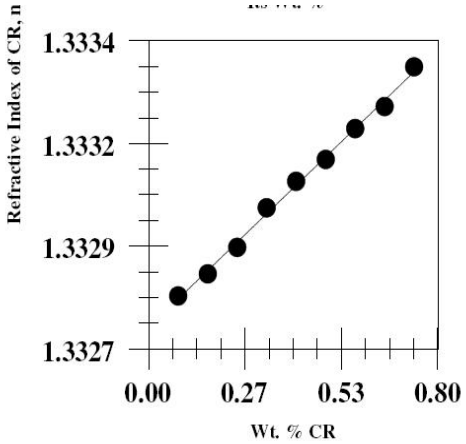

Figure 6 : The observed refractive index of cr versus its wt. %

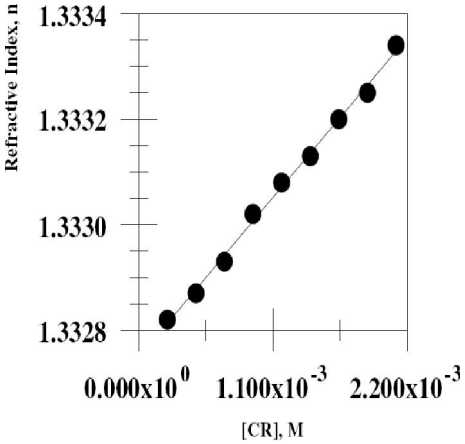

Figure 7 : Incremental refractive index of CR

using the digital Leica Abbe Auto refractometer. This instrument was supplied by Leica, Inc., New York, USA.

Methodology

Density, ρ

Congo red solutions ranging in concentration from 9.58×10^{-3} M to 4.69×10^{-2} M were prepared in seven different 25.0 mL volumetric flasks from a stock solution of 4.79×10^{-2} M. The densities of these solutions were determined using a DMA Density Meter which was calibrated using triply distilled and deionized water with a density of 0.997 g/cm^3. It was also checked with a known value of density of some common solvents. This is shown in figure 2.

It can be seen that the observed solution density correlated well with the manufacturers' claim of the solvents' density. With this fact, we are therefore sure that the Congo red solution density we obtained is reasonably accurate.

The apparent molar volume, ϕ

The partial molar volume, Vϕ, was determined at infinite CR concentration by plotting the apparent molar volume, obtained from the density data, as a function of the concentration of the CR in accordance with the literature methodology.

Refractive index, n

A stock solution of 2.34×10^{-3} M of Congo red was made. From this, concentrations ranging from 0.23 to 2.11×10^{-3} M were prepared in nine different 5.0 mL volumetric flasks. These concentrations were converted to the weight percent of the total mass of the solution. These concentrations gave the values of 0.08 to 0.73 weight percent. The refractive index of each of these solutions was then obtained from the digital Abbe Leica refractometer.

Viscosity, η

Solutions of Congo red ranging from 7.5×10^{-3} to 3.25×10^{-2} were prepared from a stock solutions of 4.79×10^{-2} M. 16.0 mL of each of these solutions were used to obtain the viscosity of each concentration.

All solutions were prepared using triply distilled water from Photronix Reagent Grade Water System. Also, all the experiments were performed at room temperature, 25 ± 0.2°C.

Treatment of density data

Although density, ρ, is an intensive property of matter, it is a ratio of the mass and volume of matter which, conventionally is given by the relation ρ = m/v in g/cm^3. m and v in this relation are extensive properties. The v in particular is both temperature and concentration dependent so also is the d. For this reason it is not expected that ρ should be linear with either m or v. When the solution density of CR obtained using the DMA density meter is plotted as function of the mass of Congo red, a non-linear curve is obtained as can be seen in figure 3.

The equation for this curve is obtained as $\rho_{obsrved} = 0.9972 + 0.0183m - 0.0077m^2$. The m in this equation is the mass of the CR. However, when the different masses of CR were fit into this equation a linear curve is obtained as can be seen in figure 4 with a regression

TABLE 1 : The observed density of Congo red at different concentrations

Concentration of CR, mM	Observed Density, g/cm^3
0	0.997
9.58	1.000
1.341	1.001
2.108	1.003
2.682	1.004
3.257	1.005
1.006	1.007

TABLE 2 : The calculated molar volume of Congo red at different concentrations

Concentration of CR, mM	Apparent molar volume, ϕ
9.580	384.6716
1.341	399.584
2.108	413.2797
2.682	436.9817
3.257	452.4024
3.832	463.1953
4.686	484.7225

TABLE 3 : The observed refractive index of Congo red at different concentrations and weight fractions

Concentration of CR, mM	Wt. Fraction, %	Observed refractive index, n
0.2343	0.0816	1.33282
0.4685	0.1632	1.33287
0.7028	0.2448	1.33293
0.9370	0.3264	1.33303
1.1713	0.4080	1.33308
1.4056	0.4896	1.33313
1.6398	0.5712	1.33320
1.8741	0.6528	1.33325
2.1083	0.7344	1.33334

TABLE 4 : The obtained viscosity of Congo red

Concentration, M	Viscosity, mPa-s
0.5250	1.01
1.0516	1.02
1.5774	1.04
2.1031	1.06
2.3963	1.05
3.1547.1	1.08
3.3548	1.10
3.6805	1.11
4.2063	1.14
6.2963	1.67

equation of y = 0.0257m + 0.9958 where y is the true density of CR.

RESULTS AND DISCUSSION

Density, ρ

TABLE 1 shows the obtained density of the solutions prepared as per the experimental section and the section of Data Treatment of Density.

When the masses of CR in grams were plotted as described above the density of CR was determined as 1.0001 ±0.0004.

The apparent molar volume, ϕ

We show in the TABLE 2 the obtained apparent molar volume, ϕ, of each solution, using the equation

$$\phi = \frac{M}{\rho_\circ} - \frac{1000(\rho - \rho_\circ)}{c\rho_\circ}$$

In this equation M is the molecular weight of Congo red, ρ_o and ρ are the densities of the solvent (water) and the solute (Congo red), respectively, and c is the solute concentration in mol/L.

When the obtained apparent molar volumes are plotted as a function of the square root of the concentration of the solute (Figure 5) and extrapolated to infinite dilution in accordance with the Mason equation[43,45,46] $V_\phi = V°_{\hat{O}} + S^*\sqrt{C}$, a linear plot is obtained with an intercept of 296.86 ± 4.16 cm^3/mol.

This value was regarded as the partial molar volume of Congo red.

Refractive index, n

In TABLE 3 we tabulated the observed refractive index of Congo red at each concentration and their respective converted weight percent.

The observed refractive indices of the solutions were plotted as a function of the weight percent of Congo red and a linear plot was obtained (Figure 6) with a regression equation of y = 0.00079 + 1.33275.

Using the application note methodology of Malvern Instruments and Savey and his co-workers[44,47], the refractive index of 100 % Congo red was determined as 1.4118 ± 0.0000 with an incremental value, dn/dc, of 0.27534 ± 0.0001 which was obtained by plotting the observed refractive index as a function of concen-

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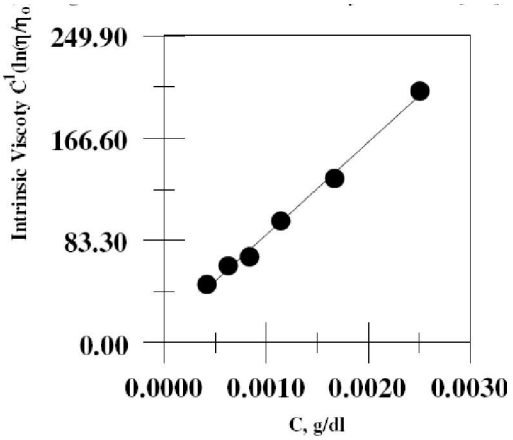

Figure 8 : Calculated intrinsic viscosity versus the [CR]

tration (Figure 7). The slope of this linear curve gives the value of dn/dc.

Viscosity, η

Viscosity is a well known physical and thermodynamic property of matter and it is a measure of the flow propensity of all matter, be it a gas, a solid, a liquid or a mixture. It is particularly important in the oil and cosmetic industries so also in pharmaceutical formulations. This property of matter is concentration dependent as can be seen in TABLE 4.

However, we evaluated the viscosity of CR in terms of intrinsic viscosity, [η], which is concentration independent. This was determined by plotting the observed the logarithm of relative viscosity, η/η_o, for different concentrations of Congo red in g/dL in accordance with the relation $C^{-1}\ln(\eta/\eta_o)$ versus C[45,46]. As can be seen in figure 8, a linear curve was obtained.

In this relationship η and η_o are the viscosity of the solution of Congo red and that of the solvent, respectively. An extrapolation of this linear curve to zero concentration of CR yields [η]. The [η] of Congo red thus obtained is 12.26 ± 3.76 g/dL. This parameter together with the other determined physico-chemical properties of Congo red are listed in TABLE 5.

CONCLUSION

As can be seen in TABLE 5 the physico-chemical properties of Congo red (the partial molar volume, density, refractive index, incremental refractive index and the intrinsic viscosity) have been determined in this work using standard techniques. It is hoped that these properties will find use in analyzing and predicting the behavior of Congo red in aqueous system.

TABLE 5 : The determined physico-chemical parameters of Congo red

Parameter	Value
Density, ρ	1.0001 ± 0.0004 g/cm^3
Partial Molar Volume, $V°_\phi$	296.86 ± 4.16 cm^3/mol
Refractive Index, n	1.4118 ± 0.0000
Incremental Refractive Index, dn/dc	0.27534 ± 0.0001
Intrinsic Viscosity, [η]	12.26 ± 3.76 g/dL

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