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ORIGINAL CONTRIBUTION

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Self-assembly of chromotropic acid – a plausible explanation for monodisperse oligomer formation

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Abstract The sodium salt of 4,5-dihydroxy-2,7-naphthalene disulphonic acid (chromotropic acid, DCA) self-assembles in aqueous media above its critical aggregate concentration (above 11 mM) to form organized aggregates as confirmed by conductometric, pH-metric and densitometric techniques. The molecular size of the aggregate was determined from gel filtration chromatographic studies. The molecular weight obtained in the postaggregate concentration region (100 mM) is about 1,660. The molecular weight of the aggregate indicates that four molecules of DCA constitute the aggregate. The results suggest that aggregate formation at high concentrations could explain the monodisperse spontaneous oligomerization in phenolic compounds.

Keywords Aggregation · Tetramer of chromotropic acid

Introduction

There is growing interest in the idea that the noncovalent assembly of small molecules may enable synthetic chemists to make aggregates of a high degree of complexity with preferred structure and function [1]. Discrete oligomeric supermolecules [2] are already being produced using such an approach. Hamaan et al. [3] synthesized molecular capsules via the dimerization of self-complementary reactants. The enhanced reaction rates in these orientation-specific systems involve templates holding the reactant in the preferred active sites [4]. We have already reported the organized aggregate formation of nonconventional surfactants such as peptide and its derivatives in aqueous and nonaqueous solvents[5]. We have also studied their influence in favouring hydrogen bonding to the preferred structure with enhanced photochemical and biological activity when compared with monomeric concentrations [5].

The proximity and orientation of surface-active molecule at the interface present an opportunity for controlled polymerization with a preferred structure [5]. Kabanov and coworkers [6] found an increase in the spontaneous polymerization of *N*-methylated 2,3- and 4-vinyl pyridinium salts in water above a monomer concentration of 3 M. The increase in the polymerization rate above 3 M concentration in this case is attributed to the formation of aggregates in which the double bonds are optimally oriented for reaction. The emulsion polymerization system involving monomers such as styrene always results in polymers with a uniform size distribution [7]. Similarly, a water-soluble craft copolymer synthesized from macromonomer methyl methacrylate in the presence of thioglycolic acid has also been reported to have a uniform size distribution [8]. Poh and coworkers [9] reported that the disodium salt of chromotropic acid (DCA) in the presence of excess formaldehyde formed a monodisperse cyclic oligomer (tetramer) at room temperature. Duda [10] has discussed the biological activity (DCA showed an antiviral effect [11]) in vitro and in vivo against the human immunodeficiency virus [12]. However, the mechanism of the formation of cyclic oligomer DCA in the presence of formaldehyde is not yet understood. Here, we report evidence for stable, tetrameric, noncovalently stabilized aggregate formation

of DCA in solution; this could explain the formation of cyclic oligomers.

Experimental

DCA was obtained from Sisco-Chem Pvt. (India) and was recrystallized from water before use. The tetramer of DCA was synthesized by the reported procedure [9]. Milli-Q water was used throughout the study. The aggregation studies of DCA were carried out using conductometric and pH-metric techniques. The conductance was measured using an Elico conductivity bridge (model CM82T) with a conductivity cell of cell constant 0.51 cm^{-1}. For conductance measurements, 10 ml water was placed in the thermostated container and a concentrated aqueous solution of DCA was added from a microburette to the water drop by drop. A magnetic stirrer was placed under the container in order to make the solution homogeneous prior to the measurements. The pH-metric measurements were made using a digital pH meter (Elico Pvt., India). The density of the DCA solutions was measured with a 25-ml pycnometer (25 ± 0.01) °C in a water bath.

The sephadex G-25 and blue dextran were obtained from Pharmacia, Sweden. The sephadex gel in water was allowed to swell overnight. The gel suspension was deaerated, poured into a column (120×1 cm) and allowed to settle down under gravity. Blue dextran and cobalt chloride were used to obtain the void volume, V_o, and the bed volume, V_t, of the column, respectively. Monomer of the DCA, bromocresol green, rose Bengal, propyl astra blue iodide, covalent adduct of poly(ethylene glycol) (PEG) (1,500) with rose Bengal and tetramer of DCA were used as molecular-weight probes in the gel filtration chromatography (GFC) studies studies [13]. The GFC fractions were monitored by UV measurements and the elution volumes were determined by the method reported earlier [14].

The total volume of the column, called the bed volume (V_t) is the sum of the volume of the swollen gel matrix, V_i, and the water outside the gel grains (V_o):

$$V_t = V_o + V_i, \quad (1)$$

where V_o is known as the void volume, which is the volume of the liquid required to elute the compounds that are completely excluded from the gel grains.

The elution volume, V_e, of the compound is the volume required to elute the compound from the column:

$$V_e = V_o + K_{av} V_i, \quad (2)$$

where K_{av} indicates the fraction of the inner volume accessible to a particular compound and is independent of the geometry of the column.

$$K_{av} = (V_e - V_o)/V_i = (V_e - V_o)/(V_t - V_o) \quad (3)$$

The K_{av} values for the molecular-weight probes such as monomer of DCA, bromocresol green, rose Bengal, propyl astra blue iodide, covalent adduct of PEG (1500) with rose Bengal and synthesized tetramer of DCA were calculated using Eq. (3).

Results and discussion

The specific conductance and H^+ ion concentration were plotted as a function of DCA concentration (Fig. 1). The abrupt change in the initial slope at a particular concentration was considered as the critical aggregation concentration (cac). The cac values obtained by these two methods are in good agreement with each other and were found to be 11 mM at 25 °C. The change in slope in the conductivity plot (Fig. 1) is indicative of aggregate formation in aqueous solution. It should be noted that the slope of the specific conductance against concentration of DCA decreases in the postaggregative region. Upon aggregation, the pK_a changes such that the proton release is hindered after the cac [15]. This could be also noted in the decrease in the slope of the post-cac H^+ concentration against DCA concentration.

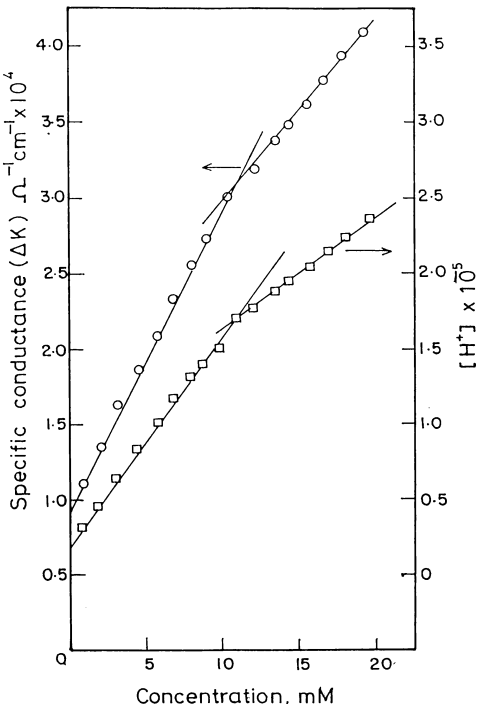

Fig. 1 Specific conductance (*circles*) and H^+ concentration (*squares*) against concentration of disodium salt of chromotropic acid (*DCA*)

From the density measurements of the DCA solution at various concentrations, the apparent molar concentration, ϕ_v, was obtained using the following relationship [16]:

$$\phi_v = \frac{M}{\rho_o} - \left(\frac{\rho - \rho_o}{c\rho_o}\right), \quad (4)$$

where M is the molecular weight of the solute, ρ is the density of the solution with molar concentration c and ρ_o is the density of the solvent. The molar volume, V_2, at different concentrations was calculated using the following equation [17]:

$$V_2 = \phi_v^o + \frac{3c^{0.5}}{2}\left(\frac{d\phi_v}{dc^{0.5}}\right). \quad (5)$$

The ϕ_v values were plotted against $c^{0.5}$. From the slope and intercept, the value of V_2 can be calculated

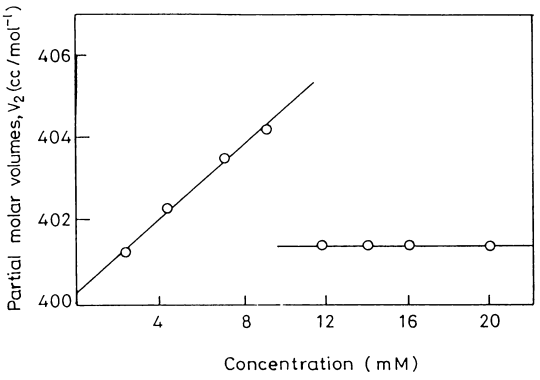

Fig. 2 Partial molar volume (V_2) against the concentration of disodium salt of DCA

using Eq. (5). V_2 was plotted against the concentration of DCA (Fig. 2). V_2 for the monomer increases with increasing concentration of DCA. This is probably due to gain of solvent molecules linked to the monomer on proton release. The changes in volume, V_A, for aggregate formation indicate that the monomer is less solvated in the aggregated state.

The molecular size of the aggregate was determined from the GFC studies. The partition coefficient K_{av} of the probes was calculated using Eq. (3) and was plotted against the molecular weight (Fig. 3). The molecular weight obtained by the K_{av} measurements of DCA in the postaggregate concentration region (100 mM) is about 1,660 (Fig. 3). The slightly higher molecular weight of the aggregate may be due to error involved in the measurement of K_{av}.

DCA contains moieties which are responsible for its aggregate formation and its numerous applications. The peri system of two hydroxyl groups determines the complex-forming properties of this compound and, on the other hand, together with the naphthalene ring makes it possible to create a hydrophobic compartment in the molecules. Sulphonic groups (or their sodium salts) confer good water solubility in DCA. All these groups make DCA as good candidate for hydrotropes with shorter hydrophobic regions (napthyl) differing from classical surfactants. However, unlike other hydrotropes, DCA forms aggregates of lower molecular weight. The GFC studies indicate that this system may represent a true tetramer above its cac. Above the cac

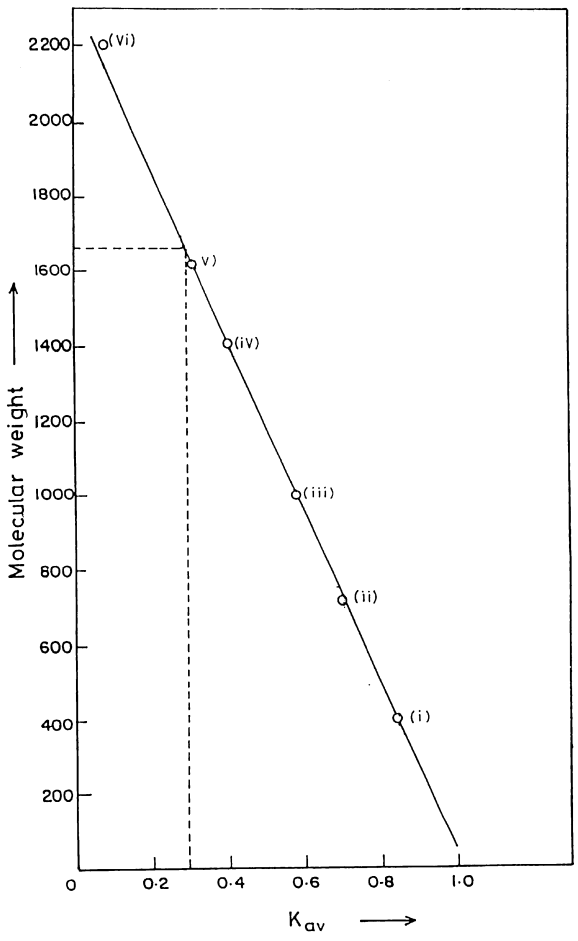

Fig. 3 Plot of molecular weight against K_{av} of DCA: *i* monomer of DCA; *ii* bromocresol green; *iii* rose Bengal; *iv* propyl astra blue iodide; *v* tetramer of DCA; *vi* covalent adduct of poly(ethylene glycol) 1,500 with rose Bengal (M_r~2,500)

this molecule self-aggregates to form an operative assembly, which in the presence of formaldehyde forms a tetrameric structure. Efforts are underway to generate higher-order cyclic aggregates and to stack these assemblies into tubes.

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