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Electrochemical Characterization, Detoxification and Anticancer Activity of Didodecyldimethylammonium Bromide

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Abstract

Quaternaryammonium compounds (QACs) are cationic surfactants with broad range applications. They enter into aquatic environment through the discharge of sewage effluents and adversely affect the ecosystem due to toxicity. Modern electrochemical methods have been used for characterization and detoxification of an extensively used QAC, didodecyldimethylammonium bromide (DDAB) in a wide pH range. The diffusion coefficient of DAAB was determined by Randles-Sevcik equation. In acidic conditions the electrochemical reduction of DAAB occurred by two electrons and two protons process. However, in neutral medium the overall reduction of DAAB followed electronation-protonation mechanism involving $3e^-$ and $3H^+$. Although DDAB is toxic yet it is bestowed with cancer preventing ability. Hence, for getting insights into the understanding of DAAB anticancer effect, its DNA binding parameters has been evaluated.

Keywords: Didodecyldimethylammonium Bromide, Detoxification, Anticancer Effect, DNA Binding Parameters
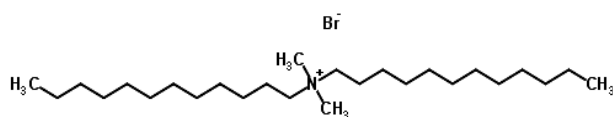
1. Introduction

Surfactants are widely used as household and industrial cleaning agents. They are classified as: cationic, anionic, non-ionic and zwitterionic. Quaternary ammonium compounds (QAC) are the most extensively studied surface active agents. They are mainly used as slimicides in swimming pools, antistatic agents in hair conditioners and wetting agents in nasal sprays [1,2]. Moreover, they also find use in preservatives, shampoos and dish washing materials [3].

The used surfactants find their way to various environmental segments and thus pose serious health hazards. They have the ability to adsorb strongly onto negatively charged suspended particulates and sludge. Intensive investigations have been carried out on their behavior, fate and biological effects [4,5]. Long exposure to QACs can cause sensitization, haemolysis and toxic effects by all routes of exposure including inhalation, ingestion, dermal application and irrigation of body cavities [6]. Their toxicity in the $mg \cdot l^{-1}$ range with a fatal dose of 1 - 3 g has been reported [7]. The concentrated solutions of QACs result in allergic reactions, hypersalivation, vomiting, haematemesis and diarrhoea [8]. In severe cases they may cause respiratory paralysis, convulsions, coma and cardiorespiratory arrest [9]. A part of the current article is devoted to voltammetric characterization and detoxification of a quaternary ammonium compound, didodecyldimethylammonium bromide (DDAB) by modern electrochemical techniques.

DDAB (**Scheme 1**) is toxic as well as cancer preventing agent [10]. Toxicity is a major nuisance of anticancer drugs. Most of the clinically used anticancer drugs have a narrow therapeutic index, with a small difference in their dose for antitumor effect and toxicity. Therefore, the doses recommended for such compounds are determined

Scheme 1. Chemical structure of DAAB.

according to their toxicity endpoints. Considerable research work has been carried out on the cancer prevention ability of DAAB but the mechanism of action has not been explored. The present work is an effort to establish a critical role of DDAB in exercising its anticancer effect.

The main cause of several diseases such as diabetes, hemophilia and cancer is related to the alteration in the replication machinery of DNA. Therefore, drug interacttions with DNA which can affect the replication processes, are potential treatments for such ailments [11-13]. Normally, cancers are difficult to be diagnosed at a stage of small hidden tumors, so the surgical eradication can not be practiced. At such an early stage, chemotherapy is the only weapon to combat with cancer. The research focused on the action mechanism of anticancer drugs is expected to accelerate the discovery of effective chemotherapeutic agents.

A variety of techniques has been employed [14-18] for the study of toxicity alleviation and drug-DNA interaction with relative advantages and disadvantages. However, most of these suffer from high cost, low sensitivity and procedural complication. Electrochemical techniques were used for the characterization, detoxification and DNA binding studies of DAAB due to their cost effecttivity, high sensitivity and ease of operation.

2. Experimental

2.1. Materials and Reagents

DDAB (98% purity) was purchased from Fluka and used without further purification. Its 5.0 mM stock solution was prepared and stored at 4°C. The working solutions were prepared in 1:1 mixed solvent of ethanol and buffer. All supporting electrolyte solutions (**Table 1**) were prepared using analytical grade reagents. Calf thymus DNA was purchased from Sigma and used as received. 2.0 mM stock solution of DNA was prepared and its concentration was determined by UV absorbance at 260 nm using a molar absorptivity (ε) of 6600 $M^{-1}cm^{-1}$ [19,20]. As the absorbance ratio of pure DNA at 260 and 280 nm (A_{260}/A_{280}) should not be less than 1.8, so a ratio of 1.85 indicated protein free DNA [21]. Doubly distilled water was used throughout.

Table 1. List of 0.1 M supporting electrolytes.

pH	Composition	pH	Composition
1.0	HCl + KCl	7.0	$NaH_2PO_4 + Na_2HPO_4$
2.0	HCl + KCl	8.0	$NaH_2PO_4 + Na_2HPO_4$
3.0	HAcO + NaAcO	9.0	$NaHCO_3 + NaOH$
4.0	HAcO + NaAcO	10.0	$NH_3 + NH_4Cl$
5.0	HAcO + NaAcO	12.0	NaOH + KCl
6.0	HAcO + NaAcO	13.0	NaOH + KCl

2.2. Equipments and Measurements

Voltammetric experiments were performed using µAutolab running with GPES 4.9 software, Eco-Chemie, The Netherlands. A glassy carbon (GC) (A = 0.07 cm^2) was used as working electrode, a Pt wire served as counter electrode and a saturated calomel electrode (SCE) was employed as the reference. The differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were carried out at a scan rate of 10 and 100 $mV \cdot s^{-1}$. Cyclic voltammetry (CV) was performed at different scan rates. Before each experiment the surface of GCE was polished with alumina powder followed by thorough rinsing with distilled water. All the voltammetric experiments were conducted in a high purity nitrogen (99.995%) atmosphere at room temperature (25°C ± 1°C).

Viscosity and density measurements were made on an Anton Paar Stabinger Viscometer SVM 3000. The dynamic laser light scattering experiment was carried out by a commercial LLS spectrometer BI-200SM motor-driven goniometer equipped with BI-9000AT digital autocorrelator and a cylindrical 22 mW uniphase He-Ne laser (wavelength = 637 nm) and BI-ISTW software.

3. Results and Discussion

3.1. Cyclic Voltammetry

Cyclic voltammetric (CV) behavior of DDAB has been examined in pH range 1 - 13. In acidic and neutral media, DDAB was found to give two cathodic peaks corresponding to two step reduction while a single anodic peak related to oxidation was registered in alkaline media. **Figures 1(a)-(c)** show typical cyclic voltammograms of 1.0 mM DDAB in different pH media. In strongly acidic conditions (pH 2.0) two close reduction peaks were recorded in the forward scan with no corresponding oxidation peaks in the reverse scan. In a medium buffered at pH 7.0, a broad reduction peak (1_c) was observed which may be due to the overlapping two close cathodic peaks appeared in harsh acidic environment. The 2nd cathodic peak appeared at high negative value. The CV of DAAB at pH 13.0 showed only oxidation. The variation in CV response indicates that the redox mechanism of DDAB is pH dependent.
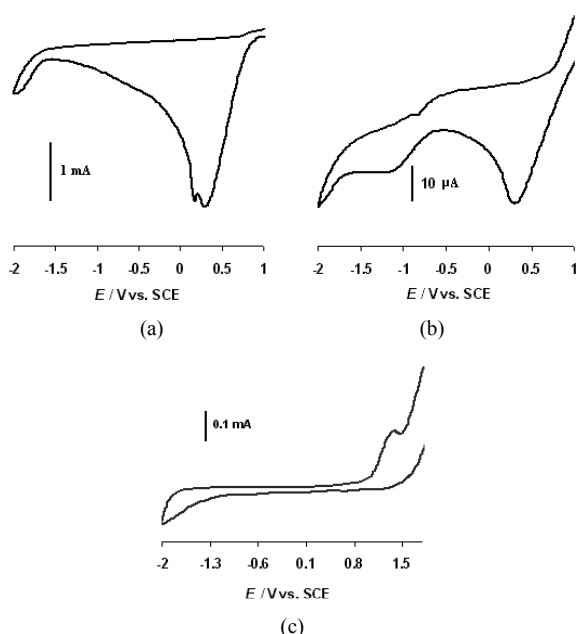
Figure 1. CVs 1.0 mM DDAB in pH (a) 2.0, (b) 7.0 and (c) 13.0 at υ = 100 mV·s⁻¹.

The dependence of peak current I_p, on the scan rate, ν, is an important diagnostic criterion for establishing the type of mechanism by cyclic voltammetry. Therefore, CVs of DDAB were recorded at different scan rates (100 - 500 mV·s⁻¹). The shift in peak potentials to more negative values with the increase in scan rate indicated the irreversibility of the reduction processes. In strongly acidic conditions (pH 2.0), the current of peak 1_c increased linearly with the square root of ν, indicating diffusion-limited reduction of analyte. The peak current in amperes for a diffusion-controlled irreversible cathodic process is given by equation [22]

$$I_{pc} = -2.99 \times 10^5 n \ (\alpha_c n)^{1/2} A C_o^* D_o^{1/2} \nu^{1/2} \qquad (1)$$

where I_{pc} is the cathodic peak current in amperes, n the number of electrons transferred during the reduction, A the geometric area of the electrode in cm², α_c the cathodic charge transfer coefficient, D_o the diffusion coefficient in cm²·s⁻¹, n the number of electrons involved in the electrochemical process, C_o^* the bulk concentration of the oxidant in mol cm⁻³ and ν the scan rate in V·s⁻¹.

The difference between peak potential E_{pc} and the potential at half peak height $E_{pc/2}$ of ~50 mV implied an irreversible 1e reduction. The $\alpha_c n$ with a value of 0.95 was calculated from the relation [23]

$$\left| E_{pc} - E_{pc/2} \right| = 47.7 / (\alpha_c n) \qquad (2)$$

The diffusion coefficient of DDAB with a value of 5.71 × 10⁻⁴ cm² s⁻¹ was evaluated from the measured slope (−2.5 × 10⁻³ A/(V·s⁻¹)¹ᐟ²) of I_{pc} vs ν¹ᐟ². The D (1.15 × 10⁻⁷

cm²·s⁻¹) of DDAB was also determined at pH 7.0. The more current carrying ability as evidenced from high peak current and concomitant D value in strongly acidic medium can be attributed to the greater charge density of cationic DDAB due to non-availability of hydroxide ions. The slightly smaller diffusion coefficient of DAAB at pH 7.0 than the D (3.70 × 10⁻⁷ cm²·s⁻¹) of a closely related surfactant, cetyltrimethylammonium bromide under similar conditions [24] may be due to its comparatively larger molecular mass, thus confirming the idea that heavy molecule diffuses slowly to the electrode surface.

3.2. Differential Pulse Voltammetry

The pH dependence of DDAB reduction was also investigated by DPV in acidic, basic and neutral media. **Figure 2** shows typical DP voltammograms of 1.0 mM DDAB. Like CV, DDAB showed two reduction peaks in neutral medium but unlike CV, peak 1_c did not split into two sub peaks at pH 2.0. The reason could be the low scan rate (10 mV·s⁻¹) at which the DPV was recorded. Thus the 2nd component of peak 1_c corresponds to the formation of an electroactive reduction product of DDAB (P_{DDAB}) that can only be observed at higher scan rates. Hence, it is necessary to scan the potential faster than the rate of the homogenous reaction (hydrolysis) that P_{DDAB} undergoes in order to observe its redox signals. The absence of peak 2_c in the DPV of DDAB at pH 2.0 and its presence in neutral buffer support the results obtained from CV. As the broad peak in DPV was due to two overlapping sub peaks (as described above) so the half peak width ($W_{1/2}$) was divided by 2. A value of 91 mV obtained in this way indicated the transfer of one electron [25].

With the increase in pH, the potential of peak 1_c was displaced to more negative values (**Figure 3**). E_{pc} varied linearly (see Inset) with increase in pH ($E_{pc} = 0.8443 - 0.0636$ pH). The slope of 63.6 mV per pH unit close to
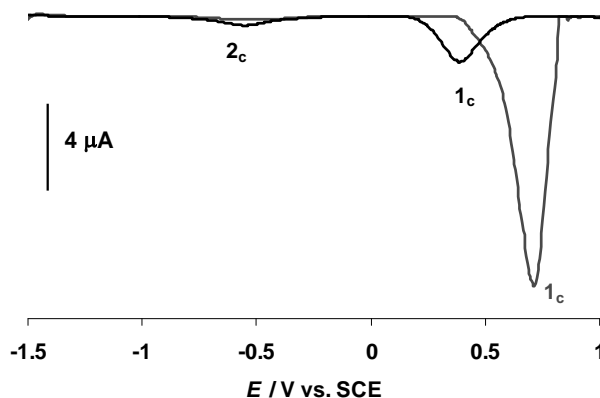

Figure 2. Base line corrected DPVs of 1.0 mM DDAB in pH 2 (—) and 7 (—) at a scan rate of 10 mV·s⁻¹.

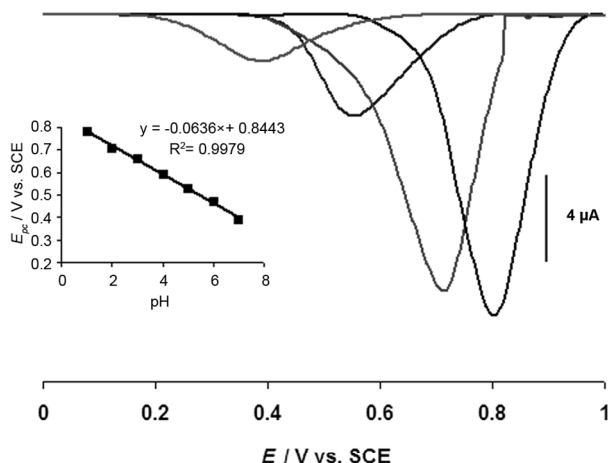**Figure 3. Base line corrected DPVs of 1.0 mM DDAB in pH 1.0 (—), 2.0 (—), 5.0 (—) and 7.0 (—) at υ = 10 mV·s^{-1}. Inset shows E_{pc} as a function of pH.**

the theoretical value of 59.5, suggested the involvement of the same number of electrons and protons in the electrochemical reduction of the analyte. Hence, the reduction of DDAB followed 1e$^-$, 1H$^+$ process in a pH dependent manner.

In strongly alkaline media the analyte registered only a single anodic peak corresponding to oxidation at E_{pa} = 1.21 V. The DPV under these conditions showed similar features to CV.

3.3. Square Wave Voltammetry

SW voltammetry is preferred over other electroanalytical techniques as it is associated with the added advantage of recording forward, backward and net current in only one scan which manifests the reversibility or irreversibility of the electron transfer reaction. Since the current is sampled in both positive and negative-going pulses, peaks corresponding to oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment. Like CV and DPV, both the reduction peaks of 1.0 mM DDAB in pH 7.0 were observed in the forward scan of SW voltammogram at E_{pc}^1 = 0. 51 V and E_{pc}^2 = –0.59 V (**Figure 4(a)**). But a dramatically different situation from CV was encountered in the backward scan which showed the reversibility of both peaks. Moreover, the reversibility of peak 1$_c$ and 2$_c$ was also evidenced by the equality of the components of total current. Due to the comparatively higher sensitivity of SWV, the reversibility of oxidation peak obtained in alkaline media (**Figure 4(b)**) was also proved in the same manner and during the same experiment.

Figure 4(c) shows the SW voltammetric behavior of 1.0 mM DDAB at pH 2.0. Like CV a couple of cathodic
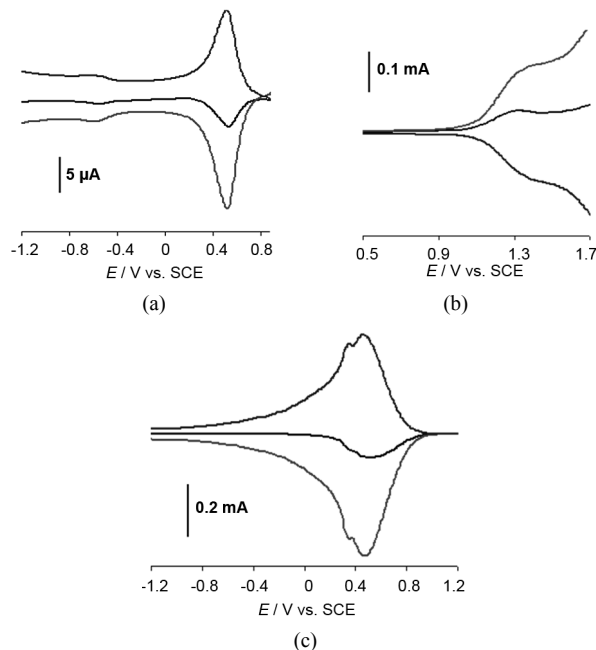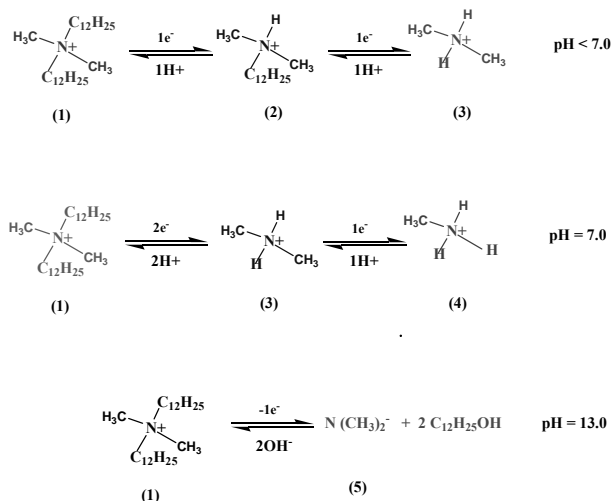

Figure 4. SWV of 1.0 mM DDAB in pH (a) 7.0, (b) 13.0 and (c) 2.0 at υ = 100 mV·s^{-1}. 1st scan in solution showing total current (—), forward current (—) and backward current (—).

peaks corresponding to two close charge transfer reactions were observed in the forward scan. But in contrast to CV, the backward scan indicated the reversibility of both the charge transfer processes. Unlike the SWV at pH 7.0, peak 2$_c$ of DDAB was not observed at pH 2.0. The behavior points to the fact that the redox reactions of DAAB follow different mechanistic pathways in acidic and neutral media.

3.4. Redox Mechanism

The results obtained from CV, SWV and DPV enabled to propose the redox mechanism of DAAB (**Scheme 2**). The appearance of cathodic peak 1$_c$ and 2$_c$ indicated the reduction of DDAB at the GCE. The splitting of peak 1$_c$ into two sub peaks at high scan rate revealed the overall reduction of DDAB to occur in three steps. In media buffered at pH < 7.0 the components of 1$_c$ are attributable to the electrochemical reduction of DDAB (**1**) to quaternary dodecyldimethylammonium ion (**2**) and quaternary dimethylammonium ion (**3**) by a mechanism involving two 1e$^-$, 1H$^+$ processes. The presence of corresponding oxidation peaks in the reverse scan (**Figure 4(c)**) suggested the reversibility of DDAB redox processes. Under neutral conditions, the components of peak 1$_c$ merged into a broad peak that can be related to the formation of reduction product (**3**) by a mechanism involving 2e$^-$, 2H$^+$ transfer. The appearance of 2nd cathodic peak 2$_c$ at pH =

Scheme 2. Proposed mechanism for the electrochemical reduction of DDAB.

Figure 5. 1st scan CVs of 1.0 mM DDAB (obtained in pH 2.0) in the absence of DNA (—) and presence of 20 (—) and 40 M DNA (—).

7.0 pointed to the formation of quaternary methylammonium ion **(4)**. The appearance of anodic peak at pH 13.0 can presumably be due to the oxidation of DAAB to product **(5)**. The mechanism suggested here is supported by the work of Nishiyama *et al*., [26] on the microbial dealkylation of alkyl trimethylammonium compounds. The presence of dealkylated intermediates of alkyltrimethylammonium salts (triethylamine, dimethylamine and methyllamine) in activated sludge obtained from municipal sewage treatment plant [27] further supported our suggested mechanism. The increase in number of non-methyl alkyl groups in QACs cause difficulty in biodegradability which in turn results in toxicity [27]. Hence, the results of our experiments reveal that QACs can be detoxified to biodegradable products by electrochemical methods.

3.5. DNA Binding Study

The effect of increasing concentration of DNA on the cyclic voltammetric response of 1.0 mM DDAB has been shown in **Figure 5**. Unlike the general trend, the peak current of DDAB increased in the presence of DNA. This anomalous behavior may be due to the dissociation of DDAB micelles by DNA and concomitant increase in number of monomers. The breakdown of micellar aggregates can be due to electrostatic interaction of anionic oxygen with ammonium moiety and the affinity of hydrophobic interior of DNA for the lipophilic hydrocarbon section of DAAB. The increase in peak current further points to the fact that the number of monomers produced by the breakdown of micelles is greater in the bulk than those attached with DNA. The peak potential shift to more negative and then to positive values is attributed to
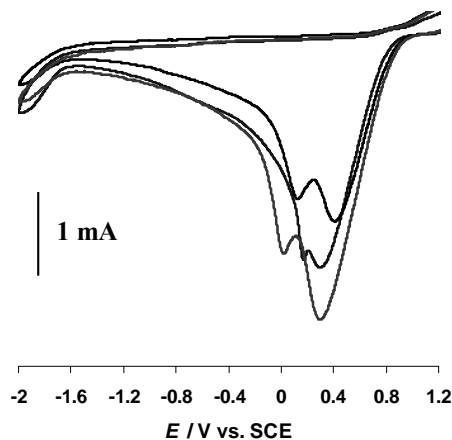
mixed binding mode. A part of the hydrocarbon tail of DDAB is suggested to intercalate and the rest may occupy the grooves. The cationic ammonium moiety may interact electrostatically with anionic phosphate backbone of DNA. The interaction is expected to effect the DNA of cancerous cells in such a way that the cell can not replicate further.

The peak current of DAAB diminished with further incremental addition of DNA. The rationale behind the diminution in peak current may be the decrease in number of free monomers due to the formation of macromolecular DAAB-DNA complex with lower current carrying ability.

The decay in peak current (I_p) of DAAB by the incremental addition of DNA was exploited for the quantification of binding constant according to the following equation [28]

$$\log\left(1/[\text{DNA}]\right) = \log K + \log\left(I/\left(I_o - I\right)\right) \qquad (3)$$

where K is the binding constant, I_o and I are the peak currents of the drug in the absence and presence of DNA. The K with a value of $3.41 \times 10^4\,\text{M}^{-1}$ was obtained from the intercept of log (1/[DNA]) *versus* $\log(I/(I_o - I))$ plot. The value is comparable with the reported K (3.60×10^4 M^{-1}) of 1,8-dihydroxyanthraquinone interacting with DNA by mixed binding mode [29].

For the determination of binding site size the following equation was used [30]

$$C_b/C_f = K\left\{[\text{DNA}]/2s\right\} \qquad (4)$$

where s is the binding site size in terms of base pairs, C_f the concentration of the free species, C_b the concentration of DNA-bound species and K the binding constant. The values of C_b/C_f were determined from the experimental peak currents by the following equation [30].

$$C_b/C_f = (I_o - I)/I \qquad (5)$$

By inserting $K = 3.41 \times 10^4 \, M^{-1}$ in Equation (4), the binding site size with a value of 0.51 bp was obtained from the slope of C_b/C_f *versus* [DNA] plot. The value of s implies that the binding parts of two molecules of DAAB can occupy one base pair.

The D of DAAB with a value of $3.22 \times 10^{-5} \, cm^2 \cdot s^{-1}$ was obtained in the presence of 100 M DNA at pH 2.0. The lower value of D in the presence of DNA corresponds to the formation of slow moving heavy DAAB-DNA adduct.

Using the values of D measured from CV data, the electrophoretic mobility (u) of DAAB was determined as 2.22×10^{-6} and $4.48 \times 10^{-10} \, m^2 \cdot s^{-1} \cdot V^{-1}$ in media buffered at pH 2.0 and 7.0 by the equation [31]

$$u = zeD/K_B T \qquad (6)$$

The lower mobility of DAAB in neutral medium can be related to its interaction with the available hydroxyl groups. In the presence of DNA the u of DAAB lowered to $1.25 \times 10^{-7} \, m^2 \cdot s^{-1} \cdot V^{-1}$ at pH 2.0. The decrease in u may be due to the formation of bulky DAAB-DNA adduct.

The radius (9.55×10^{-10} m) of the solvated DAAB was calculated by Stokes-Einstein equation

$$r = K_B T/6\pi\eta D \qquad (7)$$

where η is the viscosity of the solvent, D the diffusion coefficient, T temperature and K_B Boltzmann's constant.

The radius (5.36×10^{-10} m) of the compound in the solid state was obtained from density measurements by using the following relation

$$V = (4/3)\pi r^3 \qquad (8)$$

It can be seen that the radius of DDAB in the solvated form is greater than that in the solid form, as expected.

The mean hydrodynamic radius (1.1 nm) of DAAB was determined by laser light scattering technique. The value supports the result obtained from viscosity and CV measurements.

The critical micelle concentration (CMC) of DAAB was determined (See **Figure 6**) by conductivity method. The value of 0.48 mM ensured the micellar nature of DAAB solution used for DNA binding studies. The CMC value determined here is more than 0.05 mM and less than 2.5 mM as reported by Atkin *et al.* [32] and Antonella *et al.* [33]. The differences may be due to several factors like variation in pH, temperature, counter ion and ionic strength of the solution.

4. Conclusions

The present study has shown that DDAB can be reduced and oxidized at a glassy carbon electrode. The reduction
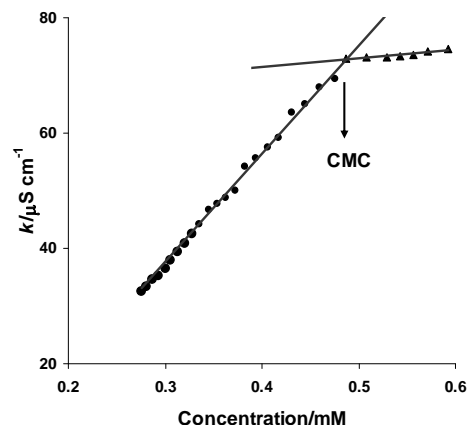
Figure 6. Specific conductance as a function of concentration.

of DDAB was found to depend strongly on the pH of the medium. In acidic conditions the mechanism involved the transfer of two electrons and two protons. However, in neutral medium the overall reduction of DAAB occurred by a $3e^-$, $3H^+$ process. Oxidation was evidenced only in strongly alkaline conditions. All the peaks were found reversible by square wave voltammetry. The diffusion coefficient in pH 2.0 was found greater than in pH 7.0. The electrochemical characterization of DDAB has the potential of providing valuable insights about the unexplored pathways by which QACs are detoxified. The binding constant, binding site size, diffusion coefficient and electrophoretic mobility were determined from cyclic voltammetric and viscosity data. Moreover, the radii of the solvated and solid DAAB were evaluated from electrochemical and laser light scattering techniques. The hydrodynamic radius of the solvated DAAB was found greater than the solid form as expected. The investigation of the electrochemical behaviour of DDAB and its interaction with DNA is expected to provide useful insights about the mechanism by which miceller solution of toxic anticancer drugs exert their biochemical action.

5. Acknowledgements

6. References

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