Thermodynamic Study of Warfarin Sodium Salt: Surface Tension, Conductivity, and Density Measurements

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The thermodynamic properties in aqueous solution of the amphiphilic anticoagulant drug warfarin sodium in the temperature range (20 to 50) °C were determined. The surface behavior and the critical micelle concentration (cmc) at 20 °C were determined by surface tension measurements. The electrical conductivity was measured as a function of the concentration at various temperatures. The Phillips and Williams methods were applied to obtain the critical micelle concentration from these data. Both methods lead to similar values of the cmc. Measurements of density in the range of concentrations from (0.005 to 0.400) mol·kg^{-1} were carried out at (20, 30, 40, and 50) °C. From these results, the apparent molal volumes have been calculated as a function of concentration and temperature.

Introduction

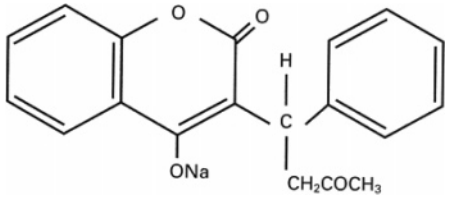
A large number of drugs have been found to exhibit typical amphiphilic behavior in aqueous solution, in that they accumulate at interfaces, depressing the surface tension, and form aggregates in solution at sufficiently high concentrations.[1] There are many types of amphiphilic drugs with different actions. Warfarin sodium salt is a widely used amphiphilic anticoagulant drug whose binding to plasma proteins as human serum albumin can be an important determinant of pharmacokinetics. In particular, warfarin sodium is 99 % bound to the protein under normal therapeutic conditions.[2] The structure of this anticoagulant drug is quite complex, with two aromatic rings in the extreme of a hydrophobic moiety with several reactive oxygens and an anionic sodium counterion as part of its hydrophilic moiety (see Scheme 1).

In the present work, we report an investigation of the properties on the surface and on the bulk of the aqueous solution of the drug warfarin sodium. The influence of the temperature on the self-association of the drug has been studied. Aggregation characteristics have been determined using density, surface tension, and conductivity techniques. In this study, we have determined the critical micelle concentration (cmc) of the drug and its temperature dependence. In addition, we have calculated the excess surface concentration, Γ_2, and the minimum area per molecule, *A*, from the surface tension data. Density data let us calculate volumetric properties of the drug in water at different temperatures.

Materials and Methods

Materials. Warfarin sodium [3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium salt], with a molar mass of 330.31 g·mol^{-1} and the molecular formula $C_{19}H_{15}O_4Na$, was obtained from Sigma-Aldrich and was used as received. Solutions for surface tension, specific conductivity, and density experiments were made up by weight at room temperature, using a METTLER AT20 balance with precision of 0.001 mg and double-distilled, deionized, and degassed water. To avoid concentration gradients, all solutions were stirred before the measurements.

Scheme 1. Chemical Structure of Warfarin Sodium



Surface Tension. Surface tension measurements were made at 20 °C with a Kruss K-12 surface tension instrument equipped with a processor to acquire the data automatically under atmospheric pressure by the Wilhelmy plate method. The instrument was connected to a HETO circulating water bath with a temperature controller to keep the temperature constant at (20.0 ± 0.1) °C. Drug solutions of known molality were progressively diluted with water using an automatic pump (Dosimat, model-655, Metrohmn AG). The platinum plate was thoroughly cleaned by washing with doubly distilled water and flame dried before each measurement. The measurements were done in such a way that the vertically hung plate was dipped into the liquid to measure its surface tension. It was then subsequently pulled out. The maximum force needed to pull the plate through the interface was then expressed as the surface tension, γ (mN·m^{-1}). Equilibrium was considered to be obtained when successive values taken at 5 min intervals agreed to within ± 0.1 mN·m^{-1}. Measurements of the surface tension of pure water at 20 °C were performed to calibrate the tensiometer and to check the cleanliness of the glassware. In all the cases, more than 10 successive measurements were carried out.

Specific Electric Conductivity. Conductivities were measured with an HP 4285A Precision LCR meter equipped with an HP E5050A colloid dielectric probe. The probe is especially designed to measure inductances and to avoid the polarization that occurs when the probe is constructed from plain condenser plates. Specific conductivities were measured

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Table 1. Surface Tension Data of Warfarin Sodium Salt in Aqueous Solution at 20 °C

m (mol·kg^{-1})	γ (mN·m^{-1})[a]
0.279	40.09
0.250	39.64
0.225	40.23
0.200	40.72
0.175	40.23
0.150	40.38
0.125	40.94
0.107	41.58
0.089	41.68
0.078	43.98
0.067	45.58
0.054	48.47
0.040	50.48
0.027	52.97
0.018	58.07
0.010	62.77
0.009	64.06
0.008	64.92
0.007	65.61
0.006	66.46

[a] Uncertainties in γ are ± 0.08 mN·m^{-1}.

Table 2. Specific Conductivity, κ, for Warfarin Sodium Salt in Aqueous Solution at Different Temperatures

	t = 20 °C	t = 30 °C	t = 40 °C	t = 50 °C
m	κ[a]	κ[a]	κ[a]	κ[a]
mol·kg^{-1}	mS·cm^{-1}	mS·cm^{-1}	mS·cm^{-1}	mS·cm^{-1}
0.300	11.02	11.54	12.06	12.45
0.275	10.41	10.81	11.3	11.71
0.250	9.74	10.08	10.46	10.91
0.225	9.01	9.28	9.63	10.08
0.200	8.22	8.47	8.80	9.21
0.175	7.37	7.61	7.90	8.30
0.150	6.51	6.70	6.94	7.34
0.125	5.60	5.75	5.95	6.34
0.100	4.64	4.73	4.89	5.23
0.080	3.81	3.90	4.01	4.29
0.060	2.83	3.02	3.14	3.38
0.040	1.978	2.09	2.16	2.33
0.020	1.042	1.087	1.131	1.211
0.010	0.535	0.565	0.590	0.638
0.009	0.485	0.510	0.532	0.576
0.008	0.434	0.455	0.474	0.516
0.007	0.379	0.400	0.416	0.454
0.006	0.326	0.344	0.358	0.390
0.005	0.273	0.288	0.299	0.327

[a] Uncertainties in κ are ± 0.03 mS·cm^{-1} between (0.300 and 0.040) mol·kg^{-1} and ± 0.005 mS·cm^{-1} between (0.040 and 0.005) mol·kg^{-1}.

at (20, 30, 40, and 50) °C. The measuring cell was immersed in a thermostat bath, keeping the temperature control to within ± 0.01 °C, and was calibrated with aqueous solutions of KCl over the appropriate concentration range using the molar conductivity data of Schedlovsky[3] and Chambers et al.[4] The procedure for measuring the cmc consisted of the addition of water to a concentrated aqueous solution of warfarin sodium of known molality under continuous stirring using a peristaltic pump (Dosimat, model-655, Metrohmn AG) under computer control.

Density. Measurements were realized using a commercial density measurement apparatus (Anton Paar DSA 5000 densimeter) equipped with a new generation stainless steel cell. Temperature control was maintained by the Peltier effect with a resolution of ± 0.001 °C, giving rise to uncertainties in density of ca. ± 1·10^{-6} g·cm^{-3}. The densimeter was calibrated using deionized and doubly distilled water, whose densities were taken from the literature,[5] and a vacuum.

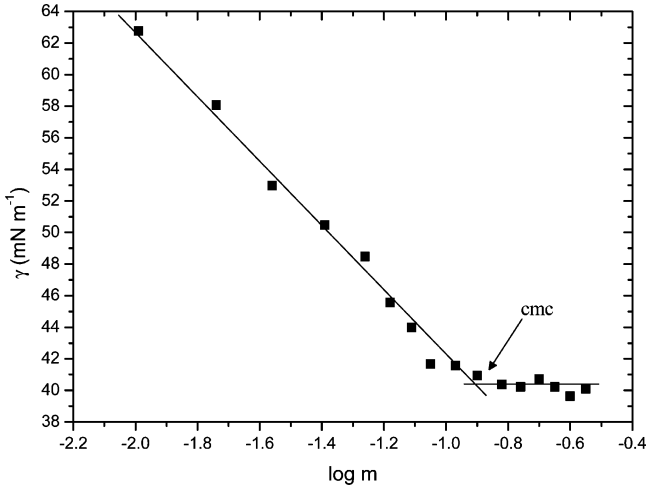

Figure 1. Surface tension, γ, against the logarithm of concentration, m (mol·kg^{-1}), for aqueous solutions of warfarin sodium salt at 20 °C. The arrow denotes the critical micelle concentration.

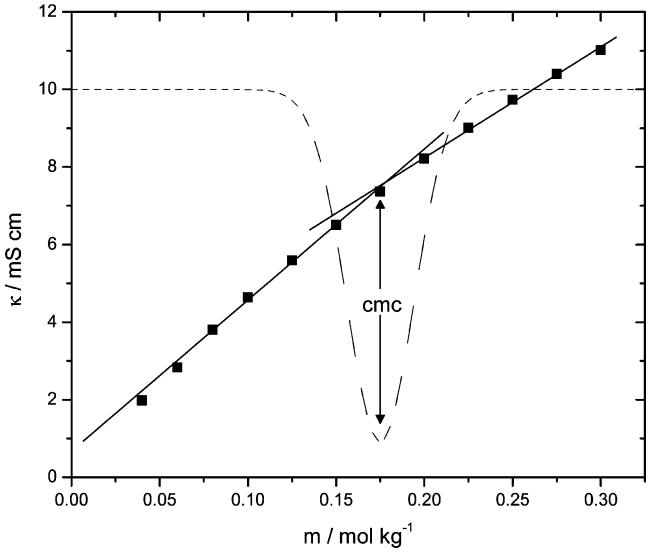

Figure 2. Specific conductivity, κ, vs concentration, m (mol·kg^{-1}), for aqueous solutions of warfarin sodium salt at 20 °C. The arrow denotes the critical micelle concentration calculated by the Williams and Phillips methods. The dotted line represents the Gaussian.

Results and Discussion

The behavior of warfarin sodium salt was studied in an aqueous medium at different temperatures. To elucidate whether this compound can form aggregates, the concentration dependence of the surface tension was determined at 20 °C. Table 1 shows the experimental surface tension data (concentration range (0.006 to 0.279) mol·kg^{-1}) at 20 °C. To obtain the critical micelle concentration, the surface tension was plotted against the logarithm of molality, log m (see Figure 1). The critical micelle concentration was calculated as the intersection point of the linear fits corresponding at each branch of the γ−log m curve, giving a value of (0.12 ± 0.01) mol·kg^{-1}.

The Gibbs surface excess concentration, Γ_2, of the monomers at the air/water interface was evaluated, assuming ideality, by application of the Gibbs equation in the form[6]

$$\Gamma_2 = -\frac{1}{RT}\left(\frac{\partial \gamma}{\partial \ln c}\right)_{T,p} \quad (1)$$

Table 3. Densities, ρ, and Apparent Molal Volume, V_ϕ, of Warfarin Sodium Salt in Aqueous Solution at (20, 30, 40, and 50) °C

m	ρ	V_ϕ	m	ρ	V_ϕ	m	ρ	V_ϕ	m	ρ	V_ϕ
mol·kg^{-1}	g·cm^{-3}	cm^3·mol^{-1}	mol·kg^{-1}	g·cm^{-3}	cm^3·mol^{-1}	mol·kg^{-1}	g·cm^{-3}	cm^3·mol^{-1}	mol·kg^{-1}	g·cm^{-3}	cm^3·mol^{-1}
	$t = 20$ °C			$t = 30$ °C			$t = 40$ °C			$t = 50$ °C	
0.40100	1.031330	240.03	0.40090	1.023307	246.40	0.40090	1.023307	246.40	0.40210	1.018322	249.49
0.37500	1.029294	240.21	0.37500	1.021403	246.58	0.37500	1.021403	246.58	0.37500	1.016469	249.45
0.35000	1.027328	240.38	0.35000	1.019563	246.72	0.35000	1.019563	246.72	0.35000	1.014678	249.59
0.32500	1.025237	240.90	0.32500	1.017603	247.22	0.32500	1.017603	247.22	0.32500	1.012769	250.07
0.30000	1.023146	241.43	0.30000	1.015641	247.73	0.30000	1.015641	247.73	0.30000	1.010859	250.57
0.27500	1.020991	242.21	0.27500	1.013620	248.47	0.27500	1.013620	248.47	0.27500	1.008890	251.30
0.25000	1.018889	242.83	0.25000	1.011637	249.10	0.25000	1.011637	249.10	0.25000	1.006960	251.92
0.22500	1.016714	243.82	0.22500	1.009611	249.97	0.22500	1.009611	249.97	0.22500	1.004990	252.76
0.20000	1.014559	244.82	0.20000	1.007590	250.91	0.20000	1.007590	250.91	0.20000	1.003022	253.68
0.17600	1.012589	245.34	0.17600	1.005743	251.38	0.17600	1.005743	251.38	0.17650	1.001220	254.36
0.15000	1.010431	246.08	0.15000	1.003715	252.08	0.15000	1.003715	252.08	0.15000	0.999248	254.81
0.12510	1.008510	245.68	0.12500	1.001912	251.62	0.12500	1.001912	251.62	0.12500	0.997493	254.33
0.09840	1.006413	245.15	0.09840	0.999945	251.12	0.09840	0.999945	251.12	0.09850	0.995582	253.83
0.07180	1.004265	244.69	0.07190	0.997928	250.71	0.07190	0.997928	250.71	0.07200	0.993609	253.51
0.04660	1.002190	244.06	0.04700	0.995977	250.58	0.04700	0.995977	250.58	0.04690	0.991708	253.04
0.02401	1.000273	243.87	0.02410	0.994171	249.84	0.02410	0.994171	249.84	0.02430	0.989947	253.01
0.00983	0.999054	243.81	0.00988	0.993022	249.42	0.00988	0.993022	249.42	0.01001	0.988824	252.85
0.00851	0.998940	243.81	0.00857	0.992916	249.28	0.00857	0.992916	249.28	0.00870	0.988722	252.64
0.00749	0.998852	243.79	0.00759	0.992836	249.24	0.00759	0.992836	249.24	0.00767	0.988642	252.42
0.00689	0.998801	243.65	0.00695	0.992784	249.16	0.00695	0.992784	249.16	0.00702	0.988591	252.31
0.00573	0.998701	243.56	0.00576	0.992687	249.02	0.00576	0.992687	249.02	0.00585	0.988498	252.24

where R is the gas constant and T is the temperature in Kelvin. The value of Γ_2 so obtained is $(3.6 \pm 0.5)\cdot10^{-6}$ mol·m^{-2}. The area per molecule, A, of the amphiphilic drug monomers at the air/water interface was calculated from eq 1 using[7]

$$A = \frac{1}{N_A \Gamma} \tag{2}$$

where N_A is Avogadro's constant. The value of A obtained, (0.46 ± 0.07) nm^2, is similar to those reported for other drug molecules; the phenothiazine drugs, for example, have areas per molecule of between (0.66 and 0.77) nm^2.[8] The uncertainty in the area per molecule was obtained using $\delta A = \delta\Gamma/\Gamma^2$.

Table 2 shows the specific conductivity of warfarin sodium as a function of concentration. As expected, the values slightly increase with an increase in temperature. The dependence of the specific conductivity, κ, on the concentration, m, of warfarin sodium salt in aqueous solution at 20 °C is shown in Figure 2. Similar plots were obtained for this anticoagulant drug at (30, 40, and 50) °C (not shown). For each temperature, the electrical conductivity increases with concentration with a gradual decrease in slope. The inflection point clearly visible in the graph corresponds to the critical micelle concentration, cmc. Critical micelle concentrations were determined from the conductometric data by an analytical method based on the Phillips definition[9]

$$\left(\frac{\partial^3 k}{\partial c^3}\right)_{c=\mathrm{cmc}} = 0 \tag{3}$$

A numerical analysis of the data was made by means of an algorithm based on the Runge−Kutta numerical integration method and the Levenverg−Marquardt least-squares fitting algorithm which allows determination of precise values (0.05 %) of the cmc of drugs and surfactants of low aggregation number.[10] Figure 2 shows both the measured conductivity and a Gaussian fit of its second derivative from which the cmc was obtained. Data for all the temperatures were plotted in a similar manner, and the critical micelle concentrations obtained were (0.170, 0.164, 0.162, and 0.162) mol·kg^{-1} at (20.0, 30.0, 40.0, and 50.0) °C, respectively. As can be seen, the value of the critical micelle concentration at 20 °C obtained from conductivity is slightly higher than the value obtained from surface tension. Different experimental methods will in most cases lead to slightly different cmc values because the concentration at which micelles initially become detectable depends on the experimental sensitivity, that is, the differently weighted averaged populations from these techniques that become significant for a weakly associating, polydisperse system such as the present one under investigation. In addition, there is a reduction in the cmc value with a temperature increase, with a possible minimum between (40 and 50) °C.

Table 4. Apparent Molal Volumes at Infinite Dilution, V_ϕ^0, Apparent Molal Volumes of Monomers in Aggregates, V_ϕ^m, Changes in Apparent Molal Volumes upon Aggregation, ΔV_ϕ^m, and B_v Parameter of Warfarin Sodium Salt in Aqueous Solution at Different Temperatures

t	V_ϕ^0	V_ϕ^m	ΔV_ϕ^m	B_v
°C	cm^3·mol^{-1}	cm^3·mol^{-1}	cm^3·mol^{-1}	cm^3·kg·mol^{-1}
20.0 ± 0.1	243.1 ± 0.2	235.9 ± 0.2	-7.1 ± 0.4	20.7 ± 1.6
30.0 ± 0.1	245.8 ± 0.2	236.7 ± 3.6	-9.1 ± 3.8	23.2 ± 1.8
40.0 ± 0.1	249.7 ± 0.2	242.5 ± 0.5	-7.2 ± 0.7	15.1 ± 1.6
50.0 ± 0.1	252.2 ± 0.1	248.4 ± 2.9	-3.8 ± 2.9	16.8 ± 0.7

Densities have been applied to study the aggregation properties of the drug warfarin sodium in aqueous solution at different temperatures. Experimental data are shown in Table 3. Apparent molal volumes, V_ϕ, were determined from those density values using[11]

$$V_\phi = \frac{M}{\rho} - \frac{10^3(\rho - \rho_0)}{m\rho\rho_0} \tag{4}$$

where M is the molar mass. Table 4 reports the values obtained. Figure 3 shows the apparent molal volumes of the drug plotted against concentration. The gradient of such plots approaches zero at high drug concentration, and many workers have subjectively chosen the approximately constant or limiting values as the apparent molal volume of the micelles, V_ϕ^m. By differentiating eq 4 with respect to m at constant ρ, an error in V_ϕ of $[(M/\rho) - V_\phi](\delta m/m)_\rho$ is obtained, which gives a maximum error of $\pm$ 0.02 cm^3·mol^{-1} in the concentration range studied [(0.006 to 0.400) mol·kg^{-1}]. If the equation of the apparent molal volume is now differentiated with respect to ρ at constant m, an error in V_ϕ of $[(1000/m\rho_0) + V_\phi](\delta\rho/\rho)_m$ is obtained that

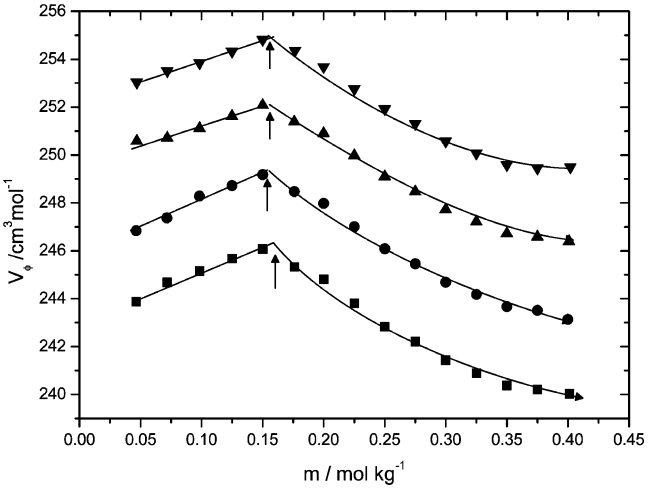

Figure 3. Apparent molal volumes, V_ϕ, vs concentration, m, for aqueous solutions of warfarin sodium salt at: ■, 20 °C; ●, 30 °C; ▲, 40 °C; and ▼, 50 °C. The arrows denote the critical micelle concentration.

will cause a maximum error of about ± 0.01 cm^3·mol^{-1} for all the data measured.

The concentration region below the critical micelle concentration for a 1:1 electrolyte may be described by the Debye−Hückel limiting law equation[12]

$$V_\phi = V_\phi^0 + A_v m^{1/2} + B_v m \qquad (5)$$

where A_v is the Debye−Hückel limiting law coefficient [(1.780, 1.952, 2.131, and 2.3205) cm^3·kg$^{-1/2}$ at (20, 30, 40, and 50) °C].[12] Values at (40 and 50) °C are interpolated from values in the reference, and B_v is an adjustable parameter related to a pair interaction and equivalent to the second virial coefficient which measures the deviation from the limiting law due to nonelectrostatic solute−solvent interactions. V_ϕ^0 is the apparent molal volume at infinite dilution and is taken as the apparent molal volume for free monomers in solution. Values obtained for B_v, V_ϕ^m, and V_ϕ^0 are listed in Table 4. Also, in this table are included the values of the volume change associated with a monomer/aggregate change, $\Delta V_m = V_\phi^m - V_\phi^0$.

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