Vapor Pressure Osmometry and Its Applications in the Osmotic Coefficients Determination of the Aqueous Monomer Glycol and Polymer Polyethylene Glycol Solutions at Various Temperature

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Based on the principle and calibration of vapor pressure osmometer and its application in the thermodynamics of the aqueous solutions, the results on aqueous solutions of monomer glycol, PEG_{200}, PEG_{400}, PEG_{1500} and PEG_{2000} over the different concentration range at various temperatures were reported. Using a linear least-square fitting routing, the osmotic coefficients were fitted by a simple polynomial equation. It was found that the relationship between the molar osmotic coefficients (Φ) and the molar concentration (c) of the solutions are in a quite good agreement with the fitted polynomial equation at various temperatures over the different concentration range. The experimental results also show that over the studied concentration range and at various temperatures, the concentration dependence of the molar osmotic coefficients of the aqueous solution systems with the solutes of PEG_{200}, PEG_{400}, PEG_{1500} and PEG_{2000} are totally presented in a rising trend, and their temperature dependence of the osmotic coefficients of the aqueous solution systems of the molar concentration exhibits their own regularities, respectively. The aqueous glycol solution system exhibits the properties of the dilute solution.

Keywords   VPO, osmotic coefficients, an aqueous polymer polyethylene solution

Vapor pressure osmometry (VPO), initiated by Hill[1] in early 1930's, is currently used to determine the osmotic coefficients of the solutions.[2] The fundamentals of the determination are based on the colligative proper-

ties of the solutions in the dynamically steady-state rather than in the thermodynamically equilibrated one. The method has demonstrated several advantages, such as simple measuring process and simple operation, quick acquiring experimental data and a wide range of experimental conditions. The measurements are performed by conversion of the thermodynamic properties of the solution into the electrical signals. Therefore, both the steadiness and the reliability of the measurements can be guaranteed. Thus, widespread applications of the method, particularly commercially available vapor pressure osmometer, are used for the osmotic coefficients determination of the solutions. Compared with the other methods, in addition to its higher sensitivity, VPO still has further advantage, for example, cryoscopy can only be used in the vicinity of the freezing point of the solvent, and ebullioscopy in the vicinity of the boiling point of the solvent. However, VPO has just filled the "gap" of the temperature range between those and can be used from 303 K to temperature below the freezing point of the solvent.

Determination of the osmotic coefficients of the aqueous polymer solutions with an improved isopiestic method and a modified isopiestic apparatus has been done by some researchers,[3-4] but the determination by VPO has only been reported in a few papers.[5-7] Most of them concern with non-aqueous and non-polar solvents, especially the work of the aqueous polymer solution was

scarcely reported.[8] Compared with the experimental methods,[3-4] both of them have high temperature stability and accuracy, the lower average relative errors (0.01%—0.02%), and the rather simple operations. However, using Ochs' method[3] the time for equilibration of the dilute or high viscous solutions is typically about one week, and using a modified isopiestic apparatus[4] the time for equilibration is still 3 or 4 d. To continue our previous work,[9-11] the present paper deals with an aqueous solutions of monomer glycol and some polymer polyethylene glycol (average molecular weights, $\bar{M}$ = 200, 400, 1500 and 2000, respectively) at various temperatures by VPO.

Experimental

All measurements were performed on aqueous solutions of the different solutes (monomer glycol, PEG$_{200}$, PEG$_{400}$, PEG$_{1500}$ and PEG$_{2000}$) at 313, 323 and 333 K over the concentration range of 0.0—1.0 mol/L for monomer glycol, 0.0—1.6 mol/L, 0.0—2.0 mol/L, 0.0—0.7 mol/L and 0.0—0.6 mol/L for PEG$_{200}$, PEG$_{400}$, PEG$_{1500}$ and PEG$_{2000}$, respectively, from which the osmotic coefficients were derived using a Knauer Vapor Pressure Osmometer Model 11.00 in combination with a digital meter and a recorder.[12] The instrument was calibrated with an aqueous standard sodium chloride solution of the known molality using the smoothed values of the osmotic coefficients reported by Herrington and Taylor.[13] The error level of the osmotic coefficients measurement was ± 0.005. Sodium chloride, analytical grade from Xi'an Chemical Works (China), was recrystallized twice in double distilled deionized water before use, and desiccated for 24 h in the vacuum drying oven at 373 K, and then prepared the standard aqueous solution with the known molality. Glycol, analytical grade from Beijing Chemical Works (China), was double distilled under the reduced pressure of 666.5 Pa prior to use, and received the distillate of the b.p. range of 347 to 349 K. Polymer polyethylene glycol from Shanghai No. 1 Reagent Works (China), are used as received without further purification. The double distilled deionized water was used for the whole preparation of the aqueous solutions.

The general principle and operation of the instrument have been described elsewhere.[2]

Results and discussion

Calibration of the vapor pressure osmometer for the osmotic coefficient determination of the aqueous solution systems

Aqueous standard sodium chloride solutions with the various concentrations were prepared first, and then their electrical signal intensities (G) at various temperatures were measured by vapor pressure osmometer; (G/c)$_{RZ32}$, the values of the ratio of the electrical signal intensity to c, and the mass molar concentration of the systems for the different concentrations at various temperatures were obtained. The experimental data are listed in Table 1.

Table 1 Relationship between (G/c)$_{RZ32}$ and c for an aqueous sodium chloride solution system at various temperatures[a]

c (mol/L)	(G/c)$_{RZ32}$ (313 K)	(G/c)$_{RZ32}$ (323 K)	(G/c)$_{RZ32}$ (333 K)
0.1043	200	220	240
0.2015	178	200	216
0.3027	176	194	212
0.3913	174	192	211
0.5046	174	190	211
0.6008	175	190	212
0.7044	176	191	213
0.8066	178	192	214
0.8985	179	193	215
0.9893	180	194	216
1.0970	181	195	217
1.2060	182	196	218
1.2960	183	197	219
1.4030	184	199	220
1.4990	186	201	221
1.5870	187	202	223
1.6930	188	203	224
1.7540	189	204	225
1.9030	191	207	226
2.0020	193	209	227
2.0990	194	211	228
2.2070	195	213	229
2.3150	195	213	229

[a] RZ32: the electrical signal intensities of the instrument were attenuated to 32 times.

The experimental data of (G/c) versus c were fitted by the following polynomial equation using a linear

least-square routine:

$$G/c = A_0 + A_1 c + A_2 c^2 + A_3 c^3 + A_4 c^4 + A_5 c^5 \qquad (1)$$

According to the experimental data in Table 1 and the above fitting polynomial equation, the concentration dependence curves of the experimental result (G/c) versus c over the concentration range of 0.1—2.3 mol/ L at 313, 323 and 333 K are represented in Fig. 1. From Fig. 1, it can be seen that the relationships between the experimental results (G/c) and c over the concentration range of 0.1—2.3 mol/L at various temperatures were in quite a good agreement with the fitting polynomial equation. Table 2 has also offered the fitting constants A_0, A_1, A_2, A_3, A_4 and A_5 obtained in Eq. (1) and their correlation coefficients R.

According to Eq. (1) and the fitting constants in Table 2, the calculated values of (G/c) at the concentrations of 0.10, 0.25, 0.50, 0.75, 1.00 and 2.00 mol/L for the various temperatures were obtained respectively, and then put the calculated values of (G/c) and Φ_{Cal} into the Eq. (2) and get the instrumental calibration constants (K_G) over the different concentration ranges at various temperatures (see Table 3).

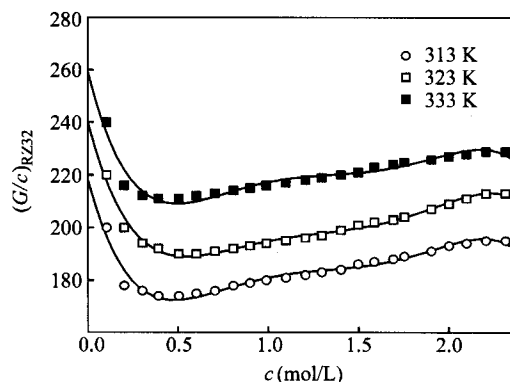$$K_G = (G/c)_{NaCl}/(\nu\ \Phi)_{NaCl} \qquad (2)$$

Fig. 1   Concentration dependence of (G/c)$_{RZ32}$ of the aqueous sodium chloride solution systems at various temperatures.

where the subscript 'NaCl' means the reference substance; ν, the number of ions generated in a complete dissociation; the other symbols are of the usual significance.

From Table 3, it indicates that the instrumental calibration constants (K_G) was only dependent upon the temperatures, and not on the concentration (actually it is almost a constant). According to the following Eq. (3).

$$K_G = (G/c)_x/(\nu\ \Phi)_x \qquad (3)$$

Table 2   Fitting constants A_i in Eq. (1) and their correlation coefficients R for the aqueous sodium chloride solution systems (reference substance) at various temperatures

T/K	A_0	A_1	A_2	A_3	A_4	A_5	R
313	218.4663	− 259.9438	519.0930	− 449.4234	179.7026	− 26.9221	0.9756
323	240.2965	− 265.4753	494.0028	− 411.1611	160.8310	− 23.7451	0.9899
333	259.8077	− 280.4486	550.8686	− 472.2478	187.2147	− 27.8687	0.9648

Table 3   Relationships between Φ_{Ref}[14] and Φ_{Cal} calculated one from Eq. (2), and the calibration constants of the instrument at various temperature over the different concentration range

$c/(mol/L)$	313 K			323 K			333 K		
	Φ_{Ref}	Φ_{Cal}	K_G	Φ_{Ref}	Φ_{Cal}	K_G	Φ_{Ref}	Φ_{Cal}	K_G
0.10	0.932	0.931	95.1	0.931	0.929	107.5	0.929	0.928	115.7
0.25	0.922	0.925	95.0	0.921	0.924	107.8	0.920	0.923	116.1
0.50	0.924	0.923	95.0	0.924	0.923	108.1	0.923	0.922	116.2
0.75	0.931	0.930	94.9	0.932	0.930	108.5	0.931	0.930	115.7
1.00	0.941	0.942	95.1	0.942	0.943	108.1	0.942	0.943	115.9
2.00	0.992	0.992	95.9	0.995	0.995	106.9	0.996	0.996	116.0

where the subscript ' x ' means the substance to-be-measured. The calibrated instrumental constant (K_G) has been obtained by using the standard aqueous sodium chloride solution systems, the values of (G/c)$_x$ for the unknown substances can also be determined using the calibrated vapor pressure osmometer, and then the values of Φ_x for the substances to-be-measured can be given at the same time by using Eq. (3).

Osmotic coefficient determination of the aqueous solution systems with different solutes at various temperatures

At 313, 323 and 333 K, the experimental results (Φ_{Exp}) of the osmotic coefficients of the aqueous monomer glycol and polymers, such as polyethylene glycol systems, Φ_{Exp} and c, are listed in Tables 4—8. For those systems, the concentration ranges of the aqueous monomer glycol systems are 0.0—1.0 mol/L, and that of the aqueous PEG$_{200}$, PEG$_{400}$, PEG$_{1500}$ and PEG$_{2000}$ systems, 0.0—1.6 mol/L, 0.0—2.0 mol/L, 0.0—0.7 mol/L and 0.0—0.6 mol/L, respectively. The relationships between the experimental values of the molar osmotic coefficients of the aqueous solutions of the studied polymer polyethylene glycol (Φ_{Exp}) and c, are fitted by the following polynomial equation using a linear least-square routine

$$\Phi = 1 + A_1 c + A_2 c^2 + A_3 c^3 + A_4 c^4 \qquad (4)$$

The relationship in Eq. (4) between Φ and c can also be represented by the curves of Figs. 2—5. From Figs. 2—5, it can be seen that the experimental values of the osmotic coefficients Φ_{Exp} are in very good agreement with the fitted polynomial equation. The calculated values (Φ_{Cal}) by Eq. (4) are also given in Tables 5—8. Table 9 gives the fitting constants A_i of the Eq. (4) and their correlation coefficients R for the aqueous systems with the different polymer polyethylene glycol at various temperatures.

From the experimental results, it can be known that the aqueous solution of monomer glycol possesses the properties of the dilute solution, *i. e.*, the relationships between the osmotic coefficients of aqueous solution and its concentration (over the studied concentration range) are basically a horizontal straight line (figure omitted). For the different polymeric polyethylene glycol, with increasing of molecular weights, their osmotic coefficients of the aqueous solution systems exhibit their own individ-

ual regularities with increasing of temperature. For PEG$_{200}$, its osmotic coefficients of the aqueous solution systems increased with increasing in temperature over the higher concentration range (> 1.0 mol/L), namely, the non-ideality of the aqueous solution systems increased with increasing in temperature. However, over the lower concentration range (< 1.0 mol/L), the temperature dependences of the osmotic coefficients of the aqueous solution system are different for different systems. As for PEG$_{400}$, its osmotic coefficients of the aqueous solution systems increased with increasing in temperatures as well. But for PEG$_{1500}$, the influence of temperatures on the osmotic coefficients of the aqueous solution systems appears interlacing. At 313 K, the osmotic coefficients of the aqueous solution systems are the biggest, and over 313 K, the osmotic coefficients of the aqueous solution systems increased with increasing in temperature (323 to 333 K). For PEG$_{2000}$, the regularities between the osmotic coefficients of the aqueous solution systems and temperatures are on the contrary to that of PEG$_{200}$ and PEG$_{400}$, that is to say, the osmotic coefficients of the aqueous solution systems of PEG$_{2000}$ decreased with increasing in temperatures. From the facts mentioned above and the analyses, it indicates that for the polymer polyethylene glycol with increasing of its molecular weight, its osmotic coefficients of aqueous solution increased more noticeably, indicating that increasing of molecular weight of polyethylene glycol can cause increasing in osmotic coefficients of its aqueous solutions, and with increasing in concentration of its aqueous solution, the increasing trend of their osmotic coefficients is also bigger and bigger.

By comparison and analysis of the experimental data and the results from our previous work,[8] it was found that there is no direct obvious relationships between the osmotic coefficients of the aqueous solution systems and the number of hydroxyl group of the solute molecules. For the systems of monomer glycol, polymer PEG$_{200}$, PEG$_{400}$, PEG$_{1500}$ and PEG$_{2000}$, polyhydroxylated compounds such as glucose, sucrose and glycerol, the factors which influence the osmotic coefficients of its aqueous solution such as the number of hydroxyl of the polymers or the molecule weight of solute molecules of aqueous polymer solution should be further studied. This work will also contribute to the studies of other thermodynamic properties of the aqueous solution of the polymer polyethylene glycol.
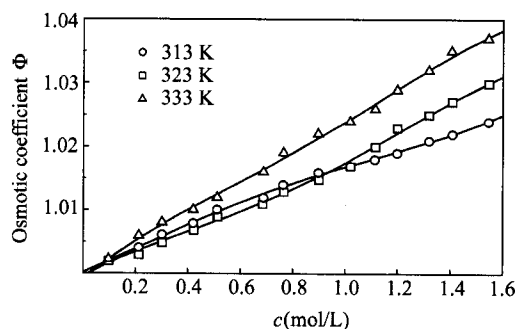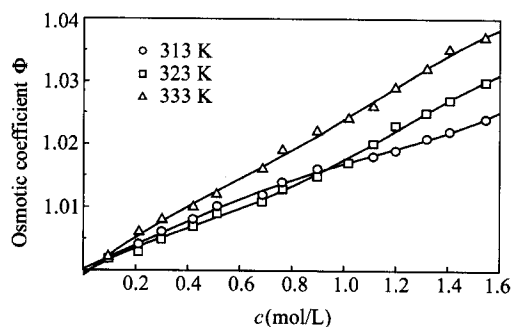
Fig. 2 Concentration dependence of the molar osmotic coefficients of the aqueous polymer PEG$_{200}$ solution systems at various temperatutes.
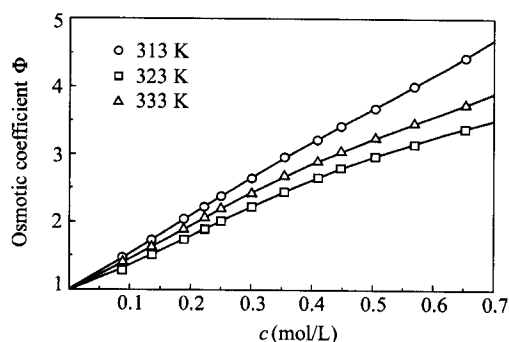
Fig. 4 Concentration dependence of the molar osmotic coefficients of the aqueous polymer PEG$_{1500}$ solution systems at various temperatutes.
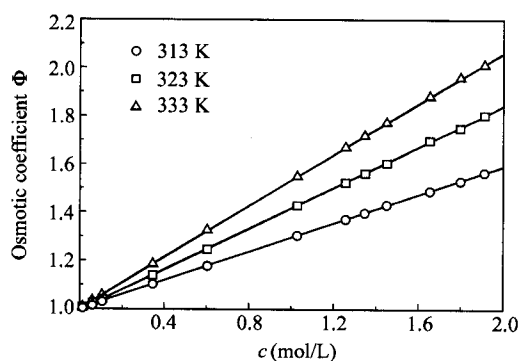
Fig. 3 Concentration dependence of the molar osmotic coefficients of the aqueous polymer PEG$_{400}$ solution systems at various temperatutes.

Fig. 5 Concentration dependence of the molar osmotic coefficients of the aqueous polymer PEG$_{2000}$ solution systems at various temperatutes.

Table 4 Concentration dependence of the osmotic coefficients of the aqueous monomer glycol systems at various temperatures

$c/(mol/L)$	Φ_{313}	Φ_{323}	Φ_{333}	$c/(mol/L)$	Φ_{313}	Φ_{323}	Φ_{333}
0.0942	0.995	0.997	0.998	1.0910	0.996	1.002	0.998
0.2171	1.001	1.000	0.998	1.2210	0.999	0.995	0.999
0.2896	1.003	1.000	0.997	1.3240	0.999	0.999	0.998
0.3888	0.998	1.000	0.997	1.4230	1.002	1.002	1.000
0.4937	1.002	1.000	0.998	1.5080	1.002	1.003	1.000
0.5881	1.006	1.000	0.999	1.5900	1.000	1.001	1.000
0.6962	1.000	1.000	0.999	1.6960	1.002	0.992	0.998
0.8184	0.995	1.000	0.998	1.8170	1.002	1.001	1.000
0.8812	1.003	1.000	0.999	1.8760	0.998	1.002	0.999
0.9971	0.999	1.001	0.998	1.9980	1.002	1.000	1.000

Table 5   Concentration dependence of the osmotic coefficients of the aqueous polymer PEG_{200} systems at various temperatures

$c/(mol/L)$	$\Phi_{Exp\,(313)}$	$\Phi_{Cal\,(313)}$	$\Phi_{Exp\,(323)}$	$\Phi_{Cal\,(323)}$	$\Phi_{Exp\,(333)}$	$\Phi_{Cal\,(333)}$
0.0951	1.002	1.002	1.002	1.002	1.002	1.003
0.2111	1.004	1.004	1.003	1.004	1.006	1.006
0.3001	1.006	1.006	1.005	1.006	1.008	1.009
0.4209	1.008	1.008	1.007	1.007	1.010	1.011
0.5121	1.010	1.010	1.009	1.009	1.012	1.013
0.6865	1.012	1.012	1.011	1.012	1.016	1.017
0.7636	1.014	1.014	1.013	1.013	1.019	1.019
0.8969	1.016	1.015	1.015	1.016	1.022	1.022
1.0181	1.017	1.017	1.017	1.018	1.024	1.025
1.1139	1.018	1.018	1.020	1.021	1.026	1.028
1.1978	1.019	1.019	1.023	1.023	1.029	1.030
1.3189	1.021	1.021	1.025	1.026	1.032	1.033
1.4072	1.022	1.022	1.027	1.028	1.035	1.035
1.5450	1.024	1.024	1.030	1.031	1.037	1.038

Table 6   Concentration dependence of the osmotic coefficients of the aqueous polymer PEG_{400} systems at various temperatures

$c/(mol/L)$	$\Phi_{Exp\,(313)}$	$\Phi_{Cal\,(313)}$	$\Phi_{Exp\,(323)}$	$\Phi_{Cal\,(323)}$	$\Phi_{Exp\,(333)}$	$\Phi_{Cal\,(333)}$
0.0119	1.004	1.004	1.007	1.005	1.007	1.007
0.0581	1.015	1.018	1.022	1.022	1.034	1.032
0.1040	1.033	1.031	1.038	1.040	1.057	1.058
0.3460	1.104	1.103	1.139	1.138	1.186	1.188
0.6038	1.178	1.179	1.247	1.246	1.326	1.323
1.0287	1.304	1.304	1.427	1.426	1.548	1.547
1.2570	1.371	1.371	1.521	1.523	1.668	1.669
1.3467	1.397	1.398	1.561	1.560	1.717	1.717
1.4505	1.430	1.428	1.603	1.604	1.773	1.773
1.6541	1.487	1.488	1.696	1.691	1.881	1.881
1.7979	1.528	1.530	1.749	1.753	1.959	1.956
1.9110	1.564	1.563	1.804	1.803	2.012	2.013

Table 7   Concentration dependence of the osmotic coefficients of the aqueous polymer PEG_{1500} systems at various temperatures

$c/(mol/L)$	$\Phi_{Exp\,(313)}$	$\Phi_{Cal\,(313)}$	$\Phi_{Exp\,(323)}$	$\Phi_{Cal\,(323)}$	$\Phi_{Exp\,(333)}$	$\Phi_{Cal\,(333)}$
0.0880	1.462	1.462	1.313	1.315	1.389	1.390
0.1354	1.729	1.728	1.513	1.511	1.626	1.623
0.1887	2.037	2.031	1.743	1.741	1.891	1.890
0.2224	2.218	2.222	1.891	1.889	2.059	2.058
0.2501	2.378	2.377	2.010	2.010	2.195	2.193
0.3010	2.646	2.658	2.219	2.227	2.422	2.433
0.3541	2.962	2.941	2.444	2.443	2.675	2.668
0.4091	3.218	3.223	2.649	2.653	2.895	2.892
0.4476	3.415	3.415	2.800	2.789	3.039	3.038
0.5039	3.687	3.691	2.974	2.973	3.236	3.238
0.5685	4.010	4.005	3.156	3.163	3.453	3.453
0.6529	4.433	4.433	3.387	3.385	3.735	3.734

Table 8   Concentration dependence of the osmotic coefficients of the aqueous polymer PEG_{2000} systems at various temperatures

$c/(\text{mol/L})$	$\Phi_{\text{Exp (313)}}$	$\Phi_{\text{Cal (313)}}$	$\Phi_{\text{Exp (323)}}$	$\Phi_{\text{Cal (323)}}$	$\Phi_{\text{Exp (333)}}$	$\Phi_{\text{Cal (333)}}$
0.0836	2.073	2.083	1.858	1.854	1.567	1.566
0.1252	2.559	2.535	2.222	2.232	1.854	1.850
0.1794	3.049	3.052	2.687	2.681	2.206	2.210
0.2002	3.227	3.232	2.844	2.840	2.341	2.342
0.2491	3.612	3.623	3.187	3.189	2.643	2.639
0.2953	3.962	3.958	3.483	3.488	2.904	2.898
0.3452	4.292	4.289	3.787	3.782	3.153	3.151
0.4207	4.761	4.749	4.179	4.182	3.482	3.489
0.4670	5.011	5.014	4.414	4.411	3.674	3.674
0.5006	5.198	5.201	4.570	4.573	3.810	3.802
0.5713	5.576	5.586	4.927	4.919	4.072	4.070
0.5901	5.697	5.687	5.009	5.014	4.143	4.144

Table 9   Constants A_i in Eq. (4) and their correlation coefficients R for aqueous polymer PEG systems at various temperatures

Systems	T/K	A_1	A_2	A_3	A_4	R
PEG_{200}	313	0.01803	0.00621	− 0.01208	0.00452	0.9985
PEG_{200}	323	0.02344	− 0.02342	0.02574	− 0.00785	0.9973
PEG_{200}	333	0.03541	− 0.03048	0.02818	− 0.00838	0.9978
PEG_{400}	313	0.30147	− 0.01120	0.00696	− 0.00157	0.9999
PEG_{400}	323	0.37862	0.07348	− 0.05019	0.01207	0.9999
PEG_{400}	333	0.56116	− 0.07181	− 0.05758	− 0.01489	0.9999
PEG_{1500}	313	4.87710	5.35910	− 13.88579	10.06445	0.9999
PEG_{1500}	323	3.05869	7.15750	− 15.09784	8.46838	0.9999
PEG_{1500}	333	3.93569	7.22567	− 18.68290	12.57115	0.9999
PEG_{2000}	313	14.59701	− 21.35447	22.49062	− 9.17417	0.9999
PEG_{2000}	323	10.96720	− 9.03958	− 0.46970	6.48886	0.9999
PEG_{2000}	333	6.53991	4.44724	− 21.73020	18.15486	0.9998

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Monitoring the Aggregation of Dansyl Chloride in Acetone through Fluorescence Measurements

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The aggregation of dansyl chloride (DNS-Cl) in acetone has been studied in detail by steady-state fluorescence techniques. It has been demonstrated that DNS-Cl is stable in acetone during purification and aggregation study processes. The aggregates are not solvolyzed in acetone, and do not take part in any chemical reactions either. It has been found that DNS-Cl tends to aggregate even when its concentration is much lower than its solubility in acetone. The aggregation is reversible, and both the aggregation and the deaggregation are very slow processes. Introduction of SDS has a positive effect upon the formation and stabilization of the aggregates.

Keywords   dansyl chloride, acetone, aggregation, fluorescence techniques

Introduction

Dansyl chloride (DNS-Cl) is widely used in labeling proteins,[1,2] carbohydrates[3,4] and synthetic polymers,[5-7] for the studies of their physical behaviors and the determination of their quantities in aqueous phase, which is due to the fact that its emission spectrum is highly sensitive to solvent polarity.[8] Based upon the properties of the fluorophore, it has been tried to label the DNS-Cl onto chitosan films and aminated quartz plates via the sulfonation of the amino groups on the surface of the substrates in order to prepare, initially, a chemical sensor for solvent polarity measurement.[9,10] The solvent used in the immobilization was acetone. It was found that DNS-Cl has a strong tendency to aggregate in acetone even though the concentration is very low. As might be expected, the ag-

gregation of the fluorophore results in an inhomogeneous labeling of the fluorophore on the film or on the plate surface, and thereby affects the sensing properties of the materials. To the best of our knowledge, there has been not any report on the aggregation of DNS-Cl in acetone. Therefore, we investigate the aggregation in detail.

Experimental

Materials and instruments

Purified DNS-Cl was obtained by extracting the reagent of DNS-Cl(Aldrich) with dry acetone in a Soxhlet's extractor. The profile of the IR spectrum of the purified DNS-Cl is basically the same as that of the standard spectrum of the reagent. Elemental analysis results (%): C 57.2, H 5.4, N 5.3, are very close to the theoretical values (%): C 57.3, H 5.2, N 5.6. Acetone used in the present study was of spectral grade, and was dried completely with molecular sieve before use.

Sodium dodecyl sulfate (SDS) of super-pure grade is a product of BDH . It was dried with anhydrous CaCl$_2$ for several days before use.

Pressed KBr disk was used for transmission infrared spectroscopy measurement, which was performed on a E-quinox 55 FTIR spectrometer.

All fluorescence measurements were conducted on a Perkin-Elmer LS 50B luminescence spectrometer. For monitoring the changes in the fluorescence spectrum, an

automatic monitoring with preset time interval method was employed. The emission was deducted to 1% of the real intensity to allow a long time monitoring.

Preparation of the saturated solution of DNS-Cl *in acetone*

An excess amount of pure DNS-Cl was put in a reagent bottle with 200 mL of dry acetone. The bottle was sealed and shaken at 25 ℃ for more than 1000 min. About 3 mL of the supernatant was carefully withdrawn from the top of the bottle, and kept in a small tube. 35 samples were collected in this way. The fluorescence intensity of each sample was measured by taking 337 nm and 520 nm as excitation and analysis wavelengths, respectively. It was found that the fluorescence intensity of the sample tends to be constant after 180 min equilibration, indicating that an solubilization equilibrium was reached. Based upon this experiment, the saturated DNS-Cl solution was prepared by filtration of the above suspension with glass sand filter after 200 min equilibration. The saturated solution prepared in this way was kept in a sealed brown bottle at room temperature and used as a stock solution during the experiment.

Solubility of DNS-Cl *in acetone*

A weighing bottle of 10 mL volume was dried at 50 ℃ for several hours till a constant weight (w_1), and then 10.00 mL of the saturated DNS-Cl solution (V) was transferred into it. The bottle was left in a dust free place at room temperature until the solvent was evaporated completely, and then the bottle was dried in an oven of a temperature of 50 ℃ (note: the m.p. of DNS-Cl is 72—74 ℃) till a constant weight (w_2). The solubility (S) of DNS-Cl in acetone was calculated by using the following equation:

$$S = \frac{w_2 - w_1}{V} \times 1000 \qquad (1)$$

The determination was repeated for three times and found that the solubility of DNS-Cl in acetone at 25 ℃ is 702 mg/L.

Concentration dependence of the fluorescence emission spectrum of DNS-Cl *in acetone*

A series of DNS-Cl solutions of different concentrations were prepared by dilution of the saturated DNS-Cl

solution with dry acetone. The solutions were shaken for more than 2 h to ensure equilibration before spectroscopic measurement. The results are shown in Fig. 1.
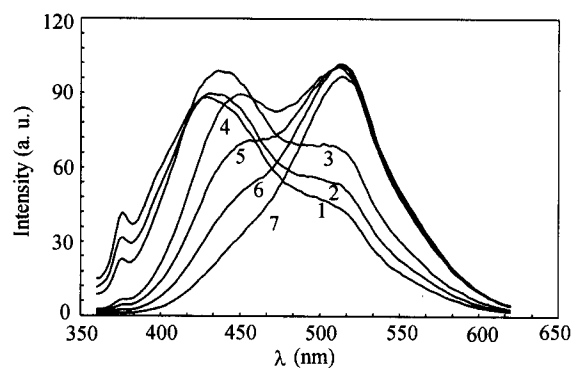
Fig. 1   Concentration dependence of the fluorescence emission spectrum of DNS-Cl in acetone (1: 5.2 mg/L; 2: 9.3 mg/L; 3: 15.6 mg/L; 4: 46.7 mg/L; 5: 56.0 mg/L; 6: 70.0 mg/L; 7: 105 mg/L).

Effect of SDS *upon the aggregation of* DNS-Cl

To 3 portions (0.6 mL each) of the saturated solution of DNS-Cl in acetone, (1) 4.4 mL of dry acetone; (2) 3.8 mL of dry acetone + 0.6 mL of saturated solution of SDS in acetone; (3) 4.4 mL of saturated solution of SDS in acetone was added, respectively. The mixtures were shaken for 2 min, and then the fluorescence spectrum of each sample was measured every hour. The fluorescence intensities at 450 nm and 513 nm, denoted as I_{450} and I_{513}, respectively, were recorded. The ratio of I_{450}/I_{513} was plotted against time (Fig. 4).

Results and discussion

Aggregation of DNS-Cl *in acetone*

Fig. 1 shows the dependence of the profile of the fluorescence emission spectrum of DNS-Cl in acetone upon the concentration of the solute at equilibrium state. With reference to the figure, it can be noted that the characteristic of the monomer emission decreases and that of the excimer emission increases with increasing the concentration of DNS-Cl. Further examination of the figure reveals that the excimer emission is becoming obvious when the concentration of the fluorophore is only 5.2 mg/ L, indicating that DNS-Cl may have a strong tendency to

aggregate. The reason for the aggregation may be attributed to the presence of the groups in DNS-Cl which are poorly compatible with the solvent, acetone. To verify if there is any significant amount of ground state associates in the solution, the excitation spectrum of DNS-Cl with a concentration of 56 mg/L was measured at the analysis wavelengths of 420 nm and 520 nm, respectively. The results are shown in Fig. 2. It is obvious that the excitation spectrum corresponding to an analysis wavelength of 520 nm is significantly red shifted compared with the one analysed at 420 nm. Based upon this observation, it may be concluded that the solubilized DNS-Cl in acetone exists not only in solvated monomer state, but also in aggregated state.
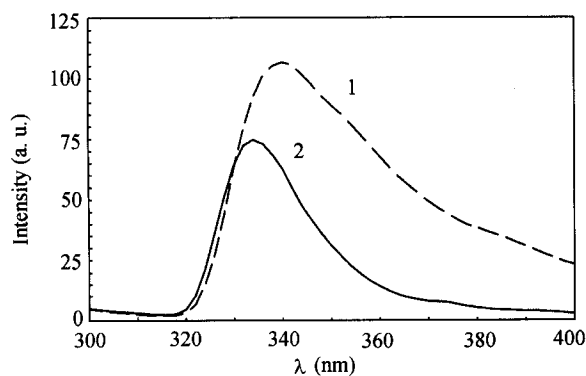

Fig. 3   3D structure of DNS-Cl in gas state (Note: the substitute group above the naphthylene ring is dimethylamino group, and the one bellow the ring is sulfonyl group).

Transformation between monomer state and association state of DNS-Cl

Change of the fluorescence emission spectrum of newly prepared DNS-Cl solution in acetone (56 mg/L) was automatically monitored with time intervals of 10 min. The results are shown in Fig. 4. With reference to the figure, it is revealed that the spectrum of the system at the beginning of the experiment is dominated by the excimer emission around 515 nm, indicating that the fluorophore exists mainly in aggregated state. With the elongation of time, the monomer emission around 440 nm increases faster than the excimer emission. Clearly, building a new equilibrium takes more than 7 h. This result shows that the aggregation of DNS-Cl and the dissociation of the aggregates in acetone are reversible and the transformation between the monomer state and the association state is very slow. Further examination of the figure reveals that the whole emission increases slowly with the elongation of time. It may be explained at the beginning of the experiment, DNS-Cl mainly exists in aggregated state and the solution was not homogeneous at a molecular level. As a result, there are some DNS-Cl rich micro-domains, in which the concentration of DNS-Cl is much higher than that in the bulk phase. Therefore, inner filtering or self-quenching effect of the system would be strong and the fluorescence emission would be weak. With the elongation of time, the aggregates of DNS-Cl dissociate gradually due to the addition of new solvent, and the solution becomes more homogeneous, and thereby the fluorescence emission increases. It is reported that hydrophobic-lipophilic interaction is one of the most important weak interactions between organic molecules and

Fig. 2   Analysis wavelength dependence of the excitation spectrum of DNS-Cl (1: $\lambda_{em} = 520$ nm; 2: $\lambda_{em} = 420$ nm).

It is to be noted that although the critical aggregation concentration ([CAgC]) of DNS-Cl in acetone could not be determined accurately, the concentration must be much lower than the solubility of DNS-Cl in the solvent. This result is in support of Jiang's statement that [CAgC] and solubility are two different concepts in nature.[11] The aggregation behavior of DNS-Cl in acetone may be explained in consideration of its molecular structure. In the gas state, DNS-Cl should adopt the structure shown in Fig. 3. The corresponding dipole moment in this structure is 7.07 D. Considering that the dipole moment of acetone in gas state is only 2.88 D and the dielectric enrichment effect,[12] it is not surprising that DNS-Cl tends to aggregate in acetone. Actually, the aggregation of organic molecules in aqueous phase and mixtures of aqueous and organic solvent have been reported widely.[13,14] However, studies on the aggregation of organic molecules in pure organic solvent are limited.
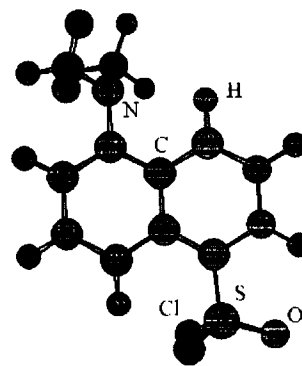
is the main driving force for their aggregations in aqueous phase and water/organic mixture systems.[11,13-15] However-er, for present system, the reason for the aggregation might be just the opposite. The liophobic-hydrophilic property of the sulfonyl chloride group on the DNS-Cl molecule might be the main reason for the aggregation of DNS-Cl in acetone even though the whole DNS-Cl is hydrophobic-lipophilic in nature. It is the weak driving force that is responsible for the slow establishment of new equilibrium and for the fact that the equilibrium state is not stable and easily affected by changes in external factors.
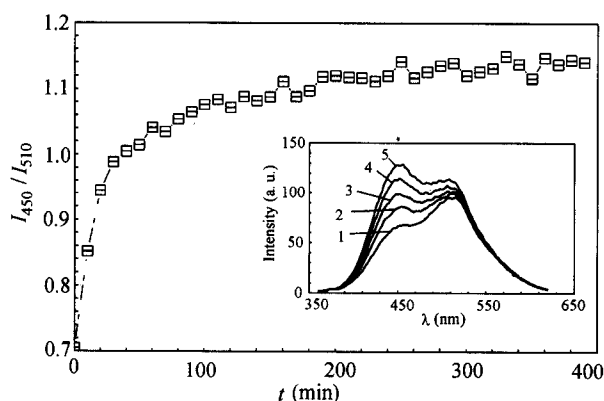

Fig. 4 Time dependence of the profile of the fluorescence emission spectrum of DNS-Cl in acetone after dilution with dry acetone (1: 0 min; 2: 10 min; 3: 30 min; 4: 110 min; 5: 390 min).

Effect of SDS on the aggregation of DNS-Cl in acetone

It has been reported that for the aggregation in aqueous phase and aqueous/organic mixture systems, introduction of some small molecules may enhance aggregation or may enhance deaggregation. The former kind of molecules is called enhancing aggregators and the latter one is called deaggregators. Normally, the enhancing aggregators are functioning by taking part in aggregation process.[11,15] As for the system of DNS-Cl in acetone, SDS shows obvious enhancing effect for the aggregation process. In Fig. 5, it may be noted that in the absence of SDS, the ratio of the monomer emission to the excimer emission (I_{450}/I_{513}) increases with time after diluting the saturated DNS-Cl solution with acetone. The increase reaches a constant value at about 16 h, indicating the establishment of a new equilibrium. For the system of high concentration of SDS (nearly saturated), the ratio decreases along with time. About 12 h later, the ratio

reaches another constant value. The system with very low concentration of SDS behaves differently. The ratio increases slightly with time after introduction of the solvent. However, 10 h later, the ratio starts to decrease and a constant value is reached about 20 h later. These results show clearly that the introduction of SDS is favorable for DNS-Cl to exist in aggregated state. More work is needed to verify if SDS stabilizes the DNS-Cl aggregates by taking part in the aggregation.
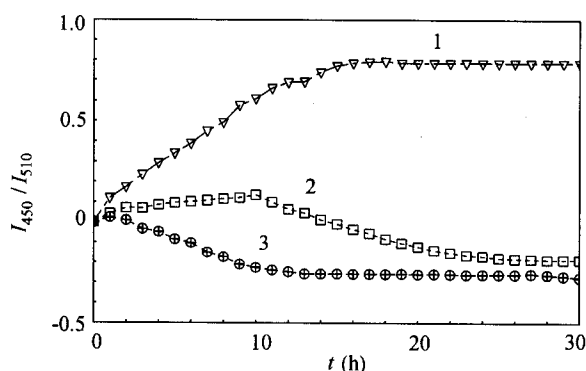
Fig. 5 SDS effect upon the profile of the emission spectrum of DNS-Cl in acetone (1: No SDS; 2: 1 : 8 saturated SDS solution in acetone; 3: 9 : 10 saturated SDS solution in acetone).

Conclusions

DNS-Cl is stable in acetone. No decomposition was observed during the process of purification and aggregation studies. DNS-Cl has a strong tendency to aggregate in acetone, and its [CAgC] is significantly lower than its solubility. The aggregation of DNS-Cl in acetone is reversible, and both the aggregation and de-aggregation are very slow. The introduction of SDS favors the formation and stabilization of the DNS-Cl aggregates.

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