Solution Structure of Detergent Micelles at Conditions Relevant to Membrane Protein Crystallization.

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In this study small angle neutron scattering was used to characterize the formation of micelles in aqueous solutions of the detergents DMG and SPC as a function of detergent concentration and ionic strength of the solvent. The effects on the micelle structure of the additives glycerol and PEG, alone as well as in combination typical for actual membrane protein crystallization, were also explored. This research suggests that the micelles are cigar-like in form at the concentrations studied. The size of the micelles was observed to increase with increasing ionic strength but decrease with the addition of glycerol or PEG.

Introduction

One of the most important classes of proteins is the integral membrane proteins. Thus, an understanding of the solvents and processes used in membrane protein crystallization is of central importance in structural biology. As these proteins are not readily soluble in water, detergent containing solutions are needed in order to dissolve them in aqueous media, separate them from the phospholipids of the cell membrane, and cause them finally to crystallize out of solution. The phase map for protein crystallization is greatly complicated by the fact that the detergent and protein solubilities are selectively altered by the chemical

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additives that are used for crystallization. Electrically neutral detergents are preferable for use in protein crystallization as they are less likely to alter the charge properties of solubilized proteins.

Previously, small angle neutron scattering (SANS) has been used to characterize the micelle structure of the detergents lauryldimethylamine-N-oxide (LDAO) and n-octyl-b-D-glucoside (OG) (Thiyagarajan&Tiede, 1994), detergents which have been used in the crystallization of bacterial photosynthetic reaction center proteins (Michel, 1982; Chang et al., 1985), at the conditions used for protein crystallization. In this paper, the micelle structures in dilute solutions of the non-ionic detergent decanoyl-N-methyl glucamide (DMG) and the zwitterionic detergent 1,2-diacyl-sn-glycero-3-phosphocholine (SPC) are studied. Recently, very dilute solutions of these detergents have been used successfully in the final stages of the crystallization of the cytochrome bc_1 complex (Xia et al, 1997), first to cause the precipitation of contaminants for removal and finally to induce the precipitation from solution and crystallization of the protein complex itself. In this work SANS is used to examine the formation of DMG and SPC micelles as a function of the respective detergent concentration and ionic strength. Furthermore, the effects of glycerol and polyethylene glycol (PEG) are studied alone as well as in combinations similar to those used for actual membrane protein crystallization.

Material and Methods

All reagents were obtained from commercial sources and used without further purification. The detergents DMG and SPC were purchased from Sigma and were 98% and

99% pure, respectively. All experiments were conducted on solutions in D_2O, which came from Aldrich and was buffered to pH 7 using 50mM MOPS and 25mM NaOH (half titration of MOPS). MOPS was obtained from Sigma and NaOH from E K Industries, Addison IL. These reagents were 99.5% pure and ACS reagent grade, respectively. ACS reagent grade potassium chloride from E K Industries and sodium chloride from Mallinckrodt AR were used study the influence of ionic strength on the micellar detergent solutions. The other additives used were 99% glycerol and SigmaUltra PEG 3350 from Sigma. 97% deuterated glycerol from Aldrich was used as well in the preparation of some DMG solutions to allow for more contrast between the detergent micelles and the glycerol containing solvent and reduce the incoherent background scattering.

The stock sample solvents were prepared by adding all of the components except for the detergents and D_2O by weight to a fixed volume of D_2O. The sample solutions containing detergents were prepared by adding the detergent by weight to a fixed volume of stock solution for a concentration of 10 mg detergent per 1 ml of stock solvent. Solutions at other detergent concentrations were prepared from these solutions by volumetric dilution with the stock solvent. The concentrations of the additives in the DMG and SPC solutions studied given in the tables below are in mg per ml D_2O or mmol per ml D_2O. All solutions were prepared fresh and SANS experiments were completed within a week.

SANS Measurements

The solutions were inserted in cylindrical Suprasil cells with 5 mm optical path length. Experiments on SPC were carried out at ambient temperature (20 – 25 °C). In case of

DMG, the temperature was kept constant at 25 °C using a water bath since DMG tended to form crystalline precipitates at lower temperatures. SANS measurements were carried out using the SAD time-of-flight small-angle neutron diffractometer at the Intense Pulsed Neutron Source at Argonne National Lab (Thiyagarajan et al., 1997). SAD uses pulsed neutrons with wavelength in the range 0.5-14 Å and a fixed sample-to-detector distance of 1.504 m. The scattered neutrons are measured by using a 20cm x 20cm area detector consisting of a 64 x 64 array of position sensitive gas filled proportional counters while the scattering patterns for neutrons of different wavelengths are measured separately by binning the neutrons counted into 67 time channels based on their time of flight t from the source to the detector. These time channels have a constant relative width $\Delta t/t = 0.05$, where Δt is the width of a time channels. The instrument provides a useful range of momentum transfer of 0.005 - 0.35 $Å^{-1}$ in a single measurement. The momentum transfer Q is related to the incident neutron wavelength λ and the scattering angle θ by the equation

$$Q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right). \qquad (1)$$

The scattering data for each sample are reduced and placed on an absolute scale following the routine procedures described by Thiyagarajan, et al. (1997).

Analysis Results

The SANS data for a typical detergent concentration series is displayed in Fig.1. The SANS scattering patterns were initially interpreted using the Guinier approximation (Porod, 1982; Guinier, 1939),

$$I(Q) = I_0 \exp\left(-Q^2 R_g^2/3\right), \qquad (2)$$

where I_0 is the intensity extrapolated to Q=0 and R_g is the average radius of gyration of the scattering particles. The R_g was observed to increase with increasing detergent concentration for all of the solution systems studied. Some of the solutions with higher DMG concentrations were also evaluated using the modified Guinier approximation for rod-like forms (Porod, 1982), given by the equation

$$QI(Q) = I_C \exp\left(-Q^2 R_C^2/2\right). \qquad (3)$$

Here, I_C is the value of the product of Q and the intensity extrapolated to Q=0 and R_C is the cross-sectional radius of gyration of the rod-like particle. This analysis showed that the larger micelles of both detergents were indeed rod-like in form with an approximate R_C of 10 Å, corresponding to an approximate radius of 14 Å if a circular cross section is assumed. An example of the modified Guinier plots for rod-like forms is shown in Figure 2. The linearity of the data in this plot demonstrates that these larger micelles formed at higher detergent concentrations are elongated rather than spherical in form.

Taken together, the results of the preliminary analysis using the Guinier approximation and the modified Guinier approximation for rod-like forms suggest that the detergents form elongated micelles that increase in length with concentration. Thus, the scattering data should be well described by either the scattering from a circular cylinder or a prolate ellipsoid of rotation. The scattering from a circular cylinder (Fournet, 1951; Pedersen, 1997) of length L and radius R is given by

$$I(Q) = I_0 \int_0^{\pi/2} \left[\frac{2J_1(QR\sin\alpha)}{QR\sin\alpha} \frac{\sin((QL\cos\alpha)/2)}{(QL\cos\alpha)/2} \right]^2 \sin\alpha d\alpha , \qquad (4)$$

where J_1 is the first order Bessel function. The scattering from an ellipsoid of rotation cylinder (Guinier, 1939; Pedersen, 1997) with two semi axes of length *a* and one semi axis of length *b* is given by

$$I(Q) = I_0 \int_0^{\pi/2} \left[\frac{3[\sin(QR(a,b,\alpha)) - QR(a,b,\alpha)\cos(QR(a,b,\alpha))]}{(QR(a,b,\alpha))^3} \right] \sin\alpha d\alpha , \qquad (5)$$

where

$$R(a,b,\alpha) = \sqrt{a^2\sin^2\alpha + b^2\cos^2\alpha} . \qquad (6)$$

The integration over α in equations 4 and 5 represents the averaging over all possible orientations of the scattering particle relative to the incident beam. The scattering curves resulting from non-linear least squares fitting of the data to equations 4 and 5 are nearly indistinguishable as can be seen from figure 3. The fit parameters and the resulting particle volumes for the DMG and SPC solutions studied are presented in Tables 1 and 2, respectively. Results for the SPC solutions with 0.2 M and 2M KCl, not included in Table 2, showed the same trends as those solutions with the same concentrations of NaCl. The radius of the scattering particles remained nearly constant for both detergents in all solutions studied. The average radii of the DMG and SPC obtained from the fits are 13.73 ± 0.03 Å and 14.58 ± 0.01 Å, respectively. With the exception of the DMG-containing solutions with both low concentration and high ionic strength, the length of the particle and thus its volume tend to increase with increasing detergent concentration. The micelle size was already quite large at low concentrations of DMG in the high ionic strength solutions.

The concentrations of the detergents in each solution and the values of I_0 and the scattering particle volume V can be used to calculate the critical micelle concentration or *CMC* for that solvent system. I_0 is related to the volume of the scattering particles by the equation

$$I_0 = nV^2(\Delta\rho)^2, \tag{7}$$

where n is the number density of the scatterers and $\Delta\rho$ is the scattering length density difference between the scatterers and the solvent. Since the mass of each micelle is Vd, where d is the density of the detergent in micelle form, the concentration of the detergent in micelles and thus contributing to the scattering in mg/ml is given by

$$c - CMC = nVd, \tag{8}$$

where c is the total concentration of detergent in the solution. Thus, the CMC is determined by fitting to the line

$$I_0(c)/V(c) = A(c - CMC), \tag{9}$$

where the slope $A = (\Delta\rho)^2/d$ includes the effects of the mass density d of the particles in solution and the contrast between the micelles and the solvent. An example of a fit to determine the *CMC* is shown in figure 4. The values for the *CMC* for the various solvent systems studied for DMG and SPC are given in Tables 3 and 4, respectively. For both detergents the *CMC* decreases with increasing ionic strength of the solvent but increases in the presence of glycerol and, in the case of SPC, in the presence of PEG.

These measurements also give information about the way in which the micelles form in solution. As seen from equation 7, the micelle number density n is proportional to I_0/V^2. Thus, the results presented in Tables 1 and 2 show that the micelle number density as well as

the micelle volume increases with increasing detergent concentration for all of the solvent systems studied. Therefore, these data show that the detergent in solution above the *CMC* is entering micelles both by lengthening existing micelles and by forming new ones.

Conclusions

The overall picture that comes out of this study is that both DMG and SPC in solution above the CMC are present in the form of elongated, rod-like micelles. The good agreement with the data of the fits to the form factors for both a circular cylinder and a prolate ellipsoid of rotation is not suprising as the true structure is probably neither but instead something intermediate between the two such as a cigar-like object or a rod with cylidrical endcaps assembeled from detergent molecules with their hydrophilic heads oriented outward toward the solvent and the hydrophobic tail groups protectively oriented inward. The observed cylindrical radius of about 14 Å is comparable to the length of a stretched detergent molecule. Detergent concentration and the presence of additives in the solvent had little effect on the measured radii of the micelles.

Above the CMC, the micelles of both detergents are observed to increase in both length and number density as the detergent concentration increased. Solvents with high salt concentrations and thus high ionic strengths enhanced micelle formation by lowering the CMC for the detergent in the solvent and producing longer micelles. In contrast, the nonionic additives PEG and glycerol appear to inhibit micelle formation, raising the CMC and causing shorter micelles to form.

These subtle changes in the structure of the detergent micelles in solution are helpful in providing a rough understanding of the role of these detergents in the crystallization

process. Since the detergents are used in this process at concentrations near the CMC, they presumably work through a selective relative solubility process similar to a solvent extraction process. With the proper choice of additives, the detergents will form mixed micelles with the protein. When the solution of mixed micelles is further diluted, the concentration is lowered below the *CMC* for the mixed micelle, causing the proteins to crystallize.

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Table 1. The effects of concentration and the presence of additives on the size and number density of DMG micelles in solution as determined from nonlinear least squares fits using the form factor for a circular cylinder.

Table 2. The effects of concentration and the presence of additives on the size and number density of SPC micelles in solution as determined from nonlinear least squares fits using the form factor for a circular cylinder.

Table 3. The effects of additives on the *CMC* of DMG solutions.

Table 4. The effects of additives on the *CMC* of SPC solutions.

Figure 1. A plot of the data for a series of concentrations of SPC in buffered D_2O with no additives.

Figure 2. Modified Guinier plot for rod-like forms for the sample containing 10 mg/ml DMG in buffered D_2O with 1 M NaCl. The linearity of the data indicates that the DMG micelles in this sample are elongated rather than spherical. The value R_C = 9.5 Å indicates a circular rod radius of 13.4 Å and is consistent with the length of a stretched DMG molecule.

Figure 3. A comparison of the fits of the form factors for a circular cylinder and a prolate ellipsoid of rotation to the data for a solution of 5 mg/ml SPC in buffered D_2O with 0.2 M NaCl.. The two fits are similar in quality, overlay nearly perfectly, and give compatible information about the micelle dimensions.

Figure 4. The plot used to determine the *CMC* of the DMG solution in buffered D_2O in the absence of other additives. The point at which the fitted line intercepts the concentration axis, 1.58±0.05 mg/ml, is the *CMC*

[DMG] (mg/ml)	I_0 (cm^{-1})	*R* (Å)	*L* (Å)	*V* (10^3 Å^3)	I_0/V^2 (10^{38} cm^{-7})
No Additives					
2	0.0349(7)	9.0(12)	53(4)	13(4)	1.9(8)
2.5	0.0701(12)	12.0(9)	57(3)	26(4)	1.07(22)
3	0.1106(15)	12.9(5)	59(2)	31(3)	1.18(15)
5	0.344(3)	13.7(2)	68.5(11)	40.4(14)	2.11(10)
7	0.612(4)	13.40(15)	79.6(9)	44.9(11)	3.03(11)
10	1.092(6)	13.46(10	85.3(8)	48.5(8)	4.64(11)
2 M NaCl					
3	0.689(16)	14.51(22)	194(5)	1289(5)	0.42(3)
7	1.278(11)	14.10(9)	141.6(15)	88.5(15)	1.63(4)
1 M NaCl					
1.5	0.275(18)	10.1(6)	232(17)	74(11)	0.51(11)
3	0.209(3)	13.1(4)	73.8(20)	40.0(25)	1.31(12)
3	0.322(5)	13.8(3)	116(3)	70(3)	0.67(4)
5	0.638(7)	13.78(18)	111.1(17)	66.3(20)	1.45(6)
7	0.924(6)	13.29(12)	103.7(11)	57.6(12)	2.79(9)
10	1.519(15)	13.70(12)	115.4(15)	68.1(15)	3.27(11)
0.182 M NaCl					
3	0.1242(15)	13.1(4)	66.0(18)	35.5(23)	0.99(9)
3	0.1212(20)	12.0(6)	60.5(25)	27(3)	1.62(25)
5	0.360(3)	13.37(22)	74.4(13)	41.7(15)	2.07(11)
7	0.657(5)	13.70(17)	83.7(11)	49.3(14)	2.70(11)
10	1.095(7)	13.61(10)	87.6(9)	51.0(9)	4.21(11)
185 mg/ml D-glycerol					
3	0.0817(10)	14.2(7)	49.1(25)	31(3)	0.84(13)
5	0.2828(20)	13.7(3)	56.9(11)	33.4(14)	2.54(15)
10	0.939(5)	13.87(11)	66.9(7)	40.4(8)	5.75(16)
185 mg/ml D-glycerol, 1M KCl					
3	0.1756(15)	14.1(3)	67.4(13)	41.9(19)	1.00(6)
10	1.187(6)	13.98(10)	86.4(8)	53.1(8)	4.21(10)
44 mg/ml PEG, 185 mg/ml glycerol, 0.182 M KCl					
5	0.158(3)	11.6(9)	44(3)	19(3)	4.5(11)
10	0.405(5)	12.3(4)	57.8(18)	27.4(19)	5.4(5)

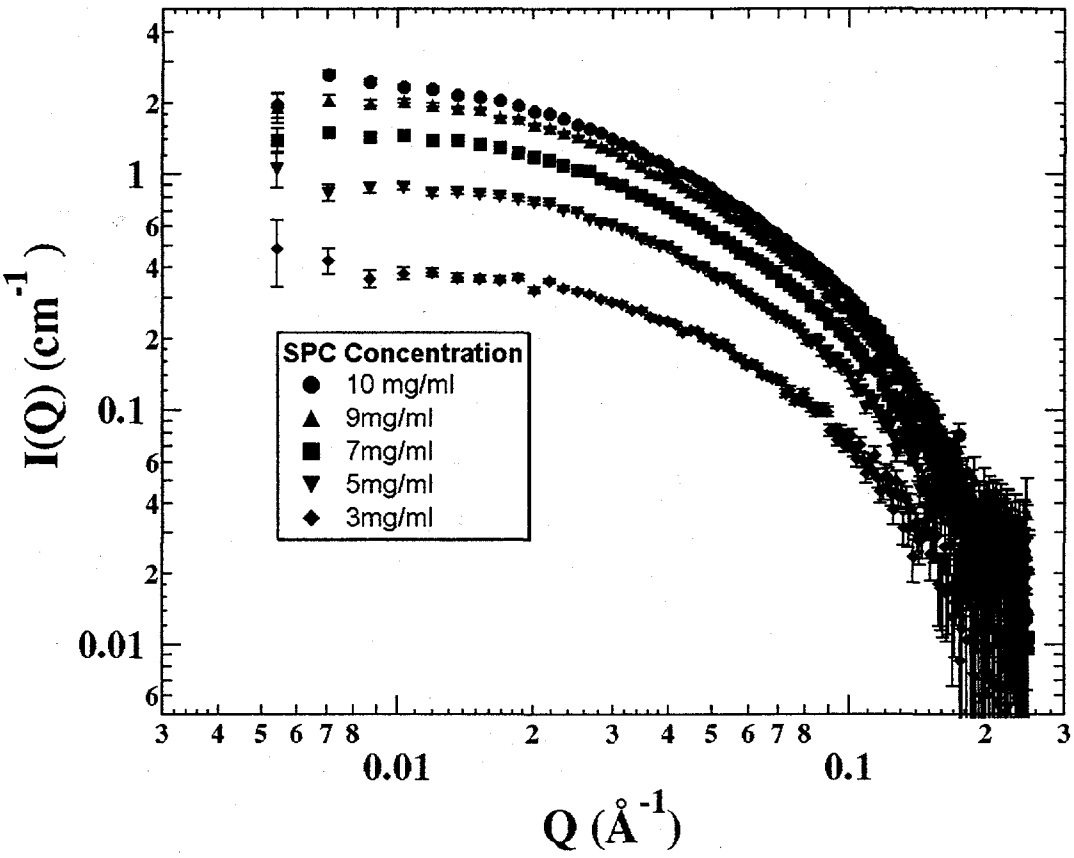
[SPC] (mg/ml)	I_0 (cm^{-1})	R (Å)	L (Å)	V (10^3 Å^3)	I_0/V^2 (10^{-38} cm^{-7})
No Additives					
0.3	0.383(4)	13.28(20)	100.3(16)	55.6(19)	1.24(6)
0.5	0.896(11)	13.74(11)	120.6(13)	71.5(14)	1.75(5)
0.7	1.472(8)	14.21(7)	133.2(10)	84.5(10)	2.06(4)
0.9	2.076(13)	14.35(8)	141.4(12)	91.5(12)	2.48(5)
1.0	2.402(14)	14.24(3)	144.9(11)	92.3(10)	2.82(5)
1 M NaCl					
0.1	0.0953(20)	13.9(7)	78(3)	47(3)	0.43(6)
0.3	0.578(4)	14.42(13)	112.9(12)	73.8(15)	1.06(3)
0.5	1.147(8)	14.61(9)	133.2(12)	89.3(14)	1.44(3)
0.7	1.718(8)	14.90(7)	142.4(11)	99.3(12)	1.74(3)
1.0	2.750(14)	14.91(5)	152.4(10)	106.5(10)	2.42(4)
0.2 M NaCl					
0.3	0.367(3)	14.26(16)	93.6(11)	56.1(22)	1.03(4)
0.5	0.854(5)	14.44(10)	113.3(11)	76.4(13)	1.55(4)
0.7	1.351(8)	14.75(8)	121.8(10)	90.9(11)	1.94(4)
1.0	2.199(10)	15.11(5)	133.3(8)	98.9(11)	2.40(3)
185 mg/ml glycerol					
0.3	0.348(3)	13.70(17)	100.3(13)	59.1(17)	1.00(4)
0.5	0.901(5)	14.16(9)	124.8(10)	78.6(12)	1.46(3)
0.7	1.362(7)	14.52(7)	133.8(9)	88.6(10)	1.74(3)
1.0	2.403(12)	14.48(5)	149.2(10)	98.2(10)	2.49(4)
44 mg/ml PEG					
0.3	0.241 (3)	13.66(24)	95.7(17)	56.1(22)	0.77(4)
0.5	0.839(6)	14.09(10)	122.4(12)	76.4(13)	1.44(4)
0.7	1.400(8)	14.47(7)	138.2(11)	90.9(12)	1.69(3)
1.0	2.291(13)	14.39(6)	151.9(11)	98.9(11)	2.34(4)

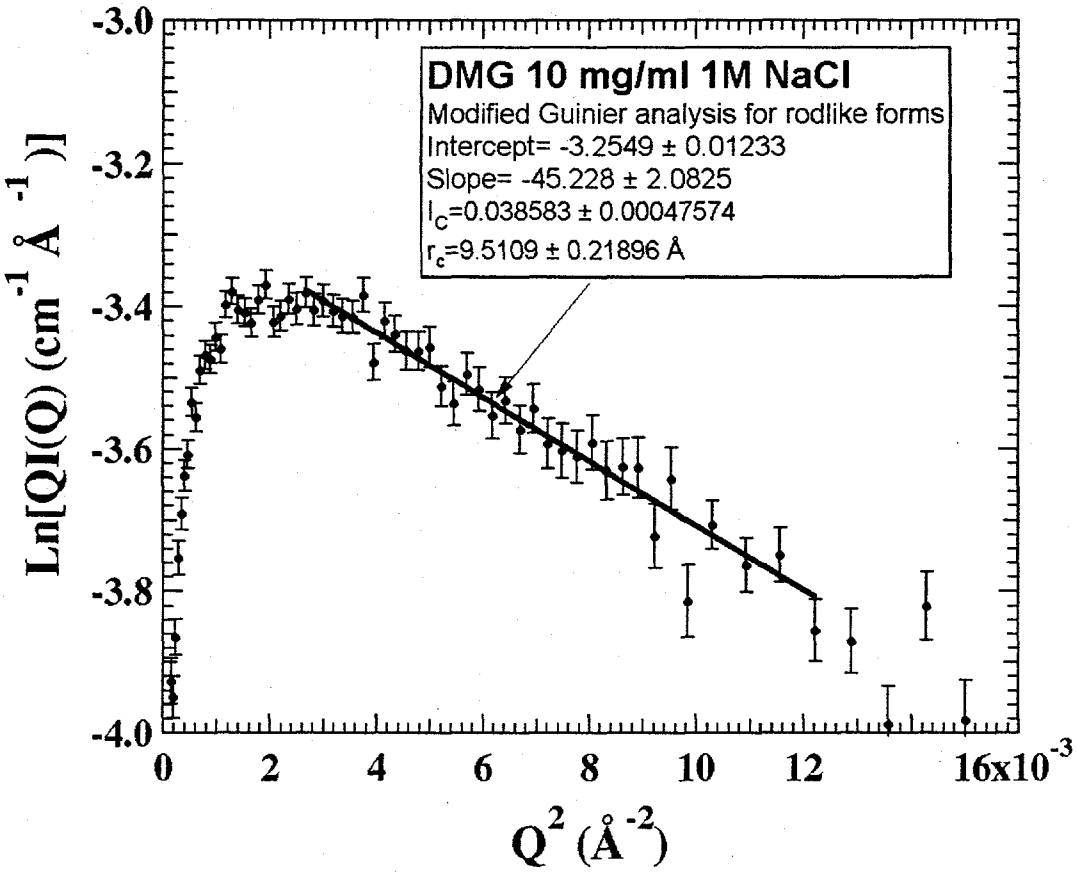
Table 3. The effects of additives on the *CMC* of DMG solutions

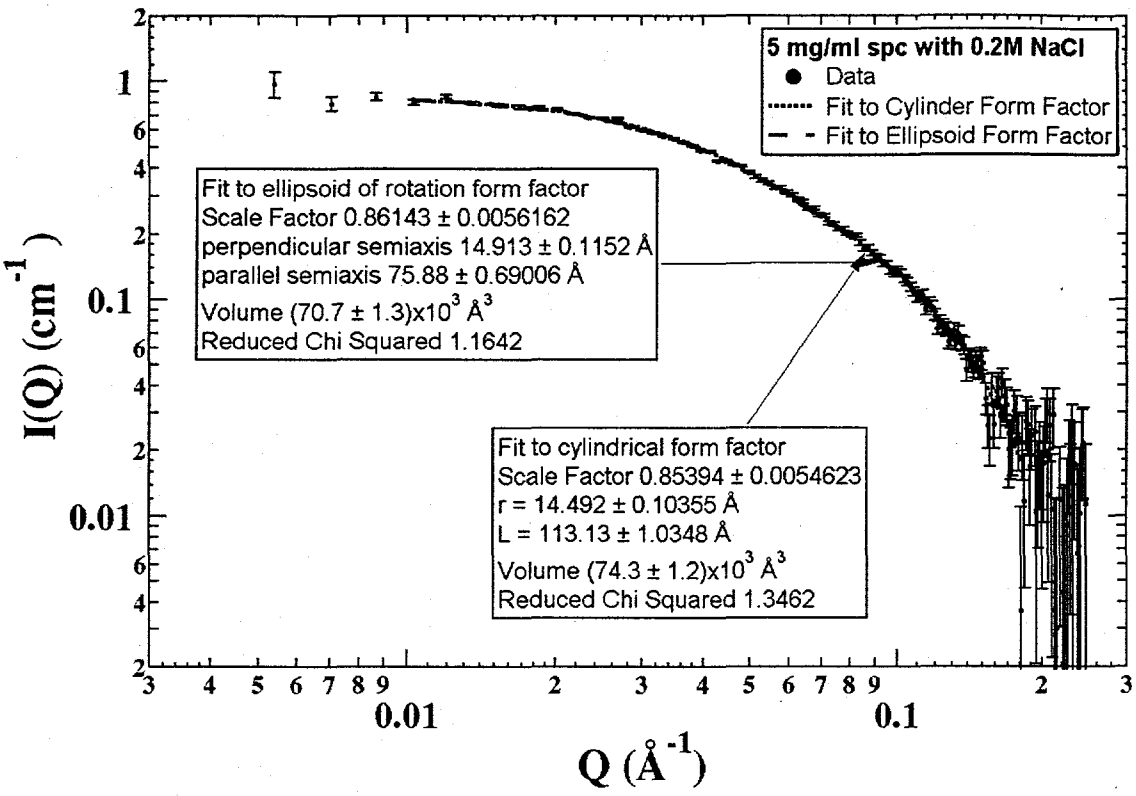
Additives	***CMC*** (mg/ml)
None	1.58±0.05
0.182 M NaCl	1.56±0.20
1 M NaCl	0.98±0.17
2 M NaCl	0.64±0.03
185 mg/ml D-glycerol	2.11±0.24
185 mg/ml D-glycerol with 1 M NaCl	1.39±0.07

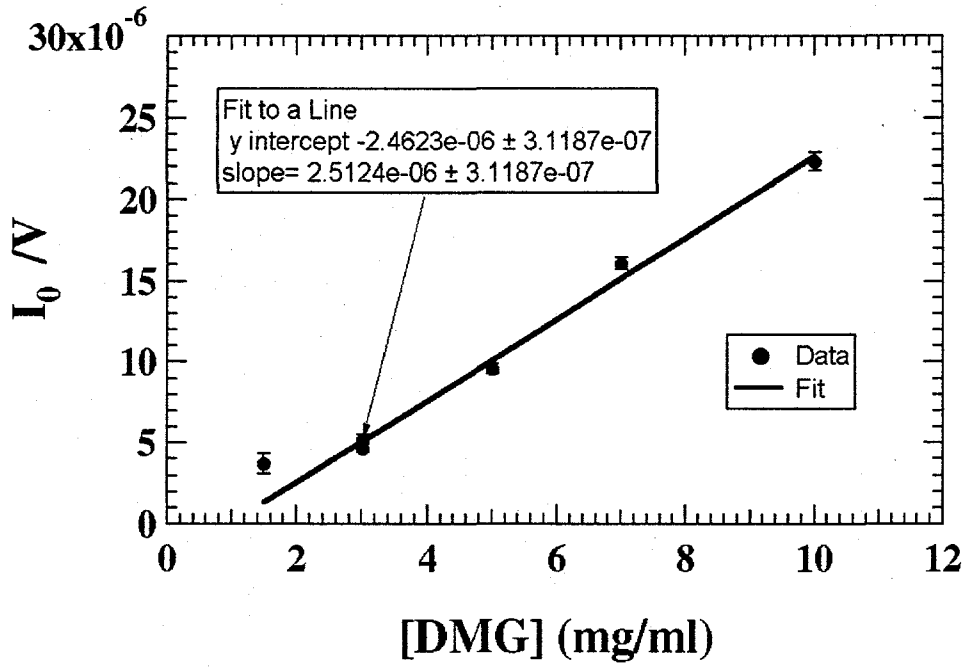
Table 4. The effects of additives on the *CMC* of SPC solutions

Additives	***CMC*** (mg/ml)
none	0.41±0.12
1 M KCl	0.26±0.11
0.2 M KCl	0.56±0.11
1 M NaCl	0.09±0.07
0.2 M NaCl	0.38±0.10
185 mg/ml glycerol	0.70±0.10
44 mg/ml PEG	1.23±0.10


SPC Concentration
10 mg/ml
9mg/ml
7mg/ml
5mg/ml
3mg/ml
I(Q) (cm-1)
Q (Å-1)
0.01
0.1
1


DMG 10 mg/ml 1M NaCl
Modified Guinier analysis for rodlike forms
Intercept= -3.2549 ± 0.01233
Slope= -45.228 ± 2.0825
I_c=0.038583 ± 0.00047574
r_c=9.5109 ± 0.21896 Å
Ln[QI(Q) (cm^{-1} Å^{-1})]
-3.0
-3.2
-3.4
-3.6
-3.8
-4.0
0
2
4
6
8
10
12
16x10^{-3}
Q^2 (Å^{-2})


5 mg/ml spc with 0.2M NaCl
Data
Fit to Cylinder Form Factor
Fit to Ellipsoid Form Factor
Fit to ellipsoid of rotation form factor
Scale Factor 0.86143 ± 0.0056162
perpendicular semiaxis 14.913 ± 0.1152 Å
parallel semiaxis 75.88 ± 0.69006 Å
Volume (70.7 ± 1.3)x10^3 Å^3
Reduced Chi Squared 1.1642
Fit to cylindrical form factor
Scale Factor 0.85394 ± 0.0054623
r = 14.492 ± 0.10355 Å
L = 113.13 ± 1.0348 Å
Volume (74.3 ± 1.2)x10^3 Å^3
Reduced Chi Squared 1.3462
I(Q) (cm^-1)
Q (Å^-1)
1
0.1
0.01
0.01
0.1


30x10^-6
25
20
15
10
5
0
I0 /V
Fit to a Line
y intercept -2.4623e-06 ± 3.1187e-07
slope= 2.5124e-06 ± 3.1187e-07
Data
Fit
0
2
4
6
8
10
12
[DMG] (mg/ml)