Dilution of protein-surfactant complexes: A fluorescence study

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Abstract: Dilution of protein-surfactant complexes is an integrated step in microfluidic protein sizing, where the contribution of free micelles to the overall fluorescence is reduced by dilution. This process can be further improved by establishing an optimum surfactant concentration and quantifying the amount of protein based on the fluorescence intensity. To this end, we study the interaction of proteins with anionic sodium dodecyl sulfate (SDS) and cationic hexadecyl trimethyl ammonium bromide (CTAB) using a hydrophobic fluorescent dye (sypro orange). We analyze these interactions fluoroumetrically with bovine serum albumin, carbonic anhydrase, and beta-galactosidase as model proteins. The fluorescent signature of protein-surfactant complexes at various dilution points shows three distinct regions, surfactant dominant, breakdown, and protein dominant region. Based on the dilution behavior of protein-surfactant complexes, we propose a fluorescence model to explain the contribution of free and bound micelles to the overall fluorescence. Our results show that protein peak is observed at 3 mM SDS as the optimum dilution concentration. Furthermore, we study the effect of protein concentration on fluorescence intensity. In a single protein model with a constant dye quantum yield, the peak height increases with protein concentration. Finally, addition of CTAB to the protein-SDS complex at mole fractions above 0.1 shifts the protein peak from 3 mM to 4 mM SDS. The knowledge of protein-surfactant interactions obtained from these studies provides significant insights for novel detection and quantification techniques in microfluidics.

Keywords: protein separation; surfactant-protein complex; microfluidics

Introduction

The interactions of proteins with surfactants have been studied extensively due to a wide range of applications in the field of separation, drug delivery, cosmetics, and detergency,^{1,2} or as a model in mimicking

the behavior of cell membrane proteins.^{3–6} Microfluidic electrophoresis is a strong tool in separation and detection of proteins.^{7,8} Fast analysis, high efficiency, small sample volume and automation are among the advantages of using these devices in protein analysis.^{9,10} Even though miniaturization has many advantages, detection of low concentrated proteins in a small sample volume is still challenging. Hydrophobic fluorescent probes are among the methods used to enhance the detection sensitivity in low concentrations.¹¹ However due to hydrophobic nature, these probes also bind to the free surfactants micelles, creating a high background signal.¹² To overcome this problem, a dilution step has been integrated on the electrophoresis microchip to reduce the fluorescence contribution of free surfactant micelles (Fig. 1). Even though detection analysis has been improved to

Abbreviations: betagal, beta-galactosidase; BSA, bovine serum albumin; CA, carbonic anhydrase; CD, circular dichroism; CE, capillary electrophoresis; CMC, critical micelle concentration; CTAB, hexadecyl trimethyl ammonium bromide; DTT, beta-mercaptoethanol; MS, mass spectroscopy; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate.

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Figure 1. Schematic of the dilution concept in protein sizing microchip. Protein–surfactant complex at concentration above the surfactant CMC enters the separation channel. Dilution is performed before the detection point in order to reduce the fluorescence of free micelles by reducing the surfactant concentration below CMC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

a great extent, no investigation has been performed on establishing an optimum dilution concentration or a correlation between fluorescence peaks (electropherograms) and protein concentration. To this end, more insights are needed in understanding protein–surfactant interactions with the hydrophobic fluorescent dye in order to improve the detection and quantification of proteins on microfluidic platforms.

Binding of ionic surfactants to proteins at a saturation concentration above critical micelle concentration (CMC) alters the native structure by initiating the unfolding or denaturing process.¹³ It is believed that at low concentrations, the binding of surfactant molecules are governed by electrostatic forces, however at concentrations above CMC hydrophobic interactions are dominant.^{14–16} Above CMC, surfactant molecules dynamically bind to protein to induce denaturation, impart additional charges and make proteins structurally similar.^{17–20} Therefore, at saturation concentration, surfactant acts as a denaturant by unfolding the protein and minimizing the influence of the structure in protein electrophoresis. By providing a constant charge/mass, separation of proteins is achieved based on the differences in molecular weight. Although, the dynamic and kinetics of surfactant–protein interactions has been studied extensively,^{21–26} to the best of our knowledge, the effect of dilution on the fluorescence intensity of protein–surfactant complexes has not been studied. Moreover, a great volume of literature on interaction of protein and surfactants is focused on single protein Bovine Serum Albumin (BSA).^{1,27–31} Due to the wide range of differences in protein structure, there is a need for analyzing these interactions in a system of multiple proteins with different molecular weights.

Understanding the nature of protein–surfactant interactions has been assisted in large by numerous structural analytical techniques such as circular dichroism (CD), nuclear magnetic resonance (NMR),

mass spectroscopy (MS), and capillary electrophoresis (CE). Although, these complex techniques have greatly advanced the field of proteomics, their application is limited to differences in protein structures and properties. Moreover, the procedure is lengthy and requires many hands-on steps. This study is aimed to address some of the key points in dilution of protein–surfactant complex through a rapid and low-tech fluorometric technique. We analyze interactions of three proteins bovine serum albumin (BSA), carbonic anhydrase (CA) and beta-galactosidase (betagal) with sodium dodecyl sulfate (SDS), using a hydrophobic dye at different dilution points. In addition, we investigate the effect of cationic surfactant additives such as hexadecyl trimethyl ammonium bromide (CTAB) added for the purpose of controlling the electro-osmotic flow in capillary electrophoresis.^{32,33} Based on our experimental results, we propose a fluorescence model to explain the interactions of the dye with free and bound surfactant micelles. The presented results on the dilution of protein–surfactant complexes significantly contribute to the development of novel detection and separation techniques in microfluidics platforms.

Results and Discussion

Optimum SDS and dye concentration

The concentration of SDS was chosen at 15 mM (4.3 mg/mL) three times more than the defined stoichiometry (1.4 g SDS/1 g protein at $[SDS] > CMC$),^{13,34} allowing for a complete binding and a full range of study with free and bound micelles (protein micelles). The optimum dye concentration was determined by measuring the fluorescence of 15 mM SDS with various dye concentrations [Fig. 2(a) inset]. A plateau at $5\times$ shows a complete uptake of the dye by the micelles. Furthermore, the fluorescence intensity of various concentrations of SDS with constant dye at $5\times$ was measured [Fig. 2(a)]. Two observations can be made from Figure 2(a), first the sudden increase in fluorescence signal at ~ 4 mM marks this concentration as the critical micelle concentration (CMC) of SDS in Tris–glycine buffer.²⁸ Upon the formation of micelles, the hydrophobic dye resides within the micelles, resulting in an increase in fluorescence. Second, the fluorescence count reaches a constant value at 15 mM SDS, due to a complete uptake of dye molecules by micelles. At this concentration, considering the aggregation number of SDS as 80 (74 in water³⁵), the number of SDS micelles present is 1.13×10^{20} .

Dilution of SDS–dye

After establishing the optimum SDS and dye concentration, the effect of dilution on the fluorescence intensity of SDS–dye complex was investigated with 15 mM SDS and $5\times$ dye as the start point of dilution. This complex is serially diluted by addition of buffer in order to evaluate the interaction of the dye with

Figure 2. (a) Uptake of dye by SDS micelles with constant dye concentration (5 $\times$). The inset shows the uptake of dye by fixed 15mM SDS at different dye concentrations. (b) Dilution of SDS-Dye with 15 mM SDS and 5 $\times$ sypro orange dye. The signal is normalized with respect to maximum and minimum values. The error bars are obtained from three consecutive measurements. Conditions: 1 $\times$ Tris-glycine buffer, pH 8.6, room temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

SDS micelles in the absence of protein. Figure 2(b) shows the normalized plot of fluorescence versus SDS concentration. Given that the emission of the dye is proportional to the number of micelles, the linear decrease in fluorescence suggests a reduction in micellar number density by dilution from 15 mM to 6 mM SDS. The fluorescence intensity is significantly reduced from 6 mM to 4 mM (CMC) suggesting that the micellar breakdown starts within this concentration range and not exactly at CMC. Fluorescence reaches zero by further dilution below CMC, where SDS molecules are present as monomers.^{36,37}

Considering the fluorescence behavior of SDS and dye, a possible model can be proposed as:

$$Fl = \sum_{i=1}^{N_{sds}} (A \times n_{dye} \times \varphi_{sds})_i \quad (1)$$

Table I. Comparison of the Proposed Fluorescence Model with the Experimental Values Above CMC

SDS (mM)	#Micelles	Fl (calculated)	Fl (experimental)	% error
15	1.13×10^{20}	1.67×10^6	1.67×10^6	–
10	7.53×10^{19}	1.11×10^6	1.13×10^6	1.8
8	6.02×10^{19}	8.92×10^5	8.82×10^5	1.1
6	4.52×10^{19}	6.69×10^5	6.60×10^5	1.4

Number of micelles is calculated based on the aggregation number and concentration of SDS.

where Fl is the fluorescence count, A is the absorption count per dye molecule, n_{dye} is the number of dye molecules in each micelle, φ_{sds} is the quantum yield of the dye molecules in the SDS micelles, and N_{sds} is the total number of SDS micelles in the solution. Since the concentration of the dye is unknown (5 $\times$), the exact number of dye molecules in the solution cannot be determined. We can estimate $\beta = A \times n_{dye} \times \varphi_{sds}$, assuming equal number of dye molecules per micelle, using the fluorescence count and the number of micelles in the solution.

With 15 mM SDS and 5 $\times$ dye as the first dilution point (15 mM SDS = 1.13×10^{20} micelles) $\beta = 1.48 \times 10^{-14}$ count/micelle.

The fluorescence count of other dilution points up to CMC (before the micellar breakdown) can be determined using constant β and the number of SDS micelles. The calculated values confirm a linear decrease in number of micelles and are in a good agreement with the experimental values as tabulated in Table I.

Dilution of protein-SDS-dye

After studying the interaction of dye with SDS micelles, the dilution of protein-SDS-dye complex was examined using three model proteins: BSA (583 amino acids), CA (259 amino acids), and betagal (1052 amino acids). The starting concentration of SDS, proteins and dye was 15 mM, 1 mg/mL, and 5 $\times$, respectively. Proteins were denatured prior to dilution by 0.2 M beta-mercaptoethanol and heated at 95°C for 5 min. Beta-galactosidase denatures into four equal subunits each at ~112 kDa.

Figure 3 shows the fluorescence intensity of protein and protein-free complexes at various dilutions. Considering the contribution of bound micelles, Eq. (1) is modified as:

$$Fl = \sum_{i=1}^{N_{sds}} (A \times n_{dye} \times \varphi_{sds})_i + \sum_{j=1}^{N_p} (A \times n_{dye} \times \varphi_p)_j \quad (2)$$

where N_p is the number of bound micelles, and φ_p is the quantum yield of the dye molecules within the bound micelles. Here, we have neglected the contribution of protein alone, assuming a complete binding

Figure 3. Dilution of SDS-protein complex. The inset shows the fluorescent profile of proteins after subtracting the background SDS fluorescence. The error bars are obtained from three consecutive measurements. Conditions: 15 mM SDS, 1 mg/mL protein, denatured by heat (5 min at 95°C) and β -mercaptoethanol (0.2M), $5\times$ sypro orange dye, $1\times$ Tris-glycine buffer at 8.6 pH, room temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of SDS molecules to the protein at saturated SDS concentration. Note that the individual values of A , n_{dye} , and ϕ cannot be determined, therefore we can only estimate the product of these factors. We have assumed equal number of dye molecules per free and bound micelle, neglecting the possible differences in micellar hydrophobicity and size. Considering constant $\beta = 1.48 \times 10^{-14}$ count/micelle, calculated in the previous section, we define $\alpha = A \times n_{\text{dye}} \times \phi$, therefore, the difference in fluorescence of proteins at 15 mM SDS in Figure 3 is attributed to variation in quantum yield of the dye with bound micelles as calculated in Table II. Number of free micelles (N_{sds}) and bound micelles (N_{p}) at 15 mM SDS was determined using the ratio of 1.4 g SDS/g protein.

Our results show that proteins with equal concentration yield different fluorescence intensities. Even though the concept of gel electrophoresis is based on the differences in size only, and dye is used as a marker of migration time, we speculate that for analysis based on fluorescence yield only in a gel-free solution, physical and chemical properties of the dye are key factors to be considered. As shown in Table

II, the dye quantum yield with bound micelles is higher than free micelles and varies for each protein.

Figure 3 shows three distinct regions in the dilution profile of protein-SDS-dye: SDS dominant region: 15–6 mM SDS, breakdown region: 6–3 mM SDS, and protein dominant region: below 3 mM SDS. In the SDS dominant region, the dilution curve of protein complex shows a similar slope as protein-free complex, suggesting a linear reduction in the number of free micelles. To evaluate this argument, the dilution of free and bound micelles is compared in Table III.

The number of free micelles at each point was determined based on the dilution factor, whereas the number of bound micelles was calculated based on the constant values of α and β and the experimental fluorescence count [Eq. (2)]. As shown in Table III, free micelles are diluted more significantly compare to bound micelles, resulting in a linear decrease in fluorescence. In the breakdown region, breaking of free SDS micelles results in the release of the dye from these micelles and its uptake by the bound micelles with higher quantum yield. This is evident by a slight increase in fluorescence count within this region as shown in Figure 3.

Finally, in the protein dominant region, with no free SDS micelles in the solution, the only fluorescence contribution is from bound micelles. Further dilution in this region results in a rapid reduction in fluorescence (increased slope) due to a decrease in number of protein molecules and consequently the number of bound micelles.

In protein electrophoresis, the concentration of SDS is above CMC to ensure a complete binding and unfolding of protein. However, a dilution step is necessary to minimize the contribution of free micelles by reducing the SDS concentration to monomers (below CMC). The electropherograms or the protein peaks are generated by subtracting the background fluorescence (SDS fluorescence). These peaks are shown as the inset in Figure 3. The maximum peak height was obtained at ~ 3 mM SDS with no free SDS micelles in the solution. By eliminating the contribution of free micelles, this dilution point is considered as the optimum SDS concentration for protein electrophoresis. It is important to note that even though the concentration of proteins is equal, the peak intensities are not identical due to a

Table II. Comparison of Quantum Yield of Sypro Orange Dye with BSA, CA, and Betagal at 1 mg/mL and 15 mM SDS

Protein	FI ($\times 10^6$)	N_{sds} ($\times 10^{19}$)	N_{p} ($\times 10^{19}$)	β ($\times 10^{-14}$) count/micelle	α ($\times 10^{-14}$) count/micelle
BSA	2.11	7.63	3.65	1.48	2.68
CA	2.30	7.63	3.65	1.48	3.20
betagal	1.80	7.63	3.65	1.48	1.84

Number of free and bound micelles was calculated using defined ratio of 1.4g SDS/1g protein.

Table III. Dilution of Free and Bound Micelles in SDS Dominant Region

SDS (mM)	N_{SDS} ($\times 10^{19}$)	% Dilution	$N_{\text{p BSA}}$ ($\times 10^{19}$)	% Dilution	$N_{\text{p CA}}$ ($\times 10^{19}$)	% Dilution	$N_{\text{p betagal}}$ ($\times 10^{19}$)	% Dilution
15	7.63	0	3.65	0	3.65	0	3.65	0
10	5.09	33	2.82	23	2.53	30	3.10	15
8	4.07	20	2.59	8	2.36	7	2.83	9
6	3.05	25	2.49	4	2.19	7	1.98	3

The number of free and bound micelles, N_{SDS} and N_{p} at 15 mM as the first point of dilution was determined using the ratio of 1.4 g SDS/1 g protein. Below 15mM N_{SDS} was calculated based on the dilution factor, N_{p} was estimated using fluorescence count and constant values of α and β [Eq. (2)]. Dilution of micelles at each concentration is with respect to the previous concentration.

difference in the dye quantum yield with each protein (Table II).

Effect of protein concentration

Effect of protein concentration on dilution of SDS–protein was studied with five concentrations of BSA ranging from 0.005 to 1 mg/mL while keeping the SDS concentration constant at 15 mM. The quantum yield of the dye with free and bound micelles was calculated based on the fluorescence counts of protein and SDS at the first point of dilution following the same procedure [Eqs. (1) and (2)]. The variation in fluorescence profiles with respect to protein concentration is shown in Figure 4. As the protein concentration increases more binding sites are available for SDS micelles. With constant number of total SDS micelles (15 mM), the number of free micelles in the solution is reduced as the number of bound micelles increases (Table IV). Higher quantum yield of the dye with bound micelles compare to free

micelles results in an increase in fluorescence at higher protein concentration. To evaluate the proposed fluorescence model [Eq. (2)] with different protein concentration, the fluorescence intensity at each protein concentration was calculated at 15 mM SDS (start point of dilution). The results are in a good agreement with the experimental values as shown in Table IV.

Figure 4 shows identical fluorescence profiles with 0.005 and 0.01 mg/mL protein as protein free complex (SDS–dye). At these concentrations, the number of free micelles is three orders of magnitude higher than bound micelles as given in Table IV. Hence, the contribution of bound micelles to the overall fluorescence is negligible due to the low concentration of protein. This is evident by an almost invisible protein peak after eliminating the SDS contribution (Figure 4, inset). It is important to note that with low protein concentration the protein peaks are shifted from 3 to 4 mM SDS as the point of micellar breakdown. This observation further emphasizes the significant contribution of free micelles at low protein concentrations. At higher concentration, a distinct protein peak is observed at 3 mM SDS. Figure 4 (inset) shows that the peak height is proportional to the protein concentration. Therefore, it can be concluded that with a single protein model with no variation in the dye quantum yield, the electropherograms can be used in quantification of the protein. We assume that the overlapping curve of 0.5 and 1 mg/mL samples at concentrations below 3 mM SDS is due to the insufficient number of dye molecules.

Figure 4. Effect of protein concentration on the dilution of SDS–BSA complex. The inset shows the fluorescence profile of the protein after subtracting the background SDS fluorescence. Conditions: 15 mM SDS, 1 $\times$ Tris–glycine buffer at 8.6 pH, 5 $\times$ sypro orange dye, room temperature. Protein was denatured by heat (5 min at 95°C) and β -mercaptoethanol (0.2M). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Effect of cationic surfactant CTAB

The CMC of CTAB in Tris–Glycine buffer was estimated to be ~ 0.4 mM following the same procedure for determination of CMC for SDS. CTAB concentrations above CMC were added to BSA–SDS complex, providing molar ratios (SDS/CTAB) from 7 to 30. Figure 5 shows the effect of CTAB addition on dilution of protein–SDS complex. The increase in fluorescence upon addition of CTAB can be explained by comparing the hydrophobicity of CTAB and SDS micelles. CTAB molecules with 16 carbons in

Table IV. Comparison of the Proposed Fluorescence Model with the Experimental Values at Various BSA Concentrations for the First Dilution Point (15 mM) SDS

BSA (mg/mL)	N_{sds}	N_{p}	Fl (calculated)	Fl (measured)	% Error
0	1.129×10^{20}	0	—	1.10×10^6	—
0.005	1.127×10^{20}	1.827×10^{17}	1.102×10^6	1.12×10^6	1.6
0.01	1.125×10^{20}	3.653×10^{17}	1.104×10^6	1.15×10^6	4
0.05	1.110×10^{20}	1.827×10^{18}	1.12×10^6	1.16×10^6	3
0.5	9.461×10^{19}	1.827×10^{19}	1.25×10^6	1.33×10^6	6
1	7.634×10^{19}	3.653×10^{19}	—	1.5×10^6	—

Values of N_{sds} and N_{p} were determined using the ratio of 1.4 g SDS/1 g protein.

the alkyl chain are more hydrophobic than SDS molecules with 12 carbons. It is possible that the quantum yield of the dye is higher with more hydrophobic CTAB micelles, or with mixed SDS–CTAB micelles. As shown in Figure 5, the fluorescence increases with higher CTAB concentration, due to a larger number of micelles present. Protein peak heights remain approximately constant at 3 mM SDS with 0.5 and 0.9 mM CTAB, however the intensity is reduced by 27 and 54% with 2 and 2.5 mM CTAB, respectively (Fig. 5, inset). Moreover, the peak is shifted to 4 mM SDS at these concentrations. Therefore, addition of CTAB with the purpose of surface modification in presence of SDS at mole fractions above 0.1 significantly affects the dynamic of protein–SDS complex in protein electrophoresis.

Conclusions

We have investigated the effect of dilution of protein–SDS complexes as an essential step for on chip protein electrophoresis by utilizing Sypro orange dye as a fluorescent hydrophobic probe. The most impor-

tant conclusion is that for protein analysis in a solution (gel-free), even though the binding stoichiometry of SDS to proteins is constant, and that the surfactant at concentrations above CMC makes proteins structurally similar, the overall fluorescence varies with different proteins. As a consequence, the fluorescence intensity of proteins with the same concentration is not equal. However, the quantification of protein based on the peak height can be achieved for a single protein where the quantum yield of the dye is constant. Moreover, the maximum peak height in electropherograms was observed at 3 mM SDS, below the SDS CMC value. At this concentration only the SDS micelles bound to protein contribute to the overall fluorescence intensity. Therefore, this concentration can be considered as the optimum SDS concentration in protein electrophoresis. The dilution profile of protein–SDS complexes shows three distinct regions: SDS dominant region above 6 mM SDS, breakdown region between 3–6 mM SDS and the protein dominant region below 3 mM SDS. In the SDS dominant region, the contribution of SDS micelles to the overall fluorescence signature is more pronounced than bound micelles. The fluorescence in this region is a linear function of free micelles number density. In the breakdown region, micelles start to break to monomers. The released dye is taken by bound micelles with higher quantum yield compare to free micelles, resulting in a slight increase in the fluorescence intensity. Finally, in the protein dominant region, with no free SDS micelles, the contribution of bound micelles becomes significant. Based on the experimental results, we propose a fluorescence model for the interaction of the dye with SDS and protein. In this model, the variation in fluorescence arises from the difference in the dye quantum yield with the micelles, assuming equal uptake of the dye molecules with free and bound micelles.

In a single protein model, quantification of protein concentration based on fluorescence intensities can be achieved with a constant dye quantum yield. This is evident by an increased in protein peak height with increasing concentration. Finally, addition of CTAB as a more hydrophobic surfactant compare to SDS at concentrations above CMC results in

Figure 5. Effect of CTAB on the dilution of SDS–BSA complex. The inset shows the fluorescence profile of the protein after subtracting the background SDS fluorescence. Conditions: 15 mM SDS, 1× Tris–glycine buffer at 8.6 pH, 5× sypro orange dye, room temperature. Protein was denatured by heat (5 min at 95°C) and β-mercaptoethanol (0.2M). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 6. (a) Agilent 2100 electropherogram and (b) pseudogel (protein 230) of BSA, CA, and betagal at 0.01 mg/mL. 4.5 kDa and 240 kDa are lower and upper markers, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

a shift in protein peak, where the mole fraction of CTAB was above 0.1. Lower concentrations of CTAB (0.5 and 0.9 mM) did not alter the dynamics of protein–SDS interactions. The presented results on the dilution of protein–surfactant complexes significantly contribute to the development of novel detection and separation techniques in microfluidics platforms, where quantification of protein can be achieved parallel to detection.

Materials and Methods

Chemicals

Bovine serum albumin (BSA, 66 kDa), beta-galactosidase from *E. coli* (betagal, 465 kDa), carbonic anhydrase from bovine erythrocytes (CA, 28 kDa), sodium dodecyl sulfate (SDS), hexadecyl trimethyl ammonium bromide (CTAB), beta-mercaptoethanol (DTT), and Tris–Glycine buffer were obtained from Sigma (St. Louis, MO). Sypro Orange protein staining dye (5000×) was purchased from Invitrogen (Carlsbad, CA). Aqueous solutions were prepared using ultra pure DI water (Elga Lab Water, Marlow, UK). All chemicals were used as received.

Protein–surfactant preparation

Solutions of SDS, CTAB, and protein were prepared in Tris–Glycine buffer (25 mM Tris/192 mM glycine, pH 8.6) and incubated at room temperature on a shaker (Innova 4080 incubator shaker, New Brunswick Scientific) for 1 h. At pH 8.6, all the proteins carry a net negative charge as required in electrophoresis separation (isoelectric point of BSA, CA, and betagal are 4.7,³⁸ 5.9,³⁹ and 4.61,⁴⁰ respectively). The protein–surfactant complexes were irreversibly denatured by beta-mercaptoethanol (0.2M) and heated (95°C for 5 min). After denaturing, sypro orange dye (5×) was added and the mixture of protein–surfactant–dye was serially diluted. All the experiments were carried out in triplicate at room temperature. The molecular weight and electropherogram of each protein was determined by gel electrophoresis on Agilent 2100 Bioanalyzer [Fig. 6(a,b)], following the manufacture protocol (Protein 230 kit).

Fluorescence measurements

The fluorescence (E_x/E_m : 470/570) of stained protein–surfactant solutions was measured at room temperature using a QM-4/2005SE spectrophotometer (Photon Technologies International, Birmingham, NJ). Sample was placed in a 10-mm length, 50 μ L quartz cuvette (Starna Cells, Atascadero, CA) and data was collected over time at 0.1 Hz. the cuvette was blanked with buffer prior to each measurement.

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