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Performance assessment of protein electrophoresis by using polyacrylamide hydrogel with porous structure modified with SDS micelles as template

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ABSTRACT: Separation processes are routinely used worldwide in biotechnology, chemical processing, wastewater treatment, and myriad other areas. Gel electrophoresis has been used for decades as a standard technique to separate charged biopolymers, such as DNA, RNA, and proteins. In this research, polyacrylamide hydrogels were synthesized in the presence of sodium dodecyl sulfate (SDS) micelles as nanotemplating agents in an effort to modify the internal porous microstructure of the gels. After removing these agents via a combination of passive and facilitated means, the gels were used during electrophoresis in order to assess the effects of its modified porous microstructure on protein separations. The results revealed that hydrogels containing 9% acrylamide and templated with SDS micelles in a 5–15% concentration range were the most effective materials in separating proteins in a range of 10–250 kDa. As expected, standard, nontemplated gels also resulted in separation of the proteins but not to the same extent as with the templated hydrogels. In summary, this research highlights the important role of the templating agent as possible useful tuning factors for achieving electrophoresis-based separation. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44063.

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INTRODUCTION

Separation processes are routinely used worldwide, and there is plethora of diverse methods of separations. As an example, gel electrophoresis is a standard technique used for separating, identifying, and purifying charged biopolymers, and it has been used for decades.[1–3] Furthermore, gel electrophoresis is a separation method essentially in biochemistry and molecular biology that distinguishes proteins or nucleic acids, such as DNA and RNA, differing slightly in the size, charge, conformation, or association degree.[2,4] Also, gel electrophoresis has been applied to a wide variety of other small-scale structures, including organelles, microorganisms, bacteria, viruses, and nanoparticles.[5–7] The most commonly used gel electrophoresis methods include polyacrylamide gel (PAGE)[1,8] and agarose gel electrophoresis.[2] Furthermore, the use of sodium dodecyl sulfate (SDS) to aid in denaturing proteins, thus minimizing the influence of shape/conformation and also coating them with a uniform negative charge for PAGE. Then, SDS-PAGE is probably the most commonly used gel electrophoretic approach for analyzing proteins to a high degree of certainty.

Polyacrylamide gels are formed when monomers are polymerized and chemically cross-linked using a cross-linking agent, such as *N*,*N*′-methylenebisacrylamide. The concentration, cross-link density and rate of formation of the gel determine the optical properties, rigidity, and pore size and hence determine the utility of the gel for the various applications.[2,9] In general, hydrogels resolve a wide range of globular solutes by simple sieving because of the broad distribution of pore sizes attributable to random wanderings and cross-linking of polymer chains. Thus, in many separations where polymer hydrogels play a role, precise and reproducible control over the pore-network architecture is demanding and in need of further research.

There are practical applications for which analyzing proteins in biological fluids, food products, agricultural products, and clinical and pharmaceutical studies are necessary.[10] Following this thinking, we found that in 1996, Rill *et al.*[11] reported an approach about the synthesis of nanostructured hydrogels for improved separations of biological macromolecules by electrophoresis or chromatography.[12] In this approach, polyacrylamide gels were synthesized using cosolutes to template internal gel

Table I. Recipes of the Different Experimental Configurations to Prepare Nanotemplated Hydrogels, Variations in Acrylamide (%T) and Nanotemplate (%S) Concentrations

Acrylamide concentration →	9%T				12%T			
Nanotemplate concentration →	0%S	5%S	10%S	15%S	0%S	5%S	10%S	15%S
Ingredients								
Protogel 30%[a] (mL)	1.5	1.5	1.5	1.5	2	2	2	2
Distilled water (mL)	2.16	1.91	1.66	1.41	1.66	1.41	1.16	0.91
Resolving buffer (mL)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
APS (μL)	50	50	50	50	50	50	50	50
TEMED (μL)	10	10	10	10	10	10	10	10
SDS (g)	—	0.25	0.5	0.75	—	0.25	0.5	0.75

[a] 37.5:1, acrylamide:bisacrylamide (National Diagnostics, USA) and purchased from Fisher Scientific.

structures with channels or pores having defined diameters, reflecting the dimensions of the templates. The pore structures of cross-linked polyacrylamide gels can be altered by polymerization in the presence of high concentrations of unreactive, micellar surfactant cosolutes, which act as "templates." Removal of surfactant after polymerization is expected to leave pores with the approximate shape and dimensions of the surfactant micelles. In consequence, the media with a pore size distribution strongly biased toward a specific size are expected to strongly influence the sieving, hence selectivity, for macromolecules with dimensions in the size range of the template pores. Those authors proved that proteins and nucleic acids are effectively separated by molecular sieving during gel electrophoresis or gel permeation chromatography[12] in hydrogels.

Thereby, a templating agent can be used to modify the hydrogel's internal structure with the objective of improving the separation of macromolecules that permeate these structures. Many efforts are being developed to improve the performance in the separation process based in electrophoresis system, such as modifications on the electrophoresis system itself[10] and the hydrogel structures used in these systems.[3,13] The SDS micelles are unreactive macromolecules inside the hydrogels and are expected to leave similarly sized pores behind after removal. Hydrogels with templated pores are expected to perform better over a narrow size range centered on the mean pore size than standard, conventional gels.[11,12] Hydrogels synthesized in the presence of surfactants have also been imaged by atomic force microscopy and shown to exhibit a different surface morphology than standard gels.[14] Chakrapani *et al.* proposed that the changes in the surface morphology were due to a percolation transition in the system of voids formed upon the surfactants' removal from the hydrogel. Other studies about the modeling of microvoid structures in hydrogels show the role of the modification in the porous structure of hydrogel in the separation process.

In this context, the general purpose of the research reported on herein was to synthesize hydrogels in the presence of SDS micelles used as molecule templates in order to modify the hydrogel's porous (internal) structure. We then evaluated the extent of separation of proteins using SDS-PAGE. In order to produce a broader understanding of the role of the templating agents and as opposed to other studies,[11] we adjusted various key parameters including the concentration of monomer, the process to eliminate the template from the gel and the technique to verify that elimination of the templating agents actually occurred. Ultimately, we achieved excellent protein separations in a weighted range of between 10 and 250 kDa using gels prepared with 9% of acrylamide (AAM) and templated with 5, 10, and 15% of SDS than with any other combination tested.

EXPERIMENTAL

Materials

All chemicals necessary for the experiments were purchased from Fisher Scientific (USA) and Bio-Rad (USA), such as SDS powder 99% purity, protogel 30% (AAM:BisAAM), ammonium persulfate (APS), tetramethylethylenediamine (TEMED), SDS 10% solution, glycine crystalline granules or powder USP aminoacetic acid, aminoethanoic acid, Gyn-hydralin, dithiothreitol, tris-HCl (tris-hydrochloride), tris-base (molecular biology grade), and protein standards (mixture of 10 proteins, Kaleidoscope Prestained Standards; Bio-Rad Catalog #161-0375). All chemicals were of analytical grade and used without further purification.

The electrophoretic system including gel box and power supply was available in the Laboratory of BioMolecular Medicine, Department of Chemical Engineering, TTU. Also, a Zetasizer ZS90 (Malvern) was available at the Department of Chemical Engineering, TTU. The Raman spectroscope (BWTEK, USA) and the spectrofluorometer were available at the Department of Chemistry, TTU. One Mini-Protean 3 multicasting chamber was acquired from Bio-Rad.

Methods

Nanotemplate Preparation and Characterization. SDS micelles to be used as nanoscale templating agents were prepared by mixing specific quantities of SDS with various liquid media. Accordingly, we evaluated different ways of preparing micelles in water solution, in acrylamide monomer solution, and in the hydrogel solution without the initiators. Also, we studied the effects of SDS concentration in order to get spherical micelles

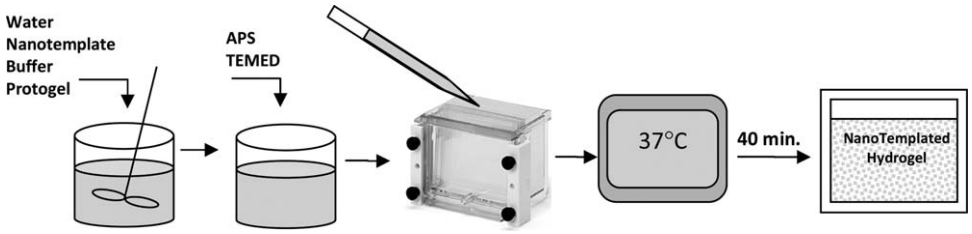

Figure 1. Templated gel preparation process. SDS-PAGE. Modified Laemmli method.

with high stability and in small sizes (on the order of 1 nm). We used the dynamic light scattering technique[15] and zeta potential (ZP) measurements to characterize the micelles.[16,17] Also, the effect of temperature on the formation of the micelles was considered important[18,19]; thus, we chose two different temperatures when characterizing the micelles: room temperature (25 °C) and the polymerization temperature typically used when preparing the hydrogel (37 °C).

Hydrogel Preparation. The hydrogel preparation procedure was based on that recommended for the SDS-PAGE method.[1] All solutions were prepared following the Laemmli method [20] before starting the preparation of the hydrogel. In our case, we called it a "modified Laemmli method" due to our modification of some steps of the original protocol (e.g., choice of running buffer, stacking buffer, and resolving buffer and the use of 10% APS and template of SDS micelles). The basic strategy followed to prepare the hydrogel with addition of the nanotemplate was initiated with the mixing of all components. After adjusting the mixing process, was verified that the better option was to add first the SDS in solid phase in the water followed for the addition of buffer and finally the protogel. The mixing process was continues until to dissolve all SDS and to form a homogeneous dissolution and stable in the time (without formation of precipitate). In this way, various concentrations were studied until to get the concentration necessary for the final formulation of the hydrogel. Table I presents a summary of all recipes used to prepare the hydrogels. When the mixture of all components was ready, it was put in the mini-gel electrophoresis system in order to obtain the thin hydrogel (thickness: 0.75 mm), and after 40 min at 37 °C, the hydrogel was completely polymerized.

Figure 1 presents the procedure for preparing the hydrogel, but this does not include the preparation of the stacking gel, which occurs after the removal process of the nanotemplate. The stacking gel is comprised of 4% of acrylamide, Tris buffer 0.5 *M* at pH 6.8, and this solution is gently pipetted onto the resolving gel.

Removal of Nanotemplate. The SDS micelles contained in the hydrogel, which was produced in the previous stage, must be removed from the matrix of the hydrogel; this step is important in an effort to leave the spaces occupied by them and yield a different porous structure in the hydrogel in comparison to the hydrogel prepared without the template. The removing procedure was initially based on two processes: passive and facilitated mechanisms.[11] The first mechanism led to diffusion of SDS out of the hydrogel. In that case the hydrogel was incubated in Tris buffer (pH 8.8) for more than 20 h with periodic changes of the buffer in order to avoid it getting saturated with SDS. The second process was "preelectrophoresis," which was basically an electrophoresis run (without any sample loading) over enough time to assure the complete elimination of the nanotemplate from the hydrogel. The evaluation of the complete elimination of the template from the hydrogel was done based on observing the SDS crystallization when the gel was stored at 4 °C[21] and via the Raman spectra of SDS[11,22,23] in the hydrogel prepared with the nanotemplate.

Separation Process. In this stage a protein standard cocktail (Precision Plus Protein Kaleidoscope Standards; Bio-Rad Catalog # 161-0375) consisting of 10 differently weighted proteins was used to test the effect that the micelle templating agent used in the hydrogel preparation had on protein separations. A vertical electrophoresis system and the SDS-PAGE method[2] were used to separate the proteins, and the bands formed by protein migration were evaluated to determine the extent of the separation. Two main hydrogel groups were tested, 9% AAM and 12% AAM (those are referred to as 9T and 12T, respectively, in the experiments). These were selected based on the protein sizes in the standard cocktail to give a hydrogel pore size suitable for the separations.[2] SDS concentrations of 5, 10, and 15% were used in the preparation of the nanotemplated hydrogels at both 9% and 12% AAM concentrations, alongside the reference hydrogels formed with the 9% and 12% AAM concentrations but no template. Basically, three parameters were compared: voltage applied on the electrophoresis system (100 and 150 V), time of running of the experiment for each group of hydrogels, and band separations of the proteins in the hydrogel.

RESULTS AND DISCUSSION

Polyacrylamide gels (both fixed and gradient compositions) are routinely used to separate mixtures of proteins up to about 250 kDa in molecular weight. Fixed percentage compositions serve well when a more narrow size range is the focus and/or the proteins are at a concentration such that band overlap is not a problem. The choice of gel and the resulting total amount of acrylamide (monomer and cross-linker) it contains is made based on the size of the target protein(s) among other factors. Gels containing the higher percentages (e.g., 20%T) are used in cases when it is important to separate proteins in the smaller size range (e.g., 10 kDa), while lower percentages such as 7.5%T result in gels with larger pores that are suitable for separating relatively larger proteins, often with increased resolution. These lower percentage gels are not effective in the separation of small proteins, while the higher percentage ones are not effective in separating larger proteins. Gradient gels (e.g., 4–20% that have

Table II. Parameters for Control of the Preelectrophoresis Process to Eliminate Nanotemplate from the Hydrogel

Hydrogel group →	9%T		12%T
SDS concentration ↓	150 V	200 V	150 V
5%	1 h 30 min	1 h	2 h 30 min
10%	3 h	2 h	3 h 30 min
15%	4 h 30 min	3 h	4 h 50 min

a gradient of monomer and cross-linker throughout the gel) on the other hand allow separation over a broader size range and are often used when the sample contents are not known. Our results showed that electrophoretic-based separations of proteins using polyacrylamide-based hydrogels formed in the presence of SDS micelles at concentrations from 5 to 15% were improved in gels containing 9%T but relatively unchanged in gels containing 12%T. Detailed results from characterizations of the gels and the resulting effects on protein separations are provided in the sections that follow.

Characterization of Nanotemplates

Measurements of nanotemplate size and stability revealed variations that were dependent on the concentrations of SDS and on the kind of solution used. Initially, aqueous solutions with 1, 5, 10, 15, and 20% (w/v) of SDS were prepared in order to evaluate their solubility and viscosity. We observed that the solution with 20% of SDS was not easy to prepare because of its high viscosity.[21] This observation and the fact that the 1% concentration (could be close to the critical micelle concentration) led us to select the middle three concentrations for the preparation of the micelle templates. Thus, SDS solutions of 5, 10, and 15% (w/v) were prepared in different media such as resolving buffer, protogel 30%, and a solution that was used to simulate the same conditions used to prepare the hydrogel [which was as mixture of protogel, buffer, and water without including the initiators (TEMED and APS)]. The results (not shown) revealed that the size of the micelles in either water or protogel was on the order of 1 nm, and the micelle stability (determined based on the ZP) depended on the media in which they were prepared. We saw that the micelles were generally stable (ZP typically >|30|) when prepared in water and less stable when prepared in other solutions such as a mixture containing the hydrogel components (e.g., water, buffer, and protogel). This loss of stability could be the result of the formation of aggregates in these solutions (which are closer to the final formulation of the hydrogel).[24] This potential aggregation is supported by the fact that micelles in Tris buffer or a mixture of Tris buffer, protogel, and water were on the order of 7 or 2–3 nm in size, respectively. In additional experiments to explore the effects of temperature, we found that temperature (in the 25–37 °C range) did not have any significant effects on the size and stability of the micelles. With those results is expected that the hydrogel prepared with 5, 10, and 15% of SDS micelles shows pore sizes in the range of 2–3 nm after removing of SDS. Those results of size could be confirmed with the analysis of the morphology of those hydrogels by micrographic techniques even though only one method is known to evaluate this kind of material and it was presented by Wang *et al.*[9] Because the complexity in the preparation of sample of this soft kind material for measuring by TEM, it was not explored in this research.

Hydrogel Preparation

The hydrogel was prepared in different conditions to control key parameters in order to obtain a hydrogel with characteristics adequate for separating macromolecules with a better resolution. We were able to introduce different concentrations of SDS-based micelle nanotemplates, and the results in testing the separation process for proteins suggest bands with better resolution in the separation. Some interesting behaviors were observed in the polymerization and after the gels were formed. For example, the polymerization rate of the hydrogels prepared with nanotemplate decreased in comparison to the reference hydrogel (0% SDS) in direct relation to the concentration of the nanotemplate.[25] In other words, the gel formed more slowly in the presence of the SDS micelles. The sequence in polymerization time was: $\text{time}_{(15\%\ \text{SDS})} > \text{time}_{(10\%\ \text{SDS})} > \text{time}_{(5\%\ \text{SDS})} > \text{time}_{(0\%\ \text{SDS})}$. A qualitative evaluation of the mechanical stability also showed a difference between the nanotemplated hydrogels and the reference hydrogels. Because of the thinness of the hydrogels (0.75 mm), the ability to manipulate them was carefully monitored all the time, and especially the nanotemplated hydrogels. We found it harder to handle the nanotemplated hydrogels.

Key interesting experimental aspects also arose regarding the stacking gel. We had initially added the stacking gel to the newly formed resolving gel before the procedure to eliminate the nanotemplate, however, some difficulties surfaced. For example, during the process of elimination of the micelles via diffusion from the gel, the hydrogels increased in size (i.e., swelled) during the time that they were soaked in buffer, and as a consequence, the wells where the proteins were to be loaded were deformed. Without addressing this situation, the deformity otherwise modified the migration of the proteins through the hydrogel. To overcome this difficulty, the addition of the stacking gel was done after removing the nanotemplate from the hydrogel.

Removal of Nanotemplate

After deciding to not continue with the diffusion-based removal of the nanotemplate, the removal of the nanotemplate was ultimately just done by a preelectrophoresis (Pre-GE) process in which the prepared hydrogel was placed in the gel box and voltage applied similar to a typical electrophoresis run. The amount of running time and the voltage applied were varied depending on the concentration of template inside the hydrogel. Table II shows the optimal parameter values that were found in each group of hydrogel (9 and 12%T) to ensure complete removal of

Figure 2. Template removal through the preelectrophoresis process.

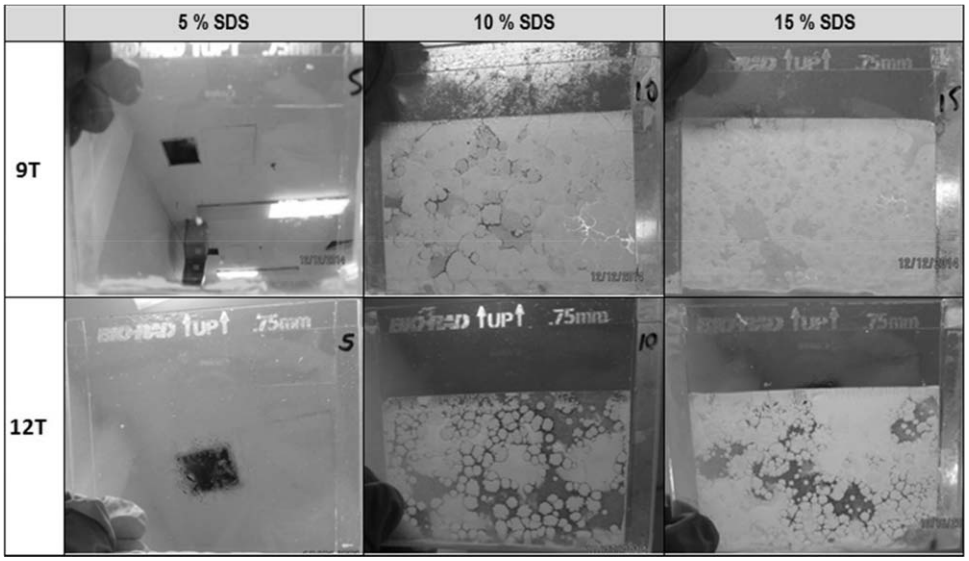

Figure 3. Pictures of the gels showing the SDS crystallized inside the gel (at 4 °C). Horizontal position: two concentrations of acrylamide (9 and 12%T). Vertical position: three concentrations of template (5, 10, and 15%).

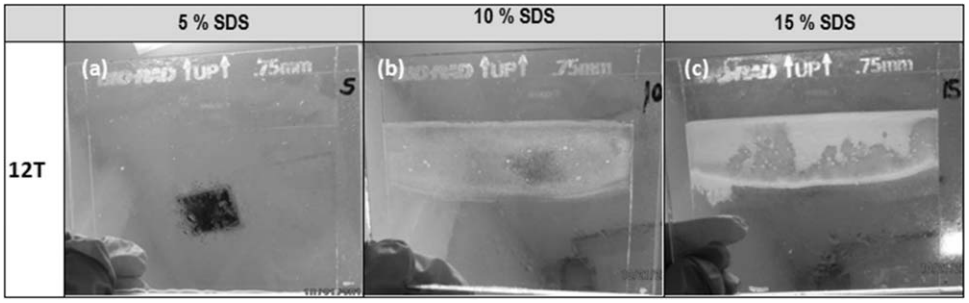

Figure 4. Pictures of the gels (12%T) showing the SDS crystallized inside the gel (at 4 °C). Pictures taken after some hours of the pre-GE elimination process. (a) Gel with 5% of template after 1 h, (b) gel with 10% of template after 2 h, and (c) gel with 15% of template after 3 h.

template as discussed further below. Figure 2 illustrates the procedure.

The desired result was basically the total elimination of the nanotemplate from the hydrogel. However, the limits of detection of concentrations of SDS inside the hydrogel measured through observation of SDS crystals that were formed when the gel temperature was reduced to ~4 °C, and Raman spectra were ~5 and 2%, respectively.[11] The results of the processes of elimination are summarized and presented in

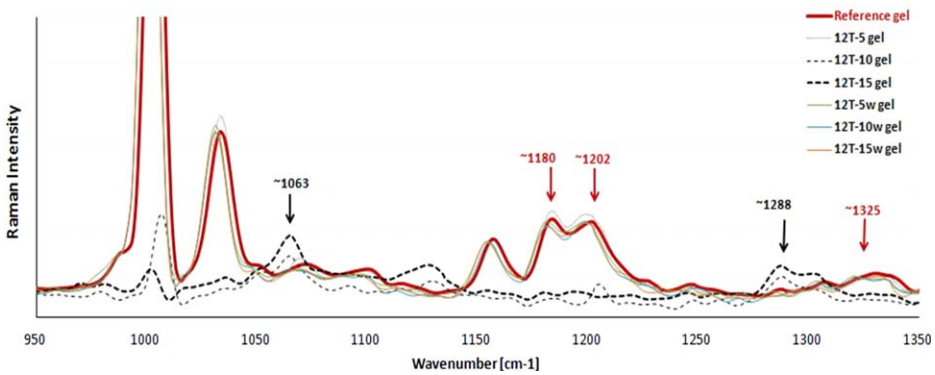

Figure 5. Raman spectra of the 12%T reference and nanotemplated gels showing characteristic peaks of the reference gel material at 1180, 1202, and 1325 cm^{-1} and of SDS (at 1063 and 1288 cm^{-1}) inside the nanotemplated gels. Dashed lines: nanotemplated gels containing 5, 10, or 15 of SDS. Continuous lines: nanotemplated gels after removing the SDS nanotemplate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

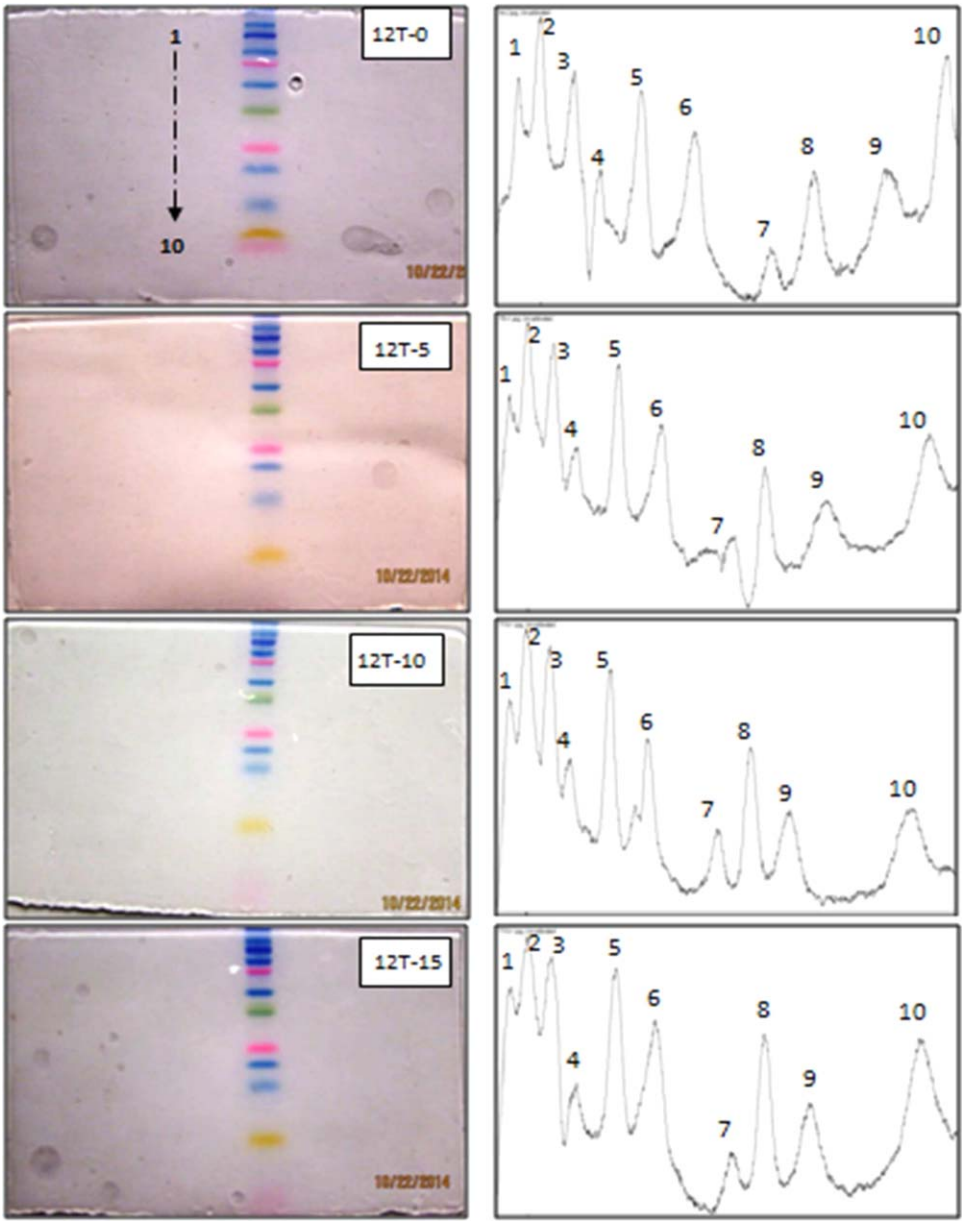

Figure 6. Qualitative measures of the separation of standard proteins in hydrogels with 12% of AAM and 0, 5, 10, and 15% of nanotemplate. Electrophoresis was run at 100 V: 12T-0 (1 h), 12T-5 (1 h 30 min), 12T-10 (1 h 40 min), and 12T-15 (2 h 15 min). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figures 3 and 4 (based on crystallization) and Figure 5 (Raman spectra). As can be seen, significant quantities of SDS crystals were observed when the gels were incubated at 4 °C and either 10 or 15% SDS concentrations were used, while the use of the 5% SDS resulted in a mostly clear or slightly cloudy gel.

Figure 5 illustrates Raman intensities collected from assessment of random areas of the hydrogels and associated with wavenumbers between 950 and 1350 cm^{-1}. Some characteristic peaks for the polyacrylamide reference hydrogel were seen next to 1180, 1202, and 1325 cm^{-1} as expected.[26] When either of the two higher concentrations of SDS micelles was introduced during the preparation of the hydrogel, the presence of the SDS masked the characteristic peaks of the reference gel. However, with low SDS micelle concentrations in the hydrogel, ~5%, it was not possible to observe significant variation in the reference gel characteristic peaks. On the other hand, the peaks observed at 1063 and 1288 cm^{-1} correspond to the presence of SDS in the hydrogel, and these peaks were progressively higher with larger amounts of SDS template. Of note, the intensity of the first peak at 1063 cm^{-1} is found in micellar solutions and is attributed to S–O vibration. Such an increase has been tentatively attributed to the hydration of the head of the SDS molecule.[23] With this information about the characteristic Raman spectra of polyacrylamide gels and similar gels templated with SDS, it was possible to determine the extent to which the SDS

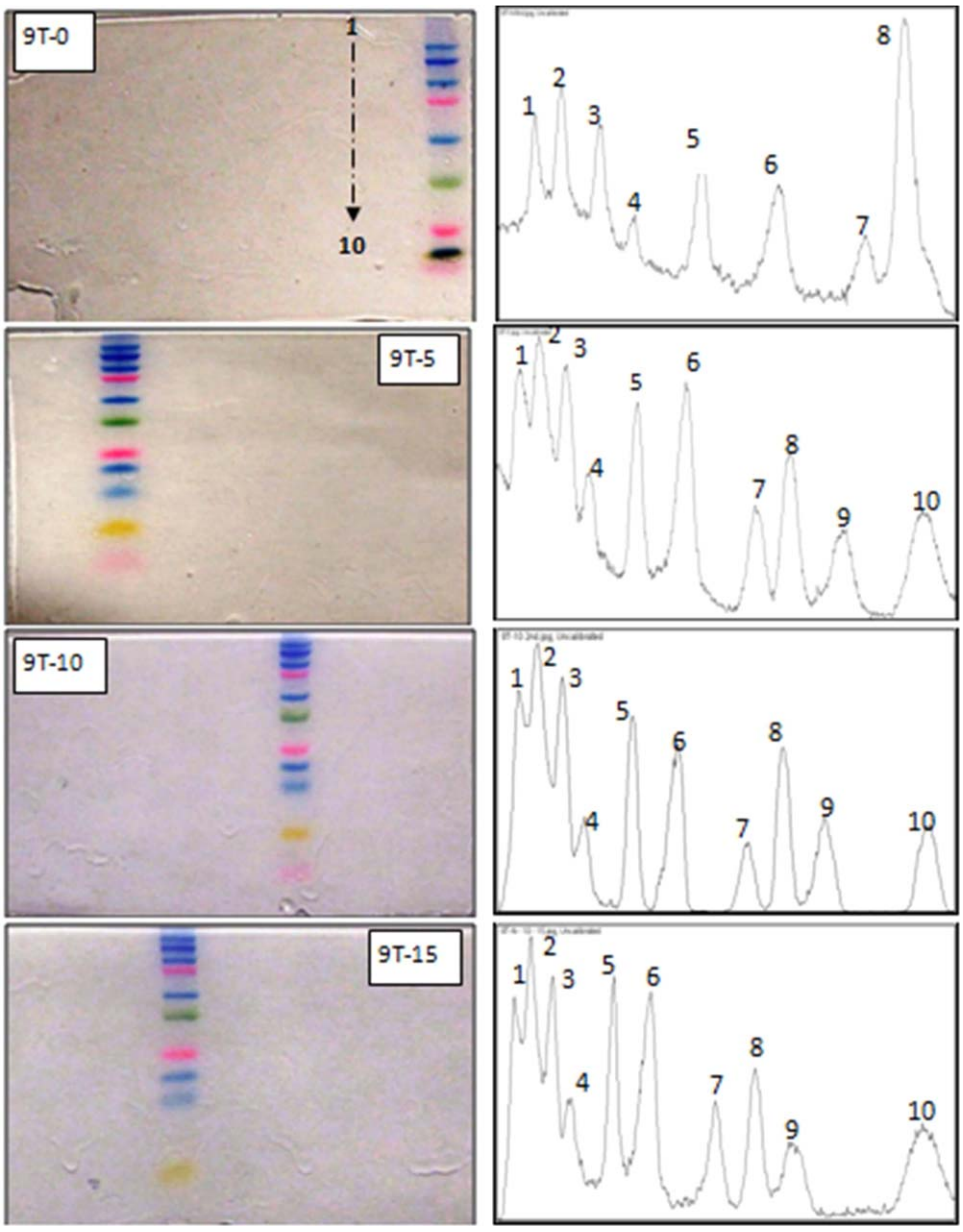

Figure 7. Qualitative measures of the separation of standard proteins in hydrogels with 9% of AAM and 0, 5, 10, and 15% of nanotemplate. Electrophoresis was run at 100 V: 9T-0 (1 h), 9T-5 (1 h 15 min), 9T-10 (1 h 30 min), and 9T-15 (1 h 40 min). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nanotemplates had been removed from the gels. After completing the process for eliminating the SDS by pre-GE, the spectra obtained from random areas of the nanotemplated hydrogels were similar to the reference hydrogel confirming the SDS elimination from the nanotemplated hydrogel.

Separation of Proteins Using Nanotemplated Hydrogels

Protein separation in 9 and 12%T hydrogels was evaluated in reference and nanotemplated hydrogels. Pictures were taken after electrophoresis and imported into ImageJ software for qualitative analysis. Each hydrogel composition was run for different times in order to distribute the bands of proteins across the full length of the gel. In Figure 6, the separation of the proteins via the nanotemplated hydrogels appears to be slightly clearer (less diffusion between peaks) than the reference hydrogel; however, in all cases, all 10 peaks can be seen. The protein molecular weights corresponding to the respective peaks are (1) 250 kDa, (2) 150 kDa, (3) 100 kDa, (4) 75 kDa, (5) 50 kDa, (6) 37 kDa, (7) 25 kDa, (8) 20 kDa, (9) 15 kDa, and (10) 10 kDa (yellow band).

Because of the previous results and in order to further seek to increase the performance in the separation of proteins using nanotemplated hydrogels, we also tested hydrogels prepared with 9% of AAM alone, and the same AAM composition combined with 5, 10, and 15% of nanotemplate. Figure 7 shows the results of the separation of proteins in this group of hydrogels. Since less AAM in the hydrogel means larger pores, smaller

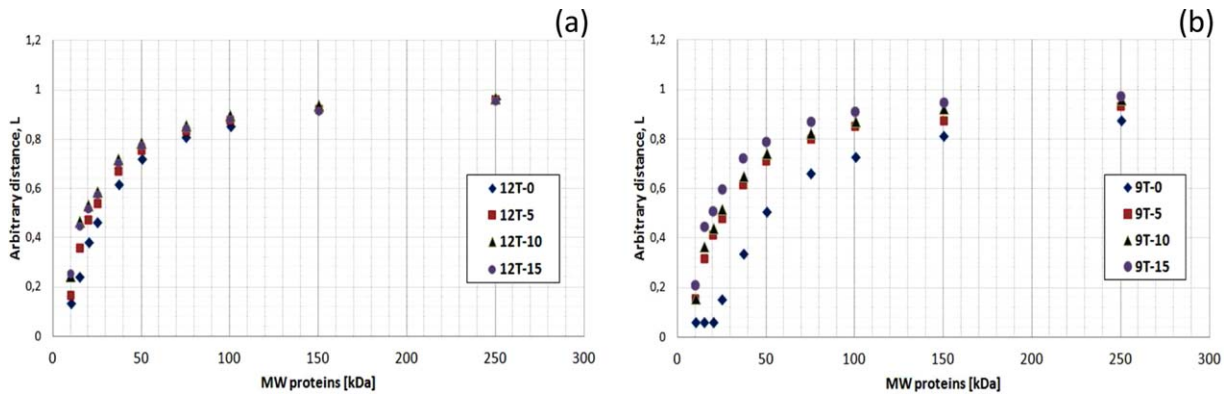

Figure 8. Plot illustrating protein mobility (measured based on migration from a reference point in each hydrogel). (a) Through the 12%T hydrogels. (b) Through the 9%T hydrogels. The *y*-axis uses an arbitrary distance scale, where $L = 1$ is at the top of the gel and $L = 0$ is the location of the pink dye reference band. Linear normalization was used to help compare gel samples since the pink dye traveled different lengths. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

proteins were expected to not separate as well, but good separation of high molecular weight proteins was still expected as with the 12%T gels. The reference hydrogel (9T-0) clearly shows only eight peaks out of ten proteins, due to poor separation of the smallest proteins. In the three nanotemplated hydrogels, all 10 proteins show up as bands/peaks.

In order to evaluate quantitatively the behavior of both groups of gels, 12 and 9%T, we prepared Figure 8, in which is illustrated the relative migration of the proteins through the hydrogels. The plots were prepared using a consistently-sized arbitrary distance scale established with ImageJ for each sample with results obtained through linear normalization of the relative distances. The results were then graphed using Excel. The plot in Figure 8(a) shows little real difference between separations in the nanotemplated and reference hydrogels (group 12T). However, in the plot of Figure 8(b) for the 9T group, the smallest three proteins in the ladder can be seen at the same distance in the reference hydrogel, while all 10 proteins can be seen as separated in the nanotemplated hydrogels. These improvements clearly show evidence of the SDS templating agent's modification of the hydrogels.

Furthermore, the time spent in the separation process by electrophoresis in both groups of hydrogels was compared. Figure 9 shows that the 9T group took less time in the separation as expected because the protein mobility inside of the hydrogels is less hindered than in the 12T hydrogels. Even with less separation time, the nanotemplated 9T gels resulted in better protein separation than those in the 12T group.

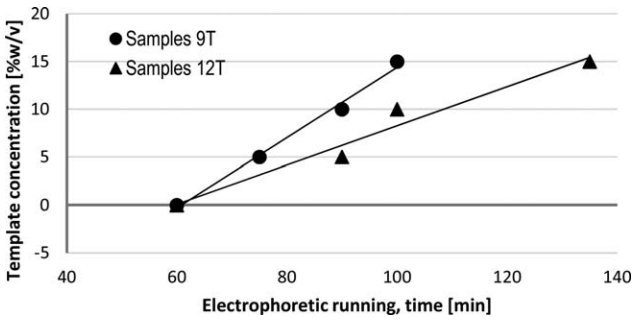

Figure 9. Plot comparing the time spent in the electrophoretic run to separate proteins using gels of group 12T and 9T. The *y*-axis shows the concentration of the template (SDS) used in each sample.

CONCLUSIONS

We synthesized polyacrylamide hydrogels via radical polymerization in the presence of SDS micelles used as a templating agent. The micelles were subsequently removed, and the hydrogels used in studies of protein separation via SDS-PAGE method. Multiple parameters such as monomer concentration (9 and 12% AAM) and nanotemplate concentration (5, 10, and 15% SDS) as well as conditions of the polymerization were examined to obtain better performance of the hydrogel in the separation process.

The preparation of the nanotemplating agent suggested the need for a more complete study of the preparation of the micelles and their characterization in size and stability because of the significant perceived influence of these characteristics on the formation of the porosity inside the hydrogel.

In regard to the performance of the nanotemplated hydrogel in the separation process of proteins, we noted that each band of proteins obtained mainly in the hydrogels with 5 and 10% of nanotemplate presented better band formation when compared with the reference hydrogels.

While the nanotemplated 12T hydrogels showed little difference from their reference hydrogel regarding separation characteristics of 10 proteins in the 10–250 kDa molecular weight size range, the use of the nanotemplated 9T hydrogels resulted in clear improvement in the separation. Because in it was possible separate the 10 proteins, while in the reference 9T hydrogel was only able to separate eight out of the 10 proteins. On the other hand, the time spent in the separation process in 9T hydrogels group was around 45% less than in the 12T hydrogels group. Furthermore, direct characterization of the hydrogel's pore structure by micrographic techniques with adequate methodology to prepare this soft kind of material should help in understanding the separation improvements seen in the 9T hydrogels.

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