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Electrophoresis in Organogels

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A new matrix for electrophoresis, a low molecular weight organogel, is described. Dansylated amino acids and peptides were separated by planar and capillary electrophoresis in acetonitrile gels of *trans*-(1*S*,2*S*)-1,2-bis-(dodecylamido)cyclohexane. The superior separation ability of the organogel over its corresponding buffer solution in capillary electrophoresis is illustrated. Organogels provide all the advantages associated with planar matrixes with 100% efficient recovery and transfer of the analytes to a mass spectrometer. We demonstrate that the planar gel can be liquefied and injected as is into an ESI-MS to identify the separands.

For the past decade or so, a new class of gels formed by small organic molecules has attracted considerable attention.[1] These organogels are formed by self-aggregation of small concentrations (typically <3%) of the gelator molecule in a solvent. The gels, which are formed in a variety of organic solvents or water, are thermoreversible. Upon heating they form liquid solutions. They are also thixotropic, becoming liquefied upon mechanical agitation. The exact requirements for gelation are still not known, and it is not possible to predict whether any particular molecule will form gels. However, most of the known gelators contain one of a number of functional groups, the most common among them being sugars,[2−5] amino acids,[6−9] amides,[10−12] ureas,[13−15] and cholesterols.[16−18] Alkanes can serve as gelators as well.[19,20] Despite the wide variety of gelators, they all have certain features in common. The aggregation occurs through noncovalent interaction, usually though not exclusively hydrogen bonding. Often one gelator combines several types of interactions such as π-stacking, van der Waals, and hydrophobic interactions with hydrogen bonding. The resulting structures are twisted fibers which intertwine to trap solvent molecules, thus causing gelation.

While much of the focus has been on discovering new gelators and understanding the process of gelation, very little success has been recorded in finding applications for these materials. Several preliminary discoveries about organogels have been made which will help lead to applications. The ability to incorporate electrolytes into gels has been shown.[6,21] Organogels have been used as templates for inorganic, sol−gel-derived chiral materials.[10,22] They have been found to have light-scattering properties which may make them suitable for electrooptical displays.[23] Gelators that can selectively gel one of a mixture of solvents have been found.[24,25] This type of gelation may be useful in dealing with oil spills. Finally, electrolytic cells incorporating gelators have been fabricated.[26] Despite this progress, none of the above advances has led to, to our knowledge, a functional material. In this paper we describe for the first time the use of an organogel as an electrophoresis matrix. The inherent thermoreversibility and thixotropy of organogels can bring about modes of MS coupling that are unattainable with any other type of solid network.

Electrophoresis, in both its planar and capillary forms, is a widely used separation technique. One of the challenges in electrophoresis is identification of the separated compounds. The advent of capillary electrophoresis allowed for direct interface with electrospray mass spectrometry (ESI-MS). However, planar electrophoresis, which remains the heart of protein analysis, is not suitable for direct MS detection. The compounds must be located by staining, cut out of the slab, extracted from the matrix, usually

(1) For recent reviews see: (a) Gronwald, O.; Snip, E.; Shinkai, S. *Curr. Opin. Colloid Interface Sci.* **2002**, *7*, 148−156. (b) Adballah, D. J.; Weiss, R. G. *Adv. Mater.* **2000**, *12*, 1237−1247.
(2) Jung, J. H.; Shinkai, S.; Shimizu, T. *Chem.—Eur. J.* **2002**, *8*, 2684−2690.
(3) Kobayashi, H.; Koumoto, K.; Jung, J. H.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1930−1936.
(4) Battacharya, S.; Acharya, S. N. G. *Chem. Mater.* **1999**, *11*, 3504−3511.
(5) Yoza, K.; Amanokura, N.; Ono, Y.; Akao, T.; Shinmori, H.; Tackeuchi, M.; Shinkai, S.; Reinhoudt, D. N. *Chem.—Eur. J.* **1999**, *5*, 2722−2729.
(6) Hanabusa, K.; Hiratsuka, K.; Kimura, M.; Shirai, H. *Chem. Mater.* **1999**, *11*, 649−655.
(7) Hanabusa, K.; Okui, K.; Karaki, K.; Kimura, M.; Shirai, H. *J. Colloid Interface Sci.* **1997**, *195*, 86−93.
(8) Ragunathan, K. G.; Bhattacharya, S. *Chem. Phys. Lipids* **1995**, *77*, 13−23.
(9) Imae, T.; Takahashi, Y.; Muramatsu, H. *J. Am. Chem. Soc.* **1992**, *114*, 3414−3419.
(10) Bied, C.; Moreau, J. J. E.; Vellutini, L.; Wong Chi Man, M. *J. Sol-Gel Sci. Technol.* **2003**, *26*, 583−586.
(11) Yasuda, Y.; Iishi, E.; Inada, H.; Shirota, Y. *Chem. Lett.* **1996**, 575−576.
(12) Hanabusa, K.; Yamada, M.; Kimura, M.; Shirai, H. *Angew. Chem., Int. Ed.* **1996**, *35*, 1949−1951.
(13) Wang, G.; Hamilton, A. D. *Chem. Commun.* **2003**, 310−311.
(14) Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447−3450.
(15) van Esch, J.; Schoonbeek, F.; de Loos, M.; Kooijman, H.; Spek, A. L.; Kellog, R. M.; Feringa, B. L. *Chem.—Eur. J.* **1999**, *5*, 937−950.
(16) Jung, H. J.; Kobayashi, H.; Masuda, M.; Shimizu, T.; Shinkai, S. *J. Am. Chem. Soc.* **2001**, *123*, 8785−8789.
(17) Lin, Y.; Kachar, B.; Weiss, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 5542−5551.
(18) Lin, Y.; Weiss, R. G. *Macromolecules* **1987**, *20*, 414−417.
(19) Abdallah, D. J.; Weiss, R. G. *Langmuir* **2000**, *16*, 352−355.
(20) Abdallah, D. J.; Lu, L.; Weiss, R. G. *Chem. Mater.* **1999**, *11*, 2907−2911.
(21) Placin, F.; Desvergne, J. P.; Lassegues, J. C. *Chem. Mater.* **2001**, *13*, 117−121.
(22) Jung, J. H.; Ono, Y.; Hanabusa, K.; Shinkai, S. *J. Am. Chem. Soc.* **2000**, *122*, 5008−5009.
(23) Mizoshita, N.; Suzuki, Y.; Kishimoto, K.; Hanabusa, K.; Kato, T. *J. Mater. Chem.* **2002**, *12*, 2197−2201.
(24) Trivedi, D. R.; Ballabh, A.; Dastidar, P. *Chem. Mater.* **2003**, *15*, 3971−3973.
(25) Bhattacharya, S.; Krishnan-Ghosh, Y. *Chem. Commun.* **2001**, 185−186.
(26) Kubo, W.; Murakoshi, K.; Kitamure, T.; Yoshida, S.; Haruki, M.; Hanabusa, K.; Shirai, H.; Wada, Y.; Yanagida, S. *J. Phys. Chem. B* **2001**, *105*, 12809−12815.

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polyacrylamide, cleaned of the stain, and only then injected into the MS for identification. The reversible nature of organogels makes them suitable for direct injection into a MS. In the simplest form of electrospray-MS coupling, which is demonstrated here for small molecules, the gel can be liquefied locally by mechanical agitation with the needle of a syringe and injected into the MS. This simple technique allows the entire matrix to be analyzed by mass spectrometry.

Another advantage of organogels is that they can be made in organic solvents in addition to water. Current electrophoresis gels are aqueous polymeric gels. The ability to create organic gels opens the door to the analysis of hydrophobic compounds which do not function in aqueous gels, as well as an entire group of new solvents which can be used as the separation matrix. Additionally, capillary electrophoresis with polymeric gels requires long and complicated procedures for in-capillary polymerization.[27] The thermoreversibilty of organogels allows them to fill the capillaries as hot solutions and then cool to form gels, in a simple, quick, one-step procedure.

In this work, we introduce for the first time the basic concepts of organogel electrophoresis and demonstrate planar electrophoretic separations of dansylated amino acids in an organogel in several different solvent systems. Amino acids are commonly used model compounds which are derivatized to allow for easier detection by UV. The gel was agitated locally and injected sequentially into an ESI-MS without separating the organic gelator from the analytes. All compounds separated were identified by their molecular ion peaks. A group of amino acids was also separated in the organogel by capillary electrophoresis and compared to the separation in the corresponding buffer. The effect of changing different parameters such voltage, ionic strength of the buffer, and gelator concentration was examined.

EXPERIMENTAL SECTION

Materials. (1*S*,2*S*)-1,2-Diaminocyclohexane, lauroyl chloride, dansyl chloride, PEG 200, triethylamine, amino acids, and peptides were purchased from Sigma-Aldrich (St. Louis, MO). Acetic acid was from Frutarom (Haifa, Israel). Ammonium acetate and THF were from Mallinckrodt (Phillipsburg, NJ). Acetonitrile, methanol, and chloroform were from J. T. Baker (Deventer, Holland). BDACH, *trans*-(1*S*,2*S*)-1,2-bis(dodecylamido)cyclohexane (**1**) was synthesized according to the procedure of Jung et al.[2] Briefly, lauroyl chloride (0.768 g, 3.5 mmol) was added to a solution of (1*S*,2*S*)-1,2-diaminocyclohexane (0.2 g, 1.75 mmol) and triethylamine (1.77 g, 17.5 mmol) in 50 mL of tetrahydrofuran in a nitrogen atmosphere. The mixture was refluxed for 3 h and allowed to cool. The solvent was removed by rotoevaporation. The remaining solid was dissolved in chloroform and washed in a separatory funnel with water. The solvent was removed, and the product dried in a vacuum desiccator overnight. The product was recrystallized from methanol. Yield: 78%. TLC (9:1 chloroform/ethyl acetate): $R_f = 0.4$. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.90$ (d, 2H), 3.65 (q, 2H), 2.10 (t, 4H), 1.81−1.49 (m, 11H), 1.25 (s, 35H), 0.88 (t, 6H). IR (KBr): $\nu = 3280$, 1638, 1549. Elemental analysis calculated (%) for $C_{30}H_{58}N_2O_4$ (478.79): C, 75.26; H, 12.21; N, 5.85. Found: C, 74.48; H, 12.12; N, 6.46. Sol-to-gel transition: 55 °C. Dansylation was performed by mixing 0.1 mL of 7.2 mM aqueous amino acid with 1 mL of 10 mM pH 9 carbonate buffer and 0.9 mL of 1 mM dansyl chloride in acetone and heating for 10 min at 70 °C.

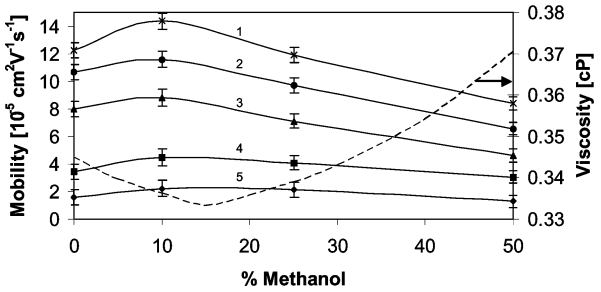

Figure 1. Dependence of the mobilities of dansylated amino acids [serine (1), phenylalanine (2), tryptophan (3), proline (4), and histidine (5)] on solvent concentration. Dotted line and right axis represent the viscosity of acetonitrile−methanol mixtures.

Instrumentation. Planar electrophoresis experiments were performed in a 12-cm long, 2.6-cm wide Teflon cell. Gel length was 6 cm. Power was supplied by a Consort (Turnhout, Belgium) E862 power supply operating between −200 and −300 V. Gels were formed by adding 6 mg of **1** to 2 mL of buffer, heating until dissolution, and casting into the cell. The buffer was 1 M acetic acid and 25 mM ammonium acetate in acetonitrile or mixtures of acetonitrile with methanol or PEG. Visual detection was performed with a UV lamp (Vilber Lourmat 215M, Torcy, France). Experiments were performed in triplicate. Samples for injection into the MS (LCQ, Finnigan, San Jose, CA) were taken by pushing a plastic grating (4-mm holes with 1-mm spacing) into the gel and withdrawing the samples with a 10-μL syringe, after gentle stirring for 1 s of the gel in each compartment by the needle of the syringe. The MS was scanned in the m/z range of 200−450 in both positive and negative ionization mode. Capillary electrophoresis was performed in a Dionex CE instrument with a 42-cm long, 100-μm i.d. capillary (Biotaq, MD). Injection was done electrophoretically. UV detection was carried out at 260 nm. Gel-filled capillaries were made by injecting hot organogel solution (60 °C) into the capillary and allowing it to cool. Relative viscosity measurements were made by flowing the solvents through an HPLC system (Finnigan) and measuring the pressure drop through the packed column. Real values were determined by correspondence to reported values of the pure solvents.

RESULTS AND DISCUSSION

Cell Electrophoresis. Figure 1 shows the mobilities of five dansylated amino acids in planar gels of different acetonitrile−methanol mixtures. The compounds all moved in the direction of the anode, indicating their negative charge in the buffers. In all cases the five compounds were resolved by visual inspection of the UV-illuminated spots. The vertical uncertainty bars were calculated from the radius of the fluorescent spots. The nonlinear dependence of the mobilities on solvent composition requires explanation. The electrophoretic mobility (μ) is the proportionality constant between the electrophoretic velocity (v) and the electric field strength (E) and is given by the Stokes equation

$$\mu = \frac{v}{E} = \frac{q}{6\pi\eta r} \qquad (1)$$

(27) Huang, A.; Chen, Y.; Zhu, T.; Fang, X.; Sun, Y. *J. Chromatogr., A* **1996**, *755*, 138−141.

Table 1. Selectivity in Acetonitrile−Methanol Mixtures

dansylated amino acid	selectivity between amino acid pairs			
	MeCN	10% MeOH	25% MeOH	50% MeOH
histidine				
	2.23	2.02	1.92	2.39
proline				
	2.30	1.97	1.74	1.51
tryptophan				
	1.35	1.31	1.36	1.42
phenylalanine				
	1.15	1.24	1.23	1.28
serine				

where q is the charge of the analyte, r is its Stokes radius, and η is the viscosity of the buffer. As the solvent accounts for 99.7% of the gel, the local viscosity encountered by the analytes is assumed to be the viscosity of the solvent. The viscosity of acetonitrile−methanol mixtures is also given in Figure 1. The minimum in the viscosity corresponds to the maximum in the mobility. According to eq 1, the relation between the mobility and the inverse of the viscosity should be linear. Indeed line fits of the $1/\mu$ versus η gave linear relationships though the correlation coefficients of the fits were rather low ranging between 0.79 and 0.93 for the five spots. This deviation can partly be attributed to the change in the Stokes radius as a function of solvent properties. It also reflects the uncertainties in measurement of the location of the spots in the 6-cm planar gel.

The selectivity between consecutive ion pairs in the different solvents is given in Table 1. These results show the versatility and adaptability of organogel systems to electrophoresis. Different solvents can be used in order to control the separation of a group of analytes or a specific analyte pair. Proline and tryptophan have the best resolution in pure acetonitrile, while phenylalanine and serine separate better in the 50% mixture. Analytes that are difficult to resolve can potentially be resolved with a change in solvent.

Poly(ethylene glycol) (PEG) was shown to improve the strength of organogels, without interfering with their conductivity.[6] Since by addition of PEG it is possible to tune the viscosity over a much larger range (compared to a mixture of solvents) we have investigated the possibility of altering the selectivity of the gel by addition of different levels of PEG. The mobilities of the dansylated amino acids were measured with differing concentrations of PEG 200 in a 0.3% gelator acetonitrile organogel. The gelator concentration was kept constant in all these tests. The observed mobilities in the planar electrophoresis configuration are shown in Figure 2 and selectivities between adjacent amino acid pairs listed in Table 2. Indeed, increasing the level of the modifier decreased the observed mobilities by up to 70%, probably due to the increased viscosity. The selectivities here too show a different dependence on the modifier for each pair of analytes. The histidine−proline separation improves with added PEG while the proline−tryptophan selectivity is worse. Specific separations can be optimized with addition of the right type and amount of oligomer modifier.

The voltage dependence in the cell was not checked due to the small voltage range (up to 300 V) in which the planar gel was stable. The dependence was checked however with capillary electrophoresis and will be discussed in that section. The mobilities of the analytes are strongly dependent on the ionic strength of the buffer, decreasing with increasing ionic strength. Over the

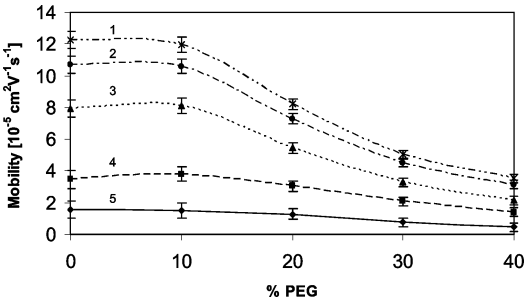

Figure 2. Dependence of mobilities of dansylated amino acids [serine (1), phenylalanine (2), tryptophan (3), proline (4), and histidine (5)] on addition of PEG 200 to acetonitrile.

Table 2. Selectivity in Acetonitrile−PEG Mixtures

dansylated amino acid	selectivity between amino acid pairs				
	MeCN	10% PEG	20% PEG	30% PEG	40%PEG
histidine					
	2.23	2.58	2.39	2.76	3.00
proline					
	2.30	2.12	1.78	1.57	1.52
tryptophan					
	1.35	1.31	1.34	1.38	1.47
phenylalanine					
	1.15	1.13	1.13	1.11	1.13
serine					

range of 0.1−1 M, the mobilities of the dansylated acids in acetonitrile buffer decreased linearly with the square root of the ionic strength. The Onsager equation expresses the mobility (μ) as a function of ionic strength (I). For univalent electrolytes it is given as[28]

$$\mu = \mu_0 - \left[\frac{8.204 \times 10^5}{(\epsilon T)^{1.5}}\mu_0 + \frac{42.75}{\eta(\epsilon T)^{0.5}}\right]\sqrt{I} \qquad (2)$$

where μ_0 is the mobility at zero ionic strength, ϵ is the dielectric constant, T is the absolute temperature, and η is the viscosity of the solvent. Plotting the mobility as a function of $I^{0.5}$ allows extrapolation to determine μ_0 and therefore the slope predicted by the Onsager equation. In very dilute solutions (up to about 0.02 M) the agreement is very good. At higher ionic strengths, the mobility is generally higher than the Onsager equation would predict.[29] Because this work was done in high ionic strength buffers, the linear relationship was observed (R^2 values ranging between 0.93 and 1), but the mobilities were much larger than those predicted by the Onsager equation.

The effect of the gelator concentration is shown in Figure 3. A clear nonlinear, nonmonotonic relationship is observed. At this stage we can only speculate about the mechanistic reasons for this dependence which may have substantial practical implications for organogel eletrochromatography. We introduce this figure here to illuminate the need for more extensive research in order to comprehend organogel electrophoresis.

Capillary Electrophoresis. The separation of a mixture of 14 dansylated amino acids was carried out in both gel-filled and

(28) Porras, S. P.; Riekkola, M. L.; Kenndler, E. *J. Chromatogr., A* **2001**, *924*, 31−42.
(29) Castellan, G. W. *Physical Chemistry*, 3rd ed.; Benjamin/Cummings Publishing Company: Menlo Park, CA, 1983.

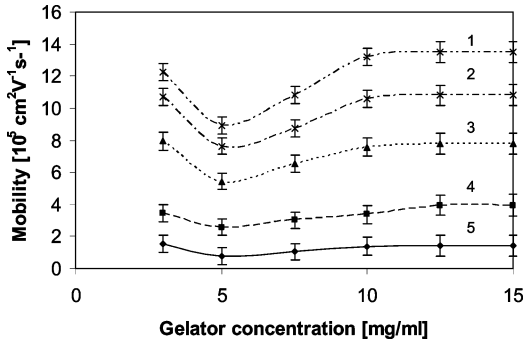

Figure 3. Dependence of mobilities of dansylated amino acids [serine (1), phenylalanine (2), tryptophan (3), proline (4), and histidine (5)] on gelator concentration. Smoothed lines were added to guide the eye, with no theoretical basis.

buffer-filled capillaries. As stated above, the dansylated acids are all negatively charged in this system. Since in the gel there is no electroosmosis, and electrophoretic mobility is the only force acting on the analytes, separations were performed in negative polarity mode (i.e., with the detector located near the anode). In the buffer-filled capillaries, electroosmotic flow marker mesityl oxide was found to have a mobility of 3.4×10^{-4} cm^2 V^{-1} s^{-1} toward the cathode, which is greater than the electrophoretic mobilities of all the amino acids toward the anode, with the exception of aspartic acid. Therefore, positive polarity was used, and the compounds were detected in the reverse order of that in the gel-filled capillaries. Figure 4 shows the electropherograms without (a) and with (b) gel. The peaks were identified by individual injection of each acid. The retention times, mobilities, and plate counts for the gel separation are given in Table 3.

The results clearly show the advantage of the organogel. Baseline resolution was achieved for 10 of the 14 compounds, whereas in the buffer several peaks fell on top of one another. Unlike polymer gel capillaries which require complicated polymerization procedures, these capillaries are made by a simple, one-step injection, and are ready in only a few minutes. The evacuation of the capillaries is also very simple. The gel can be simply pumped out of the capillary with a syringe pump, and after a short rinsing with acetonitrile a new gel can be injected into the same capillary. Organogels offer a much easier way to do capillary gel electrophoresis.

The dependence of the mobilities on voltage was also checked for a group of several dansylated amino acids. The dependence was linear with zero intercept over a range of 7−20 kV with correlation coefficients greater than 0.97. The gel-filled capillaries show stability for long periods of time. The separation of five amino acids was performed seven times over a period of 24 h. The resulting separations were essentially the same with a

Table 3. Separation Data for Gel-Filled Capillaries[a]

compound	t_r (min)	μ (10^{-4} cm^2 V^{-1} s^{-1})	N
1. dansyl chloride	4.3	3.02	16000
2. aspartic acid	5.0	2.60	14000
3. cysteine	5.2	2.47	16000
4. threonine	9.4	1.38	16000
5. serine	9.6	1.35	13000
6. glycine	12.1	1.07	20000
7. phenylalanine	12.7	1.02	22000
8. alanine	13.2	0.980	24000
9. tyrosine	14.3	0.905	28000
10. glutamine	15.0	0.866	14000
11. asparagine	17.1	0.759	24000
12. valine	17.7	0.734	19000
13. tryptophan	18.2	0.712	7000
14. leucine	19.2	0.676	8000
15. histidine	23.5	0.551	12000
16. dansyl chloride	9.3	3.10	12000
17. glutathion (glu-cys-gly)	13.0	2.22	94000
18. gly-ser	37.1	0.777	85000
19. glu-glu	47.9	0.602	35000
20. ala-gln	60.6	0.475	41000
21. phe-ala	67.5	0.427	39000

[a] N values were calculated by the formula $N = 5.54t_r^2/W^2$ where t_r represents the retention time and W is the half-height peak width.

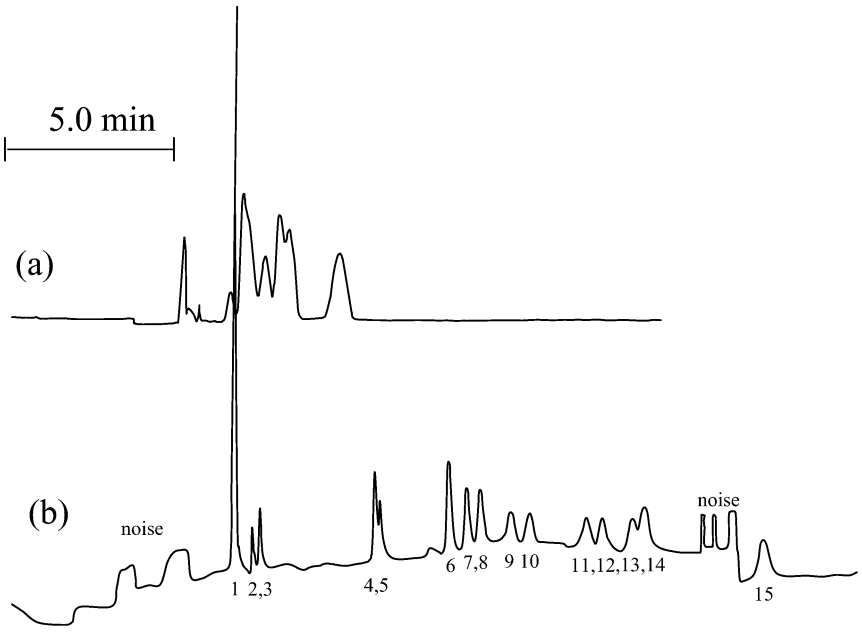

Figure 4. Electropherograms at 20 kV of 14 amino acids in (a) buffer-filled and (b) gel-filled capillaries. Peak numbers are identified in Table 3.

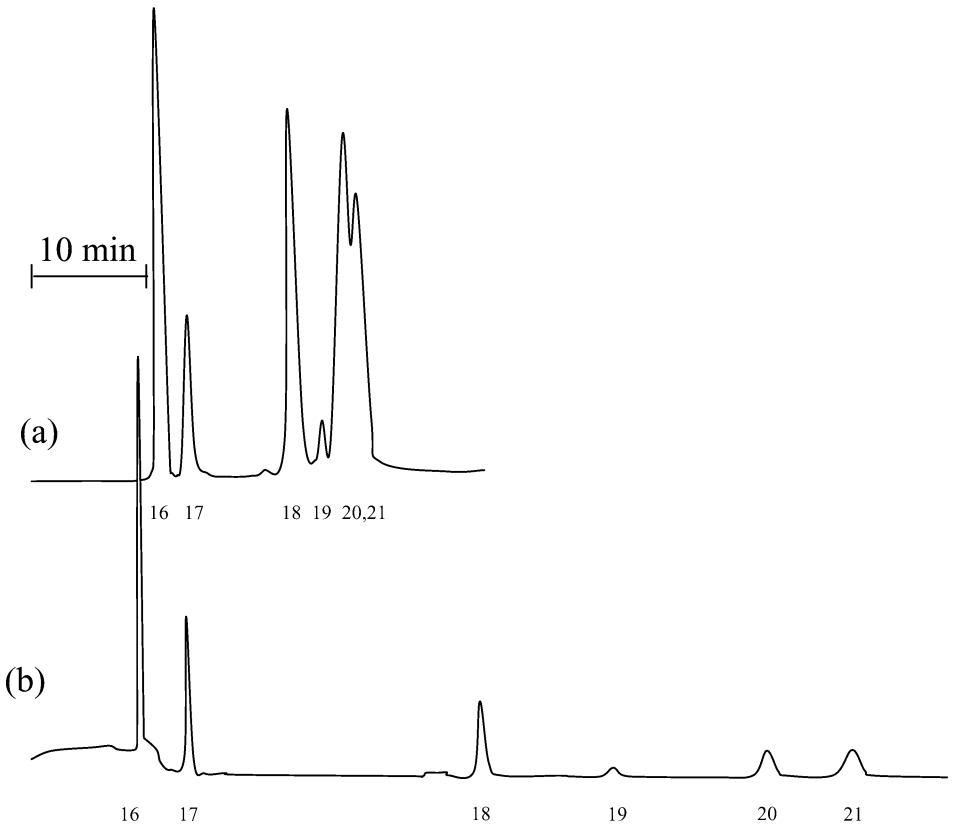

Figure 5. Electropherograms at 11 kV of five peptides in (a) buffer-filled and (b) gel-filled capillaries. Peak numbers are identified in Table 3.

standard deviation ranging between 1.5 and 4.8 in the peak retention times of the different dansylated amino acids.

In order to demonstrate the adaptability of the system to larger compounds, a series of di- and tripeptides was separated. The buffer solvent was changed from pure acetonitrile to acetonitrile−methanol (3:2) to ensure proper solubility of the analytes. The resulting electropherogram is shown in Figure 5, and the corresponding separation characteristics are delineated in Table 3. Electroosmosis in the acetonitrile−methanol gel-filled capillaries was negligible as in the acetonitrile-based gels, but the change of solvent rendered the electroosmosis in the conventional CE test (upper curve of Figure 5) negligible, and therefore the test was done in negative mode. Here too, full baseline separation was achieved in the gel, which was not possible in the buffer-filled capillaries. These results show that it is possible to modify the system to be used for larger molecules and highlight the versatility of organogels in that they permit adaptation of the solvent in order to accommodate different analytes.

Mass Spectrometry. We believe that one of the most important advantages of the organogels is the ability to inject the gel directly into the mass spectrometer, thus attaining by definition 100% recovery of the analytes. Full recovery is indeed common practice for capillary electrophoresis but not for planar gels. Theoretically, full recovery is possible also for other polymer-based gels, but in practice the polymeric matrix or its degradation products interfere with the mass spectrometric analysis. As a first step toward establishing the feasibility of direct injection of the gel we have compared the calibration curves of the different dansylated analytes in acetonitrile acetate buffer solution and organogels of **1**. The sensitivities obtained for the different analytes that were injected from the gels ranged between 73% and 85% of the respective calibration curves of the same analytes from the solution phase. Two typical examples for dansyl tryptophan and dansyl serine are shown in Figure 6. The figure also shows the mass spectrum of one of the calibration injections for tryptophan. The m/z 436 peak is the molecular ion. The m/z 223 and 250 peaks appear in all the injections and are fragments of the dansyl group, oxidized demethylized dansyl and dansylamine, respectively.

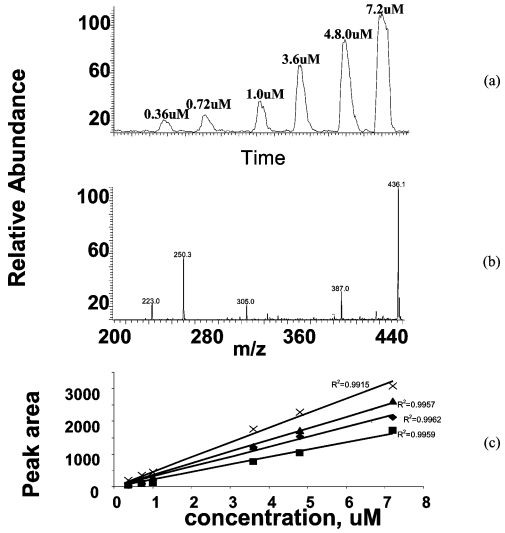

Figure 6. (a) Time trace of $m/z = 436$ for consecutive injections of 0.3% organogel of **1** in acetonitrile acetate buffer containing tryptophan at different concentrations. (b) Typical mass spectrum of injections in (a) in negative mode. (c) Calibration curves for serine in solution (×) and gel (▲) and tryptophan in solution (◆) and gel (■).

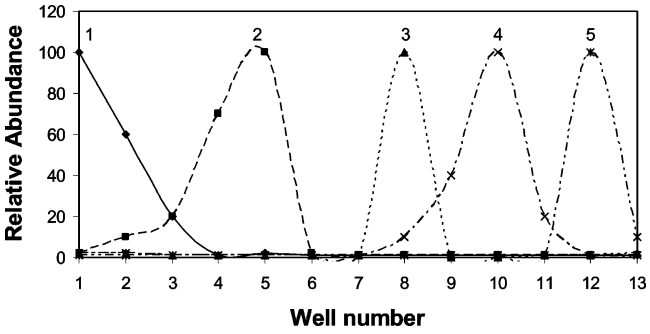

Figure 7. Relative abundances of histidine (1, *m*/*z* 389), proline (2, *m*/*z* 347), tryptophan (3, *m*/*z* 436), phenylalanine (4, *m*/*z* 397), and serine (5, *m*/*z* 337) in each of the 13 compartments. Curves were added between points to guide the eye.

We do not yet have an automated system for direct transfer of the gel to the mass spectrometer. However we could demonstrate the direct transfer by the following protocol. After the separation was completed, the gel was divided into 13 compartments by placing a plastic grating on the gel. The 4-mm size of the grating provided a sample of about 10 μL which was necessary for injection into the MS. Samples from each of the 13 compartments were mixed with the needle of the syringe for 1 s, and the slurry was injected into a sample loop of the MS. The relative abundances of each of the five acids in each of the compartments are shown in Figure 7. The different levels in each of the compartments are connected by a line to guide the eye, forming a chromatogram presentation. This mode of planar electrophoresis offers full recovery of the analytes. The reversible nature of organogels allows them to be injected directly into the MS for identification of the compounds separated on them and obviates the need for complicated and time-consuming extraction procedures.

CONCLUSIONS

Organogels provide a new and versatile matrix for electrophoresis. They are suitable for planar and capillary electrophoresis under widely different conditions. The availability of many types of organogels which can gelate a large variety of solvents differing in polarity from water to alkanes should allow for their extensive use in electrophoresis. Oligomer additives can aid resolution of hard to separate analytes. Most importantly, their reversibility allows direct injection into a MS for full recovery and identification of all compounds. This opens the way for a revolution in planar electrophoresis if the gels can be adapted to perform the separations currently carried out in polyacrylamide.

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