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Electrophoretic applications of sol–gel matrices

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Abstract

Biological application of sol–gel matrices has been extensively used through doping process. However the bioactivity of these materials is impaired mainly by uneven distribution of the biological molecules into the sol–gel matrix. The main objective of the present study was to apply electrophoretic field in order to concentrate biomolecules alongside sol–gel surface in order to increase the interaction capability with external molecules. Two potential applications of an electrophoretic field on sol–gel matrices were demonstrated. Silica gels in solid and liquid states were used as an electrophoretic matrix. In the first application, cylindrical rods made of solid silica gel and filled with tracking dye at one end, were subjected to applied electrical field. A well-defined movement of the tracking dye was attained from one edge to the other under applied electrophoretic current through the sol–gel rod pores. In the second application, accumulation of small biomolecules at one pole was obtained in a liquid pre-gelation sol–gel matrix during its polymerization. In order to optimize biologically active compounds movement in electrical field applied to sol–gel matrixes, parameters of both sol–gel and electrophoretic processes were studied. Under optimal conditions, it was feasible to increase the concentration of small biomolecules at sol–gel surface and consequently to enhance their availability towards external reactants.

Keywords: Sol–gel materials; Electrophoresis; Albumin; TEOS; MTMOS; Accumulation

1. Introduction

In the last decades sol–gel chemistry has played a major role in hybrid materials evolution by bridging organic and inorganic chemistry at molecular level. Eventually the growing interest in coupling the attractive characteristics of these materials with electrochemical science is now in progress [1–3]. For instance, modified electrode surface by sol–gel method found an application in various fields such as: electrocatalysis, electroanalysis, polymer science, protective coatings, surface analysis, electrosynthesis, molecular electronics, etc. [3]. Sol–gel technology application has been also described in the development of chemical and biological sensors, spectroelectrochemistry, electro-polymerization, batteries and fuel cells. Recently, electrophoresis of sol–gel was applied to produce nano-rods and nano-wires, as well as fabrication of nano-channels and transparent micro-patterns and electrodeposition of films with different characteristics [4–6]. Several successful attempts were undertaken in order to exploit the unique properties of sol–gel materials in electrophoresis processes [7–10]. Neiman at al. [7] used silica sols modified by sol–gel process, as an additive to a running buffer in order to modify the mobility of charged solutes in capillary electrophoresis. Sol–gel technique was also used in capillary electrophoresis detection method in order to modify the working electrode configuration [8]. Application of ceramics based on sol–gel materials in conjunction with bio-molecules were extensively studied during the past decade [11–18]. For example, such hybrid materials as organic/bio-molecule incorporated into sol–gel matrix are of interest to numerous scientific branches. The main procedure used in these applications is the simple doping process of sol–gel matrices with organic or biological reactants. A major shortcoming of this procedure is the loss of a large amount of dopants into the sol–gel matrix (not available for further surface chemical or biochemical interaction) while only external or open-pore entrapped molecules are available for further reaction [13–14,16]. It is very difficult to assess the exact amount of the reactant entrapped permanently into these matrices for every new assembly of such a system. The motivation of the present

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study was to improve the application of alkoxide sol–gel technology for bio-sensing and increased efficiency through electrophoretic process. The main task of the present study was to electro-enhance migration of dopant molecules (with electrophoretic capabilities) toward the sol–gel matrix surface in order to increase their concentration and obtain a highly reactive surface. Such approach will enhance surface reaction rate of different biosensors at extremely low concentration of reactants. Two main objectives were achieved: (a) it was shown that ormosils can be used as an electrophoretic matrix prior to final polycondensation and (b) to demonstrate the prospect of biologically active compounds migration through electrophoretic forces towards the surface of a sol–gel matrix during its polymerization.

2. Experimental

2.1. Reagents and materials

Precursors: tetraethylortosilicate (TEOS, purity 98%) and methyltrimetoxysilane (MTMOS, purity 98%) were purchased from ABCR Co. (Germany). Methanol (99.6%) was obtained from J.T. Baker (Holland) and methylene blue—from The British Drug Houses Ltd. (UK). Bromophenol blue (sodium salt), SDS (sodium dodecyl sulfate, 95%), di-mercapto ethanol and glycine were purchased from Sigma (Israel). Hydrochloric acid (36.5–38%) was obtained from BioLab Ltd. (Israel). Silver nitrate (crystals) was purchased from Spectrum Quality Products, Inc. (USA). Bovine serum albumin fraction V (98%) and tris–hydrochloride were from USB (United States Biochemical Corp., USA). Glutardialdehyde (25% solution in water) was obtained from Merck–Schuchardt (Germany). Glycerol (95%), anhydrous citric acid (analytical), ammonium hydroxide (25% NH_3, analytical), sodium hydrate (C.P.) and formaldehyde (36–38%) were all purchased from Frutarom Co. (Israel). All reagents were used in ''as received'' quality (without further purification). The solutions were prepared with triple-distilled water made with DESTAMAT'S® Bi 18F (Heraeus-Quarzglas).

2.2. Preparation of cylindrical sol–gel rods and electrophoresis conditions determination (case A)

Sol–gel stock solution was prepared by mix of 6.75 ml of TEOS (tetraethylortosilicate) with 2.1 ml of H_2O and 0.15 ml of 0.1 M HCl. The mixture was stirred for 4 h and aged at 4 °C for additional 24 h. Afterward 1 ml of the stock solution was mixed and stirred for 30 min (precisely) with 3, 4, 7 and 14 ml of 0.01 M phosphate buffer (PB) in order to obtain gels containing 18.75%, 15%, 9.35% and 5% of TEOS, respectively. The pH of prepared mixtures was 7.0 ± 0.2. Following stirring, the sol solution was poured into glass tubes (4 mm inner diameter and 12 cm in length), temporarily stopped up with tinny corks. Tubes inner surface was not subjected to any pretreatment. Subsequently, the tubes were placed vertically for mixture gelation (polycondesation) for 48 and 72 h, at room temperature (24 ± 2 °C) and 50–70% relative humidity. Previous to electrophoresis run, corks were removed from glass tubes with high caution in order to avoid cracking of formed sol–gel rods. The experimental tube containing sol–gel rod was mounted vertically between two glass containers holding PB (0.05 M) as already described [19]. Bottom end of the tube was kept in direct contact with buffer solution. On upper part of the tube a tinny glass funnel was hermetically mounted and sealed to prevent leaks. 30 μl of tracking dye stock (water solution of 0.02% bromophenol blue sodium salt) mixed with 30 μl of 5% glycerol solution was gently introduced with a Pasteur pipette through the upper tube side. Subsequently the funnel was carefully filled with 0.05 M PB solution, platinum electrodes were immersed in lower and upper buffer solution and connected to power supply. A voltage was applied by regulated dc power supply (Kepco, BHK, New York) through Pt wire immersed electrodes. Current values were measured with volt-amperometer (Zico) through voltage application during the run. The run was concluded when the tracking dye reached the bottom end of pre-cast sol–gel column.

2.3. Preparation of liquid siloxan media and electrophoresis conditions determination (case B)

For the experiments a number stock solutions were prepared:

- *Solution A*: 60.6 mg/ml of tris–HCl + 4 mg/ml of SDS (sodium dodecyl sulfate), pH 6.8.
- *Solution B*: 4 ml of solution A + 4 g of glycerol + 2 ml of di-mercaptoethanol + 1.2 g of SDS.
- *Solution C*: 2 volumes of 2% albumin + 1 volume of solution B.
- *Ammoniacal silver solution*: 1.4 ml of concentrated NH_4OH + 21 ml of 0.36% NaOH + 4 ml of 19.4% $AgNO_3$ made up to 100 ml with water.
- *Solution D*: fresh solution of 0.005% citric acid + 0.019% formaldehyde.
- *Solution E*: 1 ml of 10% albumin + 0.1 ml of 10% glutaraldehyde + 3 ml of ammoniacal silver solution + 2 ml of solution D.
- *Solution E**: Solution E in which instead albumin solution 1 ml of H_2O was added.

Three types of sol–gel compositions were prepared for the experiments.

- *Chemical set I*: 0.02 ml of methylene blue water solution (0.2%) was mixed with 3.5 ml of methanol (99.6 %) and stirred for 5 min. Afterward 2.5 ml of MTMOS were added to methylene–methanol solution and the mixture was stirred for additional 10 min. Finally, 0.5 ml of HCl (0.01 M) was added and the solution was stirred again for 10 min.
- *Chemical set II*: 3.5 ml of methanol were mixed with 2.5 ml of MTMOS and stirred for 5 min. Freshly prepared solution C (0.25 ml) was heated to 95 °C for 5 min in a water bath, and added through continuous stirring to methanol–MTMOS mixture for further 10 min. Finally, 0.4 ml HCl (0.01 M) was added and the mixture was again stirred for 10 min.

- *Chemical set III*: 3.5 ml of methanol + 2.5 ml of MTMOS were mixed by stirring for 5 min. This mixture was supplemented with 0.25 ml freshly prepared solution E and stirred for additional 10 min. Finally, 0.25 ml HCl (0.01 M) was added and mixture stirred for 10 min.

Experimental pH of mixtures was between 3.0 and 3.5. An experimental volume of 13 ml of sol–gel solution was set up for each experiment. The solution was divided equally in two separate cuvettes. One volume of 6.5 ml was used for electrophoresis process, while the second one was left on bench as control (to observe hydrolysis–polycondensation process without applied electrical field). Set III contained solution E namely silver stained albumin [19]. As control, electrophoresis was conducted in sol mixture of set III in which solution E (containing stained protein molecules) was replaced with solution E* (without protein). It should be mentioned that during sol–gel preparation, water content of the various experimental dopants was calculated (consequently water: HCl ratio varied).

The experimental electrophoretic design was very simple. Two platinum foil electrodes (13 mm × 25 mm × 0.1 mm) were placed vertically to reach the bottom of small flat plastic cuvette (H-10 mm, L-80 mm and W-35 mm) and mounted to its butt-end sides with small earth clamp crocodile type electrodes connected to an electrical circuit (Fig. 1). All electrical set ups, including the electrodes, were previously connected to power supply before pouring the sol–gel precursor sets. Power supply was switched on following cuvette loading with the experimental mixture. The experiments were carried out at an initial current value of 1.5 mA and at a potential diapason of 30–50 V. The electrophoretic run took approximately 110–120 min and was completed when the experimental sol–gel mixture turned out to be highly viscous. It should be mentioned that the observed circuit current drop off was related to the increase in silica mixture viscosity, and consequently migration process practically ended when current value reached 0.2–0.3 mA. As a result, at these current intensities the electrical circuit was turned off and the gel was left exposed to ambient air for drying until formation of a flaking film.

3. Results and discussion

3.1. Case A. Solid sol–gel matrices for electrophoresis. Optimization of sol–gel composition and electrical parameters for electrophoresis process

Primarily, the present study was aimed toward electrophoretic application in sol–gel matrices following gelation as a migration template for small charged molecules such as dyes. Electrophoretic measurements were carried out in void glass rods filled with TEOS precursor at various concentrations (5%, 9.35%, 15% and 18.75%). Gels with low TEOS concentration (5% and 9.35%) were found to be rheologically too slack for this task. The other two concentrations of TEOS (15% and 18.7%) were found to be suitable for electrophoretic migration of small charged molecules. Following voltage application to sol–gel rod the tracking dye (bromphenol blue) placed at the upper rod end instantly began to move from the positive towards the negative electrode. Fig. 2 shows the location of dye band after 40 min of electrophoretic run (at 100 V). The experiment was halted when the dye reached the bottom end of the rod (∼9 cm length). In the process of electrophoretic experiments conducted at different applied voltages a current value was measured provided that dye band reached bottom end of the rod (Table 1).

As expected, migration rate of the tracking dye was strongly dependent on applied voltage. It can be seen that tracking dye

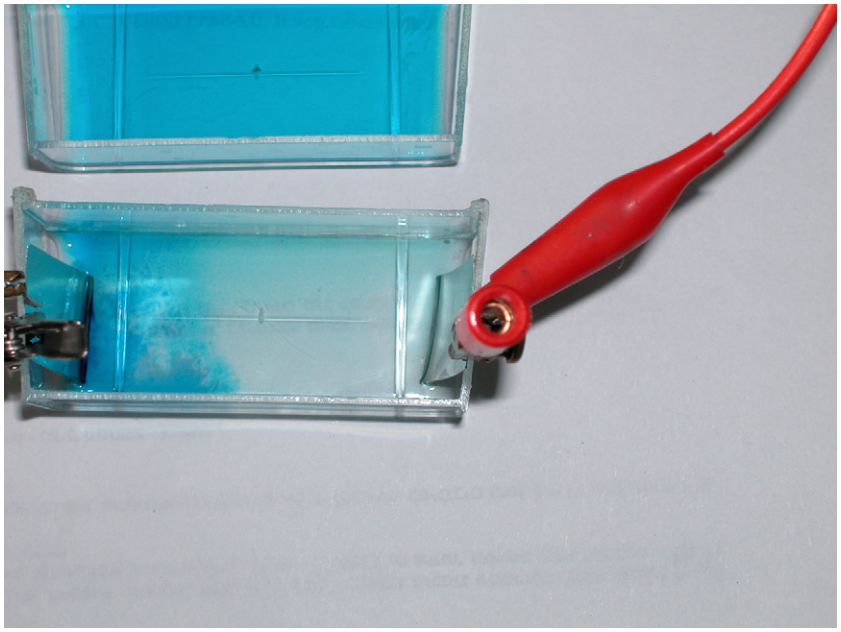

Fig. 1. Methylene blue electrophoretic mobility during current application in sol phase prior to gelation. Above the control can be seen with homogenous distribution of the dye in sol–gel solution without current application.

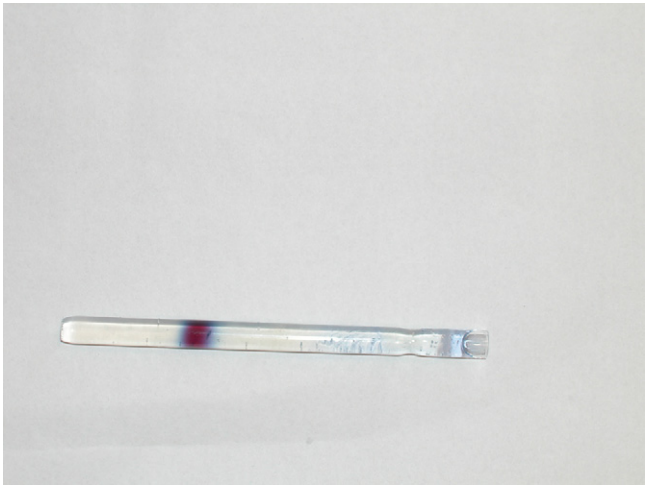

Fig. 2. Sol–gel rod with methylene blue band following electrophoretic run and drying.

migration rate increased exponentially with applied voltage. Voltage shift from 50 to 200 V resulted in approximately sixfolds increase in dye rate transport. The detected exponential relationship between dye transport rate and applied voltage (i.e. deviation from linear dependence between parameters) is clearly a result of temperature increase in sol–gel matrix attributed to current raise. In turn, increased temperature enhances ionic mobility in electrolyte, and accordingly raising dye transport rate. No significant increase in rod temperature was detected during electrophoretic runs up to 150 V. However, above 200 V substantial heat generation was measured along the run.

Control experiment was carried out with sol–gel rods loaded with tracking dye and left vertically without applied electrophoretic field. In the absence of current application tracking dye diffused slowly and uniformly from the upper to bottom end staining the whole sol–gel rod. In contrast, applied electrophoretic field carried the tracking dye and condensed it into a narrow thin disk shape inside the sol–gel rod (Fig. 2).

Table 1
Electrical characteristics of sol–gel rods during electrophoretic run

Sol–gel composition (% TEOS)	Time[a] (min)	Voltage (V)	Current (mA)
15	Start	50	1.22
	40		1.46
	75		1.7
	120 (end)		2.09
18.75	Start	50	1.5
	40		1.68
	75		1.75
	115 (end)		2.1
	Start	100	3.2
	30		3.3
	75 (end)		3.9
	Start	150	5.29
	20		6.1
	53 (end)		6.5
	Start	200	8.56
	25 (end)		10.44

[a]Time interval of tracking dye movement from the upper to bottom rod edges.

3.2. Case B. Accumulation of bio-molecules by application of electrical field to sol–gel mixture during its polymerization

In the light of preliminary results showing that electrophoretic field application can move small molecules in pre-gelated sol–gel, in the second part of the present study the feasibility to concentrate various molecules of different size by this method was tested. Larger protein molecule such as albumin (compared with bromophenol blue) did not migrated (data not shown) under conditions described above. As a result, the approach was altered and electrophoresis was performed on relatively thin layers of sol–gel solution (3–4 mm) that in consequence (after drying) were shaped into film. Pilot investigations were carried out in order to achieve the optimal sol–gel chemical ratios and electrophoretic parameters. For this task, the foremost prerequisite characteristics of sol–gel mixture composition were: (a) sol–gel gelation rate should be slower than migration and accumulation of the experimental compound at one side of the electrophoretic field; (b) sol–gel film should not be brittle and could be easily removed from cuvette, following gelation and drying for 36–48 h at ambient temperature and; (c) naked eye or optical microscopy should easily sight migration and accumulation of experimental compound at cuvette edge in case of natural chromatic/fluorescent molecules (or following staining, if colorless).

3.2.1. Optimization of sol–gel composition and electrical parameters for electrophoretic process

Preliminary experiments revealed that sol–gel at the following composition: 3.5 ml of methanol, 2.5 ml of MTMOS, 0.1 ml of distilled water and 0.4 ml of HCl (0.01 M) is the optimal composition under applied experimental conditions.

Optimization of electrophoretic parameters was carried out in chemical set I containing methylene blue tracking dye. It was shown that an efficient electrophoretic run could be conducted if applied voltage does not exceed 50 V. At higher voltage, the dye is oxidized at the positive electrode, while at the negative electrode hydrogen evolution may occur, since start pH of used sol mixture is 3.5. Moreover, when applied voltage was >50 V at cuvette cathodic region, gelation process significantly slowed down. It was empirically revealed that the most efficient electrophoresis was achieved when the initial applied current values were between 1.2 and 1.5 mA. Under these electrophoretic parameters setup (initial current −1.5 mA, initial voltage −30 V, and maximum voltage −50 V) the pervasive methylene blue concentrated in the vicinity of cathodic electrode in 90–120 min of electrophoretic run. Methylene blue migration towards cathodic electrode following 80 min of current application is shown Fig. 1 (bottom cuvette) compared with control (upper cuvette) initially en bloc blue colored. During whole experimental run (up to complete gelation) the control mixture remained uniformly blue colored.

Table 2
Current values measured during electrophoretic process in sol–gel mixture (chemical set I)

Time (min)	Current (mA)	Voltage (V)
0 (start)	1.36	30
50	0.823	45
85	0.347	50
120	0.078	50

Throughout electrophoretic run, it was noted that measured current values gradually decreased with time, indicating build up of sol–gel electrical resistance throughout the gelation process, and as such ions migration deceleration (Table 2).

Another observed feature was that the dye migration in electrical field occurred only up to a certain sol–gel consistency (critical value of sol–gel viscosity) and practically terminated with any additional electrophoretic current exposure. A current value of 0.2–0.3 mA matched this critical sol–gel viscosity point.

Consequently, these preliminary results supported the prospect of migration and accumulation of small organic compounds introduced into sol–gel under applied voltage on one region.

3.2.2. *Electrophoretic migration of albumin molecules in liquid sol–gel matrix*

Additional experiments were conducted with sol–gel prepared through chemical set II. The aim of these experiments was to show that small proteins incorporated into composition II could be concentrated at a defined zone through the electrical field application. Bovine serum albumin (60 kDa MW) acknowledged as a relatively small protein was selected for this goal. Prior to its introduction into sol–gel mixture, albumin was prepared as previously described in the protocol of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [20].

With this chemical set (II) electrophoresis was performed at electrical set up parameters identically to those used for tracking dye. The process was ended when the measured current reached the value of 0.3 mA, following an exposure time of 1.5–2 h. In order to reveal protein location after electrophoretic run, gelated and dried films containing albumin, were further stained by silver staining method (highly sensitive for proteins) as already described [19]. If present, the protein zone was stained as a dark-brown color band. Fig. 3 shows the various types of formed sol–gel films. In this figure "A" and "B" are albumin-containing films formed from sol–gel mixture of chemical set II subjected ("A") and non-subjected ("B": control) to electrophoretic run. Both films are shown after their staining by silver method, which was performed next to complete gelation and drying of experimental films. The brown zone (silver-stained proteins) could be seen on film "A" adjacent to cathode edge. Control film "B" showed no distinctive brown zone coloration, however uniformly spread brown stained albumin could be seen in pores of this film, with the help of a magnifying glass.

Improved results were obtained when electrophoresis was carried out with sol–gel prepared through chemical set III. The difference between chemical set II and chemical set III was as follows: to set III mixture, silver pre-stained albumin molecules were introduced. The staining was performed by adding to albumin solution all chemical ingredients used in mentioned above silver method in sequence of staining operations given in [19]. The obtained film following electrophoresis revealed a sharp dark-brown band located at the cathodic site end (Fig. 3C). This band was formed in liquid sol–gel during electrophoresis

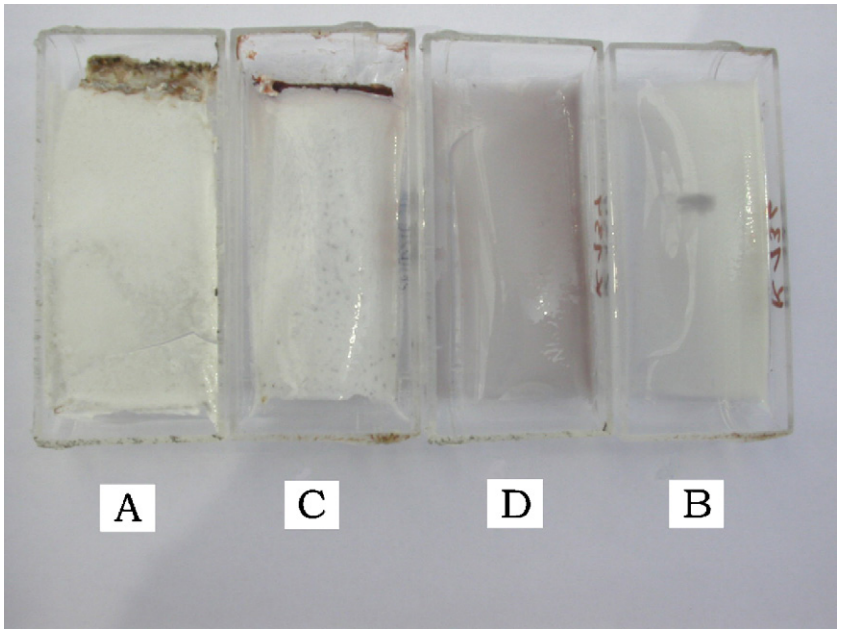

Fig. 3. Sol–gel films: (A) electrophoresis of albumin in sol–gel precursors before gelation phase with following silver staining; (B) control of "A" (the same sol–gel composition) without application of electrophoretic field; (C) electrophoresis of previously silver stained albumin; (D) control to "C"—electrophoresis of sol–gel composition used in "C" without albumin.

process. In order to make sure that produced brown band is a result of stained albumin molecules migration towards cathodic electrodes rather than staining components introduced into the sol–gel mixture together with proteins, supplementary control experiment was carried out. Electrophoresis was also conducted in sol–gel mixture containing as dopant only chemicals used for protein staining (without proteins). The control did not reveal any stained band on film made of the same sol–gel blend following electrophoresis (Fig. 3D). It is important to mention that migration of pre-stained protein molecules in electrical field occurred markedly faster compared to unstained ones. Improved electrophoresis obtained with previously stained proteins molecules may be explained as follows: positively charged Ag + ions of $AgNO_3$ salt used for the staining once incorporated into protein molecules increase their positive net charge and thus enhance the mobility of albumin molecules towards the negatively charged cathode.

4. Conclusions

In the last two decades, the sol–gel process of various ceramic materials, especially with ormosils [21,22] is widely gaining new fields in chemistry and biology. One of those aspects is production of various sensors for clinical, environmental and industrial applications. The sol–gel process is simple and because of reaction at room temperature it has a major advantage over other biosensors production. However, the doping process with various biomolecules is inefficient due to burial of many molecules into the sol–gel matrix without direct contact with the external surface, therefore inactive. The basic idea of the present study was to move those molecules from the bulk towards the outer surface and increase their concentration at site for better reaction. The present study revealed that electrophoretic field application to sol–gel process may be appropriate to achieve this task. The following conclusions can be drawn:

(a) Pre-casted gelated rods made by sol–gel technology (following hydrolysis and condensation) can be used in electrophoresis process to move small molecules from end to another (demonstrated with bromophenol blue tracking dye).
(b) Application of an electrophoretic field to a liquid sol–gel (at early stage of hydrolysis and poly-condensation) can transfer biologically active compounds and concentrate them at one of the electrodes (in our case at cathodic pole) and remain there following gelation.

In conclusion, according to the two applications mentioned above, small molecules can be concentrated in pre-casted sol–gel while large biomolecules can be concentrated at one end only in liquid sol–gel films before final gelation occurs, minimizing the loss into the matrix following random doping.

References

[1] A. Walcarius, Electrochemical applications of silica-based organic–inorganic hybrid materials, Chem. Mater. 13 (10) (2001) 3351–3372.
[2] A.R. Howells, P.J. Zambrano, M.M. Collinson, Diffusion of redox probes in hydrated sol–gel-derived glasses, Anal. Chem. 72 (21) (2000) 5265–5271.
[3] A. Walcarius, D. Mandler, J.A. Cox, M. Collinson, O. Lev, Exciting new directions in the intersection of functionalized sol–gel materials with electrochemistry, J. Mater. Chem. 15 (35–36) (2005) 3663–3689.
[4] S.J. Limner, S. Seraji, Y. Wu, T.P. Chou, C.G. Nguyen, Cao template-based growth of various oxide nano-rods by sol–gel electrophoresis, in: International SAMPE Technical Conference, vol. 33, 2001, p. 709.
[5] Y. Lin, G.S. Wu, X.Y. Yuan, T. Xie, L.D. Zhang, Fabrication and optical properties of TiO_2 nanowire arrays made by sol–gel electrophoresis deposition into anodic alumina membranes, J. Phys.: Condens. Matter. 15 (17) (2003) 2917–2923.
[6] J.S. Colson, H. Buscail, G. Bonnet, M. Lachkar, J.P. Larpin, Diffusion and defect data—solid state data Pt. B: Solid state phenomena, Diffus. React. 41 (1995) 165.
[7] B. Neiman, E. Grushka, J. Gun, O. Lev, Organically modified silica sol-mediated capillary electrophoresis, Anal. Chem. 74 (14) (2002) 3484–3491.
[8] M.-C. Millot, V.-M. Claire, Overview of the surface modification techniques for the capillary electrophoresis of proteins, Advances in Chromatography, vol. 40, Marcel Dekker, Inc., New York, 2000 pp. 427–466.
[9] J.D. Hayes, M. Abdul, Sol–gel chemistry-based Ucon-coated columns for capillary electrophoresis, J. Chromatogr. B: Biomed. Sci. Appl. 695 (1) (1997) 3–13.
[10] L. Hua, S.N. Tan, Capillary electrophoresis with an integrated on-capillary tubular detector based on a carbon sol–gel-derived platform, Anal. Chem. 72 (20) (2000) 4821–4825.
[11] D. Avnir, S. Braun, O. Lev, M. Ottolenghi, Enzymes other proteins entrapped in sol–gel materials, Chem. Mater. 6 (10) (1994) 1605–1614.
[12] D. Avnir, Organic chemistry within ceramic matrixes: doped sol–gel materials accounts of chemical, Research 28 (8) (1995) 328–334.
[13] C. Dozoretz, R. Armon, J. Starosvetsky, N. Rothschild, Entrapment of parathion hydrolase from *Pseudomonas* spp. in sol–gel glass, J. Sol–Gel Sci. Technol. 7 (1–2) (1996) 7–11.
[14] R. Armon, J.O. Starosvetsky, I. Saadi, Sol–gel as reaction matrix for bacterial enzymatic activity, J. Sol–Gel Sci. Technol. 19 (2000) 289–292.
[15] R.B. Bhatia, C.J. Brinker, A.K. Gupta, A.K. Singh, Aqueous sol–gel process for protein encapsulation, Chem. Mater. 12 (8) (2000) 2434–2441.
[16] J.D. Badjic, N.M. Kostic, Effects of encapsulation in sol–gel silica glass on esterase activity, conformational stability, and unfolding of bovine carbonic anhydrase II, Chem. Mater. 11 (12) (1999) 3671–3679.
[17] M.I. Ferrer, F. del Monte, D. Levy, A novel and simple alcohol-free sol–gel route for encapsulation of labile proteins, Chem. Mater. 14 (9) (2002) 3619–3621.
[18] M.G. Santangelo, M. Levantino, E. Vitrano, A. Cupane, Ferricytochrome c encapsulated in silica hydrogels: correlation between active site dynamics and solvent structure, Biophys. Chem. 103 (2) (2003) 99–107.
[19] A.T. Andrews, Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications, 2nd ed., Clarendon Press, Oxford, 1986, pp. 32–34.
[20] E.P. Rybicki, M.B. Von Wechmar, M. Barbara, Enzyme-assisted immune detection of plant virus proteins electroblotted onto nitrocellulose paper, J. Virol. Meth. 5 (5–6) (1982) 267–278.
[21] D. Avnir, Th. Coradin, O. Lev, J. Livage, Recent bio-applications of sol–gel materials, J. Mater. Chem. 16 (11) (2006) 1013–1030.
[22] T.M. Lopez, D. Avnir, M. Aegerter (Eds.), Emerging Fields in Sol–Gel Science and Technology, Kluwer Academic Publishers, Norwell, MA, USA, 2003, p. 454.