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Sol–gel chemistry-based Ucon-coated columns for capillary electrophoresis

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Abstract

A sol–gel chemistry-based novel approach for the preparation of a Ucon-coated fused-silica capillary column in capillary electrophoresis is presented. In this approach the sol–gel process is carried out inside 25 μm I.D. fused-silica capillaries. The sol solution contained appropriate quantities of an alkoxide-based sol–gel precursor, a polymeric coating material (Ucon), a crosslinking reagent, a surface derivatizing reagent, controlled amounts of water and a catalyst dissolved in a suitable solvent system. The coating procedure involves filling a capillary with the sol solution and allowing the sol–gel process to proceed for an optimum period. Hydrolysis of the alkoxide precursor and polycondensation of the hydrolyzed products with the surface silanol groups and the hydroxy-terminated Ucon molecules lead to the formation of a surface-bonded sol–gel coating on the inner walls of the capillary. The thickness of the coated film can be controlled by varying the reaction time, coating solution composition and experimental conditions. Commercial availability of high purity sol–gel precursors (e.g., TEOS 99.999%), the ease of coating, run-to-run and column-to-column reproducibility, and long column lifetimes make sol–gel coating chemistry very much suitable for being applied in analytical microseparations column technology. Test samples of basic proteins and nucleotides were used to evaluate the column performance. These results show that the sol–gel coating scheme has allowed for the generation of bio-compatible surfaces characterized by high separation efficiencies in CE. For different types of solutes, the sol–gel coated Ucon column consistently provided migration time R.S.D. values of the order of 0.5%. © 1997 Elsevier Science B.V.

Keywords: Sol–gel chemistry; Ucon-coated capillaries; Column technology; Proteins; Nucleotides

1. Introduction

In the recent decade capillary electrophoresis (CE) has become one of the domineering analytical techniques, especially for the separation of biomolecules including proteins, peptides, nucleotides, etc.. Because of the short analysis time and high column efficiencies inherent in CE, many researchers have been attracted to this technique. However, such problems as solute adsorption and migration time irreproducibility occur as a direct influence of the acidic silanol groups residing on the inner surface of the fused-silica capillary commonly used as the separation column. This adsorption of biomolecules, especially of those that contain basic sites, leads to considerable band broadening and asymmetry, thereby significantly reducing the column's efficiency from the theoretical predictions.

In the past, extensive research has been devoted to reducing the solute-wall interactions in CE [1–31]. Various approaches have been applied to achieve this goal including (a) the use of operating buffers with extreme pH values [1,2], (b) application of buffer additives (e.g., [6,7]) and (c) chemical modification

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of the column inner surface [8–21]. The first two approaches are easy to implement but are often coupled with adverse side effects in separations [3–6]. The third approach usually provides high efficiency surface-coated columns. However, it often involves multiple steps of surface reactions and related treatments. This increases the likelihood of column-to-column performance variability. Batch-to-batch irreproducibility of fused-silica surface characteristics is another factor that aggravates the problem.

To reduce the number of steps involved in the column making procedure, recently we developed a simple method in which the surface modification processes (derivatization, coating and crosslinking) were effectively accomplished in a single step [15]. This method allowed routine preparation of highly efficient columns (up to 1.2 million total plates) [8,15] that provided excellent separations of proteins [22,23], peptides [8] and nucleotides [24,25].

Sol–gel chemistry has the potential to provide new possibilities in achieving higher reproducibilities in column technology. Intelligent application of sol–gel chemistry should allow the creation of chemically bonded surface coatings for microcolumn separation techniques. Sol–gel technique has the potential to offer important advantages over conventional coating techniques. These include: (1) strong adhesion between the coating and the bare fused-silica surface because of chemical bonding, (2) relative ease of applying coatings, (3) enhanced coating repeatability due to the high purity of the alkoxide precursors and (4) molecular level mixing of sol–gel reaction ingredients under exceptionally mild thermal conditions. The sol–gel technique also provides an effective coverage of the exposed, adsorptive groups, and allows for operation within a wide pH range. Furthermore, sol–gel chemistry may be applied to generate thin films from various materials, organic, inorganic and composites. These features of sol–gel technology allow the experimenter to have a better control of the coating process and offer a new and promising perspective to column technology.

Until recently, only a few reports have been published in the scientific literature regarding the use of sol–gel technology for column preparation. Engelhardt and Cuñat-Walter [26] used sol–gel chemistry to create an olefinic sublayer for subsequent polymerization with acrylamide to prepare polyacrylamide coated open tubular columns for capillary electrophoresis. Guo and Colon [27] used this technique to bind retentive alkyl ligands in open tubular columns for liquid chromatography and capillary electrochromatography. Recently we reported a sol–gel method for the preparation of thermally stable open tubular GC columns with polymeric stationary phases [32].

The potential of sol–gel chemistry in creating a wide range of sol–gel composite films and coatings for analytical separations using various polymeric materials is great, and this area of column technology remains to be explored. In this paper we describe a sol–gel chemistry-based new method for creating chemically bonded Ucon coating on the inner walls of the capillary and explore capillary electrophoretic performances of such columns.

2. Experimental

2.1. Equipment

An ATI Unicam Model Crystal 310 capillary electrophoresis system (Analytical Technology, Boston, MA, USA) equipped with an ATI Unicam Model 4225 variable-wavelength UV detector was used in all CE experiments. A homemade capillary filling/purging device (Fig. 1) with gas pressurization was used for coating the fused-silica capillary by the sol–gel technique. This device allows a sol

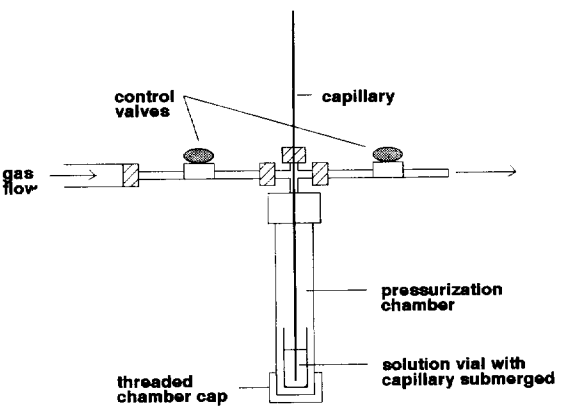


Fig. 1. Schematic of a homemade capillary filling/purging device.

solution to be pushed through the capillary using a pressurized inert gas. It can also be used to dry the capillary inner surface by passing a flow of the inert gas through the capillary. A Microcentaur Model APO 5760 centrifuge (Accurate Chemical and Scientific Corp., Westbury, NY, USA) was used to separate the sol solution from the precipitate. A Fisher Model G-560 Vortex Genie 2 system (Fisher Scientific, Pittsburgh, PA, USA) was used for thorough mixing of various solution ingredients. The electropherograms were recorded using Chrome Perfect (Version 6.07) computer software (Justice Innovations, Mountain View, CA, USA). A Chemcadet Model 5984-50 pH meter (Cole–Palmer Instrument Co., Chicago, IL, USA) equipped with a specially designed Sigma–Aldrich (St. Louis, MO, USA) pH electrode was used to measure the pH of the Trizma buffer systems.

2.2. Chemicals and materials

Anhydrous methyl formate, ethanolamine (99.5 + %), methylene chloride (HPLC Grade), tetraethoxysilane (TEOS, 99.999+%), monobasic potassium phosphate, dicumyl peroxide, dimethyl sulfoxide and hexamethyldisilizane (HMDS), and concentrated hydrochloric acid (HCl) were all purchased from Aldrich (Milwaukee, WI, USA). Basic proteins, Trizma–HCl, dibasic sodium phosphate (heptahydrate) and formamide (99.9%) were obtained from Sigma (St. Louis, MO, USA). Ucon 75-H-90000 (Polyalkylene glycol) was purchased from Alltech Associates (Deerfield, IL, USA). Deionized water (18 MΩ), used to rinse the columns and to prepare the buffer solutions, was obtained from a Barnstead Model 04741 Nanopure deionized water system (Barnstead/Thermodyne, Dubuque, IO, USA). Fused-silica capillaries of 25 μm I.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). Eppendorf 1.5 ml volume natural polypropylene microcentrifuge tubes were purchased from Brinkmann Instruments (Westbury, NY, USA).

2.3. Capillary column preparation

2.3.1. Preparation of the sol solution

The sol solution was obtained by thorough mixing of the following ingredients: (1) tetraethoxysilane (400 μl), (2) methyl formate (500 μl), (3) formamide (100 μl), (4) Ucon 75-H-90000 (20 mg), (5) hexamethyldisilizane (30 μl), 0.1 *M* dicumyl peroxide in pentane (20 μl) and 100 μl of 0.5 *M* ethanolamine solution in methanol–deionized water (6:1 v/v). Mixing was conducted by Vortex shaking for 5 min. The mixture was then centrifuged at 13 000 rpm (15 682 *g*) for 2 min. The precipitate, if any, collected on the bottom of the centrifuge tube, was discarded and the clear sol solution from the top was transferred into another clean vial for further processing.

2.3.2. Sol–gel coating of the fused-silica capillary

A 75 cm long piece of 25 μm I.D. capillary was installed into the capillary filling system (Fig. 1) by emerging the distal end through the pressurization chamber and allowing it to be submerged into a solution vial placed inside the chamber. The chamber cap (at the bottom) provides an airtight seal when the chamber is pressurized to fill the capillary with a liquid. The cap also allows for the easy exchange of vials containing liquid systems for capillary rinsing or coating.

The capillary inner surface was first cleaned by rinsing with methylene chloride and pentane solution (1:1 v/v) for 30 min, followed by purging with nitrogen at 344.7 kPa for an additional 30 min period. The capillary was then filled with the sol solution using 689.4 kPa nitrogen pressure. It was then left undisturbed for 20 min. Following this, the solution was expelled from the capillary using 689.4 kPa nitrogen pressure and purging with nitrogen for an additional 30 min. The capillary was then removed from the coating system and both ends were sealed using an oxyacetylene torch. This was followed by heating in an oven at 170°C for 1 h. Next, the capillary was removed from the oven, and both fused ends were cut open using an alumina wafer. This was followed by sequential rinsing of the capillary at 689.4 kPa nitrogen pressure for 30 min each with (a) a methylene chloride and pentane solution (1:1, v/v), (b) methanol, (c) deionized water and (d) the operating buffer. The protective polyimide coating on the outer surface of the capillary was burnt off from a 5-mm section at a distance of about 60 cm from the column inlet to prepare an on-

column detector window. This was done prior to rinsing with deionized water.

2.3.3. Preparation of buffers

A 50 m*M* Tris–HCl background electrolyte solution was prepared by dissolving 4.186 g of Trizma–HCl into 400 ml of deionized water, purified to 18 MΩ using the Nanopure water system. Dibasic sodium phosphate heptahydrate (1.608 g) and monobasic potassium phosphate (0.861 g) were added to 400 ml of 50 m*M* Tris–HCl solution to obtain the Tris–phosphate buffer. Concentrated HCl or 2 *M* sodium hydroxide solution was added drop by drop to adjust buffer pH to desired values.

2.3.4. Capillary electrophoretic operations

Following installation into the CE system, the column was rinsed with the buffer solution at 200 kPa for 2 min. Samples were prepared by dissolving the analytes in the operating buffer solution with a concentration of 1–5 mg ml^{-1}. Sample introduction into the columns was performed by electromigration injection at a constant voltage (−6 and 12 kV) for a fixed period of time (0.06 and 0.10 min). Sample injection for repeatability data was performed by hydrodynamic injection at a constant applied pressure (2 kPa) for 0.10 min.

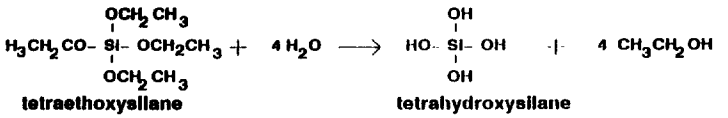
3. Results and discussion

Sample adsorption and migration time variation are two longstanding problems in capillary electrophoresis with fused-silica capillaries, commonly used as the separation column. Two main factors are responsible for this problem. These are (a) existence of surface silanol groups and (b) temporal variation of their concentrations on inner walls. Effective solution of these problems is possible through appropriate modifications of the capillary inner surfaces by chemical means. Since silanol groups are the main sites that can be used for chemical modification, a uniform distribution of these bonding sites over the surface is necessary to achieve a uniform coverage of the surface with chemically bonded organic coatings. However, an untreated fused-silica capillary is characterized by low concentrations and non-uniform distributions of the surface silanol groups that may exist in patches over the surface. This is because of the high drawing temperature (>2000°C) of the fused-silica capillary and existence of the possibility for surface siloxane bridges to undergo reaction with atmospheric moisture to generate new silanol groups. Consequently, chemical research into reproducible creation of silica surfaces with uniformly distributed silanol groups at their optimum concentrations is fundamentally important for overall development of column technology, not only for CE but also other separation techniques. A silica surface with uniformly distributed silanol groups should be very much suited for its further chemical modification using various polymeric and monomeric reagents with functional groups that can react with silanol groups. Such chemically bonded coatings will ensure effective coverage of the surface and reliably shield the residual silanol groups to prevent their participation in solute adsorption phenomena. Here we describe a sol–gel chemistry-based surface-modification scheme to achieve these goals.

3.1. The sol–gel chemistry of CE surface modification

Sol–gel chemistry provides an alternative way of surface modification for CE column technology. It is based on hydrolysis of sol–gel precursors (commonly alkoxides) and polycondensation of the hydrolyzed products. In this work, tetraethoxysilane (TEOS) was used as the sol–gel precursor. Depending on experimental conditions, the hydrolysis of TEOS may be either partial or complete. Provided ample time is allowed and sufficient quantities of water are available, the hydrolysis will proceed to completion as schematically represented in Scheme 1.

Actually, this is a complex, multi-step reaction [33,34] that ultimately leads to the formation of reactive tetrahydroxysilane species. As the reaction proceeds, the hydrolysis products undergo polycondensation reactions. The growing polymeric network will be eventually bonded to the capillary walls as a result of its condensation with the surface silanol groups. Iler has published extensive investigations involving the mechanisms of these condensations reactions [35]. The condensation reaction, occurring between the tetrahydroxysilane species and the sur-



Scheme 1. Complete hydrolysis of tetraethoxysilane (TEOS).

face silanols on the capillary inner walls is depicted in Scheme 2.

Furthermore, the hydrolyzed products may undergo condensation with themselves. This will ultimately generate a polymeric structure, which may then react with the silica surface. Because of these condensation processes, the original fused-silica surface gets covered by anchored layers of in situ created polymeric silica material. Thus, unlike conventional surface modification techniques, sol–gel column technology involves creation and chemical modification of a silica layer evolving (in space and time) on the top of the original surface. Multidimensional propagation of the sol–gel network ensures that capillaries with significantly different surface characteristics, are likely to possess very similar surface properties after sol–gel coating. High purity of the alkoxide precursors is an important factor in achieving this surface standardization. Differences in silanol group distributions on originally taken surfaces will be reflected only on the anchoring pattern of the first bottom layers of the growing sol–gel film. This difference should have little influence on the properties of the finally formed top sol–gel surface. In this respect, sol–gel technique has a significant advantage over conventional coating methods.

In the sol–gel technique, Ucon-coated surface might have been formed via the condensation processes presented in Scheme 3.

Thermal treatment of the coated capillary aims at derivatizing part of the residual surface silanol groups with hexamethyldisilazane, and simultaneously cross-linking of the Ucon coating with dicumyl peroxide. It should be recalled that both chemicals are ingredients of the coating solution.

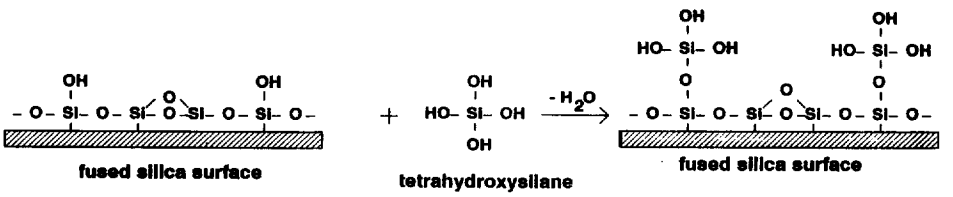
The crosslinking step was meant to provide higher operational stability to the polymeric coating. Scheme 4 illustrates the various steps involved in the crosslinking process [36].

3.2. CE characterization of the sol–gel Ucon coating

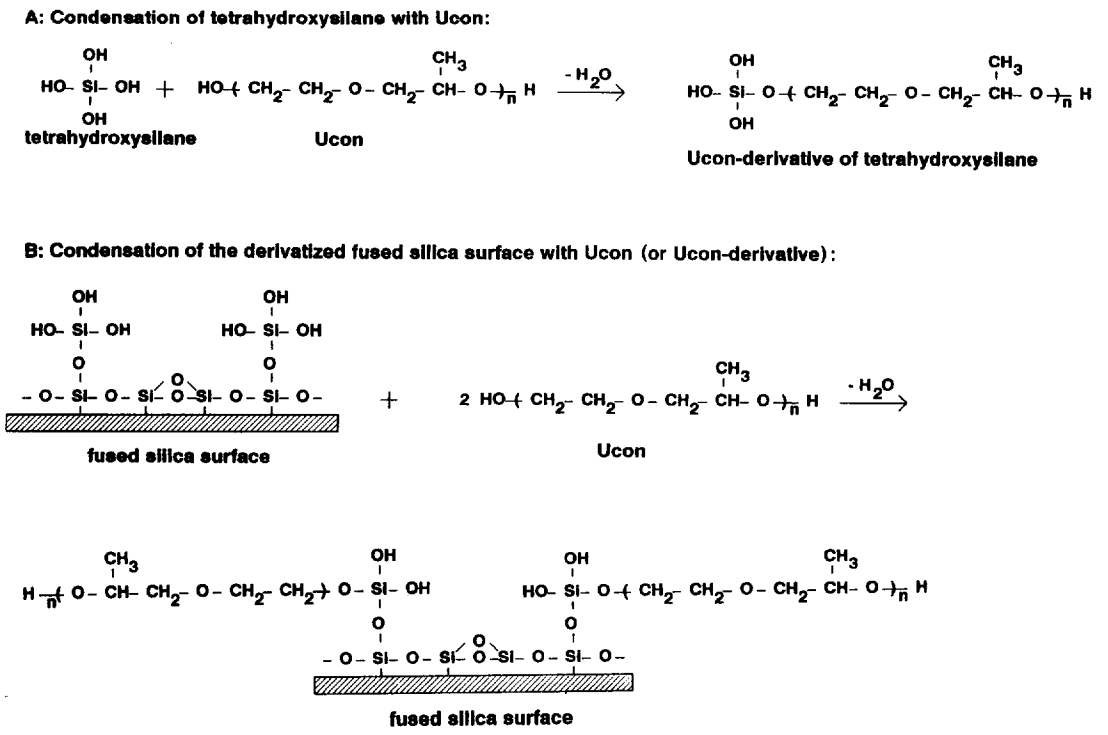
Following installation of a Ucon-coated column into the CE system, the electroosmotic flow within the column was investigated using 50 m*M* Tris–phosphate buffer (pH 5.18). Dimethylsulfoxide (DMSO) was used as the EO-flow (EOF) marker. The electroosmotic flow measurements in nine replicate CE runs, under constant conditions, had a mean migration time of 26.12 min and a relative standard deviation of 0.43%. The reproducible EOF in the sol–gel Ucon column is indicative of a stable temporal distribution of residual silanol groups along the capillary's modified inner surface.

Next, an evaluation of the column separation efficiency was performed and was evaluated in terms of theoretical plates (n). For this, bovine heart cytochrome c was used as the analytical probe, and a plate count of the order of 7×10^5 was obtained.

An analysis of column-to-column reproducibility was then evaluated using cytochrome c from horse heart as the eluting analyte. In doing this reproducibility analysis, cytochrome c was hydrodynamically injected into three different sol–gel



Scheme 2. Condensation of the fused-silica surface with tetrahydroxysilane.



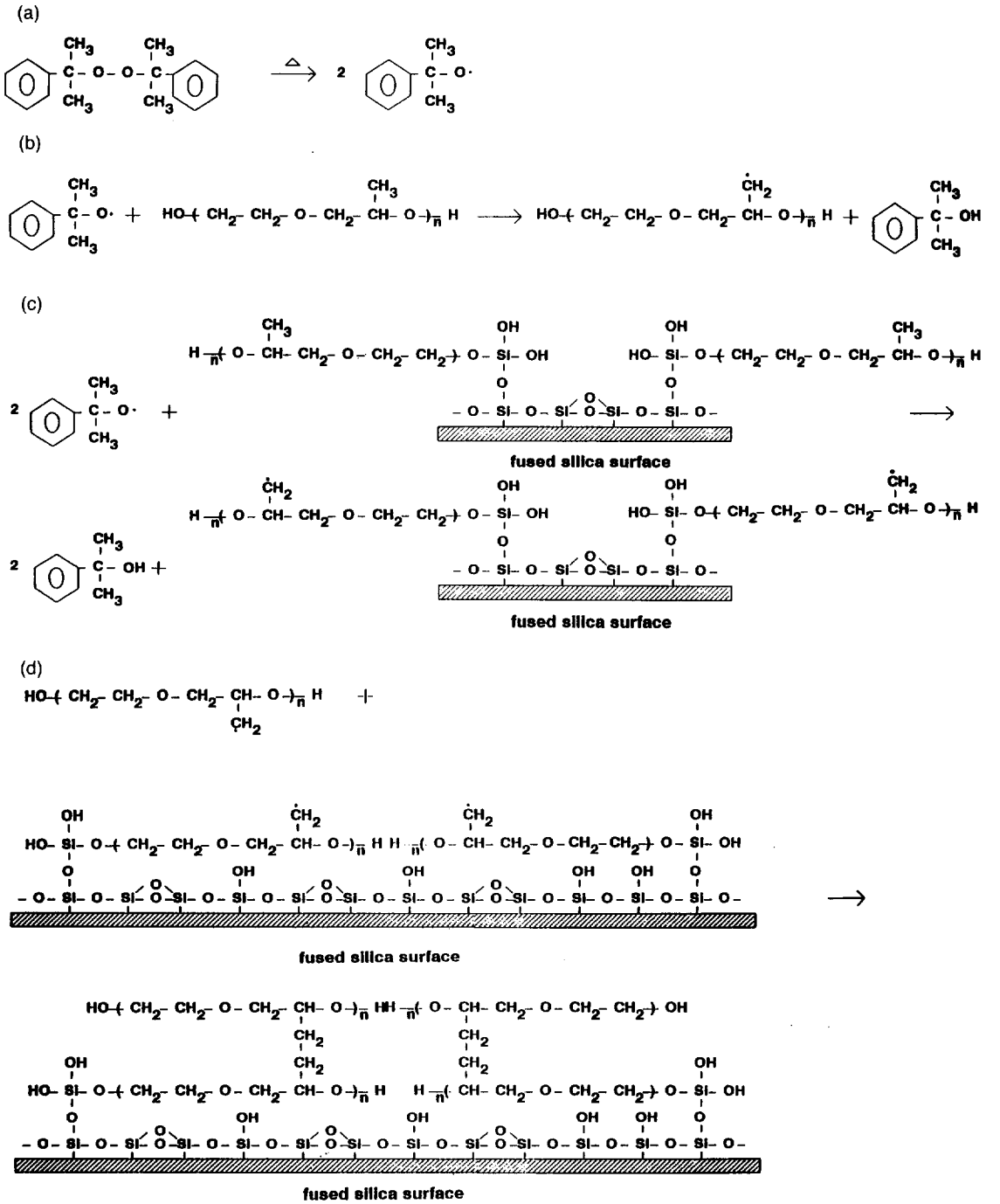
Scheme 3. Chemical bonding of sol–gel Ucon coating to fused-silica capillary inner walls.

Ucon columns, all prepared under constant conditions. Experimental results of this evaluation, using five injections into each column, showed an R.S.D. value of 1.88%.

The experimental results on the capillary electrophoresis separation of four basic proteins, on a fused-silica column with a sol–gel process mediated Ucon column, are represented in Fig. 2. In untreated fused-silica columns, interaction of basic proteins with the acidic surface silanol groups usually results in broad, tailing peaks. The sharp peaks in the presented electropherogram depict the adsorption inertness of the prepared column. This inertness may be attributed to a number of factors: (1) chemical derivation of the surface silanol groups due to their condensation reaction with the coating sol solution; (2) deactivation of the surface silanols with hexamethyldisilizane molecules during post-coating heating; and (3) creation of a bio-compatible Ucon coating that effectively shields the underlying residual silanol groups.

Experimental results on the migration time repeatability for four (basic and neutral) proteins in nine replicate runs are presented in Table 1. The relative standard deviations (R.S.D.) in migration times for these proteins were less than 0.6%. R.S.D. values of the order of 1.2–3.0% have been reported in the literature for the separation of basic proteins using dynamically coated columns [28,29]. In our previous work, static-coated Ucon columns using conventional techniques, have exhibited R.S.D. values of less than 0.7% [8,30]. Thus, the migration time repeatability data obtained on sol–gel coated columns compare favorably with analogous literature data for other types of permanently coated columns.

The capillary electrophoretic separation of seven cytochrome *c* proteins from various biological species is presented in Fig. 3. Cytochrome *c* is a very basic protein (p*I*~10.6). All of the used cytochrome *c* proteins consist of 104 amino acid residues (except cytochrome *c* from tuna with 103 amino acid residues) [37]. These cytochrome *c* proteins differ only in the identities of a small number of amino acid residues in their macromolecular primary structures



Scheme 4. (a) Production of free radicals by thermal decomposition of dicumyl peroxide (DCP). (b) Generation of free radicals on a free Ucon chain. (c) Generation of free radicals on surface-bonded Ucon chain. (d) The free radical-crosslinking of surface-bonded and free Ucon chains.

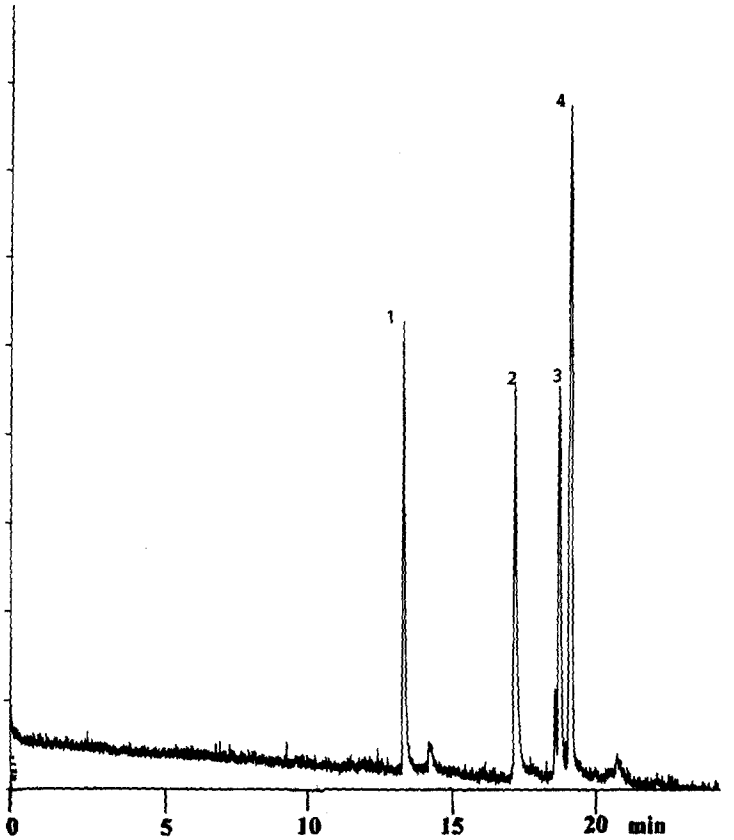


Fig. 2. CE separation of basic proteins on a sol–gel coated Ucon column. Conditions: 75 cm×25 μm I.D. sol–gel column (59 cm separation distance), coating: Ucon 75-H-90000; buffer: 50 m*M* Tris–PO_4 (25 m*M* Tris–HCl, 12.5 m*M* Na_2HPO_4 and 12.5 m*M* KH_2PO_4 pH 5.18, adjusted with HCl); injection: 5 kPa, 0.1 min; operating voltage: 30 kV, current: ≈11.6 μA; detection: UV at 214 nm. Peaks: (1) cytochrome *c* (horse heart); (2) ribonuclease A (bovine pancreas); (3) α-chymotrypsinogen A (bovine pancreas); (4) myoglobin (horse heart).

Table 1
Repeatability data on a sol–gel mediated Ucon column using a test sample of four basic proteins[a]

Protein	M_r (Da)[b]	p*I* (25°C)[c]	t_R (min)	*s*	R.S.D. (*n*=9)
Cytochrome *c* (horse heart)	12 384	10.6	13.221	0.07842	0.59%
α-Chymotrypsinogen A (bovine pancreas)	23 700	9.6	18.452	0.10872	0.59%
Ribonuclease A (bovine pancreas)	13 700	7.8	16.968	0.10232	0.60%
Myoglobin (horse heart)	17 500	7.0	18.796	0.10611	0.56%

[a] Conditions: buffer: 50 m*M* Tris–PO_4 (25 m*M* Tris–HCl, 12.5 m*M* Na_2HPO_4 and 12.5 m*M* KH_2PO_4 pH 5.18) adjusted with HCl; injection: 5 kPa for 0.1 min; operating voltage: 30 kV; current: ≈13.1 μA.
[b] Taken from 1996 Sigma catalog.
[c] Taken from Ref. [37], page 81.

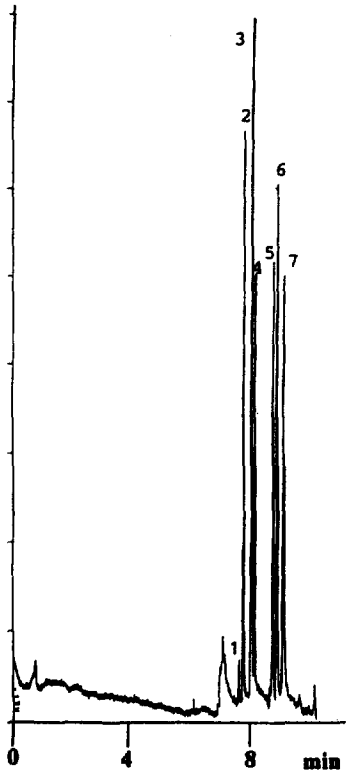


Fig. 3. CE separation of cytochrome *c* proteins from different biological species. Conditions: 75 cm×25 μm I.D. sol–gel column (59 cm separation distance), coating: Ucon 75-H-90000; background electrolyte: 50 m*M* Tris–HCl (pH 5.18); injection: electromigration (6 kV, 0.04 min); operating voltage: 30 kV; current: ≈11.4 μA; UV detection at 214 nm. Peaks: (1) horse (heart); (2) dog (heart); (3) pigeon (breast); (4) cow (heart); (5) chicken (heart); (6) tuna (heart); (7) pichia membranefaciens.

[4]. CE separation of these closely related basic proteins as sharp, narrow peaks illustrates the high efficiency and inertness of the sol–gel coated Ucon column. Table 2 represents the migration time repeatability data for these proteins obtained in nine successive runs. The R.S.D. values for these solutes fall within the 0.58–0.74% range.

Fig. 4 shows the CE separation of a standard mixture of eight 2′-, 3′- and 5′-monophosphate ribonucleotides on a sol–gel coated Ucon column. All of the components eluted within thirty minutes. This highly efficient separation is additional evidence for the generation of a biocompatible Ucon coating. CE separation of six 2′-, 3′- and 5′-monophosphate deoxyribonucleotides are depicted in Fig. 5.

Table 2
Repeatability data on a sol–gel mediated Ucon coated column using cytochrome *c* samples from various species[a]

Source	t_R (min)	*s*	R.S.D. (n=9)
Horse heart	12.545	0.07329	0.58%
Dog heart	12.613	0.08548	0.68%
Pigeon breast	12.976	0.08052	0.62%
Cow heart	13.235	0.08896	0.67%
Chicken heart	13.705	0.08401	0.61%
Pichia membranefaciens	14.536	0.10809	0.74%

[a] Conditions: buffer: 50 m*M* Tris–PO_4 (25 m*M* Tris–HCl, 12.5 m*M* Na_2HPO_4 and 12.5 m*M* KH_2PO_4 pH 5.18) adjusted with HCl; injection: 12 kV for 0.04 min; operating voltage: 30 kV; current: ≈10.6 μA.

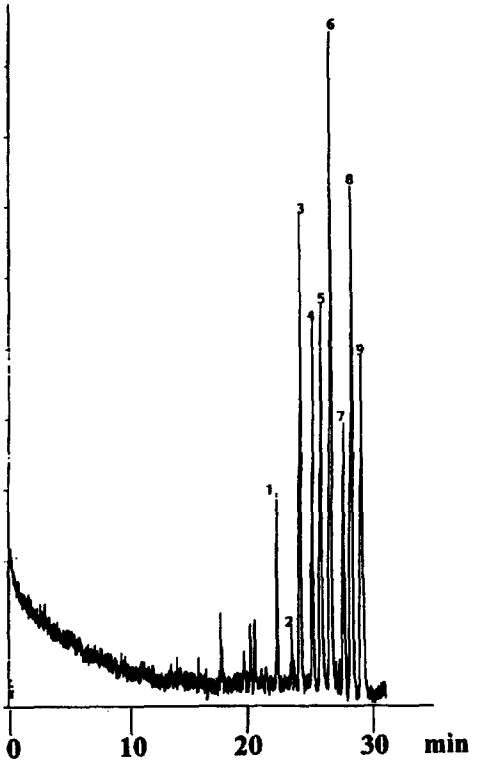


Fig. 4. CE separation of ribonucleotides on a sol–gel coated Ucon column. Conditions: 75 cm×25 μm I.D. sol–gel column (59 cm separation distance), coating: Ucon 75-H-90000; buffer: 80 m*M* Tris–PO_4 (50 m*M* Tris–HCl, 15 m*M* Na_2HPO_4 and 15 m*M* KH_2PO_4 pH 6.76), injection: electromigration (−6 kV, 0.05 min), operating voltage: −30 kV, current: ≈13.2 μA, UV detection at 254 nm. Peaks: (1) 2′-UMP; (2) unidentified; (3) 3′-UMP; (4) 2′-CMP; (5) 2′-GMP; (6) 3′-CMP; (7) 5′-UMP; (8) 5′-CMP; (9) 5′-GMP.

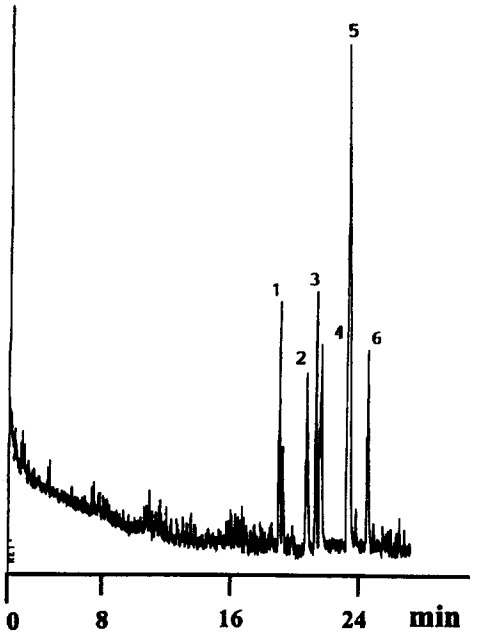


Fig. 5. CE separation of deoxyribonucleotides on a sol–gel coated Ucon column. Conditions: 75 cm×25 μm I.D. sol–gel column (59 cm separation distance); coating: Ucon 75-H-90000; buffer: 80 m*M* Tris–PO_4 (50 m*M* Tris–HCl, 15 m*M* Na_2HPO_4 and 15 m*M* KH_2PO_4 pH 6.76); injection: electromigration (−6 kV, 0.05 min); operating voltage: −30 kV; current: ≈12.9 μA; detection: UV at 254 nm. Peaks: (1) dUMP; (2) dCMP; (3) dAMP; (4) 2′-deoxyinosine 5′-monophosphate; (5) 2′-deoxyadenosine 3′-monophosphate; (6) dGMP.

The sol–gel technology evidently provides highly efficient Ucon columns with consistency in EOF and migration times for various types of solutes. This is indicative of the effective shielding of residual silanol groups with the Ucon coating, and stable temporal distribution of the residual silanol groups. Sol–gel technology also provides tools to control the concentration of the residual silanol groups by using sol–gel end-capping reagents. In our laboratory, we use trimethylmethoxysilane (TMMS) for this purpose. TMMS has only one methoxy functionality to undergo hydrolysis. The hydrolyzed product can undergo condensation with a residual surface silanol group and provide coverage of the column surface with its three methyl groups. Unlike polyhydroxysilanes, the surface-bonded TMMS residue is unable to undergo further condensation processes, and thus provides a means for effective endcapping. Results on the use of end-capping chemistry in sol–gel column technology for capillary electrophoresis will be published elsewhere [38].

4. Conclusion

The advantages obtained from the sol–gel chemistry are far too extensive to be neglected in capillary electrophoresis column technology. A fast, simple and efficient method for the preparation of Ucon columns for capillary electrophoresis using sol–gel technology was developed. This new technology provides columns that show adsorption inertness to basic proteins and other biomolecules, while still providing significant EOF. The low migration time R.S.D. values for various types of biomolecules (<0.7%) suggest effective coverage of the column inner surface with the biocompatible Ucon coating. Additionally, consistent EOF of the sol–gel columns (R.S.D.=0.43%) points to the stable temporal distribution of the surface silanol groups and their effective shielding with the Ucon coating. Low column-to-column variation in solute (cytochrome *c*) migration time (R.S.D.=1.88%) is evidence for the method's ability to create CE surfaces in a reproducible manner.

Acknowledgments

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