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ω-Iodoalkylammonium salts as permanent capillary silica wall modifiers
Comparative analysis of their structural parameters and substituent effects

Roberto Sebastiano[a], Cecilia Gelfi[b], Pier Giorgio Righetti[c], Attilio Citterio[a,*]

[a]*Department of Chemistry, Politecnico of Milano, Via Mancinelli 7, Milan 20131, Italy*
[b]*ITBA, CNR, L.I.T.A., Via Fratelli Cervi 93, Segrate 20090, Milan, Italy*
[c]*Department of Agricultural and Industrial Biotechnology, University of Verona, Verona, Italy*

Abstract

Following previous work on the modification and inversion of electroendoosmotic flow (EOF) of naked silica by a cyclic diamine [1-(4-iodobutyl)-1,4-dimethylpiperazin-1-ium iodide] [J. Chromatogr. A 894 (2000) 53], the present report considerably expands previous data by describing additional compounds of the same series of ω-iodoalkylammonium salts. Four of them are able to instantaneously reverse the EOF, thus producing a cationic surface with a highly stable reverse EOF. All these compounds are believed to become covalently attached to the silica surface via alkylation occurring by nucleophilic substitution of ionized silanols on the silica wall by the ω-iodo functionality in the modifier. The unique advantage of such compounds, as compared to adsorbed polymers or oligoamine EOF quenchers, is that they are not needed any longer in the background electrolyte, after the initial conditioning step inducing the covalent bond. It is additionally demonstrated, by running a mixture of cinnamic acid compounds, that some of the ω-iodoalkylammonium salts can act as modulators of analyte migration, thus inducing separations of otherwise identical compounds, such as isomeric species. Such interactions can only occur when the analytes drift close to the silica wall, and must be rapidly reversible, since no peak tailing or broadening is experienced.

Keywords: Electroosmotic flow; Capillary columns; Iodoalkylammonium salts; Cinnamic acid

1. Introduction

For many applications, control and/or suppression of electroendoosmotic flow (EOF) is necessary for exploiting the full potential of capillary zone electrophoresis (CZE). Several approaches have been employed for controlling and possibly eliminating EOF. In general, the various methods can be divided into three main groups [1]:

(a) changes of the buffer pH, concentration and composition through the addition of organic solvents or additives;
(b) dynamic modification of the wall surface, via adsorption of neutral or charged polymers, including surfactants;
(c) chemical derivatization of surface silanols with concomitant covalent binding of a variety of polymers (e.g., polyacrylamides, celluloses).

Figures of merits have recently been given for the

*Corresponding author. Tel.: +39-2-239-93082; fax: +39-2-239-93082.
E-mail address: attilio.citterio@polimi.it (A. Citterio).

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first two groups of methods for EOF control [2–4]. It was generally concluded that there was a hydrophobic leitmotif underlying the mechanism of action of such additives: although it might sound strange, it appeared that their efficacy was closely related to their hydrophobicity. This was quite clear in the case of amines, where the CH_2/N ratio seems to be the dominant theme [2]; it became then apparent in the case of neutral polymers, where the most effective one was found to be poly(*N*,*N*-dimethylacrylamide) (a quite hydrophobic polymer) [3] and it is true also in the investigation on detergents, in which it was clearly demonstrated that the shielding efficacy of surfactants closely follows a hydrophobicity scale [4].

Chemical derivatization of the silica wall, via covalent attachment of polymers to the surface silanols, is perhaps the best method for neutralizing the wall, in that, even if not 100% of all possible reacting sites are extinguished, nevertheless EOF would still be fully suppressed and macromolecule interaction with the wall abolished, since the high viscosity in the wall proximity would both quench EOF and prevent drift of macromolecules close to the few, unreacted silanols. However, proper wall derivatization with stable links, such as direct carbon–silicon bonds, as obtained via Grignard reaction, requires a rather complex chemistry and cumbersome manipulations [5].

In an attempt at finding other ways for modulating the charge on the silica wall, we have recently described a unique compound, an ω-iodoalkylammonium salt, in fact a quaternarized piperazine [1-(4-iodobutyl)-1,4-dimethylpiperazin-1-ium iodide] (QPzI), able to fully quench and even reverse the EOF of naked silica [6]. Unlike standard oligoamines (like spermine and tetraethylene pentamine, TEPA) which are very efficient in quenching macromolecule interaction with the silica wall, but only in acidic pH ranges, QPzI was found to act all along the pH scale, including alkaline pH ranges. It is believed that QPzI behaves like a trifunctional derivative: it interacts ionically with dissociated silanols via its quaternary nitrogen, by hydrogen bonds via its tertiary nitrogen and, most importantly, by a covalent bond via alkylation of ionized silanols through the terminal iodine atom in the butyl chain. Excellent separations were obtained with a variety of organic compounds, such as aromatic carboxylic acids, tryptophan metabolites and arylalkanoic acids. Such separations could not be obtained in naked capillaries in presence of oligoamines (e.g., spermine, spermidine, TEPA) and in some occasions not even with capillaries coated with a covalent layer of neutral polymers. In separations taking place in alkaline media, QPzI is not added to the background electrolyte, but is used simply in the capillary preconditioning step, a unique feature strongly supporting the hypothesis of its covalent binding to the silica surface. This notion was supported also by other indirect evidence, such as the fact that, if separations were performed at acidic pH values, QPzI had to be always present in the background electrolyte but, if pre-conditioned at alkaline pH values and then equilibrated into low pH buffers, no QpzI addition would be required [7]. In addition, QpzI was found to slowly alkylate, at pH ca. 10, Cys groups in proteins, suggesting an analogy between this reaction with free, ionized –SH groups of cysteines and ionized –OH groups onto the silica surface [8]. The fact that alkylation of proteins by QpzI in solution had very slow kinetics, further reinforced the notion that QpzI had to first dock and form a complex with the silica surface in order to exhibit such vastly improved reaction rate in presence of ionized silanols.

Moreover, in difficult separations, such as in the case of 2-methoxyphenylacetic and 4-methoxyphenylacetic acids or nicotinic/picolinic acid, which would not normally occur under standard conditions, it was found that QPzI would act as a discriminator, thus playing an active role in the separation process, rather than simply modulating the EOF. Also in the case of protein separations QpzI was found to give much improved results, often even better than those of polymer-coated capillaries, suggesting that this compound could have a broad range of applications [9].

In view of the unique performance of this chemical, we have synthesized, and we here report, a series of analogous compounds, in an attempt at obtaining a better insight on their behaviour and for exploring the possibility of further improving the performance of this family of chemicals.

2. Experimental

2.1. Reagents

A series of cinnamic acid derivatives (**1**, **2**, **3**, **4**, **5**, **6**, and **7** (Fig. 5)) were obtained from Aldrich. Fused-silica capillaries (50 μm I.D.×375 μm O.D.) were from Polymicro Technologies (Phoenix, AZ, USA) and were used as such, without inner coating.

2.2. Synthesis of 1-(4-iodobutyl)-1,4-dimethyl-piperazin-1-ium iodide (QPzI)

N,*N*′-Dimethylpiperazine (11.4 g, 0.1 mol) is dissolved in acetone (100 ml) and the resulting solution is added to a stirred mixture of 1,4-diiodobutane (31 g, 0.1 mol) in acetone (100 ml). The reaction is run for 24 h. The precipitate formed is filtered, washed with acetone and dried under vacuum at 0.5 mmHg for 3 h to yield QPzI (38.2 g, 90%, m.p. 278–280°C with decomposition) (1 mmHg=133.322 Pa). ^{1}H NMR (dimethyl sulfoxide, DMSO) δ (ppm): 1.71–1.88 (m, 4 H), 2.28 (s, 3 H), 2.58–2.68 (m, 2 H), 2.68–2.78 (m, 2 H), 3.05 (s, 3 H), 3.3 (t, 2 H), 3.35–3.45 (m, 6 H). MS (matrix-assisted laser desorption ionization, MALDI): 296 (M^+-I, 100), 169 (28).

The other compounds listed have been synthesized in an analogous manner from suitable precursors.

2.3. Capillary electrophoresis

When operating with a new capillary, it is necessary to perform a washing with 0.1 *M* NaOH for 2 h and water for 3 h. Then, a brief pre-conditioning, consisting in a few washing cycles, as described below, is applied until reaching constant (inverted) EOF flux values. Pre-conditioning: washing (5 bar for 2 min) with a modifier solution (2–4 m*M* in 25 m*M* sodium tetraborate buffer, at pH 9.0), followed by a brief washing (5 bar for 4 min) with running buffer. Sample analysis is performed according to the following procedure: washing (5 bar for 2 min) with the modifier solution (2–4 m*M* in 25 m*M* sodium tetraborate buffer, at pH 9.0), followed by a washing (5 bar per 4 min) with running buffer, sample injection (10–20 mbar for 10–20 s), injection of a running buffer plug (10 mbar for 5 s). Analysis have been performed with a Hewlett-Packard ^{3D}CE instrument, in capillaries of 50 μm I.D., typical length of 50 cm, applied voltage: −25 to +25 kV; detection at 210 nm, temperature of 25°C.

3. Results

Fig. 1 gives the formula of the 10 different compounds synthesized and tested in the present work. They can be divided into two classes: those with a butyl chain (indicated by *C4*) and those with an octyl chain (indicated by *C8*) terminating with the reactive iodine. In addition, two sub-classes can be evidenced: the compounds with a piperazine ring, and those in which the tertiary nitrogen is absent and/or substituted with an oxygen atom. In addition, other structural motifs are readily visible: single ring monomers; three-ring compounds and a star-shaped species (M5C4). In this way, we aimed at exploring the behaviour of these species not only in terms of substituent effects, but also as a function of structural parameters.

Fig. 2 shows how the different modifiers can modulate the charge (and thus the EOF) on a naked silica wall. We can distinguish three types of behaviour: those which have a modest influence on the EOF profile probably due to a very slow reaction kinetics with the wall (M4C4 and M5C4); those which substantially quench the negative EOF flux (M2C4, M6C4 and M6C8) and, finally, those which are fully capable of reversing the EOF, from negative to positive, while maintaining this reversed flux at a constant level upon repeated runs (M1C4, M1C8, M7C4 and M7C8). It is noted that the first class comprises those species that do not have a tertiary amino group in the ring (M4C4) or do not even have a ring (the star monomer M5C4). The second class (intermediate reacting compounds) is again made up of species lacking the tertiary amino group in the ring, typically substituted by a carbon atom with or without an oxygen bound to it. Both classes of compounds show slow reaction kinetics with the wall. Finally, the third class of chemicals is made up exclusively of bi-basic compounds, either with a piperazine or with a three-ring structural

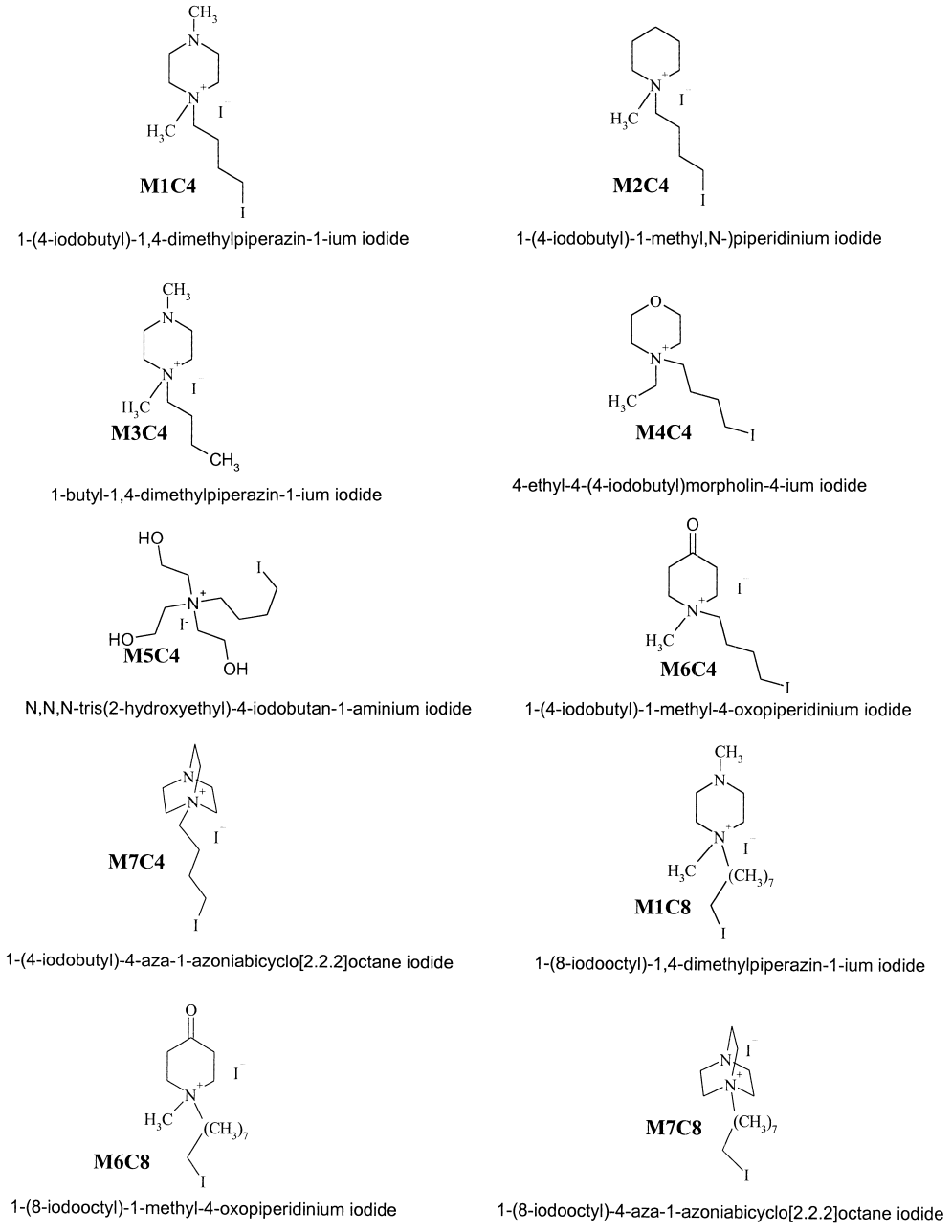

Fig. 1. Chemical formulas of the 10 ω-iodoalkylammonium salts synthesized and used in the present study.

motif. This last class appears to react swiftly, reverse immediately (at the very first, brief 2-min conditioning) the EOF flux and maintain it stable for a number of subsequent runs.

Fig. 3 offers a better insight onto the stability of the silica wall modification, by measuring the EOF flux in a series of subsequent runs. It is here assumed that all modifiers are covalently linked to the wall, since they are not present in all subsequent runs, after the first one, in the background electrolyte. It is

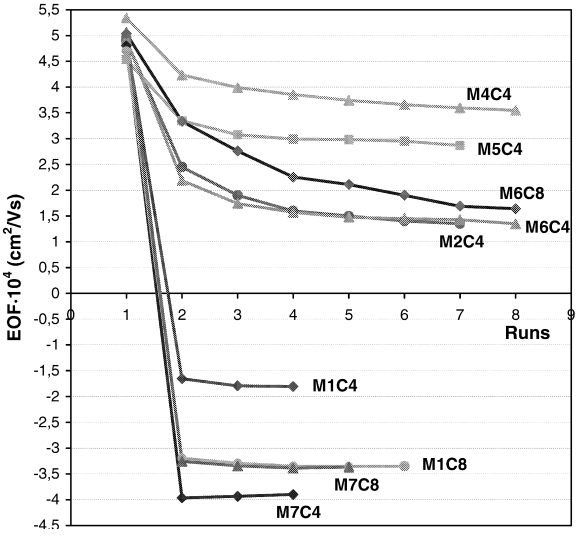

Fig. 2. Efficiency of the capillary silica wall interaction and EOF modification, depending on the different ammonium salts tested. Procedure 1: washing (5 bar for 2 min) with the proper modifier solution (4 m*M* in borate buffer, 25 m*M*, at pH 9.0), followed by further washing (5 bar for 4 min) with running buffer (borate 25 m*M*, pH 9.0), then EOF marker injection (acrylamide) and injection of a running buffer plug. The run number 1 gives the EOF value for the untreated capillary.

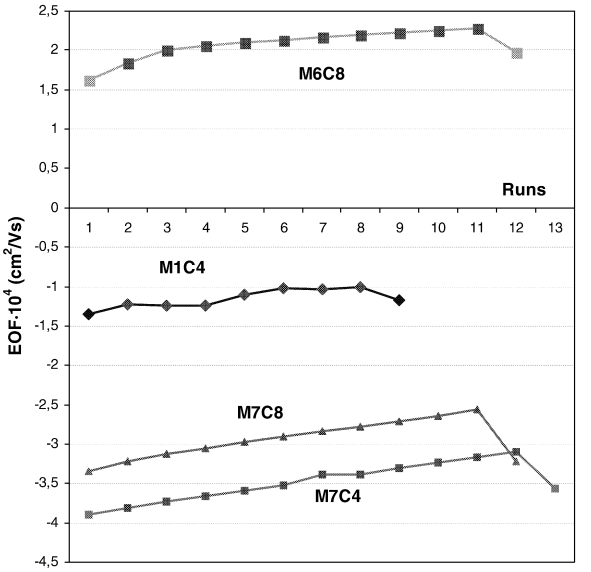

Fig. 3. Stability assessment of the silica wall modification by EOF measurements in absence of the modifier solution washing step. Each capillary was first conditioned with the appropriate modifier (see the caption to Fig. 2) until the stable EOF value was obtained (run number 1). The subsequent EOF measurements were achieved *without* performing the washing of the capillary with the modifier solution. The last EOF value was obtained by performing again the capillary washing with the modifier solution.

noted that the EOF flux is quite constant, although it tends to vary a bit from run to run. This variation is minute, though, and it is estimated to be about 2% per run, as calculated from the slope of the curves of the two compounds M7C4 and M6C8. The interpretation: either there is a slow hydrolysis of the siloxane bond linking them to the wall (such a bond is known to be unstable at alkaline pH values) or, by the same token, there could be a slow generation of new free silanols (again due to the action of the pH 9.0 buffer). Whichever the explanation, though, it should be noted that, in all cases, if, after a series of runs in the absence of modifier, the capillary is briefly rinsed again with 4 m*M* of the relevant piperazine, the EOF is immediately brought back to that obtained at the start of this series of runs (see the drop in each curve in between the two last runs).

Fig. 4 gives an example of the kind of separations obtainable when running a cinnamic acid mixture, having the chemical formulas drawn in Fig. 5. In particular, we will focus our attention on the splitting of compounds **5** and **6**, which have identical formulas and are simple isomers. In a control run (uncoated capillary) only species **4**, **7** and **1** are well separated: the other four are essentially eluted in a single, poorly resolved peak in spite of the fact that EOF and electrical field work in opposite directions. Similar results are obtained with the modifiers M1C4, M1C8, M7C4 and M7C8, with a clustering of three to four analytes in a single peak (note, though, the reversal of the order of elution due to the EOF reversal); but in this case the bad results can be related to the strong EOF pushing past the analytes to the detector.

Best results, in resolving all seven compounds and especially the two isomers, are obtained with the M6C4 and M6C8 modifiers. It must be noticed that in these cases: (a) the EOF is only reduced, non inverted, and the analytes migrate to the detector because of the electrical field [inlet (−)], against the electroosmotic flow, (b) the migration times of the analytes are very different in spite of the EOF values produced by these two modifiers being comparable.

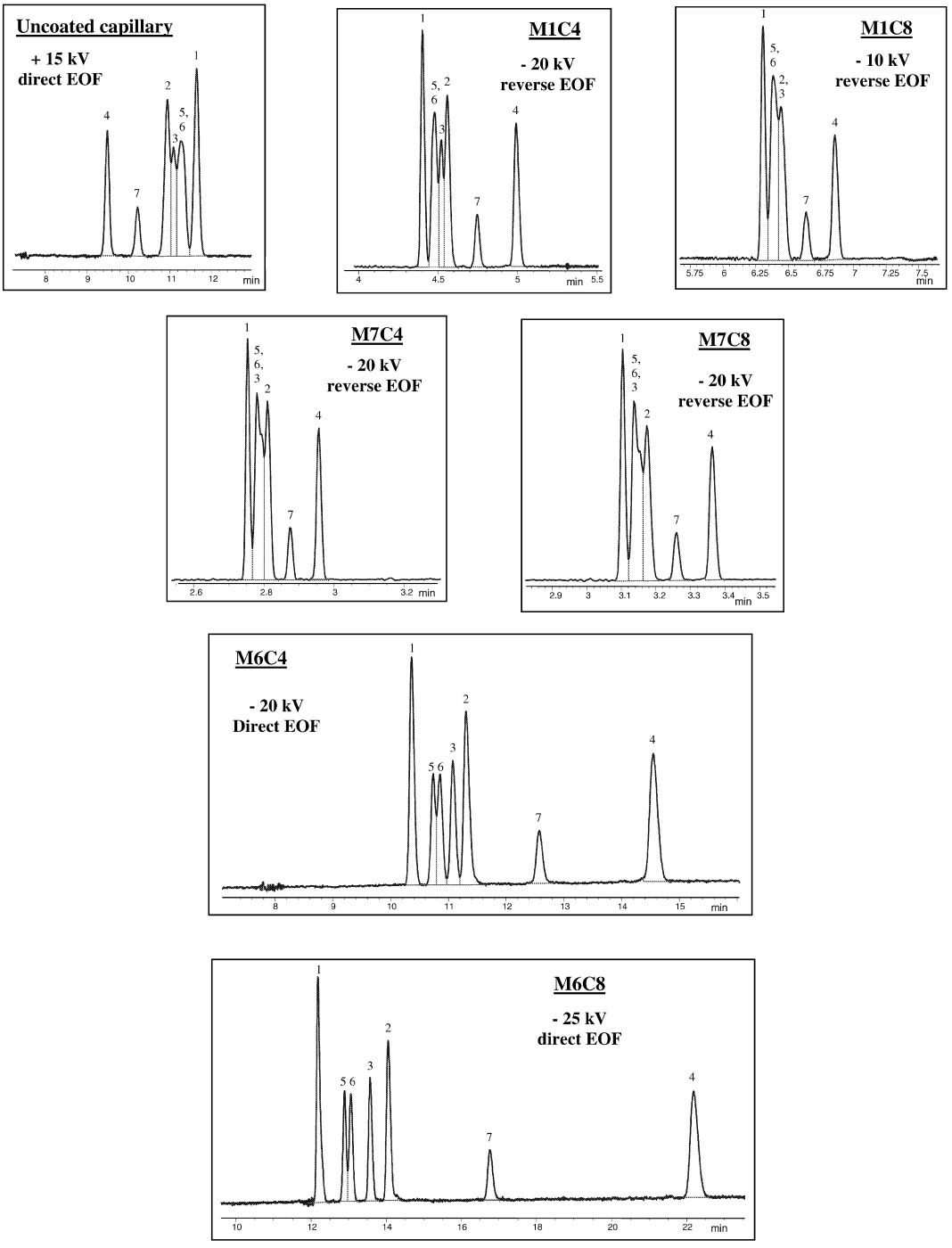

Fig. 4. Comparative analysis of cinnamic acid mixtures with coated and uncoated capillaries. Pre-conditioning: all the capillaries were pre-conditioned by washing (5 bar for 2 min) with the proper modifier solution (4 m*M* in 25 m*M* borate buffer, pH 9.0) until a stable EOF was obtained. Analysis procedure: washing (5 bar for 2 min) with the modifier solution (4 m*M* in 25 m*M* borate buffer, pH 9.0), followed by a further washing (5 bar per 4 min) with the running buffer solution (25 m*M* borate, pH 9.0), then sample injection (10 mbar for 10 s) and injection of a running buffer plug (5 mbar for 5 s). Fused-silica capillary, 50 cm total length×50 μm I.D., T=25°C.

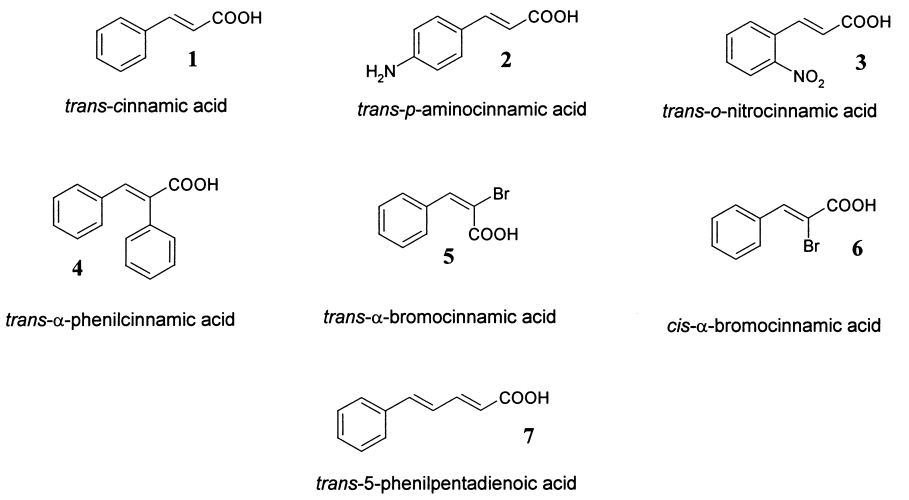

Fig. 5. Formulae of the seven cinnamic acid derivatives used in the separations of Fig. 4.

We have next run a series of tests in order to gain some insight on the efficacy of silica wall modification as a function of the structural parameters of the various modifiers. Fig. 6 gives comparative runs of M4C4 versus M2C4 (Fig. 6A) and M5C4 versus M2C4 (Fig. 6B). In both cases it is noted that M4C4 and M5C4 (the star monomer) are slow-reacting species as compared with M2C4. This suggests that steric hindrance on the quaternary ammonium, higher in M4C4 and M5C4, can be the parameter that reduces the efficacy of interaction of such compounds with the wall. We hypothesize that a firm docking of such compounds (primarily driven by the quaternary nitrogen) should occur prior to the alkyla-

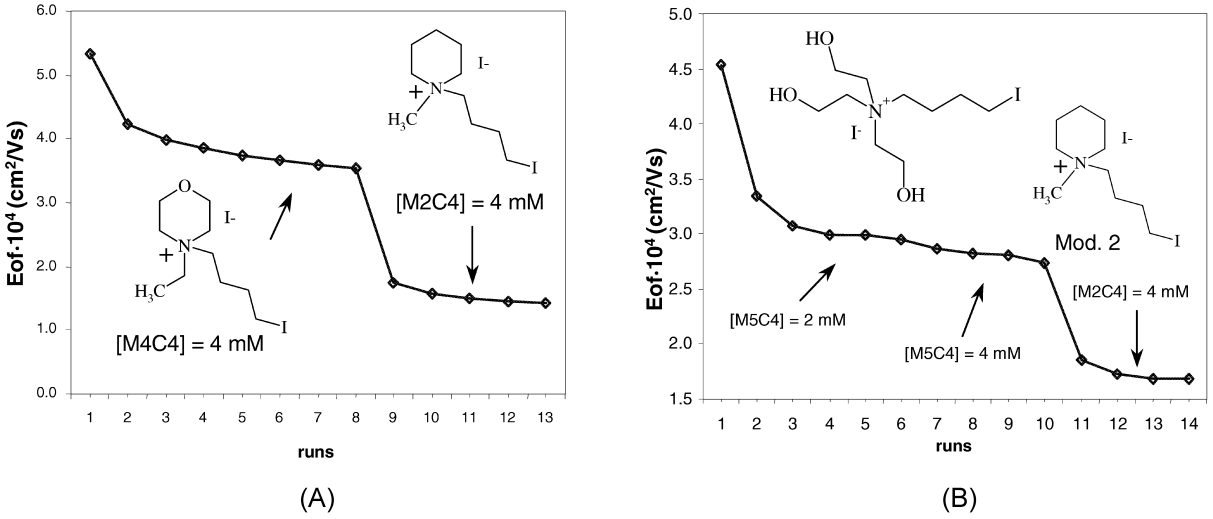

Fig. 6. Correlation between the chemical structure of the ammonium salts and their efficacy in modifying the silica wall. The data reported in (A) and (B) indicate that the steric hindrance on the quaternary ammonium, higher in the compound M4C4 and M5C4, can be a parameters that reduce the efficacy of the silica wall interaction. All the tests were performed following the general procedure 1, using acrylamide as EOF marker. The measurements reported in the single were obtained using the same capillary and only changing the modifier washing solution.

tion event. If that is the case, it is clear that M2C4 should have an advantage even over the closely related M4C4, the latter having an ethyl, instead of a methyl, substituent onto the quaternary nitrogen.

Another chemical composition motif important for the action of these modifiers on the wall is shown in Fig. 7, which displays a competitive binding test between M1C4 and M2C4. It is recalled here (see Fig. 2) that the first belongs to the class of the 'fast', whereas the second belongs to the class of 'intermediate', alkylating agents. Indeed, the presence of M2C4 (even at double molarity) on the washing mixture, induces only a short delay towards the establishment of the reversed EOF flow typical of M1C4. This experiment shows the importance of the tertiary nitrogen present only in M1C4 for establishing an efficient absorption to the wall, prior to the alkylation event. Conversely, the modest ability of M2C4 in establishing an analogous complex render him a poor competitor for the binding sites on the silica wall.

Finally, in Fig. 8, we explore the influence of washing times on the alkylating efficiency of the modifiers. If a compound belongs to the class of 'slow' reacting species, such as M4C4, not much is gained in switching from brief (2 min) to very long

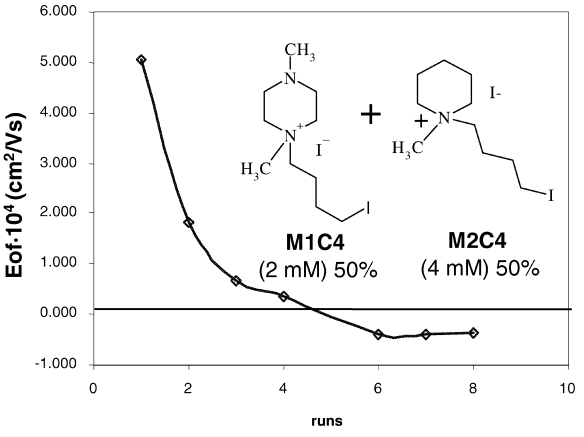

Fig. 7. EOF data measured by performing a competitive test between the M1C4 and M2C4 modifiers. The results show the importance of the basic nitrogen atom present in the M1C4 salt on the silica wall coating ability. The presence of the M2C4 ammonium salt in the washing mixture induce only a short 'delay' on the reaching of the negative EOF but the value is lower, in absolute, than the EOF value obtained using the pure M1C4 modifier.

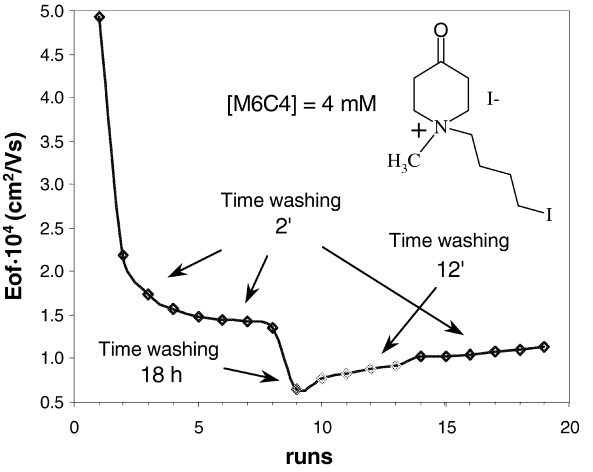

Fig. 8. Dependence of the silica wall modification magnitude on the length and number of washing cycles. It is shown that, is the modifier is a moderate alkylating agent, such as M6C4, not much is gained in prolonging the washing times up to much extended periods.

(18 h) washing (or incubation) cycles. In the latter case, only a modest, further decrement of EOF is obtained. Interestingly if, after the 18-h incubation time, 12-min, followed by 2-min, washing cycles are resumed, the final EOF profile tends to reach the same final value it would have attained by simply continuing the original 2-min washing routine. This again points to the relevance of structural parameters on the reaction kinetics of each individual compound.

In conclusion, Fig. 9 gives some of the electronic/steric motifs underlying the reactivity of these compounds. Here, the electronic density clouds and Van Der Waals radii are shown for two of them, the M1C4 first described by us [6] and one of the novel species, M7C4. Although they are both good alkylators, it appears that M7C4 is more powerful than M1C4. This can be explained by the following reasons: (a) in the tertiary nitrogen, the lone pair of electrons is more exposed in M7C4 as compared with M1C4, this permitting a better hydrogen bonding interaction and also an easier deprotonation of the SiO–H groups present on the silica surface. The additional SiO^- so generated can undergo a nucleophilic substitution on the iodine atom of the alkyl chain of the modifier giving a new covalently bonded positive charge. Additionally, due to the bridged

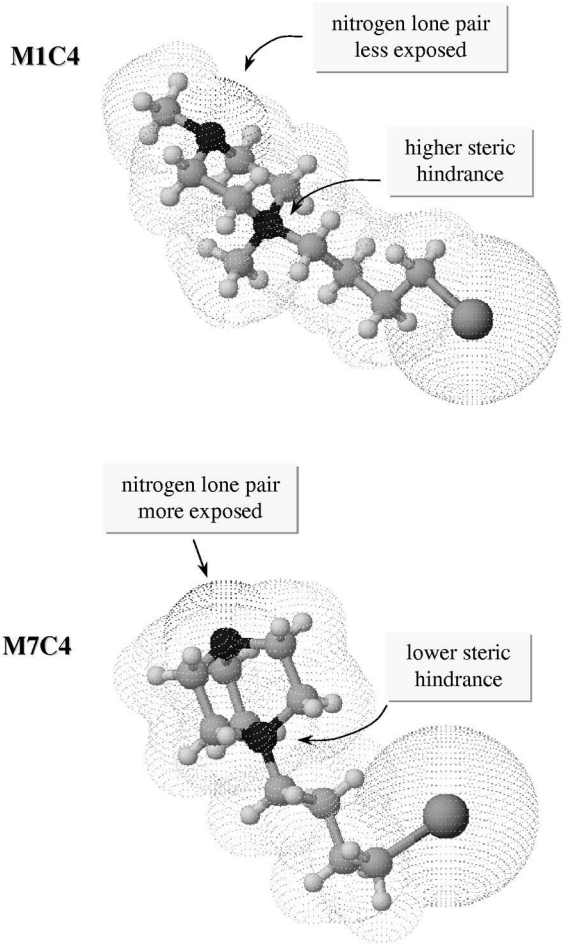

Fig. 9. Proposed mechanism for the different efficacy of the various ω-iodoalkylammonium salts as wall modifiers. When comparing the M7C4 and M1C4 compounds, it is seen that the former, due to electronic and steric reasons, should have a higher binding capacity, as compared with M1C4, and thus a higher capability in forming a covalent bond with the silica surface.

nature of M7C4, the steric hindrance around the nitrogen atom is reduced, as compared with M1C4, helping further in forming a firm binding via charge-charge interaction of this quaternary nitrogen with the silica wall.

4. Discussion

Our results suggest that we could now have a new, powerful tool for control and/or reversal of electro-osmotic flow in the analysis of metabolites as well as of macromolecules. The advantages of the present series of modifiers can be summarized as follows:

4.1. EOF control

As shown in Figs. 1 and 2, EOF can be substantially reduced with a series of modifiers or completely reversed with four of them (M1C4, M1C8, M7C4 and M7C8). In both cases, constancy of EOF is obtained, slowly with the first two classes of modifiers, but rapidly (just one equilibration step) with the last class of compounds. This constancy is maintained over several runs, with the proviso that a ca. 2% drift is measured from run to run. Nevertheless, it is seen that a single conditioning step at any time after the first one fully restores the original conditions (see Fig. 2). This appears to offer unique advantages, not only when compared with dynamic coatings with oligoamines, but also with poly-cationic coatings. In the first case, it is known that free oligoamines, no matter how efficient, are always needed as additives to the background electrolyte. In addition, most of them are ineffective at alkaline pH values, due to progressive deprotonation of the oligoamino backbone. Both shortcomings do not apply in the present case. But the situation is even more contrasted if one compares the present coating with dynamic adsorption of polyamines, as described in a number of reports. Deacylation of chitosans, for instance, produces a polyamine (p*K* ca. 6.3) which can be adsorbed onto the capillary wall [10]. Polyvinylimidazole (p*K* ca. 6 in the monomeric state) is another such a cationic polymer [11]. Another quite popular one is polyethyleneimine (PEI), available in a wide range of molecular masses, up to 1 million. PEI adsorbs tenaciously to silica surfaces and exhibits a broad titration curve, ranging from 5 to 10 [12]. Even basic surfactants, such as the cationic fluorosurfactant Fluorad FC134 [13], can reverse the EOF flux. Flow reversal can also be obtained by covalently anchoring to the activated silica wall a series of basic Immobiline chemicals (p*K* values ranging from 6.3 up to 10.3) [14], the same ones used for isoelectric focusing in immobilized pH gradients [15]. In most of these cases, especially with polymers having low p*K* values, EOF control will not apply any longer at basic pH values. In addition,

adsorption of these polymers could be erratic and not reproducible among different dynamic coating procedures. When growing such polymers as strings covalently bound to the silica wall, such as in [14], there is the added risk of poor control on the length of the polymer chain. In addition, the vast excess of positive charges onto the wall might adversely affect a number of separations, especially when dealing with intermediate isoelectric point (p*I*) proteins. In the case of our modifiers, the excess of positive charges is only modest and, in any event, it occurs evenly along the silica surface, since we deal with simple monomers bound to the wall. Thus, even when dealing with protein separations, we have not experienced any tailing or disappearance of peaks, suggesting that polypeptide adsorption onto the coated silica should be minimal [9].

4.2. Charge neutralization

By looking at Fig. 1, it is also anticipated that one should be able to find a point of zero net charge onto the silica surface. It is in fact seen that the first two classes of compounds only lower the magnitude of EOF, but never bring about a reversal. On the contrary, the last class of modifiers fully and rapidly reverses the EOF. A judicious blend (or a sequential coating procedure) of class 1 and 2 with class 3 compounds should enable one to reach a state of true zero charge condition on the capillary wall, a state rarely reached in most coating procedure (work in progress).

4.3. Modulation of analyte mobility

An extra bonus of our modifiers is that a number of them can enter as an active player onto the separation process. This is nicely shown here, in the case of cinnamic acid derivatives mixtures, with the M6C4 and M6C8 modifiers. In fact, comparing the two relative electropherograms, it is evident the strong difference of the migration time values in spite of electroosmotic flows being very similar. Since our modifiers are only present onto the wall surface, this means that, as the analytes, on their way to the opposite-charge electrode, drift by diffusion in the proximity of the wall, they interact preferentially with such modifiers. This interaction is not detrimental to the separation, in fact it highly enhances it, suggesting that it is fully reversible and it has very fast kinetics. The same phenomenon could be exploited in the case of *o*-/*p*-OMe-phenylacetic acids or nicotinic/picolinic acid, which would not normally occur under standard conditions. In these cases, QPzI acted as a discriminator, thus playing an active role in the separation process [6].

5. Conclusions

The results on the inner capillary wall modification by the studied compounds support the cooperative mechanism already proposed by us [6] and show its generality. As proved by the analysis of the compounds M2C4 to M6C4 and M6C8, a permanent silica wall capillary adsorption is realized if the molecule is a ω-iodoalkylquaternary ammonium salt. Indeed, the charge interaction between the ammonium cation and the negatively ionized silanol groups allows to obtain a strong adsorption of such molecules on the capillary silica wall; this increases the rate of nucleophilic substitution between the SiO^- groups and the terminal carbon atom bearing the iodine, present in the modifier. The strong and fast EOF inversion produced by the M1C4, M1C8, M7C4 and M7C8 derivatives is due to a larger amount of positive charge present on the capillary silica wall. This result is related to the basic, tertiary nitrogen atom present in these compounds, which increase the binding on the capillary silica surface. The different magnitude of the reverse EOF observed in this class of compounds can be related with four parameters that influence the physico-chemical interaction with the silica: (1) the basicity of the free nitrogen atom; (2) the steric hindrance on the positively charged quaternary ammonium atom; (3) the lipophilic component related to the alkyl chain, and (4) the distance between the positive charge and the terminal carbon atom bearing the iodine.

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