

Review

Surfing silica surfaces superciliously[☆]

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Available online 2 July 2004

Abstract

The present mini-review summarizes the experience gathered by our group in developing different classes of novel quaternized heterocyclic compounds able to modulate and reverse the electroosmotic flow (EOF) in a most peculiar manner. The first class comprises mono-salt compounds, with the determinant ω -iodoalkyl chains of different lengths (typically C₄–C₈), able to be adsorbed by silicas, at alkaline pH, and spontaneously alkylate ionised silanols, thus becoming covalently affixed to it. The second class is constituted by di-salt compounds, attached at the termini of an alkyl chain of variable lengths (here too, typically, C₄–C₈). This second class is unable to bind covalently silica surfaces, although, in thin-layer chromatography, it exhibits an extraordinary affinity for silica beads, contrary to the first one. On the basis of the strikingly different behaviour, structural rules are derived for the minimum requirements for general classes of amines to bind to silica walls and modify EOF. For compounds unable to bind covalently to the wall, the most important structural motif is two quaternary nitrogens spaced apart by a C₄ chain: this seems to be the average distance (i.e., 0.8 nm) between two adjacent, ionized silanols for a snug fit. The other structural binding motif is the “hydrophobic decoration”, i.e., the ratio of charged groups to alkyl residue in the various amines; amines with high levels of such alkane groups (i.e., with higher hydrophobicity), seem to bind more tenaciously to the wall, probably due to hydrophobic interaction not to the wall but among the amine derivatives themselves, when carpeting the silica.

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Keywords: Reviews; Silica; Surface coating; Electroosmotic flow; Piperazines; Quaternary ammonium compounds

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1. Introduction

In fact, the Beach Boys could have hardly surfed silicas in 1964, when very few cared about this material, but certainly their surfing the strong waves of the Californian Pacific coast must have been just as “perilous” as for us scientists trying to surf and master the hidden currents criss-crossing silica surfaces under an electric field. So much has been stated about

[☆] The Beach Boys, Surfing Safari, 1964, unpublished.

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silica properties in the field of capillary zone electrophoresis (CZE) and so many reviews written that we want to avoid inundating the field with yet an *n*th review increasing the entropy of the system. We will briefly mention here some old and robust reviews amply covering the field [1–3] and a book dedicated to adsorption phenomena on silica surfaces [4], which contains a wealth of information useful to those fighting in the CZE arena. We will only give here a brief survey on how we tackled the problem, in the last few years, and what novel solutions we could devise to an old problem. May be the readers will not accept this self-inflicted mutilation, but may be some of them might find solutions to their problems.

2. Re-exploring the silica surface

Fig. 1 offers a glimpse at the silica surface. When viewed at room temperature (panel C), silica can be seen coated by a mono-molecular layer of water molecules, which tightly cling to this surface via extended hydrogen bonds to adjacent silanols, in agreement with the high hydrophilicity of this material. Silica has a moderately strong acidity ($\alpha = 1 \pm 0.1$); negligible basicity ($\beta = 0$) and moderately strong dipolarizability ($\pi^* = 1 \pm 0.1$) [5]. These data are in agreement with independent work performed by users of CZE, who have assessed the average pK of silanols at 5.3 [6] or 6.3 [7]. The mean surface density of silanols at pH 7.7 has been estimated at $5/\text{nm}^2$ [8], whereas the mean surface density of deprotonated silanols at pH 10 has been assessed as $2\text{--}3/\text{nm}^2$. When silica is heated at 150°C , the surface is dehydrated and is now seen with sparse hydrogen bonds among

vicinal silanols (panel B). Upon further heating at 400°C , the surface changes morphology, exhibiting now regions of dehydrated silica residues interspersed among areas fully devoid of silanols, displaying only siloxane bridges (panel A) [9]. Also, strong pH changes can modify irreversibly the surface, as shown by the hysteresis loop in the right panel of Fig. 1, pertaining to silicas titrated from acidic to basic milieu, and vice versa; the electroosmotic flow (EOF) profile, in both directions, follows different paths.

Among all possible additives, for manipulating EOF and “surfing safely the silica surface”, perhaps the class which has received the greatest attention is that of the amine modifiers, ever since Nahum and Horváth [10] recommended them as additives to the mobile phase employed in reversed-phase chromatography of ionogenic substances, in order to suppress the untoward effect of the residual unmasked silanolic groups in the stationary phase. This class comprises a vast number of compounds, starting from mono-, di-, tri-, tetra- and oligoamines (for reviews, see [1,3,11]). It is surprising that, in most of these methods, weak amino groups (comprising primary to tertiary nitrogens) have been adopted, since their use is limited to acidic to neutral pH values of the background electrolyte, because at high pH values these substances would be progressively deprotonated and thus unable to bind to ionised silanols. In contrast, quaternary amino derivatives have a vast range of applications, as illustrated in Fig. 2. For example (see left column in Fig. 2), cationic surfactants, such as benzalconium and cetylpyridinium chlorides, are extensively used in laundering and fabric softeners; phase-transfer catalysts, such as salts of tetraalkylammonium (upper central column in Fig. 2) are employed

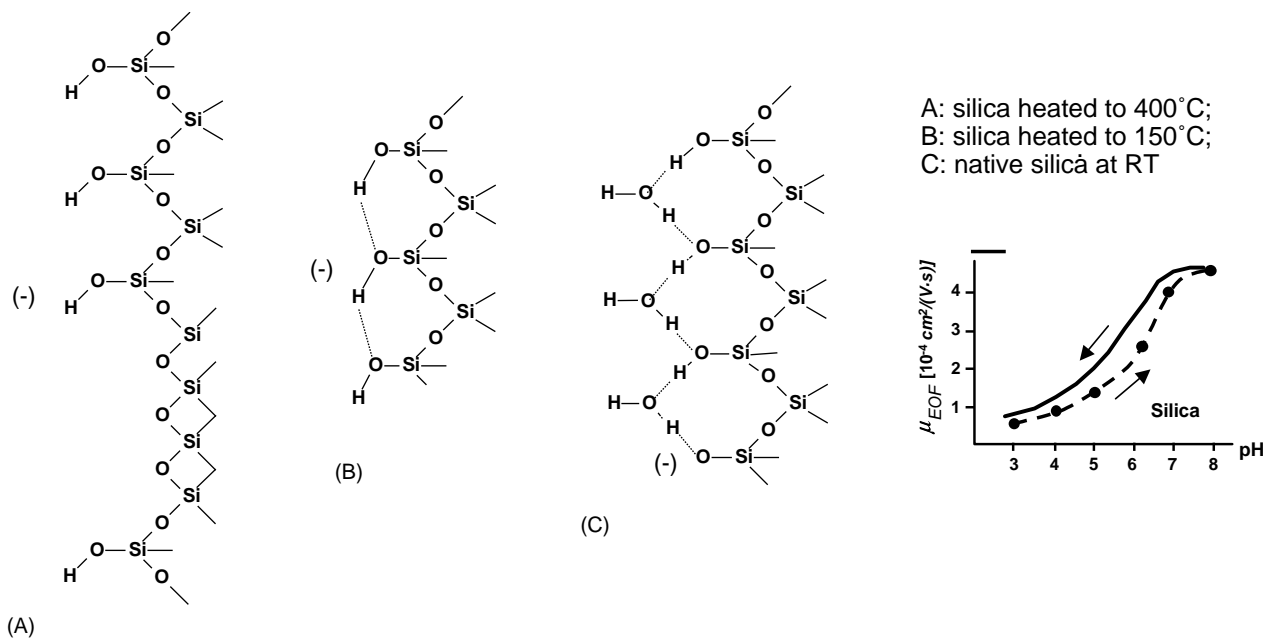
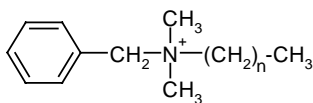
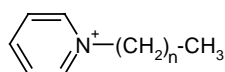


Fig. 1. A pictorial representation of the silica surface after heating at 400°C or at 150°C or at room temperature (native silica). Right panel: EOF profile when titrating a capillary from acidic to basic conditions and vice versa.

Cationic surfactants
Fabric softeners and
toilettries (antimicrobial)

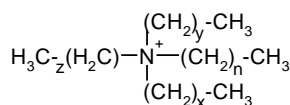


Benzalconium Chloride (BKC)



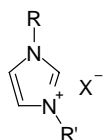
Cetylpyridinium Chloride (CPC)

Phase-transfer
catalysts



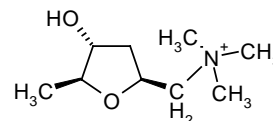
Tetraalkylammonium

- Adhesion promoters
- Corrosion inhibitors

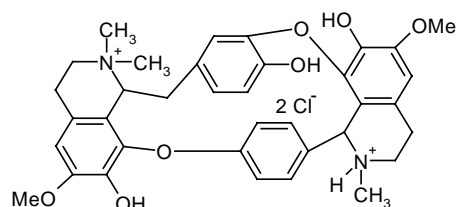


Liquid salts

Biologically active
compounds



Muscarine



(+ Tubocurarine chloride

Fig. 2. A panel of different quaternary amino derivatives with their potential industrial and biochemical applications.

as adhesion promoters and corrosion inhibitors; such as *N*-methyl-*N'*-butylimidazolium tetrafluoroborate, are suggested as green solvents (lower central column in Fig. 2); without mentioning the class of biologically-active compounds, such as the highly-poisonous alkaloids muscarine and tubocurarine, to name just a few.

The interaction between silica surfaces and cationic surfactants has been well documented in several publications, and could be depicted in the series of events illustrated in Fig. 3. At extreme dilutions (equilibrium 1), only a few silanolic sites will be occupied by the quaternarized nitrogen compounds. As the concentration is increased (equilibrium

2), additional sites will be engaged, thus progressively carpeting the silica surface. Above the critical micellar concentration (equilibrium 3), two phenomena will occur: the micelles will tend to fully capture the surface, and in this process they can assume the configuration of a lamina, in fact of a double layer, having an excess of positive charges on both surfaces. Thus, as shown in the reaction step (4), the original negative surface charge of silanols at alkaline pH values will be reversed, thereby allowing for binding of negatively-charged compounds and for EOF reversal [12]. Similar results can be obtained via polyethyleneimine (PEI) coatings, although through a different mechanisms: PEI is

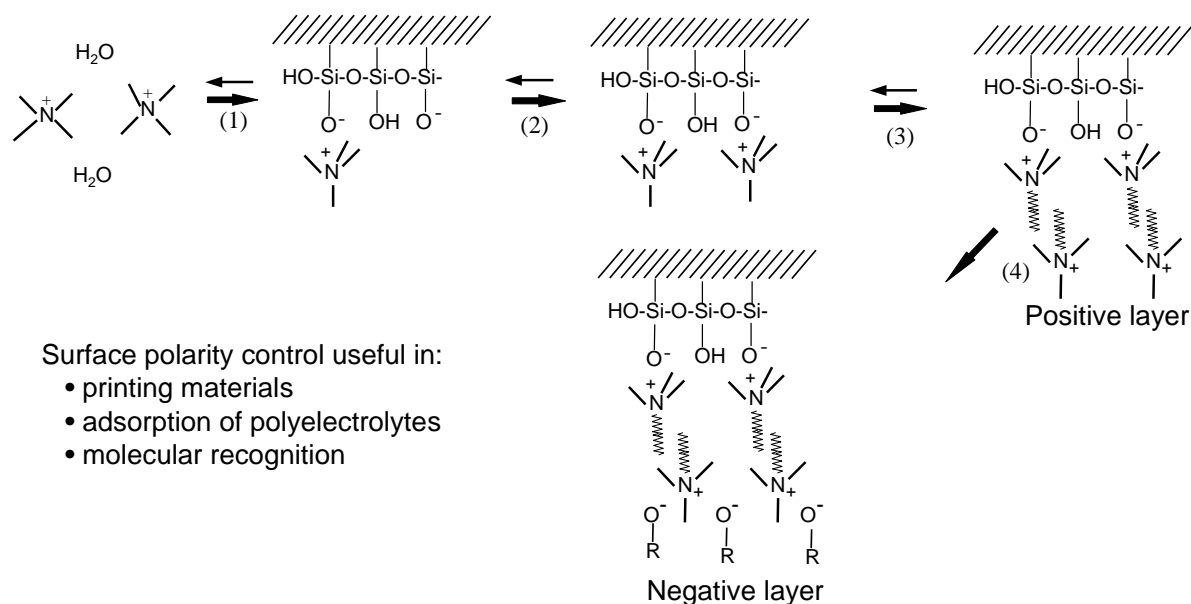


Fig. 3. Pictorial representation of the binding of cationic surfactants to silica at progressively higher concentrations, till surface charge reversal [reaction step (4)].

endowed with mostly secondary and tertiary amino groups, which must encompass pK values over a few pH units, and cannot possibly form a double layer, as in the previous case. The EOF reversal then must be due to loose ends and loops of polymer hanging freely from the silica surface [13].

3. The “Skorprios”: how to bite the wall without being bitten

Not that quaternary ammonium salts had not been used, previously, for EOF control, mind you. For example, α,ω -bis-quaternary ammonium alkanes, such as hexamethonium and decamethonium bromides, had been reported for EOF modulation [14]. They were also found to be exceptionally active, since they could almost zero the EOF at milli-molar and sub-milli-molar levels. Yet, their binding was transient, as easily deduced by the fact that they had to be constantly present in the background electrolyte and electroic reservoirs. Things changed abruptly when we synthesized the first compound of the series nicknamed “Skorprios”, namely the quaternarized piperazine (*N*-methyl,*N*-4-iodobutyl)-*N*'-methylpiperazine iodide (M1C4I) [15] (see the formula in the insert in Fig. 4). By serendipity (i.e., because a technician had forgotten to dissolve the compound in the running buffer, the same serendipity that helped Porath's group to discover molecular sieving, because they had forgotten to turn on the electric current in the column filled with cross-linked dextran; a cute anecdote narrated in [16]), we found out that when the capillary is briefly pre-conditioned with a milli-molar solution of M1C4I (at alkaline pH), washed with the running buffer and then the run continued in its absence, the EOF

modulation would still be active, even when working with acidic buffers.

Such a behaviour suggested that this compound had to be covalently affixed to the wall, via a reaction mechanism depicted in Fig. 4. We hypothesize that this molecule first docks onto the silica wall via the net positive charge of the quaternary nitrogen. Once hooked onto the wall, M1C4I is further pasted to it via hydrogen bonding occurring on the deprotonated tertiary nitrogen. Finally, given the close surface contact, the iodinated tail is able to react and form a covalent link with a neighbouring silanol anion, thus inducing the alkylation of the surface through a carbo-siloxane bond. This last reaction is enormously accelerated because of the extended contact between the silanols and the ω -iodoalkyl chain present in the modifier molecules. An important property of this class of compounds would be that, by properly dosing its amount during the pre-conditioning step, or better by using an appropriate mixture of them, one can achieve full control of the silica surface charge: partial quenching of its negative charge, achievement of a null-charge point and charge reversal, with progressively higher positive charge. This property renders these molecules highly attractive for properly modulating the surface state of silica, all of this without resorting to the very complex and lengthy procedures typically needed when, e.g., trying to anchor covalently neutral polymers to the silica surface.

It might sound strange that we can achieve even charge reversal, considering that M1C4I-like compounds are simple diamines and that, at alkaline pH values, only the quaternary nitrogen is protonated. We can explain this behaviour by considering that any molecule of quaternary ammonium salt bonded onto silica surface replaces a negative charge with a positive one and when the average number of these charges

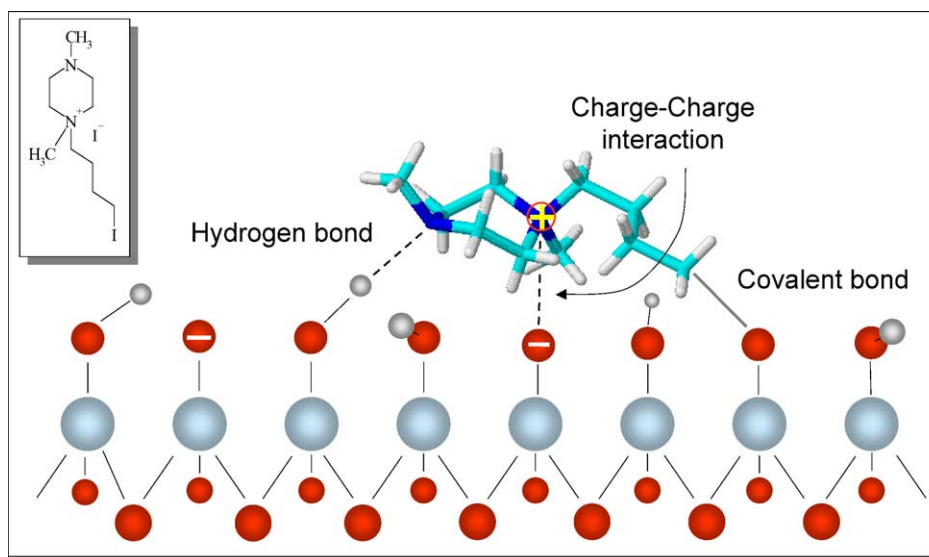
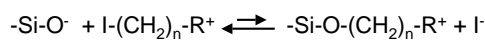
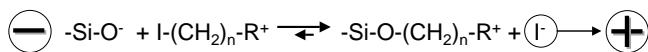


Fig. 4. Proposed mechanism of binding of the quaternarized piperazine M1C4I (formula in the upper left insert) to the silica wall. The hydrogen bond is formed by the tertiary amino group, the ionic bond by the quaternary nitrogen and the covalent bond by the iodinated alkyl tail.

Thermodynamic contribution:

Assisted
Nucleophilic
Substitution:
equilibrium
shifted to the left

Application of the electric field:



Equilibrium shifted to the right

Kinetic contribution:

Effect of the electric field on the *transition state polarity* of the reaction

Fig. 5. Mechanisms of formation of a covalent bond between M1C4I and silanols. Thermodynamic and kinetic contributions.

is larger than the number of the negative ones we obtain the reversal of electroosmotic flow.

There still remains to explain the reaction mechanism, for which we hypothesize the scheme depicted in Fig. 5. From a thermodynamic point of view, it is known that this nucleophilic substitution is highly unfavourable, so that the equilibrium is shifted to the left, indicating absence of reaction. We have direct evidence by two observations: (1) in silica TLC experiments, using appropriate eluent, the M1C4I-like compounds show the same behaviour of the corresponding quaternary ammonium salts without the alkyl iodine function; (2) when trying an analogous alkylation between M1C4I and –SH groups in proteins, in solution, under the same alkaline pH conditions, no reactivity could be observed up to one week of incubation [17]. Here, however, we believe that another factor comes into play: the contribution of the electric field on

the equilibrium, thus shifting the reaction to the right. Since any iodine ion produced by the reaction would be immediately harvested by the voltage gradient, this drives efficiently the reaction to the right, allowing proper silanol alkylation.

4. Take another bite, if you please

Encouraged (and puzzled) by these results, we synthesized a number of derivatives, whose formulae and abbreviations are depicted in Fig. 6 [18]. They can be divided into two classes: those with a butyl chain (C₄) and those with an octyl chain (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 (M5C4I). 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. 7A shows how the different modifiers can modulate the charge (and thus the EOF) on a naked silica wall. We can distinguish four types of behaviour: those which have not influence on the EOF value. This is the case of the M3C4 compound, which has the same chemical structure of the M1C4I, except for lack of the iodo alkyl function. Those which have a modest influence on the EOF profile, probably due to the modest weak interaction established with the silica surface (M4C4I and M5C4I); those which reduce or substantially quench the negative EOF flux (M2C4I, M6C4I

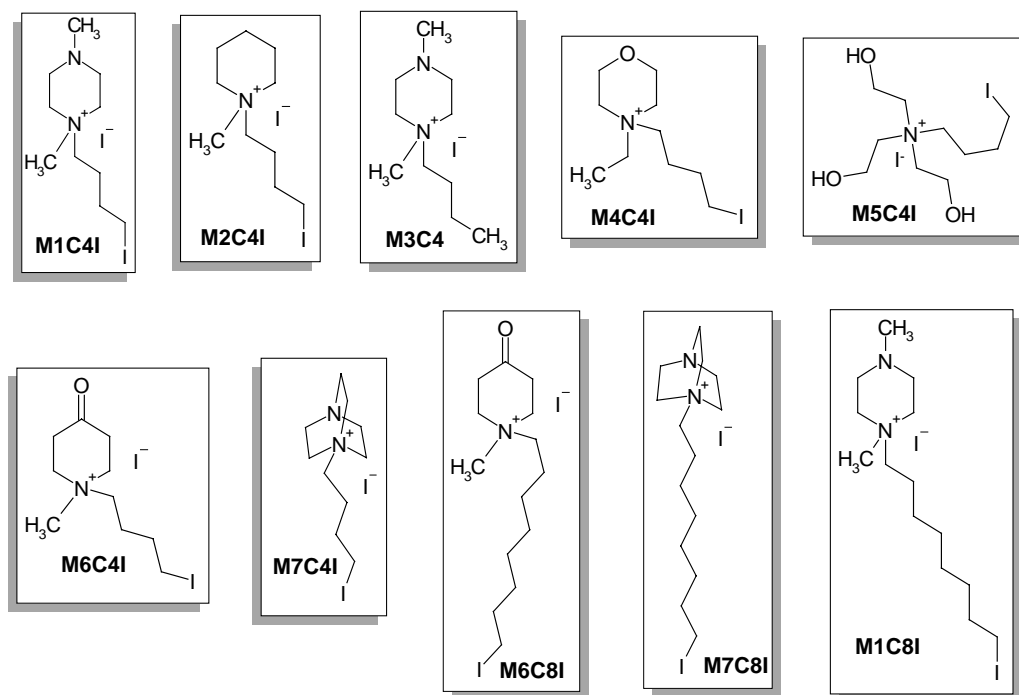


Fig. 6. Chemical formulae of 10 different quaternary mono ammonium salts used as models for the study.

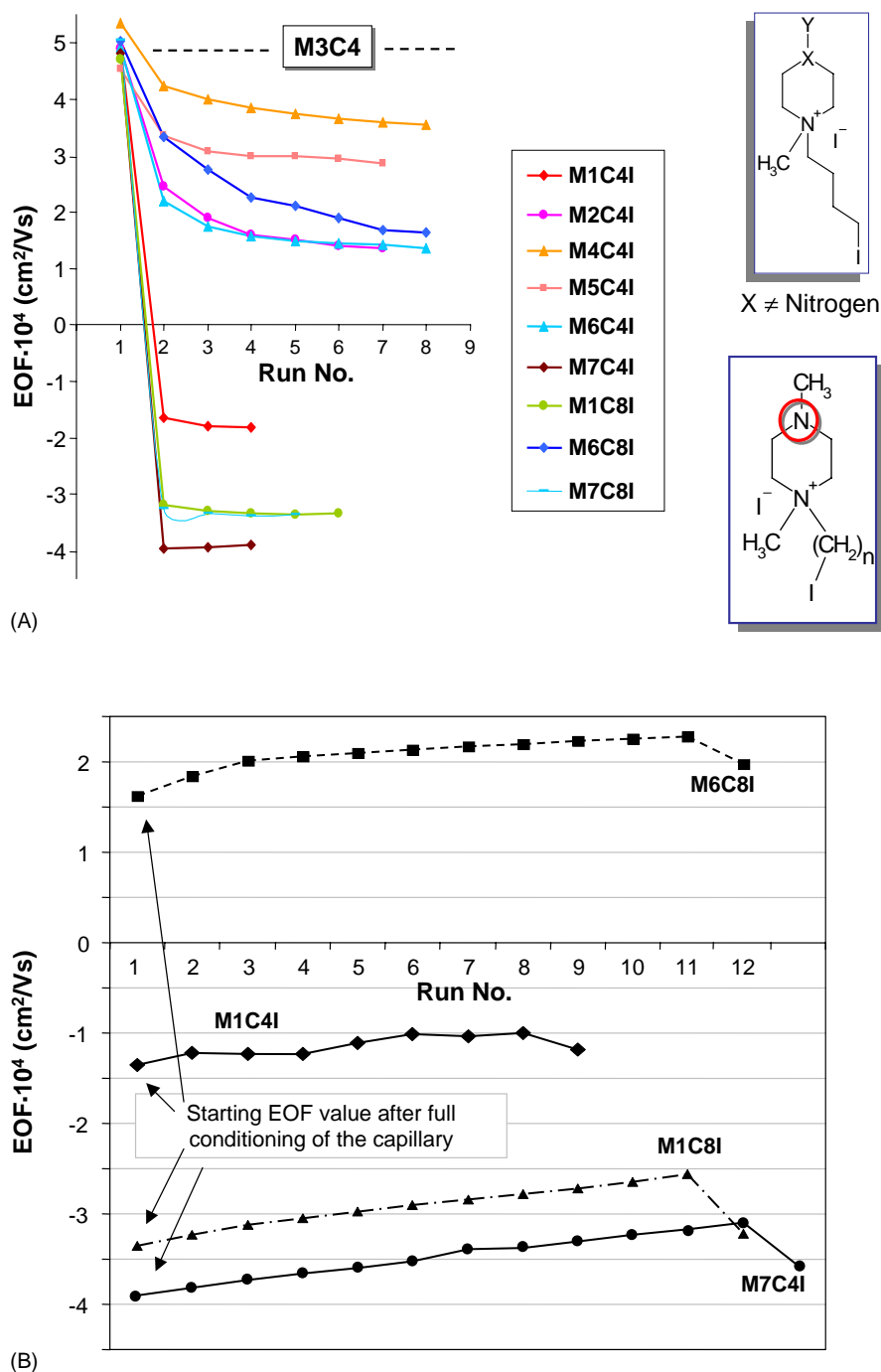


Fig. 7. (A) Ability of the various compounds listed in Fig. 6 to modulate and/or reverse the EOF in CZE. Preconditioning step followed between any runs: washing (5 bar \times 2 min) with the appropriate modifier solution (4 mM in 25 mM borate buffer, pH 9); washing (5 bar \times 3 min) with the running buffer solution (25 mM borate buffer, pH 9). (B) Stability assessment of the silica wall modification. Preconditioning step followed between any runs after full conditioning of the silica capillary: washing (5 bar \times 3 min) with the running buffer solution (25 mM borate buffer, pH 9). The last EOF values were recorded after restoration of the capillary washing with the appropriate modifier solution.

and M6C8I) 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 (M1C4I, M1C8I, M7C4I and M7C8I). It is noted that the second class comprises those species that do not have a tertiary amino group in the ring (M4C4I) or do not even

have a ring (the star monomer M5C4I). The third 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 require several cycles of washing-run for achieving a stable EOF value. Finally, all

the compounds of the fourth class are characterized to the presence in the molecule of a quaternary and a ternary basic nitrogen comprised inside of a single or a tree-ring structural 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.

It has to be remarked that in all the cases, when the stable EOF value is obtained, the silica modification is quite stable also when omitting the washing step of the capillary between any runs. Fig. 7B shows the variation of the EOF values when the capillary washing step with the modifier solution is omitted. It should be noted, additionally, that the small loss of modifier effect (3–4% per run) is the same for the different compounds (same slope of each curve): this indicates that the mechanism by which the different molecules are removed from the silica surface has to be the same. This common process should be the slow hydrolysis reaction of the Si–OR bond always present in a watery medium.

The results on the inner capillary wall modification by the studied compounds support the cooperative mechanism already proposed by us [15] and show its generality. As proved by the analysis of the compounds M2C4I to M6C4I and M6C8I, 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 M1C4I, M1C8I, M7C4I and M7C8I 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 increases their adsorption on the capillary silica surface (as highlighted in the left panel in Fig. 7A). 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 positive charged quaternary ammonium atom; (3) the lipophilic component related to the alkylic chain; and (4) the distance between the positive charge and the terminal carbon atom bearing the iodine. Interestingly, the modulation of the EOF profile, as shown in Fig. 7A, can be reproduced theoretically by calculating the zeta potential (ζ in mV) as a function of the log of the molar activity coefficient of the compound adsorbed onto the silica surface, as suggested by Spange et al. [19]. The results of these calculations are shown in Fig. 8. Basically any compound, when adsorbed by silica, will alter its ζ -value. However, species which have a very modest affinity, such as (C₆H₅)₃CBr, are unable the reverse the ζ -potential, and the sigmoidal transition reaches a plateau at molar activity coefficient values of the order of mM. Conversely, our compounds M1C4I and M7C8I, which had been shown, experimentally, to be able

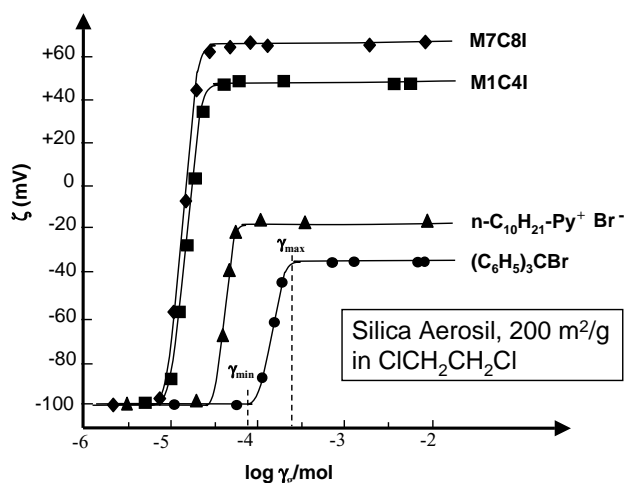


Fig. 8. Theoretical calculations of the ζ -potential profiles of different chemicals adsorbed onto silica surfaces, as a function of the log of their respective molar activity coefficients.

to fully reverse the EOF flow (cf. Fig. 7A) are here shown to be able to affect the same transition on the ζ -potential as well, and at much reduced concentrations.

5. Other subtle coating ways

In CZE technology, another strategy for full EOF control is to try to affix covalently a layer of neutral polymer to the wall (as reviewed in [1–3]). Among the various approaches, the use of polyacrylamide, or of some of its stable and hydrophilic *N*-substituted derivatives [20,21], is privileged, since this coating results in a highly viscous layer essentially zeroing EOF and preventing protein adsorption to silica. A classical approach to this reaction is depicted in Fig. 9: it consists in first chlorinating the wall and then further processing it with Grignard reagent, so as to form a direct carbon-silicon bond with an unsaturated ethylene group. At this point, filling the capillary lumen with a solution of acrylamide monomer, properly admixed with catalysts [persulphate and *N,N,N',N'*-tetramethylethylenediamine (TEMED)] will result in a growth of polymer covalently affixed to the silica surface. The practical aspects, though, are not as easy as jotted down; in fact, this procedure is fraught with difficulties. Due to the high sensibility to the oxygen and humidity of the reactant and to the peculiarity

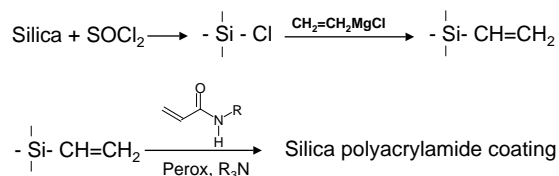


Fig. 9. Example of a traditional permanent coating with a polyacrylamide layer of the silica wall via activation of silanols through a Grignard reaction.

of the substrate (the silica capillary), all the procedure must be executed by expert hands for obtaining reproducible and stable silica surface coating; in any case requiring no less than a couples of working days [22].

In general, in aqueous solutions, a polyacrylamide gel is obtained by mixing monomers, cross-linkers, the radical reaction initiator (persulphate) and an appropriate catalyst (the ternary amine TEMED). The reaction occurs following the steps show in Fig. 10. The key step is the oxidation of the tertiary amine by the persulphate radical to generate an aminium radical cation, which, by losing a proton in the methylene group, is transformed into an alfa amino alkyl radical [23]. This last species is the true radical chain initiator able to add to the activated double bond of the acrylamide by subsequent generation of the alkyl radical adduct that continues the radical chain reaction (upper reaction pathway). Now, the role of the ternary amine can be carried out by modifiers like the M7C4I, where a ternary nitrogen atom is present, susceptible to be oxidized to radical cation by the persulphate and continue the reaction, just as previously described, to produce the acrylamide polymerisation. The big advantage offered by these compound is that they can be easily and rapidly bonded onto silica surface, thus substituting the hard process of functionalizing silica with the vinyl groups and, at the same time, giving the necessary surface anchorage points for the polymer, since the polymerization process starts from such cations, as indicated in the lower reaction scheme of Fig. 10. A Fata Morgana? Not quite, judging by the excellent separations of both, proteins and DNAs, as shown in the two bottom panels [24]. Here too,

one should appreciate how easily this reaction can be done, since both the covalent binding of M1C4I to the wall and the subsequent growth of the polymer chain occur spontaneously.

6. A kaleidoscope of chemicals and related activities

Quite thrilled by the results obtained so far, we decided to push the exploration deeper into the *terra incognita*, by synthesizing a number of quaternary diamino compounds, i.e., species having two rings at the extremities of an alkyl chain of variable length [25,26]. We wanted to understand the structural motives enabling these compounds to interact with the silica wall. A summary of the formulae of some of these (and previously described) compounds, together with an assessment of their behaviour in presence of silica powder, is shown in Fig. 11. It is seen that all these new bicyclic compounds bind so strongly to silica that they cannot be eluted from the application point in thin layer chromatography. At the opposite extreme of this scale, a mono-cyclic derivative, containing only tertiary nitrogens in the ring, moves with an R_F close to unity, indicating that the contribution of the neutral nitrogen atom to the silica adsorption process is very low compared with the one of the quaternary ammonium.

In the middle, we have the quaternary monoammonium salts with and without the iodo alkyl function: all these compound can be eluted and the R_F value are essentially not depended on this chemical function. All these results are

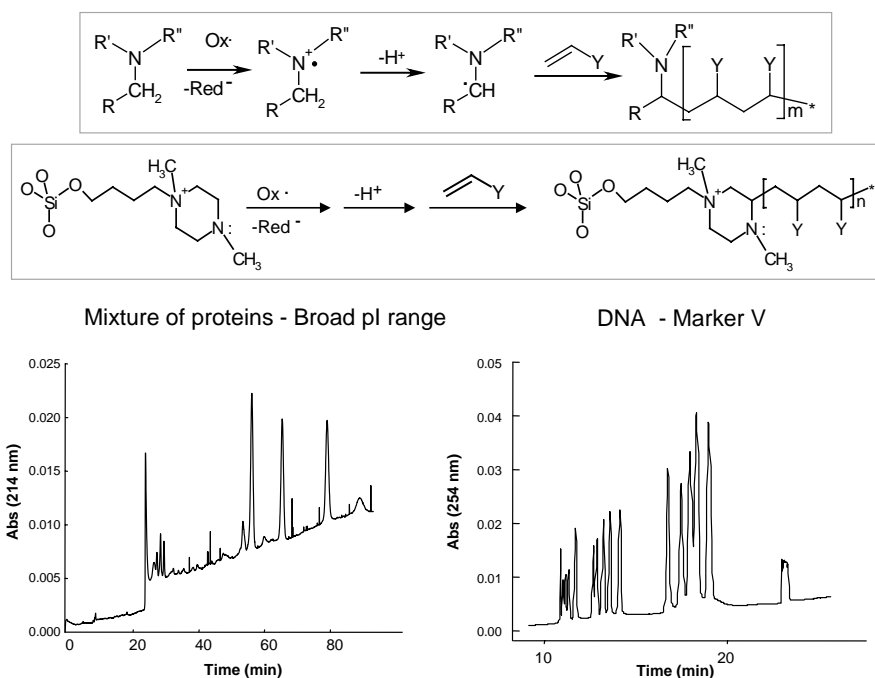


Fig. 10. Upper row: Activation of TEMED during radical polymerisation of acrylamide (note that the growing chain will contain a charged TEMED residue at its extremity). Mid row: proposed mechanism of covalently affixing a polyacrylamide string to silica via a quaternized piperazine bound to the wall. Two bottom panels: examples of separations of proteins and DNA fragments in capillaries coated via the M1C4I bridge.

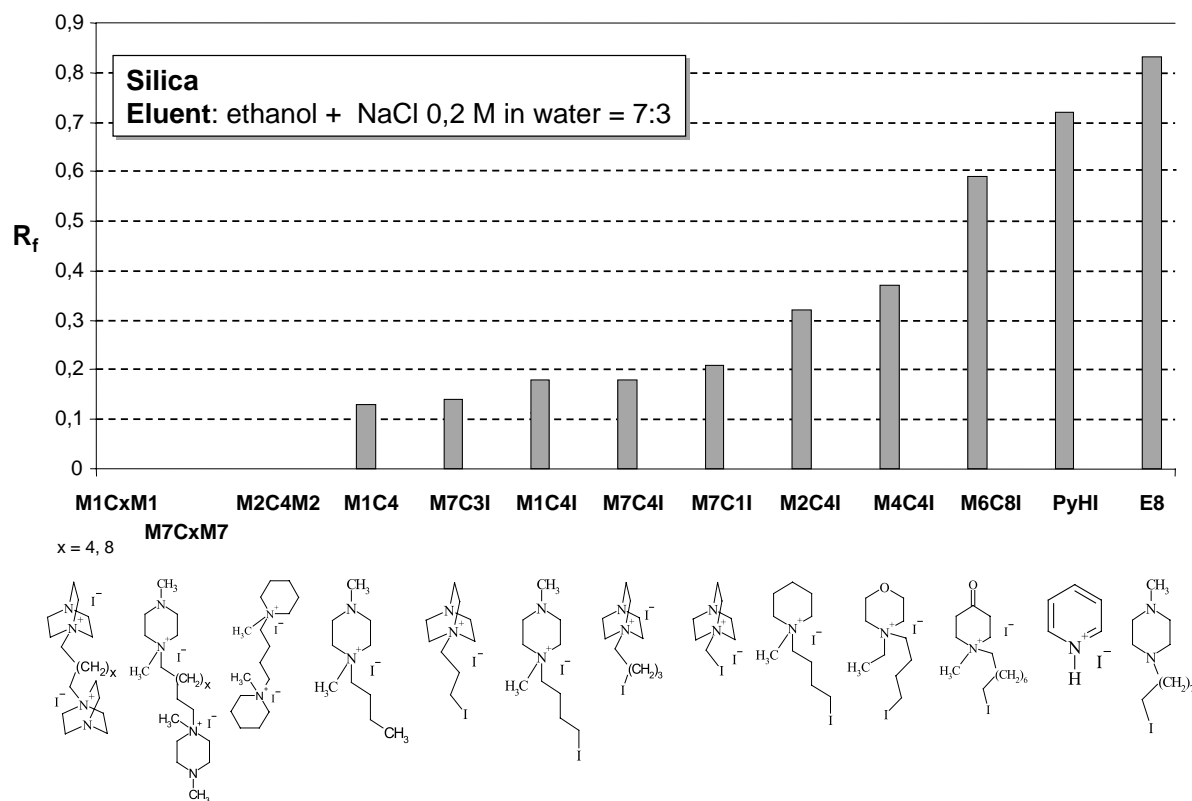


Fig. 11. Interaction of bicyclo and monocyclo derivatives with silica particles. The chemical formulae of each compounds are drawn underneath the bars representing their respective R_f values (note that the first three chemicals cannot be eluted from their application point, thus no R_f bars are shown).

easily explicable considering the physico-chemical properties of the compounds tested.

Switching to CZE, we found a relative behaviour of these compounds apparently not in agreement with the TLC experiments: all the bicyclic compounds are hardly able to modify the EOF value as shown in Fig. 12. Conversely, as already mentioned, the monocyclic derivatives, endowed with an iodinated tail, such as M7C4I, M1C4I and the like, fully reverse the EOF: these results are a significant support to the “Skorpio” mechanism of action proposed. Nevertheless, even M7C4M7 (and other members of the family as well), when present in the background electrolyte during the CZE run, are able to amply modify EOF at low levels and, at higher concentrations, also reverse it.

7. Emergence of a structural motif

At the end of this excursus, we will try to summarize our data by bringing in a general view on all possible amino quenchers and trying to derive some general structural motives for their mechanism of action (see also Table 1). It is striking that, notwithstanding the large number of publications dealing with the use of amino buffers (mono-, di-, oligoamines) in CZE, no model on their action and no possible structural motifs have emerged up to the present. We will try to build such a model, based on the following consider-

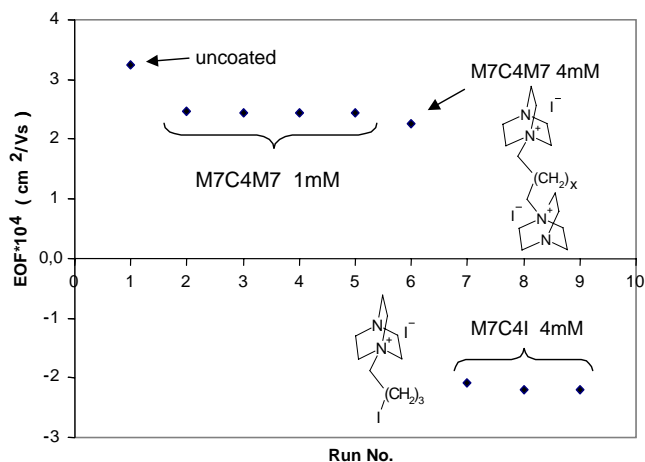


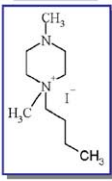
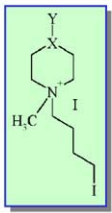
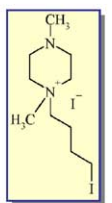
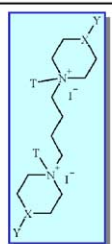
Fig. 12. EOF flow values of, respectively, an untreated silica wall and as treated with two different molarities of M7C4M7 and with M7C4I. Preconditioning step followed between any runs: washing (5 bar \times 2 min) with the appropriate modifier solution (1–4 mM in borate buffer 25 mM pH 9); washing (5 bar \times 3 min) with the running buffer solution (borate buffer 25 mM, pH 9). Note that only M7C4I, endowed with an iodinated alkyl tail, is able to reverse EOF.

ations, as gathered from our (and others) previous research:

- (i) Monoamines (e.g., ethylamine, triethylamine, triethanolamine, glucosamine, galactosamine) are very poor quenchers of protein interaction with the silica wall (e.g., in order to bring about 90% binding inhibi-

Table 1

Comparative table of some properties of the salts studied in the TLC and CZE experiments

Modifier type	TLC experiment	CZE experiment
 <p>M3C4 like</p>	<ul style="list-style-type: none"> • R_f comparable with the other quaternary mono ammonium salts. 	<ul style="list-style-type: none"> • <u>No EOF modification.</u>
 <p>M4C4I like</p>	<ul style="list-style-type: none"> • X ≠ Nitrogen • R_f comparable with the other quaternary mono ammonium salts. • Not relevant effect of the alkyl iodine function 	<ul style="list-style-type: none"> • Sensible <u>reduction</u> of EOF. • No EOF reversion. • Stable EOF value after <u>several</u> washing – run cycles. • <u>Hard restoration</u> of the capillary surface.
 <p>M1C4I like</p>	<ul style="list-style-type: none"> • R_f comparable with the other quaternary mono ammonium salts. • Moderate effect of the ternary nitrogen atom present. • Not relevant effect of the alkyl iodine function. 	<ul style="list-style-type: none"> • Sensible <u>reversal</u> of EOF. • Stable EOF value after <u>a few</u> washing – run cycles. • In appropriate mixture with the M4C4I like compounds allows EOF modulation. • Works also in <u>acidic media</u>. • <u>Hard restoration</u> of the capillary surface.
 <p>M7C4M7 like</p>	<ul style="list-style-type: none"> • X = CH₂; T = CH₃ • X = N; Y = CH₂CH₂- • $R_f = 0$. 	<ul style="list-style-type: none"> • <u>Modest reduction</u> of EOF. • X = CH₂; T = CH₃ in <i>dynamic coating mode</i> able to <u>reduce</u> the EOF. • X = N; Y = CH₂CH₂- in <i>dynamic coating mode</i> able to <u>reverse</u> the EOF. • Easy and <u>fast restoring</u> of the capillary surface by washing with diluted H₃PO₄ solution. • Usable as silica modifier in LC.

tion they have to be used at a ca. 200 mM level, and this at pH 5.0, where they are fully protonated) [27];

- (ii) Diamines (e.g., cadaverine, putrescine) fare slightly better, but here too 90% binding inhibition is brought about by ca. 120–150 mM levels) [28];
- (iii) A triamine (spermidine) suddenly offers a much improved binding inhibition, being effective at only ca. 20 mM [27];
- (iv) Oligoamines, such as spermine and tetraethylenepentamine) offer a dramatically higher performance, being active at levels of ca. 1–2 mM [27];
- (v) Two α,ω -bis-quaternary ammonium alkanes (hexamethonium chloride and decamethonium bromide) were found to be extraordinarily effective additives in resolving ovalbumin glycoforms in uncoated capillaries in borate buffers at pH 8.4 at levels of only 100–200 μ M [14]. Nevertheless, although they could

strongly reduce the EOF, they could not reverse it even up to 3 mM;

- (vi) 1,4-Diaminobutane was found to be effective (at ca. 1 mM concentration) in resolving glycoforms of ovalbumin [14] as well of erythropoietin [28];
- (vii) A *p*-(quaternary ammonium)calix[4]arene was found to be effective in reversing the EOF flow, in the 4–8 mM concentration range [29].

When comparing all the above data, the following structural motifs seem to emerge as minimum necessary requirements for the strongest possible affinity of amino compounds to the silica wall:

- (1) The presence of two quaternary nitrogens;
- (2) An optimal distance, for a snug fit to the wall, of a C₄ chain;
- (3) Various hydrophobic “decorations” onto the molecule.

The first assumption seems well documented: the most effective compounds, in all cases reported, are only those containing two quaternary nitrogens. In addition, they are obviously active all along the pH scale, contrary to tertiary amines, which, when exhibiting some wall affinity, they do so only at acidic pH values (pH 5 and below). The second “structural motif” requires some additional explanations. Our compounds, which seem to tightly bind to the wall to the point of reversing the EOF at only ca. 0.1–0.3 mM levels, contain two quaternary nitrogens held apart by a butyl chain (C₄). On the contrary, hexamethonium chloride (C₆Met) and decamethonium bromide (C₁₀Met), which appear to be just as effective in maximizing glycoprotein separations in CZE, are nevertheless unable to reverse the EOF up to at least 3 mM [14]. Notwithstanding the claims of Oda et al. [14] that C₁₀Met is more efficient than C₆Met, in reality they show the same protein profiles at 300 μM levels; moreover, in terms of reducing EOF, C₆Met appears to be decidedly superior than C₁₀Met, indicating that the former compound could cling somewhat more strongly to the silica wall. Thus, it would appear that lengthening the distance between the two quaternary nitrogens from a C₄ to a C₆ up to a C₁₀ diminishes the affinity of such compounds to the wall, since only the structural motif N⁺–C₄–N⁺ appears to be able to bring about EOF reversal, and that at only sub-milli-molar levels. Some additional calculations could also be made. It is known that a silica surface should contain 5 silanols/nm² [9,30]. Assuming a quincunx distribution, this suggests that, under ideal conditions, two neighboring silanols should be spaced apart by ca. 0.4 nm. This might be a bit too naive, considering that the silanol distribution on the surface is not homogeneous, since there exist, e.g., geminal silanols. Moreover, not all silanols can be fully ionized; according to some authors, even at pH 10 maximum silanol ionization reaches only 50% of the total [30]. Thus, it is reasonable to assume that the average distance between two neighboring silanols could be just about twice the level reported above, i.e., ca 0.8 nm. Given a C₄ distance between the two quaternary nitrogens in our M1C4M1 and M7C4M7 compounds, and considering that, due to the strong positive charge on the two adjacent nitrogens, the C₄ bond will be fully stretched, the average distance between the two charged groups would amount to ca. 0.75 nm (assuming an average length between a C–C or a C–N bond of 0.15 nm). Thus, such a distance would be just the ideal one for forcing a snug fit between the two quaternary nitrogens and two charged silanols on the silica wall. The third point on “hydrophobic decoration” of the molecules also needs some explanations. There is ample evidence in the literature, that the efficacy of all additives, dissolved in the background electrolyte for minimizing protein–silica interaction, is closely related to their hydrophobicity [31]. In the case of amines, thus, the dominant theme would be the CH₂/N ratio, which, in our compounds (neglecting the tertiary nitrogen) is of the order of 8 carbon atoms per quaternary nitrogen. We believe that the strong adsorption of

our M1C4M1 and M7C4M7 species are primarily driven by the two strong charges of the quaternary nitrogens, seeking two negatively ionized silanols for charge neutralization. Once hooked onto the wall, the hydrophobic leitmotiv would then be the dominant theme for holding them in place. Such additional interaction would be hydrophobically driven, but in the sense that it is vectorially oriented parallel to the silica surface, i.e., it occurs among the various M1C4M1 and M7C4M7 molecules deposited onto the surface, interlocking them via, precisely, hydrophobic bonding.

One last comment is here due on EOF reversal. This phenomenon, most probably, is brought about by those molecules of modifier which are only loosely (and sparsely) bound to the surface via only one quaternary nitrogen, hooked onto the remaining population of free ionized silanols not yet occupied by the bulk of modifiers lying flat on the surface and tightly held via two salt links. These former molecules, thus, are oriented quasi perpendicular to the surface, rendering available their free positive charge to the bulk solvent and thus reversing the surface polarity. This population must be more loosely held to the surface and possibly helped to remain in situ via hydrogen and/or hydrophobic bonds with surrounding modifier molecules tightly clinging to the silica surface. If this model is correct, we should indeed expect, as a corollary, the following phenomena: (a) the modifier should be present in the background electrolyte during each run, to prevent a massive loss of molecules from the surface; (b) the EOF reversal should be modest, as compared to the forward EOF. Both conditions are well verified by our experiments.

8. Conclusions

It is hoped that this mini-review will broaden the knowledge of the potential readers on the various mechanisms of action of quenchers and modulators of EOF. The EOF, per se, could be a blessing or a disaster, depending on the aims and scopes of a CZE run and on the nature of the analytes. In general, though, for macromolecules the EOF is anathema, since the extensive ionisation of silanols, responsible for this phenomenon, will be accompanied by severe adsorption, in case of proteins and peptides, and dramatic loss of resolution. Being able to “tame the shrew” has been the dream of almost of generation of scientists working with CZE and the object of countless publications. Whether this nth one will just increase the entropy in Babel tower or bring some useful, novel information, remains for the readers to emit the verdict. For those of you who enjoyed the review, well take out your surfing board and let us meet outside the San Diego Bay, on the Pacific Ocean, waiting for the “Big One”, like those characters in *Big Wednesday*.

Acknowledgements

This work has been supported in part by grants from MURST (Coordinated Project 40%, 2003, FIRB-2001, no. RBAU01JSC-0058 to CG and no. RBNE01KJHT to PGR) and by ASI (Agenzia Spaziale Italiana, grant no. I/R/294/02).

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