

# Cation exchange contribution to the retention of specific quaternary ammonium compounds in reversed-phase high-performance liquid chromatography

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**Abstract:** The influence of electrolytes on the retention of organic cationic solutes in reversed-phase high-performance liquid chromatography (RP-HPLC) has been investigated. The effects of the nature and concentration of electrolytes and mobile phase pH on the retention of two model quaternary ammonium compounds were studied on  $\mu$ -Bondapak C<sub>18</sub> stationary phase with aqueous methanolic eluents. The nature and concentration of inorganic cations added to the mobile phase modified the retention of the solutes. The counter anion of the added electrolyte did not perceptibly influence solute retention at constant mobile phase pH, although it did significantly influence solute retention when the electrolytes were added to unbuffered mobile phases. The retention data are consistent with the inclusion of an ion exchange contribution to the retention of cationic solutes in the systems investigated.

**Keywords:** *Quaternary ammonium compounds; reversed-phase HPLC; effect of electrolytes on retention; bonded phase silica gels.*

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## Introduction

The presence of electrolytes has been shown to markedly influence the retention and peak shape of amines and quaternary ammonium compounds when these solutes are separated by chromatography on chemically-bonded stationary phases in reversed-phase high-performance liquid chromatography (RP-HPLC) [1–9]. These effects have often been attributed to the reduction of solute interactions with residual silanols on the stationary phase [1, 3–5, 7–9], as verified in a recent study [10].

Since silica gel has been shown to exchange cations in aqueous eluents [3, 10–12], it is reasonable to expect that cationic solutes may similarly show cation exchange behaviour at the residual silanol sites on chemically-bonded stationary phases. Evidence supporting this hypothesis has been presented by Thurman [13] and Van der Houwan *et al.* [14], who have reported the exchange of inorganic and organic cations on  $\mu$ -Bondapak C<sub>18</sub>.

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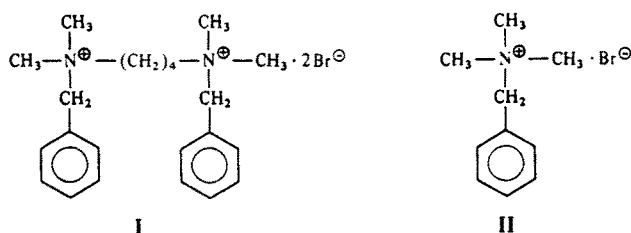
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However, others have suggested that ion exchange does not influence the retention of cationic solutes on bonded reversed-phase materials [3].

The ubiquitous occurrence of amine functionalities in drug molecules prompted further investigations of variables that could be used to modify their retention. Amines are most commonly separated as ammonium ions in RP-HPLC due to the improved column stability [15, 16] and reduced solute reactivity at these pH values.

Quaternary ammonium compounds (I and II) were chosen for this study to maintain the cationic character of the amine nitrogen with changes in mobile phase pH, ionic strength and organic modifier concentration. If a cation exchange contribution to retention is operative, retention may be controlled by changing mobile phase pH and the nature and concentration of various electrolytes added to the eluent. The influence of such variables on the RP-HPLC of a model *bis*-quaternary ammonium compound (I) has been investigated. A di-cation was studied because of its structural similarity to the anthracycline type antibiotics [17, 18] under investigation in our laboratories. These solutes are generally separated as protonated polyamines [17].



## Materials and Methods

### High-performance liquid chromatography

The chromatographic system consisted of a Waters (Milford, MA, USA) Model 6000A pump, Model U6K injector and a Model 440 absorbance detector. Column temperature was maintained at  $30.0 \pm 0.2^\circ\text{C}$  with a circulating water bath (Haake, Saddle Brook, NJ, USA) and a column water jacket (Alltech Assoc., Deerfield, IL, USA).

HPLC columns ( $4.6 \times 100$  mm) were slurry packed with  $10\text{-}\mu\text{m}$   $\mu$ -Bondapak  $\text{C}_{18}$  (Waters Associates) stationary phase, according to the method described by Bristow *et al.* [19]. A flow rate of  $2.0$  ml/min was maintained throughout these experiments, and the column effluent was monitored spectrophotometrically at  $254$  nm.

Retention measurements were obtained in triplicate using a Model 8678 Digital Stopwatch (Cole Parmer, Chicago, IL, USA) and capacity factors were calculated using the conventional relationship. The column dead-volume ( $t_0$ ) was determined by six replicate injections of HPLC-grade water ( $10$   $\mu\text{l}$ ) and could be measured to within  $0.2$  sec. The poor chromatographic behaviour of the quaternary ammonium compounds necessitated working with mobile phases giving  $k' \leq 5$ . In many instances,  $k'$  values less than  $1$  were found, and these could be determined with a relative standard deviation of  $\leq 5\%$ . Solutions containing I and II were prepared at  $1 \times 10^{-3}$  M and triplicate  $10$   $\mu\text{l}$  injections of each solute were made.

The mobile phases were prepared on a volume fraction ( $\phi$ ) basis, by mixing known volumes of methanol ( $V_{\text{om}}$ ) with HPLC water ( $V_{\text{aq}}$ ) according to equation (1).

$$\phi = \frac{V_{om}}{(V_{om} + V_{aq})} \quad (1)$$

### Materials

Benzyltrimethylammonium bromide (Aldrich Chemical Co., Milwaukee, WI, USA), HPLC grade methanol (Fisher Scientific, Fair Lawn, NJ, USA) and sodium 1-hexanesulphonate (Eastman Kodak, Rochester, NY, USA) were used as received. All water was distilled from glass following mixed-bed deionization/reversed osmosis treatment.

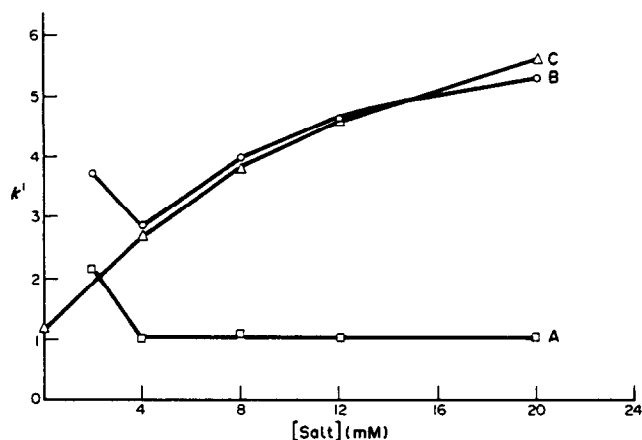
Bis-(*N*-benzyl-*N,N*-dimethyl)-1,4-butanediammonium dibromide (I) was synthesized according to the procedure described elsewhere [20]. All other chemicals were of reagent grade and were used without further purification.

### Results and Discussion

The bis-quaternary ammonium compound (I) failed to perceptibly elute from an RP-18 column when using aqueous methanolic eluents ( $\phi = 0.0$ – $1.0$ , where  $\phi$  = volume fraction, equation 1) at temperatures of  $50^\circ\text{C}$ . Similar effects have previously been attributed to strong interactions between the quaternary ammonium ion and the residual silanols on the stationary phase [4, 5].

#### *The influence of salts on the retention of solute I*

The addition of  $\text{NaNO}_3$  to the mobile phase results in an initial decrease in the retention of I with increasing  $\text{NaNO}_3$  concentration at  $[\text{NaNO}_3] < 4 \text{ mM}$  (Fig. 1, curve A). An eluent containing methanol ( $\phi = 0.55$ ) provided acceptable  $k'$  for I in the presence of alkali metal salts and was used for all further studies. Facilitation of elution by  $\text{NaNO}_3$  may be explained in terms of a cation exchange mechanism in which the sodium ion competes with I for the residual silanol sites, thereby reducing solute retention. At  $\text{NaNO}_3$  concentrations greater than 4 mM, the retention of I is essentially independent of  $\text{NaNO}_3$  concentration, suggesting that sodium ion has effectively



**Figure 1**

The relationship between the retention of I and the concentration of (a)  $\text{NaNO}_3$  ( $\square$ ), (b) HSA ( $\circ$ ), and (c) HSA in the presence of  $\text{NaNO}_3$  (20 mM) ( $\triangle$ ) in 55% methanol–water mobile phase. Stationary phase:  $\mu$ -Bondapak  $\text{C}_{18}$  ( $100 \times 4.6 \text{ mm i.d.}$ ). Temperature:  $30^\circ\text{C}$ .

eliminated the cation exchange contribution to the retention of **I** and that **I** does not ion pair with nitrate ion at  $[\text{NO}_3^-] \leq 20$  mM. Additionally an ion exchange contribution can also arise through surface charge created on the stationary phase by anion adsorption (Donnan penetration). However, the rigidity of the support phase and the low external molality of salt in the mobile phase makes Donnan invasion unlikely [21, 22].

The substitution of the ion-pairing agent sodium 1-hexane-sulphonate (HSA) for  $\text{NaNO}_3$  resulted in a similar decrease in the retention of **I** at  $[\text{HSA}] \leq 4$  mM, followed by increasing retention with increasing HSA concentration (Fig. 1, curve B). This behaviour results from the superimposition of a cation exchange contribution and an ion-pair contribution to the retention of **I**. At low HSA concentrations, the cation exchange mechanism dominates the retention of **I**, whereas at HSA concentrations greater than 4 mM, the ion-pair contribution enhances retention [23].

When mobile phases containing both  $\text{NaNO}_3$  (20 mM) and varying HSA concentrations were employed (Fig. 1, curve C), the retention of **I** increased with increasing HSA concentration in accordance with ion-pair theory [23], suggesting that the addition of higher concentrations of sodium ion effectively eliminates the ion exchange contribution to the retention of **I**. The non-zero intercept (Fig. 1, curve C) most likely reflects the hydrophobic contribution to the retention of **I**.

#### *The influence of mobile phase pH on the retention of solute I*

The effects of mobile phase pH on the retention of **I** were studied in 55% methanol/dilute  $\text{H}_2\text{SO}_4$  mobile phases. Dilute  $\text{H}_2\text{SO}_4$  solutions were used as the aqueous mobile phase component to minimize salt contributions to retention.

The addition of acid to the eluent resulted in greatly improved peak shapes for **I**, thus allowing the measurement of  $k'$  values greater than 5. The retention of **I** increased with increasing pH of the aqueous mobile phase component with  $k' = 0.86, 1.44$  and  $6.95$  and pH 3.0, 3.5 and 4.0, respectively. Similar results were obtained when  $\text{H}_3\text{PO}_4$ ,  $\text{HNO}_3$  or  $\text{HCl}$  were substituted for  $\text{H}_2\text{SO}_4$  in these mobile phases. Since the pH of these mobile phases was more than two pH units below the  $\text{pK}_a$  of silica gel (6.5, ref. [24]), the silanols should remain unionized and not offer an opportunity for ion exchange. The increased retention observed with pH elevation may, however, arise from the presence of isolated strongly acidic sites on the silica surface [10, 12], which can participate in cation exchange at the pH values used ( $\text{pH} \geq 3$ ), and increase in ion exchange capacity with increases in pH.

#### *Influences of various chromatographic variables on the retention of solutes I and II*

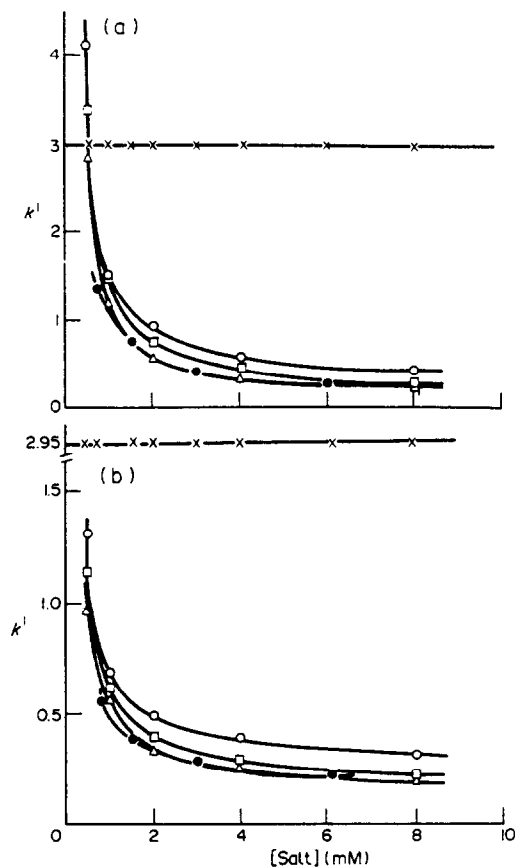
To determine whether the cation exchange contribution to retention was more generally applicable to quaternary ammonium compounds, a mono-quaternary ammonium compound (benzyltrimethylammonium bromide, **II**) was introduced in these studies for purposes of comparison with the model compound **I**.

*The effect of the nature and concentration of inorganic cations on retention.* The abilities of inorganic cations to modify the retention of **I** and **II** were investigated by the addition of  $\text{LiNO}_3$ ,  $\text{NaNO}_3$ ,  $\text{KNO}_3$  or  $\text{CsNO}_3$  to the aqueous methanolic eluent. Results are shown in Fig. 2 (a and b) for solutes **I** and **II**, respectively. Several features are apparent from Fig. 2:

(1)  $k'$  decreases for both **I** and **II** with increasing concentrations of the four salts employed;

**Figure 2**

The relationship between the retention of **I**(a) and **II**(b) and salt concentration in methanol–water mobile phase ( $\phi = 0.55$ ). Key:  $\text{LiNO}_3$  (○),  $\text{NaNO}_3$  (□),  $\text{KNO}_3$  (△) and  $\text{CsNO}_3$  (●). Conditions as in Fig. 1. The retention of benzene (×) is also shown.



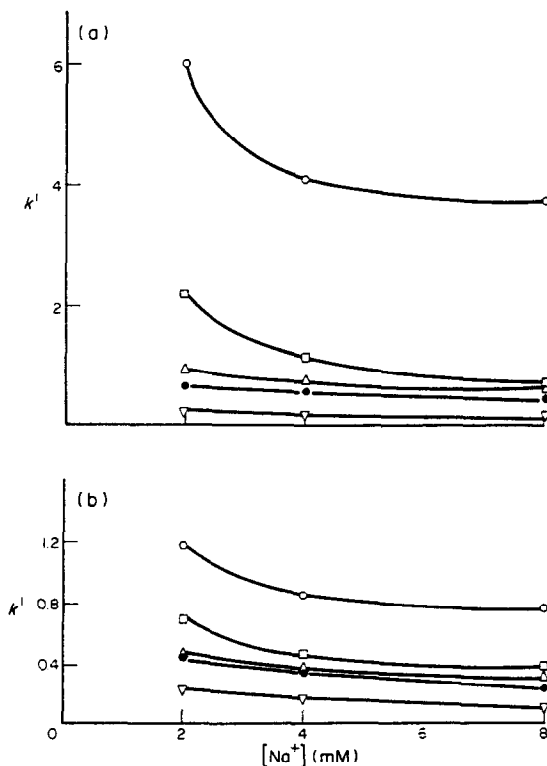
(2) The added salt is more effective in reducing  $k'$  for the di-cationic solute **I** than the mono-cationic solute **II**;

(3) The nature of the cation affects the retention of both **I** and **II**;

(4) The retention of benzene remains constant for all salt solutions. These results (and in particular the eluent strength of the electrolytes studied) are consistent with a cation exchange mechanism. The silanol group is a polarizable soft acid (as a result of charge delocalization through the siloxane bonds [12]), at which alkali metal cations will interact in the order  $\text{Cs}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$ . Thus, eluent strength of alkali metal salts increases in the same order. The bis-quaternary ammonium compound (**I**), being a larger more highly charged molecule than the mono-quaternary ammonium compound (**II**), interacts more strongly with the silanol sites and is thus retained to a greater extent (Fig. 2). Since benzene is not ionizable and therefore is not subject to ion exchange, the added salts do not affect its retention.

The effect of the concentration of the added alkali nitrate salts on aqueous solution pH was also studied. Although small changes in the pH of these salt solutions were observed when the salt concentration was increased from 2 to 8 mM, they were not consistent with the retention order of **I** and **II** in the presence of these four salts. Therefore, the addition of alkali metal nitrates affects the retention of **I** and **II** (Fig. 2) by their influence on the cation exchange contribution to their retention.

*The nature and concentration of anions.* To better define the influence of electrolytes on the retention of cationic solutes, the effect of the anionic component of the added salt was also investigated. Only sodium salts were employed in this portion of the study to maintain constant cation exchange contributions. The retention behaviour of I and II in eluents containing sodium fluoride, sulphate, nitrate, chloride or bitartrate is shown in Fig. 3. Decreasing  $k'$  with increasing salt concentration was observed for both solutes in



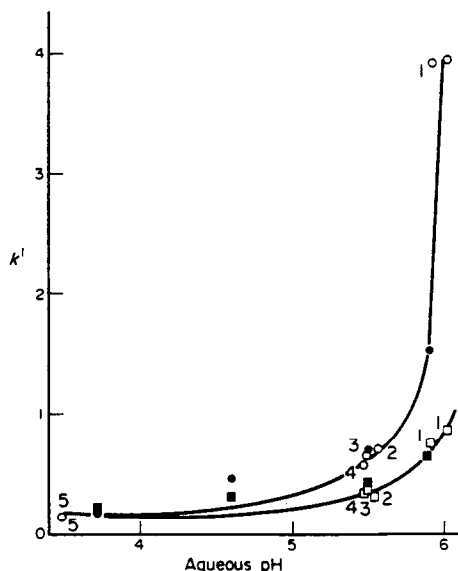
**Figure 3**

The effect of various mobile phase anions on the retention of I(a) and II(b) in methanol-water mobile phase ( $\phi = 0.55$ ). Key: NaF (O),  $\text{Na}_2\text{SO}_4$  (□),  $\text{NaNO}_3$  ( $\Delta$ ), NaCl (●) and Na bitartrate ( $\nabla$ ). Other conditions as in Fig. 1.

all five salt solutions employed in the mobile phase. This trend is again accounted for in terms of the cation exchange behaviour of  $\text{Na}^+$ . Since the lines are not superimposable, the anionic component of the salt does influence solute retention. Although this behaviour could result from ion pair contributions to retention, this seems unlikely for the following reasons: I does not ion pair with nitrate ion at concentrations  $\leq 20$  mM (Fig. 1, curve B), and retention decreases with increasing concentration for all anions in Fig. 3. Rather, this effect appears to be related to the acid-base properties of the salts employed. Solutions of the more basic salts (i.e. NaF) promote the ionization of the residual silanols thereby increasing solute retention, whereas solutions of the more acidic salts (i.e. sodium bitartrate) suppress silanol ionization and thereby lessen the ion exchange contribution to retention. As shown in Fig. 4 (open symbols), a general relationship relating  $k'$  and the pH of the aqueous component of the mobile phase was established for both I and II for all five salts (all at  $[\text{Na}^+] = 4$  mM) used to modify solute

**Figure 4**

The relationship between the retention of I (○) and II (□) and aqueous salt solution pH for mobile phases containing (1) NaF, (2) Na<sub>2</sub>SO<sub>4</sub>, (3) NaNO<sub>3</sub>, (4) NaCl, (5) Na bitartrate, or sodium formate buffer. Key: open symbols represent the data for salts 1–5, whereas closed symbols represent the data for sodium formate buffer. All electrolytes were present at [Na<sup>+</sup>] = 4 mM. Other conditions as in Fig. 1.



retention. A similar relationship was established for  $k'$  and the apparent measured pH of the mobile phase (data not shown). The anion of the modifying salt alters the eluent pH to a varying extent, based on the  $pK_a$  of its conjugate acid.

The experiment was repeated with sodium formate buffer ( $[Na^+] = 4$  mM) over the corresponding pH range, and the retention data thus obtained (closed symbols, Fig. 4) fall on the lines previously generated from the data for the inorganic salts and sodium bitartrate. Thus, the influence of the salt on the mobile phase pH is responsible for the differences in retention behaviour observed with changes in anion. Further evidence substantiating the pH effect of the electrolyte anion on retention was obtained by separating I and II in eluents containing Na<sub>2</sub>SO<sub>4</sub> and NaCl, in which the aqueous mobile phase component was adjusted to pH 5.0 with conc. HCl. Similar retention behaviour was observed for each solute in both eluents, suggesting that the nature of the anionic component has no direct influence on the retention of the cationic solutes other than its effect on mobile phase pH.

The pH of the aqueous solutions of all five salts employed in Fig. 3 increases as the salt concentration increases from 2 to 8 mM, with NaF exhibiting the largest increase of 0.5 pH units and NaNO<sub>3</sub> showing the smallest increase of 0.1 pH unit. Therefore, as the salt concentration increases, two opposing effects contribute to solute retention: (1) increasing pH increases the number of sites available for cation exchange ( $k'$  increases); and (2) the cation exchange contribution to solute retention is reduced as the sodium ion concentration increases ( $k'$  decreases). These opposing forces are evident in Fig. 3, where at high salt concentration the pH contribution to retention is approximately equal to the cation exchange contribution, as reflected by the approach to constant  $k'$ .

## Conclusion

The existence of a cation exchange contribution to the retention of singly- and doubly-charged quaternary ammonium ions in RP-HPLC has been demonstrated.  $\mu$ -Bondapak C<sub>18</sub> has been shown to exhibit a cation exchange selectivity similar to that of a strong

cation exchange resin [25], on which the more polarizable multivalent cations are retained more strongly and thus have greater eluting strength. These results also suggest that multivalent cations may be more effective in eliminating residual silanol sites than the singly charged ions commonly used, although this argument fails to consider the hydrophobic contribution to silanol masking.

The use of unbuffered eluents in the RP-HPLC of cationic solutes is not recommended, since salts added to the mobile phase can influence solute retention not only through cation exchange contributions but also by pH effects. The use of eluents buffered at pH 2–3 is recommended to eliminate the cation exchange contribution of the residual silanol sites to retention. However, the presence of highly acidic sites on the stationary phase has been observed, which still may offer the potential for cation exchange at lower pH. The existence of a cation exchange contribution to the retention of cationic solutes in RP-HPLC can be exploited to control their elution.

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## References

- [1] A. Sokolowski and K. G. Wahlund, *J. Chromatogr.* **189**, 299–316 (1980).
- [2] K. G. Wahlund and A. Sokolowski, *J. Chromatogr.* **151**, 299–310 (1978).
- [3] K. Sugden, C. B. Cox and C. R. Loscombe, *J. Chromatogr.* **149**, 377–390 (1978).
- [4] M. G. M. De Ruyter, R. Cronnelly and N. Castagnoli, Jr., *J. Chromatogr.* **183**, 193–201 (1980).
- [5] F. P. B. Van der Maeden, P. T. Van Rens, F. A. Buytenhuys and E. Buurman, *J. Chromatogr.* **142**, 715–723 (1977).
- [6] J. Knox and J. Jurand, *J. Chromatogr.* **186**, 763–782 (1979).
- [7] J. H. Knox and J. Jurand, *J. Chromatogr.* **142**, 651–670 (1977).
- [8] A. Tilly-Melin, M. Ljungcrantz and G. Schill, *J. Chromatogr.* **185**, 225–239 (1979).
- [9] K. E. Bij, C. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.* **203**, 65–84 (1981).
- [10] D. L. Dugger, J. H. Stanton *et al.*, *J. Phys. Chem.* **68**, 757–760 (1964).
- [11] B. A. Bidlingmeyer, J. K. Del Rios, J. Korpi and J. N. Little, in *Advances in Chromatography* (A. Zlatkis, Ed.), pp. 437–448. Chromatography Symposium, Department of Chemistry University of Houston, Houston, TX (1981).
- [12] D. N. Strazhesko, V. B. Strelko, V. N. Belyakov and S. C. Rubanik, *J. Chromatogr.* **102**, 191–195 (1974).
- [13] E. M. Thurman, *J. Chromatogr.* **185**, 625–634 (1979).
- [14] O. A. G. J. Van der Houwen, R. H. A. Sorel *et al.*, *J. Chromatogr.* **209**, 393–404 (1981).
- [15] C. Horváth, W. Melander and J. Molnár, *Anal. Chem.* **49**, 142–154 (1977).
- [16] K. K. Unger, *Porous Silica*, p. 207. Elsevier, New York (1979).
- [17] D. L. Reynolds, L. A. Sternson and A. J. Repta, *J. Chromatogr.* **222**, 225–240 (1981).
- [18] R. K. Y. Zee-Cheng and C. C. Cheng, *J. Med. Chem.* **21**, 291–294 (1978).
- [19] P. A. Bristow, P. N. Brittain, C. M. Riley and B. F. Williamson, *J. Chromatogr.* **131**, 57–64 (1977).
- [20] D. L. Reynolds, Ph.D. Dissertation, The University of Kansas, Lawrence, Kansas, USA (1983).
- [21] G. E. Boyd, S. Lindenbaum and G. Myers, *J. Phys. Chem.* **65**, 577–586 (1961).
- [22] G. E. Boyd and S. Lindenbaum, *J. Phys. Chem.* **69**, 2378–2382 (1965).
- [23] L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd edn., pp. 453–482. Interscience, New York (1979).
- [24] R. K. Iler, *The Chemistry of Silica*, p. 660. Interscience, New York (1978).
- [25] R. Kunin, *Ion Exchange Resins*, p. 30. Interscience, New York (1958).

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