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EFFECT OF SOME OPERATIONAL VARIABLES ON THE EFFICIENCY OF ION CHROMATOGRAPHIC SEPARATIONS

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SUMMARY

The influence of injection mass, eluent composition and operating temperature on the efficiency of six commercially available ion chromatography columns was examined. Efficiency was linearly related to injection mass even at masses well below the column overload limit. The linear relationship was maintained at injection masses approaching the method's quantitation limit. Injection mass had little influence on the nature of the efficiency *versus* linear velocity relationship. Changing the concentration of the eluent ion (ionic strength) in the mobile phase influenced efficiency; however, the nature of the effect was analyte and column specific. Modifying the speciation of the mobile phase at constant ionic strength produced a marked effect on efficiency with the multivalent form of the eluent ion producing a better efficiency. For silica based stationary phases, addition of up to 10% by volume methanol had no impact on chromatographic performance. Increasing the column temperature increased efficiency at high linear velocities but decreased efficiency at low linear velocity in accord with theoretical expectations.

INTRODUCTION

Optimization of separation efficiency in the application of a chromatographic methodology is critical in situations involving multi-solute separations in complex matrices wherein the assurance of specificity and/or accurate and reproducible quantitation is paramount. The utility of ion chromatography (IC), as it matures as an analytical technology, will, in many applications, depend on the optimization of operational variables with respect to separation efficiency. In a previous study, it was observed that mobile phase linear velocity had some influence on the efficiency of IC separations for representative commercially available stationary phases[1]. In this study, the effect of eluent ion charge and concentration, injection mass, organic modifier content and operating temperature on efficiency for six commercially available stationary phases was examined.

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EXPERIMENTAL

Apparatus

The chromatographic system consisted of the following components: ABI (Ramsey, NJ, U.S.A.) Model 400 pump and 757 UV detector, Alcott (Norcross, GA, U.S.A.) Model 728 autosampler, Rheodyne (Cotati, CA, U.S.A.) Model 7010 electronically actuated injector and an appropriate stripchart recorder. Columns examined are described in Table I. All components between the injector and detector were connected with the minimum possible length of 0.007 in. I.D. stainless-steel tubing to minimize extra-column void volume and heat transfer. The chromatographic system was unchanged in any respect over the entire course of this experimentation. In the experiments designed to evaluate temperature effects, the mobile phase reservoir was placed in a water bath from which heated water was circulated into a column jacket. Approximately 3 m of tubing, also in the water bath, was placed between the pump and the injector. Such an approach should minimize the generation of radial thermal gradients, which may influence the efficiency–temperature relationship[2,3], in the column.

Operating conditions

The mobile phases used in all experiments were potassium hydrogenphthalate (KHP) buffers and, prepared from reagent or HPLC-grade chemicals and were filtered through 0.2-μm filters prior to use. Analytes were detected indirectly using a "vacancy" chromatographic approach at wavelengths of either 250 or 270 nm. The samples used throughout contained from 5 to 600 ppm each of chloride, nitrate, and sulfate and were prepared from their sodium salts. The sample injection size was 10 μl and all flow-rates were measured throughout the course of the experimentation.

Design

Each column evaluated was used as received by the vendor and exhibited performance consistent with the supplied test chromatograms. Columns were equilibrated with a particular mobile phase at a particular operating temperature for a

TABLE I

COLUMNS EXAMINED

Designation	*Supplier*	*Backbone type*	*Length (mm)*	*I.D. (mm)*	*Particle size (μm)*	*Functional group*[a]
269-013	Wescan	Silica	100	4.6	5	QA
269-031	Wescan	Polymer	100	4.1	10	QA
301TP	Vydac	Silica	250	4.6	10	QA
PRP-X100	Hamilton	Polymer[b]	150	4.1	10	QA
AS-5A	Dionex	latex[c]	150	4.0	5	P
IonPak A	Waters	polymethacrylate[d]	50	4.6	10	QA

[a] QA = Quaternary amine; P = propriatary.
[b] Copolystyrene divinylbenzene.
[c] Agglomerated.
[d] gel.

period of not less than 3 h prior to the initiation of any efficiency evaluation. After equilibration, each column was "conditioned" by making five injections of a sample containing 100 ppm of each analyte while the system was operating at a flow-rate of 1 ml/min. After conditioning, the individual column were equilibrated to a particular flow-rate (ranging from 0.1 to 4.0 ml/min) and the appropriate samples were injected in replicate. Where appropriate, the experimental conditions were then changed, the column re-equilibrated as necessary and more injections were made. Flow-rate changes were made in a random order for each column tested. Appropriate properties of the resultant chromatograms were then measured from the stripchart recordings. Under the chromatographic conditions used in this study, the measured peak asymmetry was less than 1.1 in all cases and the signal to noise was (usually) greater than 100; thus these two quantities will not greatly influence the accuracy of the efficiency calculations[4,5].

Calculations

The following equations were used to calculate chromatographic performance and operating parameters:

$$N = 5.545(t_1/w_{1/2})^2 \tag{1}$$

$$R = 2(t_2 - t_1)/(w_{b,2} + w_{b,1}) \tag{2}$$

$$h = L/(Nd_p) \tag{3}$$

$$v = (Ld_p)/(D_m t_0) \tag{4}$$

$$A_s = a/b \tag{5}$$

where N = number of theoretical plates; R = resolution; A_s = peak asymmetry factor; h = reduced plate height; v = reduced velocity; t = retention time or distance; a/b = ratio of the distances from a perpendicular (dropped from the apex of the peak to the baseline) to the rear side and front side of the peak, measured at 10% peak height; $w_{1/2}$ = peak width at half peak height; w_b = peak width at the baseline; L = column length; d_p = particle size of the stationary phase; t_0 = retention time of an unretained solute; D_m = solute diffusion coefficient in the mobile phase (taken as $5 \cdot 10^{-6}$ cm^{-2} s^{-1} as being typical of low-molecular-weight solutes in water[6]); and 1 and 2 refer to two analytes for which the retention time of 2 is greater than that of 1.

RESULTS AND DISCUSSION

Effect of injection mass

The ion-exchange capacity of the stationary phases evaluated is generally quite small (0.03 to 0.2 mequiv./g) and since the columns employed are relatively short, the total exchange capacity of these columns is not large. Thus one expects that the efficiency of IC separations would be greatly influenced by the amount of analyte injected, especially as this amount approaches a significant fraction of the total exchange capacity and column overloading occurs. Undeed, Haddad *et al.*[7] observe

that at a sample loading of 70 μg, most IC columns are greatly overloaded and that even at a loading of 7 pgl some peak distortion may occur. Similar peak shape distortions as a function of sample loading are reported by Jenke and Pagenkopf[8]. In this research, column overloading was minimized by using injection masses of 10 μg or less per analyte and the absence of apparent overloading was demonstrated (by a peak shape evaluation) in peak asymmetry values of 1.1 or less for all peaks evaluated. However, it is clear from the efficiency evaluation that some overloading, as a manifestation in a reduction in efficiency, was produced at injection masses greater than approximately 2–4 μg. The typical effect of sample injection mass on the measured efficiency at constant linear velocity for the different columns evaluated is shown in Fig. 1; in all cases a linear relationship exists between these two quantities. Correlation coefficients for the best fit lines in all cases are 0.99 or greater. In general, the effect of injection mass on efficiency is decreased as analyte retention (capacity factor, k') increases, although no simple correlation between these quantities can be made from the data generated in this study. This finding is in partial agreement with theoretical relationships established for chromatographic separations in general. In these models, based on the assumption of a Langmuir interaction isotherm and the absence of mixed isotherm effects, the relationship derived between efficiency, injection mass and solute retention (capacity factor) can be written[9]:

$$1/N = 1/N_0 + \tfrac{3}{8}[k'/(1 + k')]^2(W_x/W_s) \quad (6)$$

where: N_0 = efficiency at low injection mass; W_x = injection mass; and W_s = column capacity. Clearly the data in Fig. 1 reflects the linear relationship (with an intercept

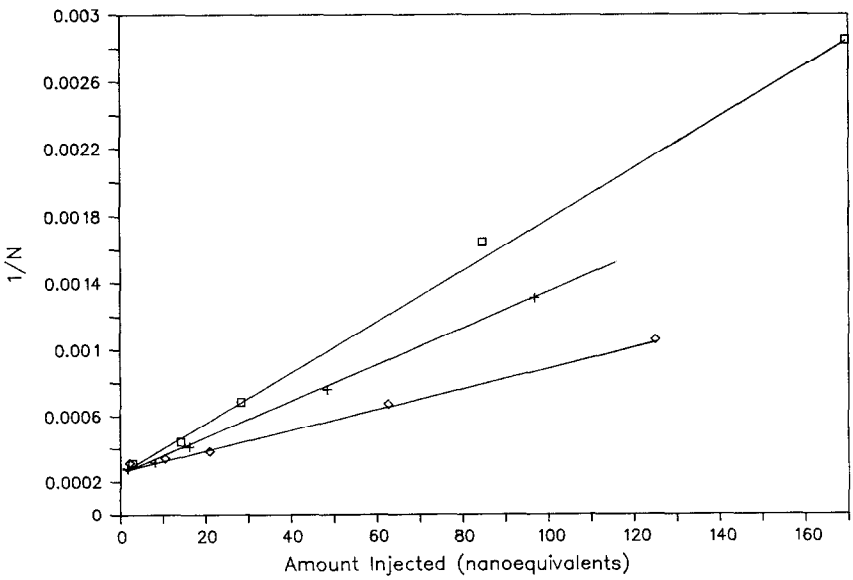

Fig. 1. Effect of injection mass on column efficiency. Column, Wescan 269-013; mobile phase, 1 m*M* KHP, pH 6; flow-rate, 1 ml/min. Approximate capacity factors are: chloride (□), 2.0; nitrate (+), 3.2; and sulfate (◇), 9.0.

independent of analyte identity) between $1/N$ and W_x predicted by this equation; however, equally clear is that the observed effect of k' on efficiency is not only different but in fact the opposite of that predicted by eqn. 6. That is, while the equation predicts an increase in efficiency as k' decreases, the observed behavior is that efficiency increases as k' increases. In general, for the IC column examined herein, the effect of injection mass on efficiency is decreased as analyte retention increases (and the absolute efficiency increases as retention increases) although no simple correlation between capacity factor and efficiency can be generated from the current data base. Current research is focusing on the establishment of the experimental and theoretical nature of this relationship. However, one can use the current data base to generate an empirical expression defining the relationship between capacity factor, efficiency and injection mass. The resulting empirical expression takes the form:

$$1/N = 1/N_0 + (C/k')(W_x/W_s) \tag{7}$$

where C is a constant.

Figs. 2–4 document the fit of the efficiency/injection mass/capacity factor data which exists for three IC columns; the correlation obtained between eqn. 7 and the observed data (three solutes per column, multiple injection masses) is typified by $r^2 > 0.98$ and represents adequate agreement between the actual and predicted behavior. Finally, it is interesting to note that the linear relationship between injection mass and efficiency holds true even at sample loadings approaching the mass detection

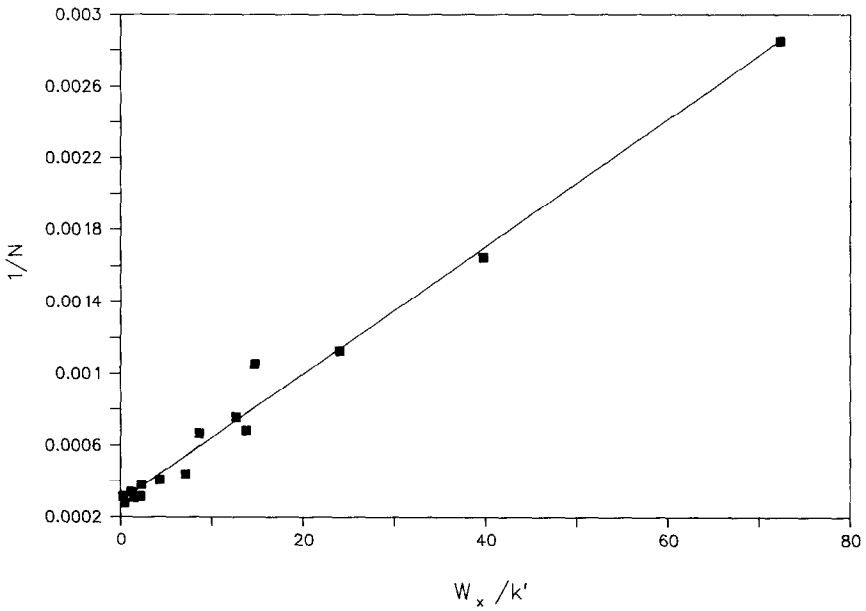

Fig. 2. Relationship between efficiency, injection mass and analyte capacity factor. Column, Wescan 269-013, same mobile phase as in Fig. 1. The data represents three solutes (chloride, nitrate and sulfate), injected over the mass range of 0.1–6 μg per analyte and representing a capacity factor range of 1.9–9.5. r^2 for the fit of this data to eqn. 7 (represented by the solid line) is 0.986.

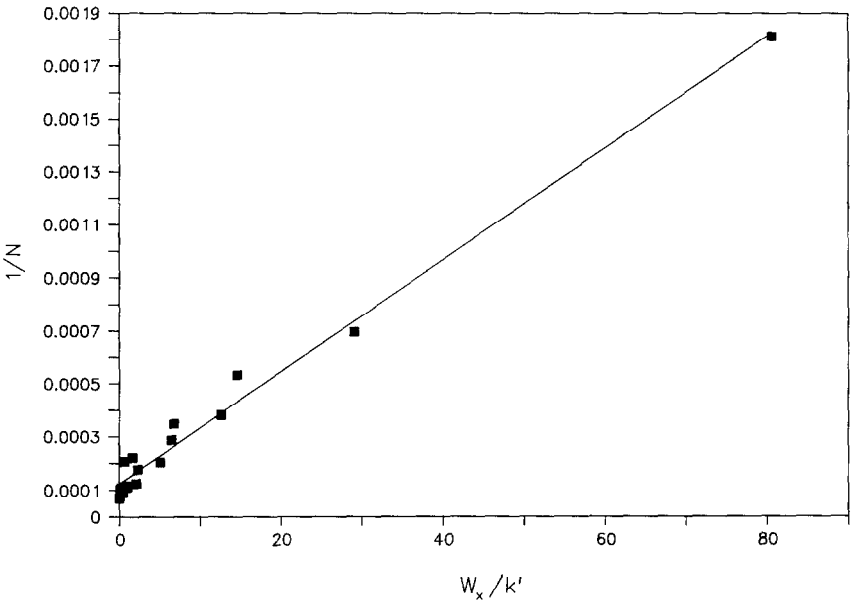

Fig. 3. Relationship between efficiency, injection mass and analyte capacity factor. Column, Dionex AS-5A; mobile phase, 1 m*M* KHP, pH 6; flow-rate, 1 ml/min. The data represents three solutes (chloride, nitrate and sulfate), injected over the mass range of 0.02–3 μg per analyte and representing a capacity factor range of 0.9–9.3. r^2 for the fit of this data to eqn. 7 (represented by the solid line) is 0.986.

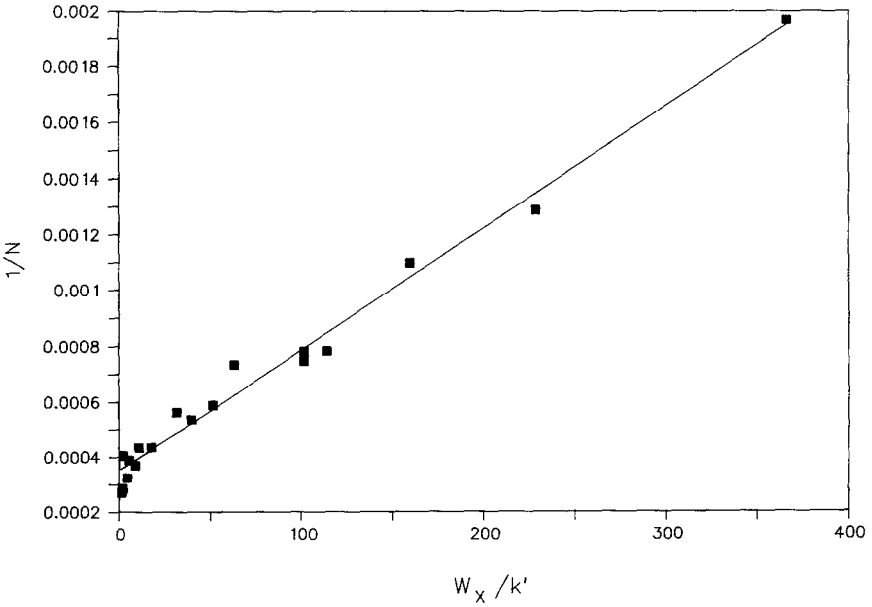

Fig. 4. Relationship between efficiency, injection mass and solute capacity factor. Column, Vydac 301TP; mobile phase, 2 m*M* KHP, pH 6; flow-rate, 1 ml/min. The data represents three solutes (chloride, nitrate and sulfate), injected over the mass range of 0.1–10 μg and representing a capacity factor range of 0.6–2.1. r^2 for the fit of this data to eqn. 7 (represented by the solid line) is 0.984.

limit for the technique (especially true for the 0.02-μg injection used in the evaluation of the AS-5A column).

The effect of injection mass on efficiency at various flow-rates was examined for the AS-5A column and is illustrated in Fig. 5. Essentially the shape of the reduced parameter plot, relating efficiency with linear velocity, is not influenced by injection mass and the magnitude of enhanced efficiency obtained when lower sample loadings are used is not flow-rate dependant. The implication of Figs. 1–5 is clear, efficiency can be maximized by decreasing the sample load, independent of other operating conditions, even to the point of approaching the method's quantitation limit.

Effect of mobile phase composition

The influence of three perturbations of mobile phase composition on efficiency was examined in this study; the effect of increasing the eluting ion concentration (ionic strength) at constant pH, the effect of changing pH (and thus eluent ion charge) at constant ionic strength, and the effect of adding an organic modifier (methanol) to the mobile phase. Considering the first of these, the effect of changing mobile phase ionic strength at constant pH on column efficiency is both column and analyte specific. For example, in Figs. 6–8, one observes that for the 269–013 (silica based) column the shape of the reduced parameter plot is essentially unaffected by changing phthalate concentration in the mobile phase. However, efficiency improves as the concentration of the eluting ion increases. The magnitude of the increase decreases with increasing solute retention (capacity factor) and thus is largest for chloride and smallest for sulfate. However, as shown in Fig. 9, while the shape of the reduced parameter plot for the IonPak A column is again unaffected by the concentration of the eluting

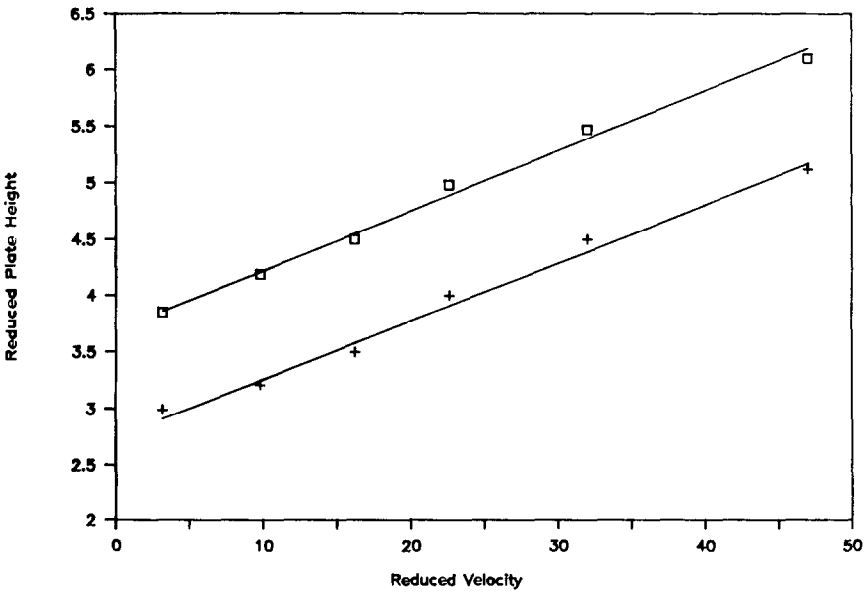

Fig. 5. Effect of injection mass on the reduced parameter plot. Column, Dionex AS-5A; mobile phase, 1 m*M* KHP, pH 6. □ = 100 ppm; + = 20 ppm.

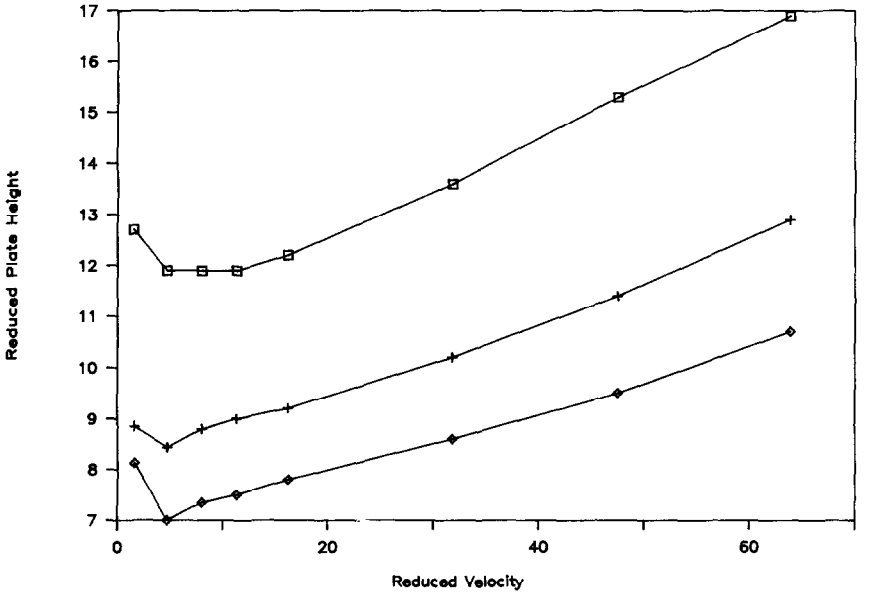

Fig. 6. Effect of mobile phase ionic strength on column efficiency. Concentrations of phthalate ion: □ = 1 mM; + = 2 mM; ◇ = 3 mM. Column, Wescan 269-013; analyte, chloride; injection mass, 1 μg; mobile phase pH = 6.

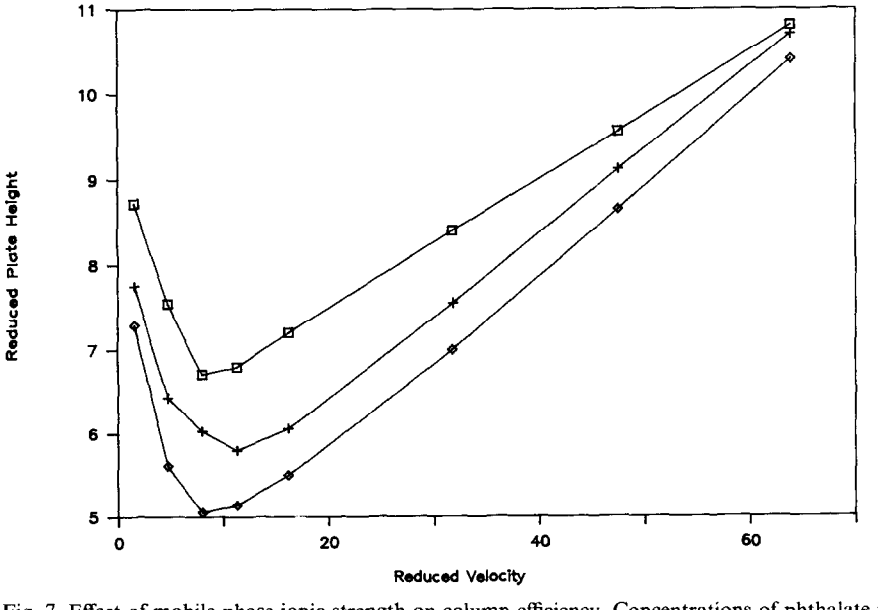

Fig. 7. Effect of mobile phase ionic strength on column efficiency. Concentrations of phthalate ion as in Fig. 6. Same conditions as in Fig. 6; analyte, nitrate.

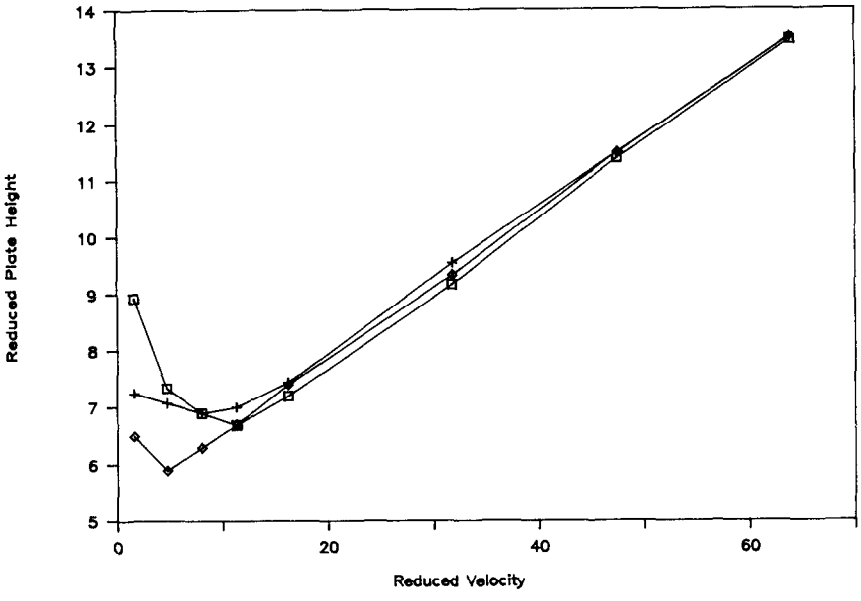

Fig. 8. Effect of mobile phase ionic strength on column efficiency. Concentrations of phthalate ion as in Fig. 6. Same conditions as in Fig. 6; analyte, sulfate.

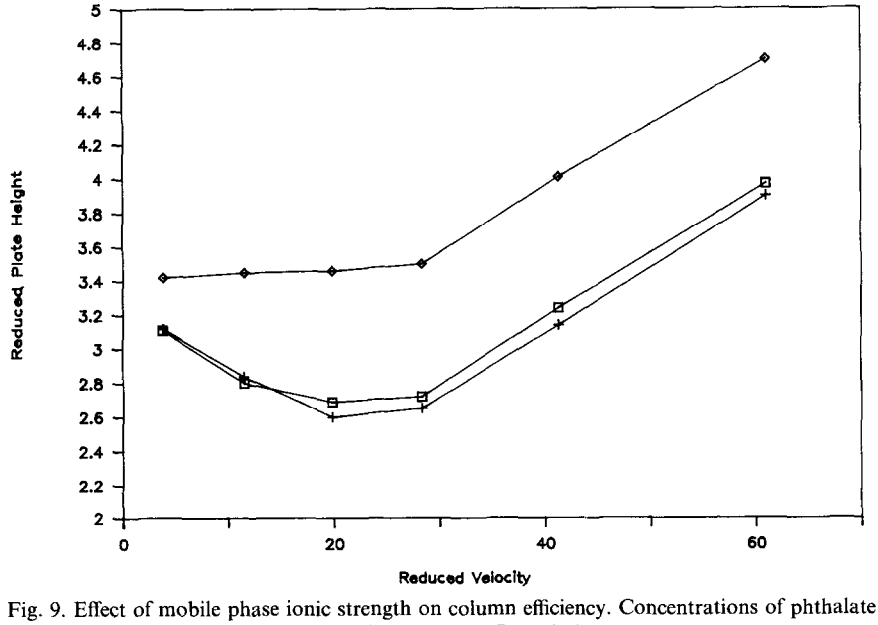

Fig. 9. Effect of mobile phase ionic strength on column efficiency. Concentrations of phthalate ion as in Fig. 6. Same conditions as in Fig. 6; column, Waters Ionpak A.

ion, the most efficient separation is achieved with the mobile phase with the lowest ionic strength (in which the solutes have the largest capacity factor). A similar relationship is observed to occur in the 269-031 column as well (Fig. 10). The behavior exhibited by the latter two columns is consistant with eqn. 7 is terms of the relationship predicted between efficiency and capacity factor. These data serve to illustrate that the mobile phase ionic strength, which impacts the physico-chemical nature of the mobile and stationary phases as well as the equilibrium thermodynamics and reaction kinetics controlling the interaction between the two, has an influence on separation efficiency which is dictated essentially by the nature of the test system and cannot readily be generalized.

In an attempt to evaluate the effect of charge (identity) of eluent ion on efficiency, mobile phase pH and total salt content were manipulated to produce mobile phases in which the total ionic strength was similar but eluent ion speciation was different. Thus the mobile phases used in this portion of the study (1 m*M* total salt at pH 6 and 2.5 m*M* total salt at pH 4.1) have virtually identical ionic strengths but the former is dominated by divalent phthalate ions while the latter is dominated by the singly charged species. Specifically, the phthalate speciation in these two mobile phases, assuming phthalate dissociation constant of 3.1 and 5.4[10], are: 2.5 m*M* total salt, pH 4.1; 0.24 m*M* undissociated, 2.18 m*M* monovalent and 0.13 m*M* divalent; 1 m*M* total salt, pH 6.0; 0.01 m*M* undissociated, 0.19 m*M* monovalent and 0.80 m*M* divalent. These mobile phases were used with the PRP-X100 column and the resultant efficiencies were evaluated at a variety of flow-rates. While the selectivity of the column was not influenced by the mobile phase difference (selectivity versus chloride of 1.7 and 5.1 for nitrate and sulfate for both conditions), the pH 4.1 mobile phase was

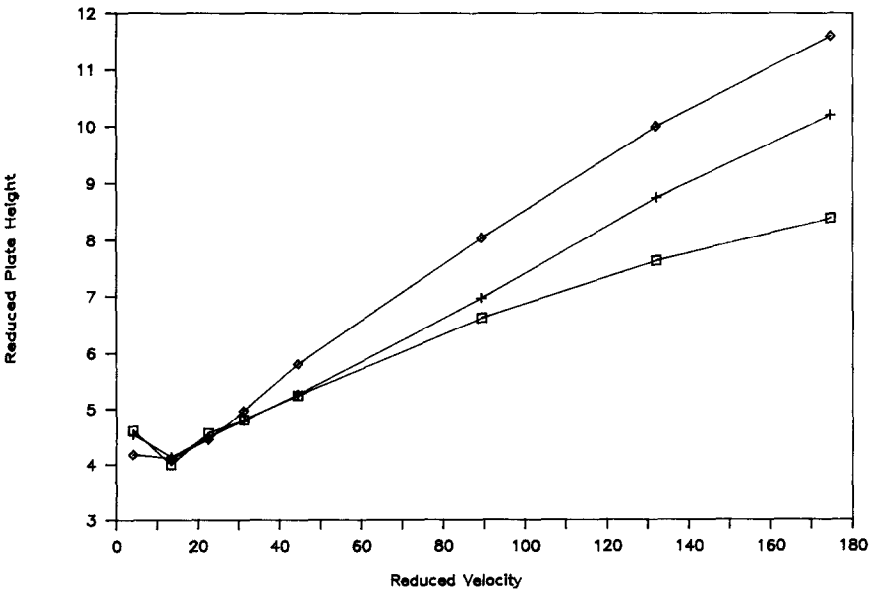

Fig. 10. Effect of mobile phase ionic strength on column efficiency. Concentrations of phthalate ion as in Fig. 6. Same conditions as in Fig. 6; column, Wescan 269-013.

actually "stronger" in terms of producing smaller capacity factors or all three analytes (1.8 *versus* 2.3, 3.2 *versus* 3.8 and 9.5 *versus* 11.3 for chloride, nitrate and sulfate, respectively). However, as shown in Figs. 11 and 12, the pH 6 mobile phase improved efficiency in two ways. At the flow-rates examined, the absolute efficiency was better with the pH 6 mobile phase; additionally, this mobile phase decreases the magnitude of the flow-rate influence on efficiency at higher flow-rates. Clearly the mobile phase speciation influences mass transfer properties which essentially mediate the efficiency at higher flow-rates. Again this behavior is consistant with the capacity factor–efficiency relationship expresed in eqn. 7.

The addition of an organic modifier to an IC mobile phase has been used to impact the performance characteristics of inorganic and organic analyte separations[7,11–13], although the exact mechanism of influence is unclear. In this study, the influence of 0–10% methanol on the chromatographic performance of the silica based stationary phases (301TP and 269–013) was examined. As is shown in Table II, the performance of the 269–013 (which was typical for the 301TP as well) was only minimally impacted by the presence of up to 10% (by volume) organic modifier in the mobile phase.

Effect of temperature

Historically, an increase in column temperature is generally expected to improve column efficiency in ion-exchange separations[14,15]. However, it is fair to say that most stationary phases used in modern IC are significantly different from those for which such generalizations were valid. While reports of the influence of column

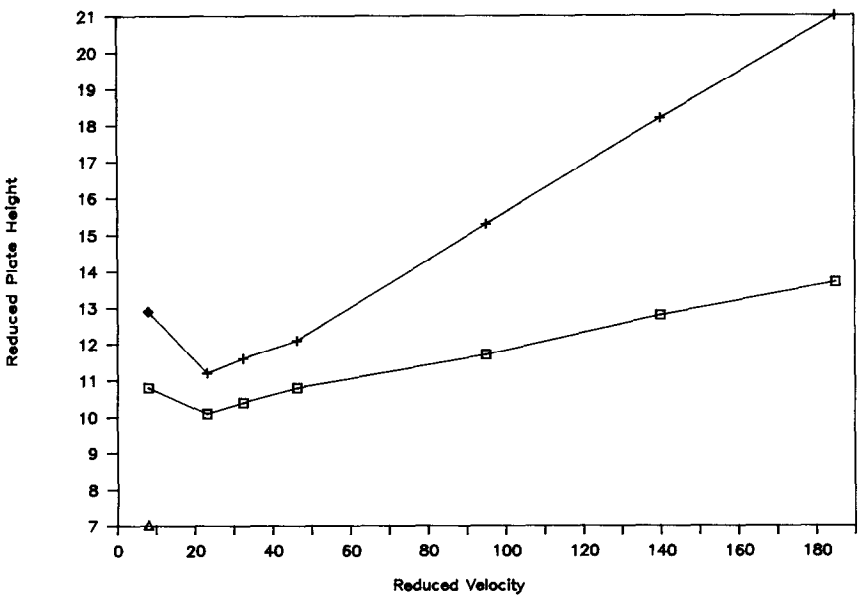

Fig. 11. Effect of mobile phase speciation (at constant ionic strength) on column efficiency. Column, Hamilton PRP-X100; sample size, 1 μg; analyte, chloride. Mobile phases used were (□) 1 m*M* total phthalate at pH 6 and (+) 2.5 m*M* total phthalate at pH 4.1.

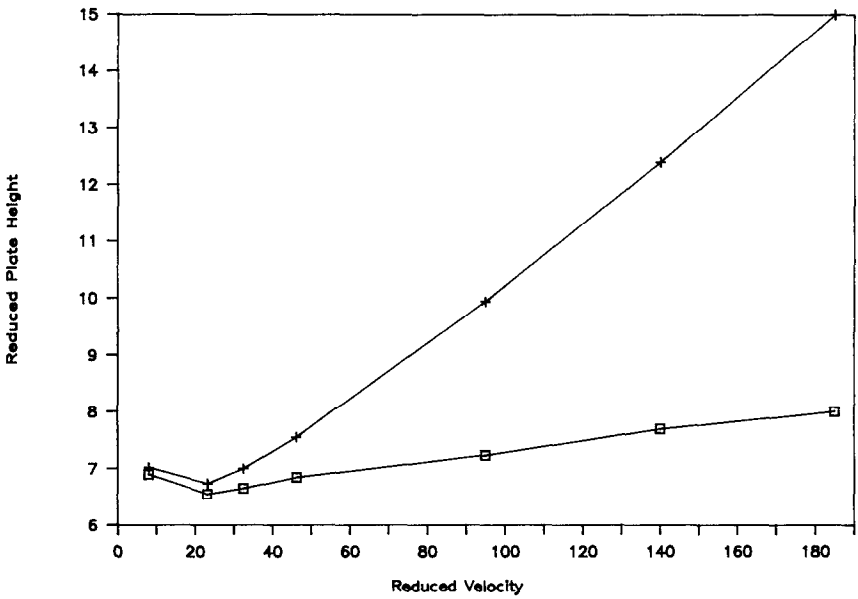

Fig. 12. Effect of mobile phase speciation (at constant ionic strength) on column efficiency. Same conditions and key to symbols as in Fig. 11; analyte, sulfate.

temperature on retention times (and resolution) in IC appear in the literature (for example, see refs. 16 and 17), a full plot of efficiency *versus* linear velocity was not determined as a function of temperature. In this study, the effect of temperature on the reduced parameter plot for the PRP-X100 column was examined. Column temperature was accurately determined by measuring the temperature of the mobile phase entering and leaving the column (which in all cases was equivalent within experimental precision). As shown in Figs. 13 and 14, the nature of the temperature influence itself is impacted by the reduced linear velocity. At higher reduced velocities (flow-rates of 0.5ml/min or greater) efficiency is improved at elevated temperature. The magnitude of the improvement is impacted by the flow-rate (improvement is

TABLE II

EFFECT OF METHANOL ON COLUMN PERFORMANCE

Column, 269-013. Mobile phase, 2 m*M* KHP (pH 6) with varying amounts of methanol (%, v/v). Injection size: 1 μg of each analyte.

Methanol (%)	*Chloride*		*Nitrate*		*Sulfate*		*Resolution*	
	k'	*N*	*k'*	*N*	*k'*	*N*	Cl^-/NO_3^-	NO_3^-/SO_4^{2-}
0	1.18	2153	2.04	2835	4.01	2271	4.03	5.99
3	1.22	2270	2.10	3011	4.17	2400	4.10	6.22
7	1.22	2106	2.14	2750	4.31	2251	4.15	6.23
10	1.20	2103	2.07	2913	4.16	2296	4.05	6.26

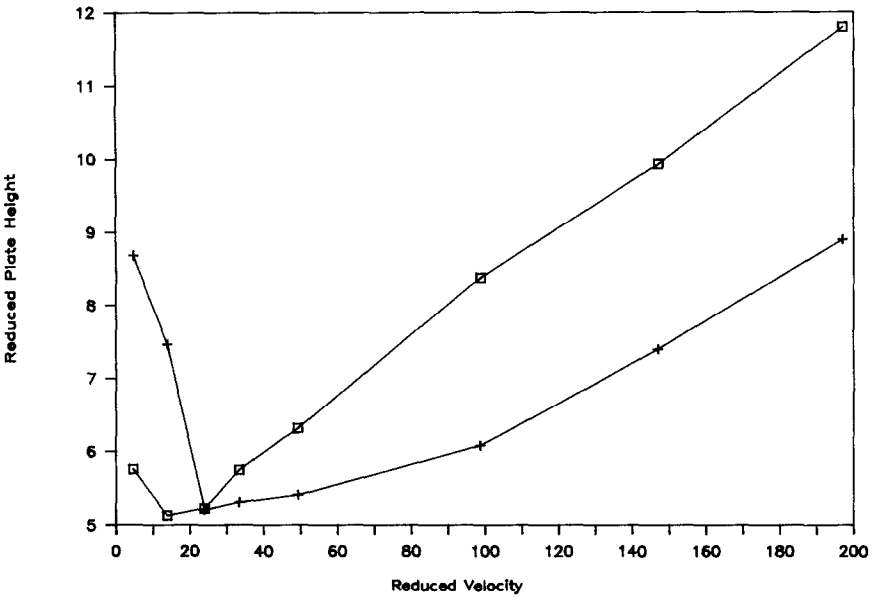

Fig. 13. Effect of column temperature on effiency. Column, Hamilton PRP-X100; mobile phase, 1 m*M* KHP, pH = 6; injection mass, 0.5 μl per analyte; analyte, chloride. □ = 25°C; + = 55°C.

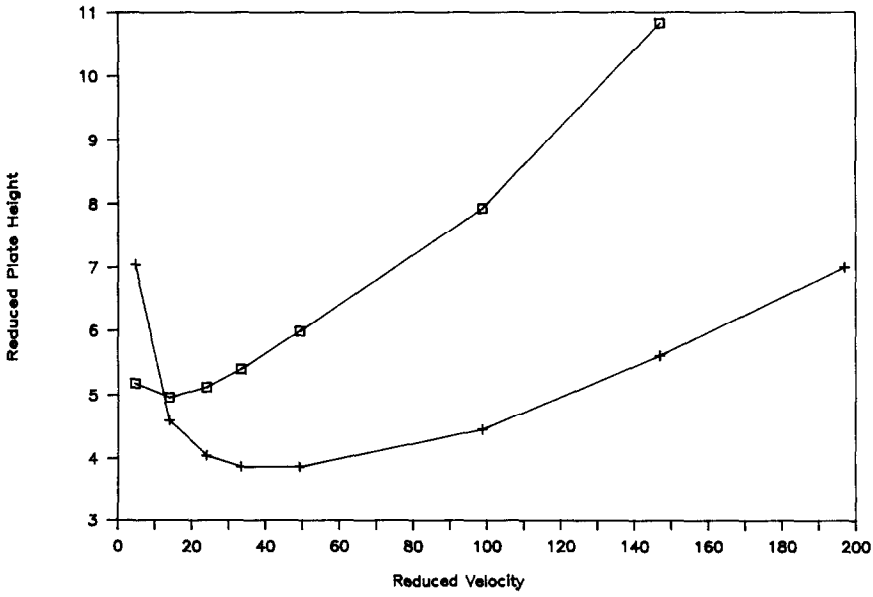

Fig. 14. Effect of column temperature on efficiency. Same conditions as in Fig. 13; analyte, sulfate. Key to symbols as in Fig. 13.

more pronounced at higher flow-rate) and by the capacity factor of the analyte (improvement is more pronounced as capacity factor increases). At low reduced linear velocities, however, an increase in column temperature produces a corresponding decrease in efficiency. This type of relationship between linear velocity and column temperature has been predicted for modern reversed phase stationary phases[18]. It is noted in passing that while the retention of chloride is immune to changing column temperature, nitrate retention is decreased with increasing temperature while sulfate retention exhibited the opposite trend. This observation is consistent with suggestions made elsewhere in the literature that the retention mechanism for nitrate in ion exchange columns is somewhat different than that for other inorganic ions[12].

CONCLUSIONS

The operational efficiency of stationary phases used in modern ion chromatography is greatly influenced by the operating conditions. For all columns studied, efficiency is improved, independent of operating flow-rate, as sample injection mass is decreased, even if the solute concentration is well below the column overload limit and approaches the mass sensitivity of the method. The chemical nature of the mobile phase impacts the efficiency both in terms of the absolute concentration of the eluting ion (ionic strength) and the absolute speciation of a multi-protic eluent. The influence of mobile phase composition on efficiency is generally stationary phases dependent although in general greater efficiencies are achieved when the net charge of the eluting ion is increased. The performance of silica-based stationary phases is independent of methanol concentration in the mobile phase at concentration up to 10% when inorganic species are separated. Increasing the column temperature improves efficiency at higher linear velocities and decreases efficiency at lower linear velocities in accord with theoretical expectations if proper thermal control is used to avoid the generation of thermal gradients in the column.

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