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THE OCCURRENCE AND ORIGIN OF SYSTEM PEAKS IN NON-SUPPRESSED ION CHROMATOGRAPHY OF INORGANIC ANIONS WITH INDIRECT ULTRAVIOLET ABSORPTION DETECTION*

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SUMMARY

Chromatograms obtained for inorganic anions using non-suppressed ion chromatography with indirect UV absorption detection show two extraneous peaks; a non-retained peak (called the injection peak) and a late eluting peak (called the system peak). Experimental studies are reported which show that the system peak is governed by the sample concentration, the injected volume of sample, the nature of the sample ion, the sample pH and the eluent concentration and pH. A mechanism for the generation of injection and system peaks is presented which proposes that the injection peak is due to eluent dilution effects combined with the displacement of adsorbed eluent anions by the injected sample. The system peak is attributed to adsorption and desorption of neutral eluent molecules from the column surface which are retained by a reversed-phase mechanism on the unfunctionalised portions of the anion-exchange column. Evidence is presented to support this proposal. System peaks may be eliminated using an eluent pH such that no neutral eluent molecules are present.

INTRODUCTION

The determination of inorganic anions by non-suppressed ion chromatography generally involves the use of a low-capacity anion-exchange column combined with an eluent consisting of a dilute solution of an aromatic acid anion such as benzoate or phthalate[1,2]. Detection methods include conductivity[3] and indirect UV absorption[4,5]. In the latter approach, the absorbance of the eluent is monitored and solute ions are detected by the decrease in absorbance resulting from displacement of eluent ions from the mobile phase by eluted ions. For convenience, the recorder polarity is usually arranged so that a positive peak corresponds to a decrease in absorbance. Indirect UV absorption detection has been shown to be a very sensitive detection

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mode[5] and has the advantage of being applicable to conventional high-performance liquid chromatographic (HPLC) instrumentation.

Chromatograms obtained with indirect UV absorption detection are characterised by the presence of two extraneous peaks. The first of these peaks elutes early in the chromatogram and is generally referred to as the "injection" or "solvent" peak[6], whereas the second peak is later eluting and is often described as a "system" peak[7]. The system peak can cause several chromatographic problems including co-elution with solute peaks[8], incorrect assignment of peak identities, unnecessarily lengthy run times or ghosting in subsequent chromatograms[9] and erroneous quantitation of some solutes[8,10]. The outcome of these effects is that the appearance of a system peak imposes a severe limitation on the utility of indirect UV absorption detection in ion chromatography.

In this paper, we report a study of the parameters which influence the retention time and magnitude of the system peak. A mechanism for the formation of the system peak in non-suppressed ion chromatography with indirect UV absorption detection is proposed. The results described were obtained using phthalate eluents in conjunction with several different low-capacity anion-exchange columns.

EXPERIMENTAL

Instrumentation and reagents

The liquid chromatograph used consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model M6000 pump, Model U6K injector, Model M450 variable-wavelength UV detector and Model M730 data module. Three low-capacity anion-exchange columns were used: a Vydac 302 IC 4.6 anion chromatography column (Separations Group, Hesperia, CA, U.S.A.), 250 × 4.6 mm I.D.; a Bio-Gel TSK IC Anion PW column (Bio-Rad Labs., Richmond, CA, U.S.A.), 50 × 4.6 mm I.D.; and a PRP-X100 ion chromatography column (Hamilton, Reno, NV, U.S.A.), 150 × 4.1 mm I.D.

All reagents were of the highest available purity. Standard solutions (1000 ppm) of inorganic anions were prepared by dissolving weighed amounts of the sodium salts in water purified on a Millipore (Bedford, MA, U.S.A.) Milli-Q water purification system. The standard solution (1000 ppm) of nitric acid was prepared by dilution of the concentrated acid.

Chromatographic procedures

Mobile phases were prepared using analytical grade phthalic acid or potassium hydrogen phthalate, dissolved in water from the Milli-Q water purification system. The pH of each mobile phase was adjusted by dropwise addition of 1 *M* sodium hydroxide. All mobile phases were filtered through a 0.45-μm filter and degassed in an ultrasonic bath prior to use. Further chromatographic conditions are given in the figure captions.

RESULTS AND DISCUSSION

Occurrence of system peaks

A typical chromatogram obtained for a mixture of inorganic anions separated

on a low-capacity anion-exchange column with phthalate as eluent and using indirect UV absorption detection is shown in Fig. 1. This chromatogram exhibits decreases in eluent absorbance (displayed as positive peaks) corresponding to elution of the solute ions and also contains two further peaks, namely the injection peak and the system peak. Both of these latter peaks may be positive or negative in direction, depending on the conditions used.

In the experimental work described in this paper, the characteristics of the late eluting system peak were studied and qualitative information on the characteristics of the injection peak was also obtained. A quantitative study of the injection peak observed with conductivity detection has previously been reported by Hershcovitz *et al.*[6].

Effect of eluent parameters on the system peak

If the eluent concentration was maintained at a constant value, changes in the eluent pH produced marked changes in the system peak. The retention time of the system peak showed a general increase with increasing pH (Fig. 2) and the height of the system peak became progressively smaller. At pH 7 and higher pH values, no system peak was observed. With the phthalate eluents used, it is noteworthy that above pH 7, the eluent contained only doubly ionised phthalate ($pKa_1 = 2.95$, $pKa_2 = 5.41$). The above observations are in agreement with those reported by Okada and

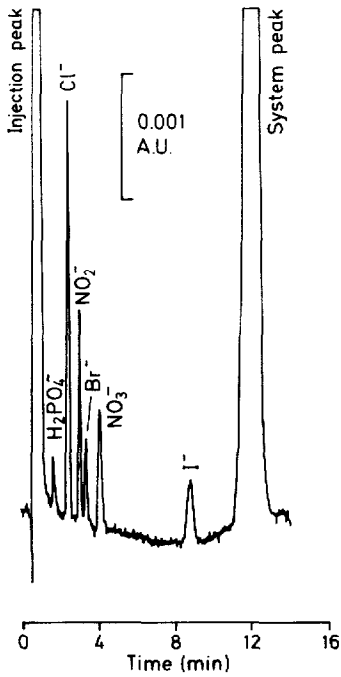

Fig. 1. Ion chromatogram showing injection and system peaks observed with indirect UV absorption detection. Conditions: Column, Vydac 302 IC 250 × 4.6 mm I.D.; eluent, 2.5 m*M* potassium hydrogen phthalate at pH 4.0; flow-rate, 2.0 ml/min; injection volume, 250 μl; detection by UV absorption at 285 nm; solute concentrations, 100–400 ppb. [The American billion (10^9) is meant.]

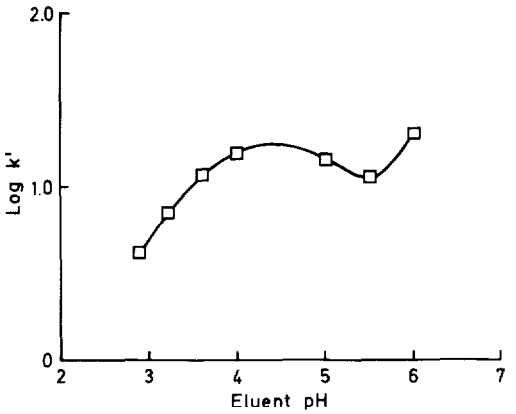

Fig. 2. Variation of the logarithm of capacity factor for the system peak with mobile phase pH. Conditions: column, Bio-Gel TSK Anion PW, 50 × 4.6 mm I.D.; eluent, 1.2 m*M* potassium hydrogen phthalate; flow-rate, 1.4 ml/min; detector sensitivity, 0.04 a.u.f.s.

Kuwamoto[11] for tartrate eluents (pKa_1 = 3.04, pKa_2 = 4.37), which showed no system peak at pH values of 5 or higher.

A plot of the logarithm of the capacity factor of the system peak *versus* the logarithm of eluent concentration was constructed at constant eluent pH. This plot is given in Fig. 3, from which it can be seen that a linear relationship existed between log k' and log [eluent]. This result agrees with previous measurements made with a silica based anion-exchange column using phthalate eluents[12].

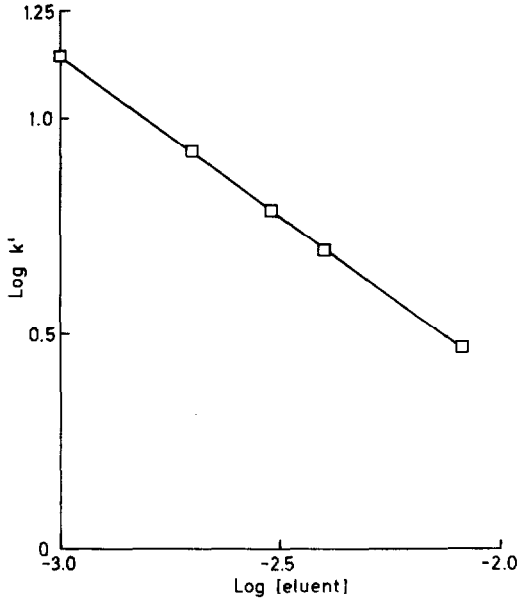

Fig. 3. Effect of eluent concentration on the capacity factor of the system peak. Conditions: as for Fig. 2 except that a mobile phase pH of 5.5 was used.

Effect of sample parameters on the system peak

When deionised water was injected, a positive system peak was observed, with the magnitude of this peak being proportional to the injected volume. The tendency for the system peak to increase with larger injection volumes was also observed when a solute was injected. Fig. 4 illustrates the effect of injecting water and also 1 μg of nitrite ion, using injection volumes of 10 μl or 100 μl. The height of the nitrite peak was the same for both injection volumes, but a larger system peak was observed for the 100-μl injection.

Fig. 5 shows the effect of increasing the sample concentration whilst maintaining a constant injection volume of 10 μl. As the concentration of nitrite ion was increased from 100 to 1000 ppm, the system peak changed from a slight positive peak to a small negative peak. These results indicate that increased sample concentrations tended to result in a more negative system peak.

The nature of the solute anion used was also found to have a significant effect on the system peak. For example, at identical concentrations of 1000 ppm, the height of the system peak for various univalent anions followed the order chloride > nitrite > nitrate > bromide > iodide. It is noteworthy that the heights of the analytical peaks for these solutes followed the same order, suggesting that this effect results chiefly from the differing molecular weights of the species involved. Indeed, the trend

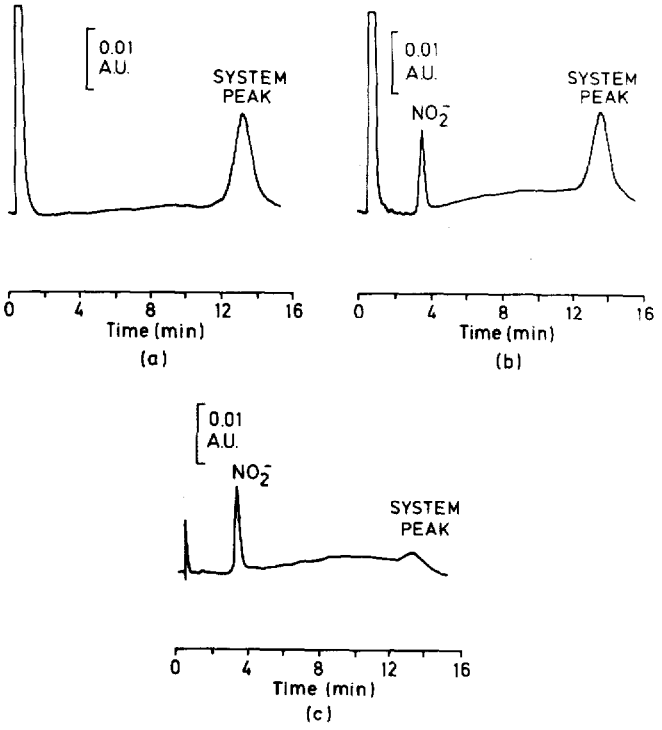

Fig. 4. Effect of sample injection volume on the system peak. (a) 100 μl deionised water, (b) 100 μl 10 ppm nitrite, (c) 10 μl 100 ppm nitrite. Conditions: column, Bio-Gel TSK Anion PW, 50 × 4.6 mm I.D.; eluent, 3.2 m*M* potassium hydrogen phthalate at pH 4.2; flow-rate, 1.2 ml/min; detection, UV absorption at 295 nm, 0.1 a.u.f.s.

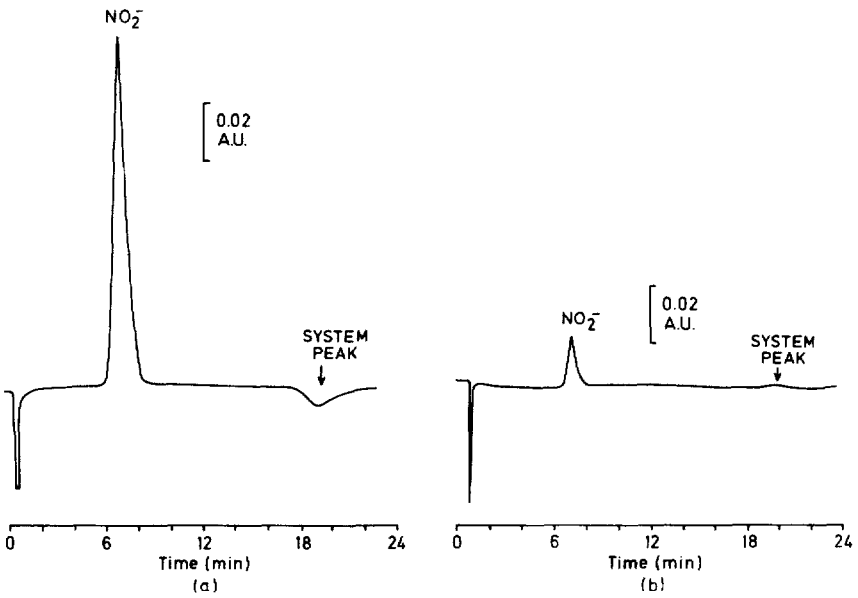

Fig. 5. Effect of sample concentration on the system peak. (a) 100 ppm nitrite, (b) 1000 ppm nitrite. Conditions: eluent, 1.2 m*M* potassium hydrogen phthalate at pH 4.2; flow-rate, 1.2 ml/min; detection, UV absorption at 265 nm, 0.2 a.u.f.s.; injection volume, 10 μl; other conditions as for Fig. 2.

described above was not apparent when solutions of identical molar concentrations were used.

A further aspect pertaining to the nature of the solute ion was the tendency for some solutes to exhibit retention times close to that of the system peak. Sulphate ion was most troublesome in this regard and under a wide range of mobile phase conditions, sulphate partly co-eluted with the system peak (Fig. 6). Resolution of

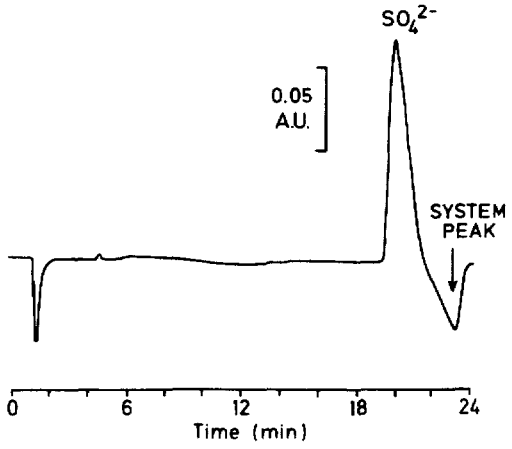

Fig. 6. Co-elution of the system peak with sulphate. Conditions: column, Vydac 302 IC 250 × 4.6 mm I.D.; eluent, 4.0 m*M* potassium hydrogen phthalate at pH 4.2; flow-rate, 2.0 ml/min; detection, UV absorption at 295 nm, 0.4 a.u.f.s.; sample, 10 μl 1000 ppm sulphate.

sulphate and the system peak was possible only at relatively high values of eluent pH, under which conditions sulphate could be eluted before the system peak.

The pH of the injected sample was found to exert a large effect on the system peak. The factor of importance here was the disparity between the pH values of the eluent and sample. When the sample was more acidic than the eluent, the height of the system peak was decreased, whilst samples more alkaline than the eluent caused an increase in the system peak. The greater the disparity between the sample and eluent pH values, the greater was the observed effect.

This behaviour was illustrated by injection of carbonate, phosphate, nitrate and nitric acid into a phthalate eluent at pH 4.2. Both carbonate and phosphate gave large, positive system peaks (Fig. 7) since both samples (pH 11 and 12, respectively) were considerably more alkaline than the eluent. It is also noteworthy that no analytical peak was observed for carbonate and an early eluting peak was observed for phosphate. Consideration of the buffering capacity of the eluent and the acid dissociation constants for carbonic and phosphoric acids suggests that carbonate would be fully protonated and phosphate would be partly protonated to form dihydrogen phosphate. The protonated carbonic acid would be expected to elute with the injection peak and would not be detected using indirect UV absorption detection. Similarly, dihydrogen phosphate would be expected to elute early in the chromatogram, as shown in Fig. 7. This figure also highlights the possibility of incorrect assignment of peak identity resulting from the appearance of a system peak. Since divalent carbonate ion was injected in Fig. 7a, it would not be unreasonable for the late eluting system peak to be assigned to carbonate.

When nitrate ion and nitric acid were separately injected into a phthalate eluent

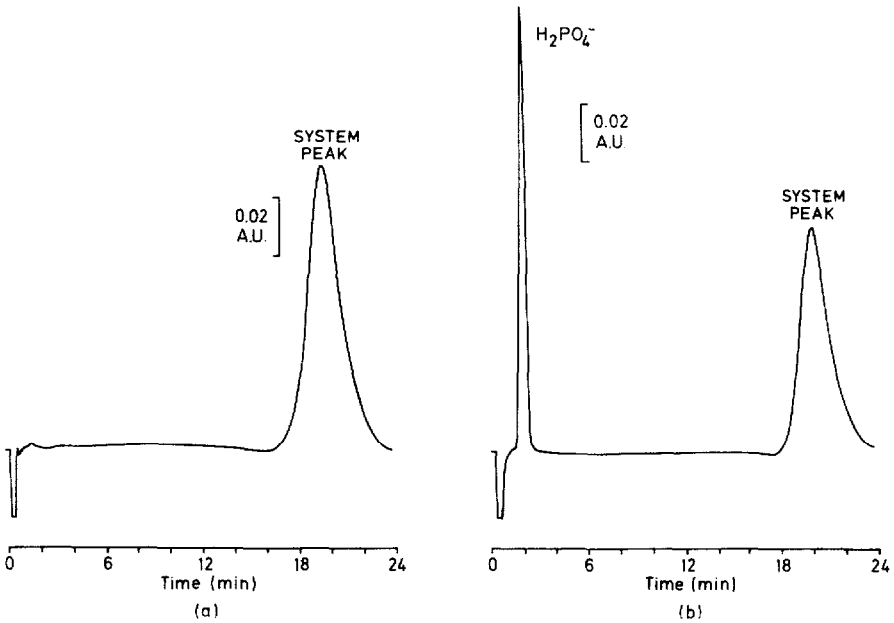

Fig. 7. System peaks resulting from injection of basic solutes. (a) 10 μl 1000 ppm carbonate (b) 10 μl 1000 ppm phosphate. Conditions: as for Fig. 5.

at pH 4.2, the system peak for the acidic sample was considerably more negative than that observed for nitrate ion (Fig. 8). The analytical peak for nitrate was independent of the sample pH.

The effect of sample pH on the system peak was also evident when solutions of phthalate were injected into the same phthalate eluent used in the above studies. If the pH of the phthalate sample was identical to that of the eluent, no system peak was observed. Alternatively, samples more acidic (*e.g.* phthalic acid at pH 3) or more alkaline (*e.g.* phthalate at pH 7.0) produced negative and positive system peaks, respectively, when injected into a phthalate eluent at pH 4.2.

Possible models for system peak formation

The preceding discussion has illustrated that the system peak was influenced by a considerable number of factors, and these effects may be summarised as follows: (1) The retention time of the system peak showed a general increase with eluent pH. (2) The height of the system peak reduced with increasing eluent pH. Eluents of pH greater than 6.5 gave no system peak. (3) The logarithm of the capacity factor for the system peak was linearly related to the logarithm of the eluent concentration, at constant pH. (4) For constant amounts of sample, an increase in the injected volume of sample produced a positive change in the system peak. (5) At constant injection volumes, an increase in sample concentration produced a negative change in the system peak. (6) Samples more alkaline than the eluent produced positive system peaks, whereas samples more acidic than the eluent gave negative system peaks. The magnitude of the system peak produced by this effect was dependent on the pH difference between the sample and eluent.

It is clear that these effects could combine to produce either positive or negative

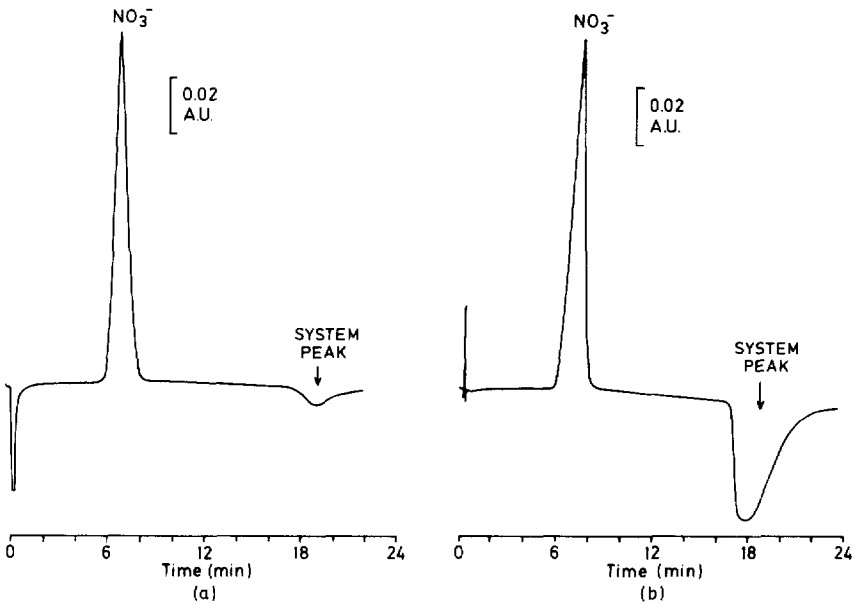

Fig. 8. System peak resulting from injection of an acidic sample. (a) 10 μl 1000 ppm nitrate (b) 10 μl 1000 ppm nitrate injected as nitric acid. Conditions: as for Fig. 5.

system peaks and under conditions where opposing effects were counterbalanced, the system peak could be absent.

General trends in the behaviour of the injection peak were also apparent from the studies conducted on the system peak. These trends are summarised below: (1) The retention time of the injection peak was independent of the sample pH and the eluent concentration. (2) For constant amounts of sample, the height of the injection peak increased with increasing injected volume of the sample. (3) At constant injection volumes, an increase in sample concentration produced a negative change in he injection peak.

As with the system peak, the injection peak resulting from these trends could be positive or negative in direction (see Figs. 4–8), or absent from the chromatogram.

Two somewhat opposing models have been advanced to explain the injection and system peaks. The first model proposes that the injection peak results from ion-exclusion of sample cations from the anion-exchange column and the system peak is due to an "anion absent effect" which compensates for disturbance of the eluent–column equilibrium caused by passage of the injection peak through the column[11]. An essential feature of this model is that the eluent ions displaced from the column after injection of sample anions do not travel through the column with the injection peak. In contrast, the second model proposes that these displaced eluent anions are unretained on the column and elute with the injection peak[6]. The height of the injection peak is determined by a combination of the displaced eluent anion effect and the dilution factor resulting from the sample injection volume. The system peak may be considered to be due to elution of the neutral, protonated form of the eluent which traverses the column by a reversed-phase mechanism[2].

A detailed study of the "anion absent effect" has been reported for tartaric acid eluents used with conductivity or indirect UV absorption detection[11]. On the other hand, the alternative mechanism proposed above has also been supported by a quantitative study of the injection peak[6] in which the calculations made were based on the assumption that displaced eluent anions eluted with the injection peak.

The results of the present study are generally supportive of the proposal that both eluent anions and neutral eluent molecules are displaced from the column on injection of the sample. The eluent anions are unretained on the column and elute with the injection peak, whereas the neutral eluent molecules elute slowly under a reversed-phase mechanism and appear as a positive or negative system peak. The only evidence contrary to this proposal is Fig. 3, where the linear relationship observed between log [eluent] and log k' for the system peak is similar to that reported for anionic solutes retained via an ion-exchange mechanism[12]. On the other hand, it is important to note that the system peak was found to disappear at eluent pH values of about 6.5; that is, under conditions where the eluent was completely ionised. At these pH values, the absence of any neutral eluent molecules would account for the lack of a system peak under the mechanism proposed above, whereas the presence of an abundance of eluent anions would suggest that if an "anion absent effect" model was in operation, a system peak should still occur. An additional factor was that only a single system peak was observed for eluents containing both divalent and monovalent eluent anions. Under these conditions, two system peaks might be expected with the "anion absent effect" model.

The eluent acid effect

In order to investigate further whether the system peak was the result of reversed-phase elution of the neutral (*i.e.* acidic) form of the eluent, additional experiments were undertaken. These experiments were based on the assumption that a system peak resulting from reversed-phase retention would exhibit strong dependence on the nature of the packing material and would show predictable retention behaviour when organic modifiers were added to the mobile phase.

The dependence of system peak retention on the nature of the packing material was evaluated using three different low-capacity anion-exchange columns. Each column contained quaternary ammonium ion-exchange functionalities bonded to different types of material: silica (Vydac 302 IC), polymethacrylate (Bio-Gel TSK Anion PW) or styrene–divinylbenzene copolymer (Hamilton PRP X100). Of these materials, the styrene–divinylbenzene copolymer ion-exchange resin was expected to show the greatest reversed-phase characteristics since the neutral polymer has frequently been used as a reversed-phase packing[13]. Using phthalate eluents of identical pH, the eluent concentration and flow-rate was adjusted so that chloride ion showed equal retention on each column. In this way, the balance between eluent strength and the ion-exchange capacity of the column was approximately equal for the three columns. Table I gives the chromatographic conditions used and also lists the retention time for the system peak observed with each column. The styrene–divinylbenzene copolymer column showed much greater retention of the system peak than the other two columns.

The effect of addition of organic modifiers to mobile phases used with the styrene–divinylbenzene copolymer column was studied in a similar manner to that described above. Here, a series of phthalate mobile phases at constant pH and containing increasing percentages of methanol were prepared, with the phthalate concentration for each eluent being adjusted so that chloride ion gave an identical retention time with each eluent. In this way, the ion-exchange elution strengths of all of these eluents were equivalent. A plot of the logarithm of the capacity factor for the system peak *versus* the percentage of methanol was prepared (Fig. 9), revealing that a linear relationship was followed. This behaviour was indicative that the retention of the system peak was subject to a reversed-phase mechanism[14].

The effect of injection of organic modifiers onto a column equilibrated with an aqueous phthalate eluent was also studied. Fig. 10 shows the system peaks ob-

TABLE I

RETENTION TIMES FOR SYSTEM PEAKS ON VARIOUS LOW CAPACITY ANION-EXCHANGE COLUMNS

Phthalate eluents (pH 4.2) were used at the concentrations and flow-rates indicated.

Column	*Eluent concentration (mM)*	*Flow-rate (ml/min)*	*Retention time (min)*	
			Chloride	*System peak*
Vydac 302 IC	3.0	2.0	4.37	19.00
Bio-Gel TSK Anion PW	3.0	0.8	4.43	20.39
Hamilton PRP X100	1.5	1.9	4.34	47.67

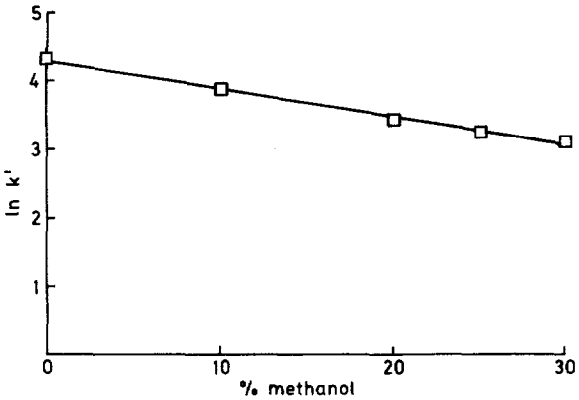

Fig. 9. Variation of the capacity factor for the system peak with the percentage of methanol in the mobile phase. Conditions: column, Hamilton PRP X100, 150 × 4.1 mm I.D.; eluent, potassium hydrogen phthalate at pH 4.2 containing the indicated percentages of methanol. The eluent concentrations were adjusted so that chloride ion gave the same retention time for all eluents.

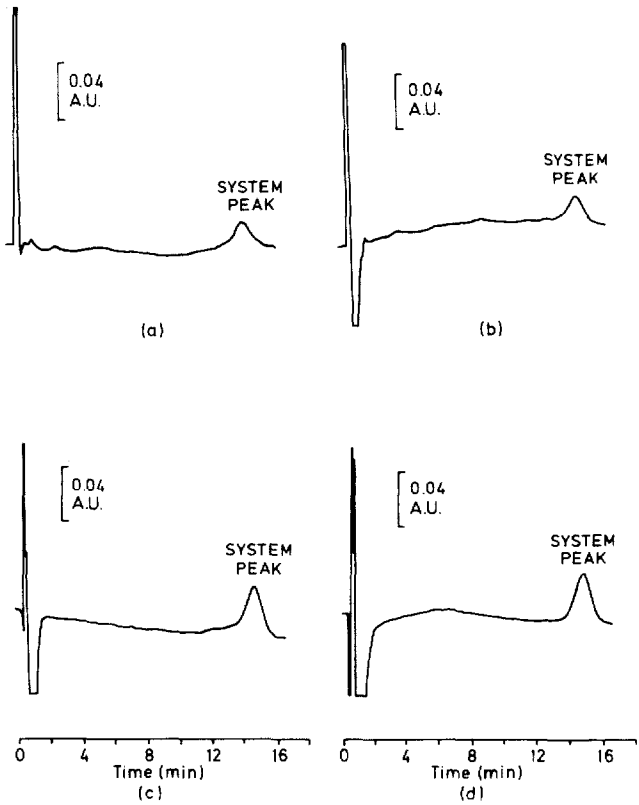

Fig. 10. System peaks induced by injection of organic modifiers. (a) 25 μl deionised water. (b) 25 μl methanol, (c) 25 μl acetonitrile, (d) 25 μl tetrahydrofuran. Conditions: column, Bio-Gel TSK Anion PW, 50 × 4.6 mm I.D.; eluent, 2.0 m*M* potassium hydrogen phthalate at pH 4.2; flow-rate, 1.2 ml/min; detection, UV absorption at 265 nm, 0.4 a.u.f.s.

tained when 25-μl volumes of water, methanol, acetonitrile and tetrahydrofuran were injected onto the polymethacrylate column. This figure illustrates that the size of the system peak increased when the organic modifiers were injected and this effect may be attributed to greater desorption of bound neutral eluent molecules from the column surface.

CONCLUSIONS

The injection peak in non-suppressed ion chromatography using indirect UV absorption detection is considered to arise from a combination of a decreased absorbance due to eluent dilution resulting from the injected sample solvent and an increased absorbance caused by eluent anions displaced by initial binding of the sample anions onto the column. This peak elutes at the unretained volume of the column and its height is dependent on sample concentration and the injected sample volume. The injection peak may be positive or negative.

The system peak is considered to arise from the elution of neutral, protonated eluent molecules desorbed from the column surface during sample injection. These eluent acid molecules traverse the column by a reversed-phase mechanism in which they are continually adsorbed onto and desorbed from the unfunctionalised regions of the packing material. With the low capacity columns used, as much as 85% of the surface area of the packing material is free of anion-exchange functionalities[15]. If the eluent pH is raised to a level where the eluent is completely ionised, then the system peak is eliminated.

Injection of water causes both dilution of the eluent and desorption of the eluent acid. The resultant system peak is therefore positive (*i.e.* decreased absorbance) since the eluent acid concentration resulting from this effect will never reach the equilibrium level found in the bulk eluent. When a solute ion is injected, both of the abovementioned effects occur, however the effect of the ionic strength of the sample on the initial desorption of eluent acid must also be considered. High ionic strength samples will tend to favour increased adsorption of eluent acid molecules on the column. The resulting system peak can therefore be positive or negative, depending on the injection volume, the sample concentration and the nature of the injected anion.

Sample pH effects result from changes in the mobile phase concentration of eluent acid arising from acid–base reactions. Samples more alkaline than the eluent will cause an initial rapid decrease of protonated eluent molecules in the mobile phase, leading to a positive system peak. The opposite effect occurs when an acidic sample is injected and a negative system peak results.

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