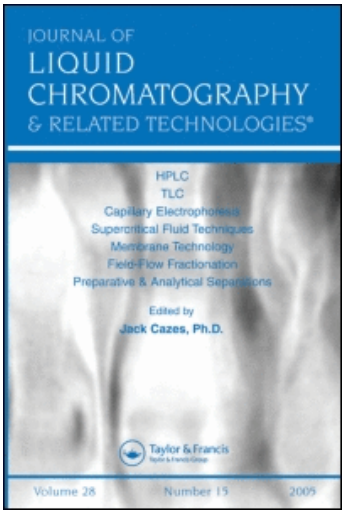

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ORTHO-PHOSPHATE OF AROMATIC AMINES AS THE INTERACTION REAGENTS IN REVERSED-PHASE HPLC CHROMATOGRAPHY. DIRECT SPECTROPHOTOMETRIC DETECTION OF NON ABSORBING ANIONS

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ABSTRACT

This paper shows that in the ion-interaction-reagent reversed-phase HPLC chromatography here employed anions too, like amines, are retained through the formation of ion-pairs and that under this form they are also released.

Experiments were performed with spectrophotometric detection and with the use of interaction reagents composed by an anion which does not absorb at the detection wavelength and an amine which on the contrary does.

The formation of the characteristic system peaks was discussed and explained, in agreement with previously obtained results.

The method here developed permits the spectrophotometrical detection of non-absorbing anions, through the absorbance properties of the hetaeron amine.

INTRODUCTION

Works performed in this laboratory have been devoted to the separation of anions and amines [1-5] by means of reversed-phase ion-interaction-reagent HPLC chromatography .

New separation methods have been developed and information has been collected in order to better understand the discussed [6-9] interaction mechanisms which govern retention in the ion-interaction-reagent chromatography. The interaction reagents are salts composed of suitable acids and amines with pH values at which the acids are present as their anions and the amines as their protonated forms . Interaction reagents constitute the mobile phase which, when flowing in isocratic conditions, simultaneously dynamically functionalizes the stationary phase (an ODS-2 reversed-phase) ,so modifying its interaction properties.

The results up to now collected agree with the model [6] which assumes the formation of an electrical double layer on the surface of the stationary phase : retention of analytes is due to electrostatic forces associated with sorption effects. Such a chromatographic system is usefully employable in the separation of species which are able to give rise to ion-pairs with the

cation (the protonated amine) or the anion of the interaction reagent, respectively.

In particular, it has been shown that amines are released as the same ion-pairs (formed with the anion of the interaction reagent) under which they were retained. Therefore also amines which do not absorb at a certain wavelength can be detected spectrophotometrically, if the anion of the interaction reagent does absorb . Thus , for example, the use of octylamine salicylate as the interaction reagent at a wavelength of 254 nm (at which salicylate is characterized by a positive value of absorptivity) permitted the direct spectrophotometric detection of non-absorbing aliphatic amines [1].

As concerns the retention and the elution behaviour of anions, it is likely that a similar mechanism is taking place: therefore anions should be retained and released as ion-pairs formed with the amonium ion of the interaction reagent . However, no experimental evidence has been presented up to now and no example can be found in literature.

In this paper the mechanism of retention of anions is investigated through the use of interaction reagents formed by an anion which does not absorb and an amine which, on the contrary, does so. If the mechanism is as expected ,also the analyte anions which do not absorb at a certain wavelength will be spectrophotometrically detected as positive peaks, the measured absorbance being due to the hetaeron amine.

Non-absorbing anions have already been spectrophotometrically detected by other authors and ourselves

(10-21) by indirect spectrophotometry, making use of absorbing species as the interaction reagent.

In this paper the direct spectrophotometric detection of non-absorbing anions is presented.

EXPERIMENTAL

Apparatus

Analyses were carried out with a Merck-Hitachi Lichrograph chromatograph mod. L-6200, equipped with a two-channel Merck-Hitachi Model D-2500 Chromato-Integrator . The integrator is interfaced both with an UV/UV-Vis Detector L-4200 and a Wescan 213 A conductometric detector. It is so possible to compare, for the same injection, the spectrophotometric and the conductometric responses .

For pH measurements,a Metrohm 654 pH-meter equipped with a combined glass-calomel electrode was employed and for absorptivity values a Hitachi 150-20 spectrophotometer was used.

Chemicals and reagents.

Ultra-pure water from Millipore Milli-Q was used for the preparation of solutions.

2-phenylethylamine, 3-phenylpropylamine and aniline were "Fluka" analytical grade reagent.Benzylamine was a "Merck" reagent. All other reagents were "C.Erba" analytical grade chemicals.

Chromatographic conditions.

A Phase Separation Spherisorb ODS-2 5 μm packed in a 250 x 4.6 mm column was used as the stationary phase. This column is characterized by a carbon load equal to 12% (0.5 mM/g m/m) and is fully endcapped .

The salts for use as the interaction reagent were prepared as described elsewhere [1-5] by dissolving the weighed amount of the amine in ultra-pure water and adjusting the pH value of the solutions to 6.4 ± 0.4 through additions of ortho-phosphoric or hydrochloric acid. The solutions were freshly prepared each third day.

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained (a minimum of 1 hour was necessary). The repeatibility of measurements was within 2% for sequential measurements (with the same conditions of eluent preparation and column conditioning) and the reproducibility (for different eluent preparations) was within 4% . Table II lists the average data and reproducibilities.

Between one utilization and another, the column was regenerated by passing ultra-pure water (10 minutes, flow-rate = 0.3 ml/min) and then a water-methanol mixture 1/1 v/v overnight, flow-rate = 0.1 ml/min). Before being put to a new use ultra-pure water (10 minutes ,flow-rate 0.3 ml/min) was again flowed, because of a possible insolubility of the eluents in methanol. By adopting these treatments, no particular

TABLE I

Absorptivity Values evaluated at Different Wavelengths for the Amines used in the Interaction Reagents .

λ	210 nm	250 nm	270 nm
Benzylamine	8400 ± 21	153 ± 9	46 ± 5
2-phenylethylamine	5509 ± 18	163 ± 12	62 ± 7
3-phenylpropylamine	7721 ± 31	219 ± 14	82 ± 9

deterioration in the column was observed, with respect to its use in other RP-HPLC chromatographic techniques.

RESULTS AND DISCUSSION

As mentioned, in order to gain experimental evidence that in the chromatographic system here employed anions too are retained and released as ion pairs, spectrophotometric detection was used together with interaction reagents composed of a non-absorbing anion and an absorbing amine . Different systems were studied and compared. Ortho-phosphate and chloride were chosen as the anions. Benzylamine , 2-phenylethylamine and 3-phenylpropylamine were the absorbing amines : their absorptivity values evaluated for some typical wavelengths are listed in table I.

As concerns the choice of the aromatic amine and of detection wavelengths , at first glance it would seem that (if the proposed

mechanism holds) the highest sensitivities have to be expected for the highest absorptivities of the amines to be used as the cation of the interaction reagent. Unfortunately, practical limitations arise because of the difficulty of obtaining a good baseline when too high an absorbance of the eluent must be subtracted in the autozering process. This operation generally results in a very high noise, so that the signal to noise ratio and, of consequence, the detection limit is very unfavourable. The situation does not improve if eluent concentration or flow-rate is decreased ; the noise decreases but sensitivity does also .

2-phenylethylamine ortho-phosphate, 3-phenylpropylamine ortho-phosphate and benzylamine chloride at a wavelength of 270 nm were employed as the interaction reagents. It was possible to detect as positive peaks a series of anions such nitrite, nitrate, sulphate, malate, succinate,acetate -which are all characterized by null absorptivity at 270 nm. As mentioned, the peaks observed are due to the ion pair which these anions form with the aromatic amine, the measured absorbance being due to the amine. These results were also confirmed by conductometric detection performed for the same injection of analyte.

The obtained retention times are listed in table II and some typical examples are here reported.

2-phenylpropylamine as the interaction reagent

Figure 1 reports the chromatograms recorded for the injection of 100 µl of chloride (at concentration of 50.0 ppm)

TABLE II

Typical Retention Times obtained for the investigated Ion-Interaction Reagents.
Stationary Phase : Phase Separation Spherisorb ODS-2 , 250 x 4.6 mm , 5 µm.
Flow-rate : 1.0 ml/min

	2-Phenylethylamine o-phosphate 0.005 M	3-phenylpropylamine o-phosphate 0.005 M	Benzylamine chloride 0.005 M
Injection peak	1.9 ± 0.1	1.9 ± 0.1	
System peak 1	6.0 ± 0.4 (o-phosphate)	8.0 ± 0.5 (o-phosphate)	2.0 ± 0.4 (benzylamine)
System peak 2	18.5 ± 1.3 (2-phenylethylamine)	32. ± 2. (3-phenylpropylamine)	4.0 ± 0.5 (chloride)
Bromide	3.8 ± 0.2	4.6 ± 0.2	
Chloride	4.1 ± 0.2	5.0 ± 0.2	4.0 ± 0.5
Acetate	4.1 ± 0.2		2.7 ± 0.2
Carbonate	4.3 ± 0.2	5.0 ± 0.2	4.2 ± 0.2
Nitrate	5.0 ± 0.3	6.3 ± 0.3	4.6 ± 0.2
Ascorbate		6.5 ± 0.2	5.1 ± 0.3
o-Phosphate	6.0 ± 0.4	8.0 ± 0.5	5.5 ± 0.2
Malonate	12.8 ± 0.4	24.9 ± 0.4	
Sulphate		29.1 ± 0.4	9.9 ± 0.3
Malate			10.9 ± 0.2
Succinate	16.5 ± 0.5		11.5 ± 0.3
Hexylamine		15.0 ± 0.5	

with spectrophotometric detection at 270 nm : the interaction reagent is 0.0050 M 3-phenylpropylamine. The injected chloride gives rise to a positive peak at 5.04 min and the chromatogram is also characterized by the presence of injection peak (j) and of two system peaks (s_1 and s_2), which will be discussed later.

Figure 2 shows the chromatogram recorded, by using the same nteraction reagent, for the injection of sulphite (100.0

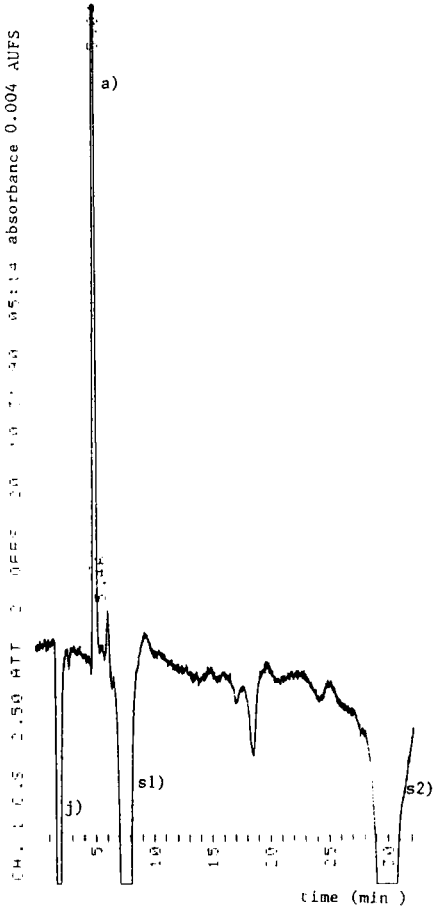

FIGURE 1.
Chromatogram recorded for injection of chloride (50.0 ppm).
Conditions :
Ion interaction reagent : 0.0050 M 3-phenylpropylamine ortho-phosphate.Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm. Spectrophotometric detection : 270 nm.
Peaks :
j) injection peak , a) chloride (50.0 ppm), s1) system peak due to ortho-phosphate , s2) system peak due to 3-phenylpropylamine.

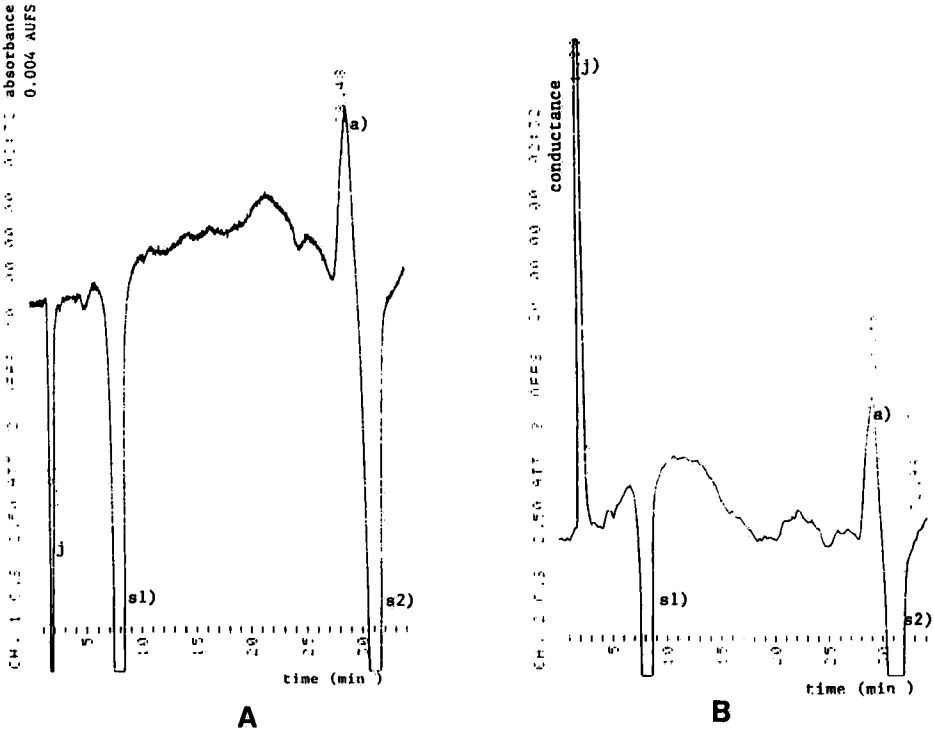

FIGURE 2.
Chromatograms recorded for injection of sulphite (100.0 ppm).
Conditions :
Ion interaction reagent : 0.0050 M 3-phenylpropylamine ortho-phosphate.Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm. Spectrophotometric detection : 270 nm.
Peaks :
j) injection peak , a) sulphite (100.0 ppm), s1) system peak due to ortho-phosphate , s2) system peak due to 3-phenylpropylamine.

A) Spectrophotometric detection , λ= 270 nm
B) Conductometric detection.

ppm) with spectrophotometric at 270 nm (figure 2A) and conductometric detection (figure 2B). Besides the peak a) due to sulphite, also here we can observe the two system peaks and the injection peak (j). The latter is due to unretained species and in this example to the Na^+ ion which (being characterized by a null value of absorptivity at 270 nm and a positive value of equivalent conducibility) comes out as a negative peak in spectrophotometry and as a positive one in conductance detection.

Other experiments were performed in order to obtain further confirmation of the proposed mechanism. So, the injection of hexylamine (which does not absorb and forms an ion-pair with the non-absorbing ortho-phosphate) gives rise to a negative peak, its absorbance being less than that which gave rise to the recorded baseline : hexylamine therefore can be detected by indirect spectrophotometry.

On the contrary, much higher sensitivites than those of non-absorbing anions can be obtained for acids which absorb at 270 nm ,such as the ascorbic acid : the measured absorbance is in fact due to the contributions of both the anion and the amine.

2-phenylethylamine ortho-phosphate as the interaction reagent.

With 2-phenylethylamine ortho-phosphate as the interaction reagent, typical chromatograms are presented for injection of

anions which do not absorb in the detection conditions (266 nm), namely: malonic acid (figure 3) and chloride with spectrophotometric (figure 4A) and conductometric (figure 4 B) detection.

In all of them , in addition to the positive peak due to the analyte, two system peaks (respectively at 5.9 ± 0.5 and 18 ± 1 min) and the injection peak can be observed.

Benzylamine chloride as the interaction reagent.

Figures 5 shows an example of positive detection of sulphate when benzylamine chloride is the interaction reagent. Two negative system peaks (details will be given later) are here also present.

In conclusion, these results give confirmation that in these experimental conditions injected anions give rise to ion-pairs with the interaction reagent (and in particular with its amonium ion) and that they are also eluted under this same form.

Such an interesting result may offer many new possibilities for anion analysis. However , detection levels (which at the moment are of the order of 3-5 ppm) still have to be improved. It can be observed that sensitivity in spectrophotometric detection (for the same interaction reagent) is nearly the same for all the anions investigated, in agreement with the proposed mechanism .

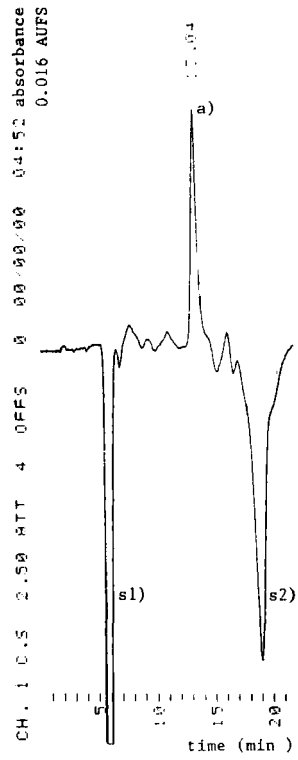

FIGURE 3.
Chromatogram recorded for injection of malonic acid (50.00 ppm).
Conditions :
Ion interaction reagent : 0.0050 M 3-phenylethylamine ortho-phosphate. Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm. Spectrophotometric detection : 266 nm.

Peaks : s1) system peak due to ortho-phosphate ,a) malonic acid (50.0 ppm), s2) system peak due to 2-phenylethylamine.

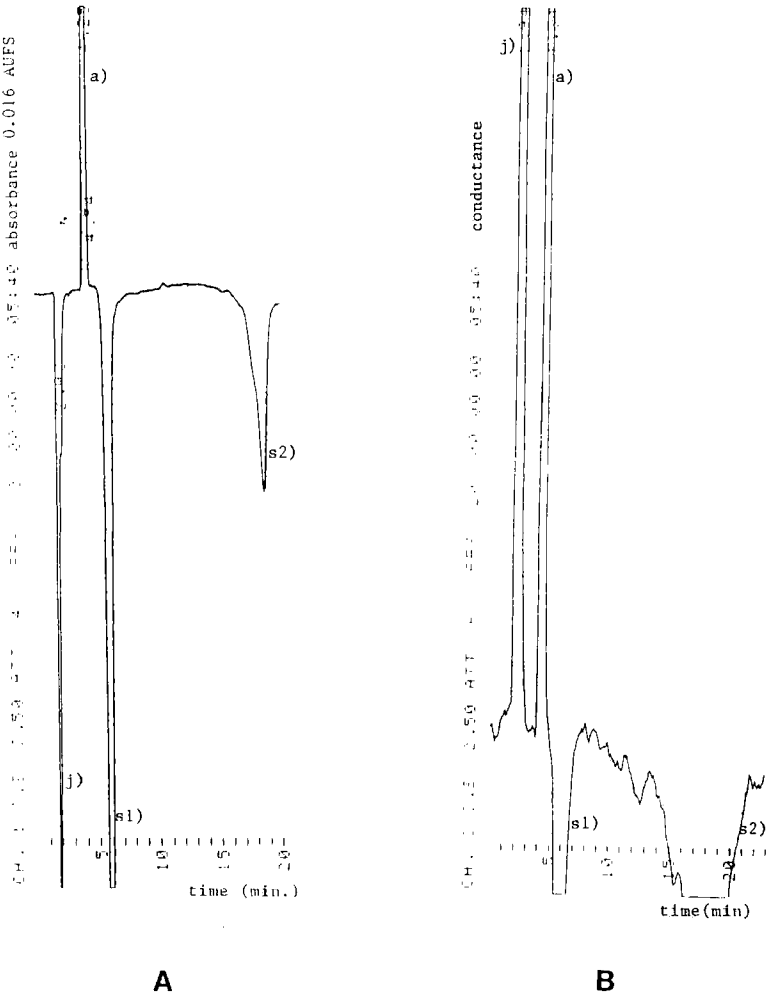

FIGURE 4.
Chromatogram recorded for injection of chloride (50.00 ppm) with :
A) spectropthotometric detection (λ = 266 nm) and
B) conductometric detection.

Conditions :
Ion interaction reagent : 0.0050 M 3-phenylethylamine ortho-phosphate. Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm.

Peaks : j) injection peak , s1) system peak due to ortho-phosphate , a) chloride (50.0 ppm), s2) system peak due to 2-phenylethylamine.

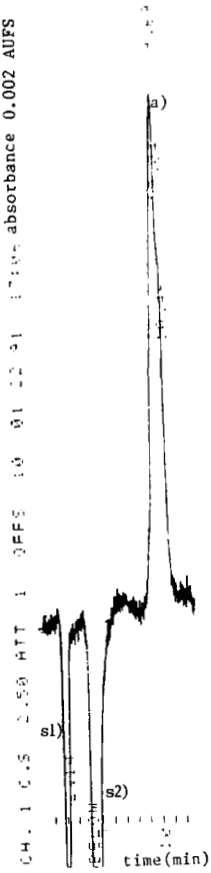

FIGURE 5.
Chromatogram recorded for injection of sulphate (50.00 ppm).
Conditions :
Ion interaction reagent : 0.0050 M benzylamine chloride .
Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm. Spectrophotometric detection : 268 nm.

Peaks : s1) system peak due to benzylamine,a) sulphate (50.0 ppm), s2) system peak due to chloride.

System peaks.

As previously mentioned, it is worth stressing that all the above presented chromatograms are characterized by the presence of two system peaks.

A previous work of ours published in this journal [22] was devoted to a discussion, based on experimental results, about the formation of the system peaks,which are characteristic of this experimental technique. Retention of analytes onto the stationary phase involves a corresponding amount of the interaction reagent (which had been initially absorbed onto the stationary phase) being released and when the retention time of the interaction reagent anion itself is reached, its concentration is lower than initially (when the baseline was adjusted) . When conductometric detection is used, this decreased concentration can always be detected as a negative peak with respect to the baseline which was recorded for the conductance of the interaction reagent (which is always non null). When the detection is spectrophotometric , the visualization of the system peak depends on the spectral properties of the interaction reagent at that wavelength.

In the systems here investigated (formed by an absorbing amine and a non-absorbing anion) two different system peaks can be observed. It can be shown that these two peaks are due to the amine and to the anion of the interaction reagent. respectively. When new species are retained onto the stationary phase, the concentrations of both the anion and the cation are

lowered onto it and when their retention times are reached, each of them gives rise to a negative peak.

Evidence of this hypothesis was gained through experiments in which the anion and the amine of the interaction reagent were independently injected at concentrations higher than in the mobile phase . In both cases, not only did the corresponding system peak disappear as negative peak but, in addition, a positive peak was formed at the same retention time. When the amine is injected, the observed absorbance increase is due to its own absorbance; when the anion is injected , the observed absorbance is due to the ion-pair which the anion forms with the absorbing amine of the eluent.

We can again consider figure 1, which shows the shape obtained for injection of chloride when the chromatographic system is composed of 0.0050 M phenylpropylamine ortho-phosphate and spectrophotometric detection at 270 nm -the baseline being recorded by autozering for the absorbance of the 2-phenylpropylamine ortho-phosphate.

It can be shown that the system peak s1) is due to ortho-phosphate and the system peak s2) to 3-phenylpropylamine by the following experiments . When orthophosphate (at concentration of 600.0 ppm) is injected , the peak s1) changes its sign (figure 6) : the injected ortho-phosphate forms a ion-pair with the absorbing 3-phenylpropylamine and, being its concentration greater than that in the eluent, gives rise for the same retention time, to a positive peak . The other system peak (s2) is unaffected by this process.

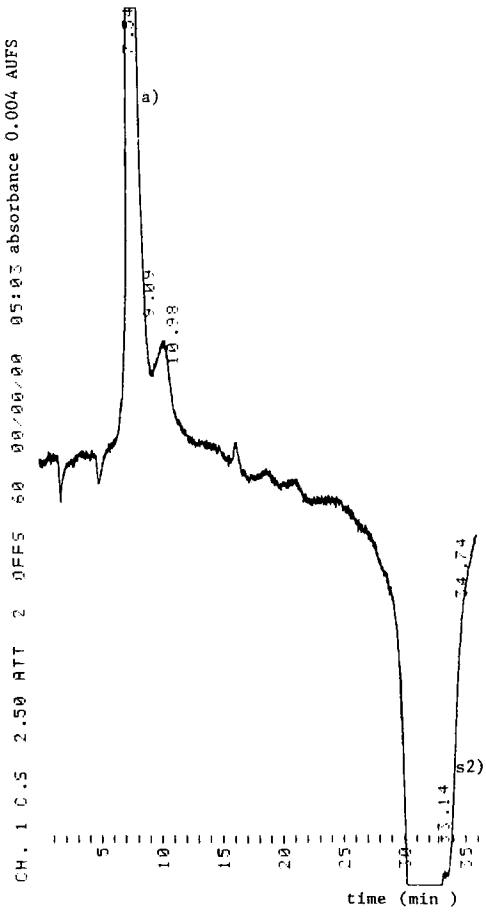

FIGURE 6.
Chromatogram recorded for injection of 100 µl of a solution 600.0 ppm of ortho-phosphate.
Conditions :
Ion interaction reagent : 0.0050 M 3-phenylpropylamine ortho-phosphate.Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm. Spectrophotometric detection : 270 nm.
Peaks :
a) ortho-phosphate , s2) system peak due to 3-phenylpropylamine.

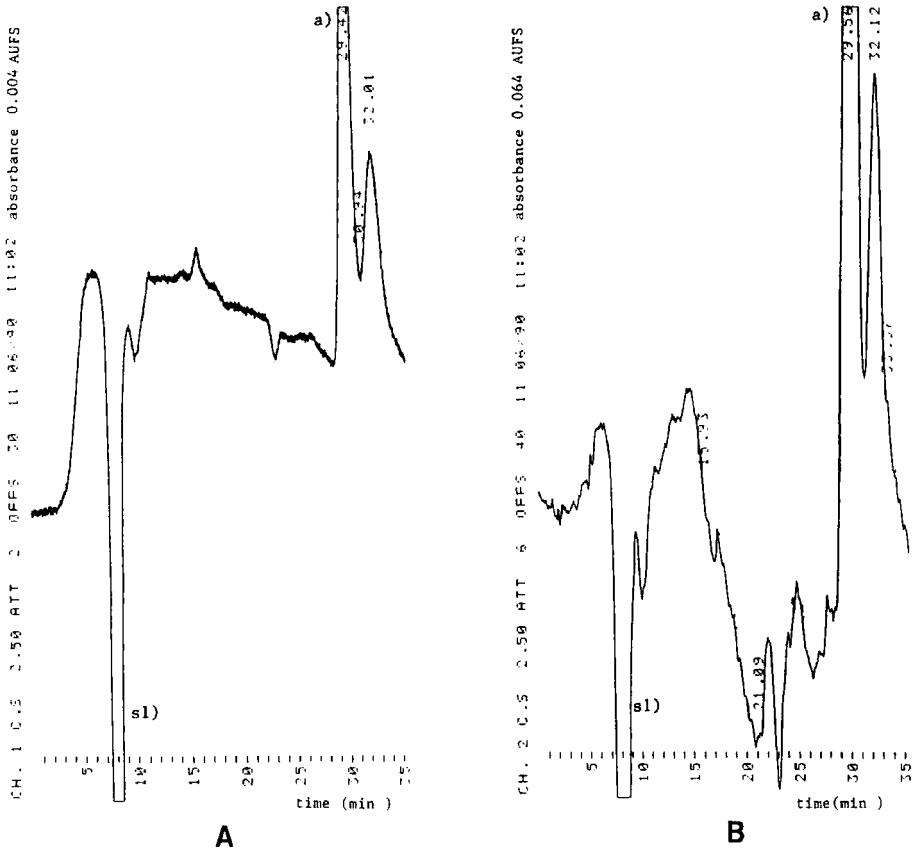

FIGURE 7.
Chromatogram recorded for injection of 100 µl of 500.0 ppm of 3-phenylpropylamine with
A) spectrophotometric detection (270 nm)
B) conductometric detection.
Conditions :
Ion interaction reagent : 0.0050 M 3-phenylpropylamine ortho-phosphate.Flow-rate = 1.0 ml/min. Injection : 100 µl
Stationary phase : Phase Separation Spherisorb ODS-2 (250 x 4.6 mm), 5 µm.
Peaks :
s1) system peak due to ortho-phosphate , a) 3-phenylpropylamine.

On the other hand, when 500 ppm (figure 7) of 2-phenylpropylamine are injected , whilst the peak s1) due to ortho-phosphate remains unchanged , the peak s2) due to 3-phenylpropylamine changes its sign.

Similar results have been obtained also for the system peaks observed for 2-phenylethylamine ortho-phosphate and benzylamine chloride and permitted to ascribe the peaks respectively to the anion and the amine. It can be stressed that, whilst when 3-phenylpropylamine ortho-phosphate and 2-phenylethylamine ortho-phosphate are the eluents, the system peak due to ortho-phosphate is the less retained ; the contrary happens with benzylamine chloride , for which the system peak due to benzylamine corresponds to a less retention .

Furthermore, at the moment we can not still explain why the system peak due to 2-phenylpropylamine is a twin peak, as evidenced also by conductometric detection (figure 7 B) .

CONCLUSION

As a conclusion, this paper has shown that the mechanism of retention of anions is the same as that previously evidenced for amines. Anions and amines are retained as ion-pairs and under this same form they are also eluted.

Furthermore, the formation of the system peaks in these new systems can be explained through the mechanisms already proposed [22].

From a practical point of view, the method here proposed may offer enormous potentiality in spectrophotometrical detection of non-absorbing anions , provided that sensitivity can be improved.

REFERENCES

1. Gennaro M.C., Bertolo P.L., Simultaneous determination of nitrites, nitrates and amines by ion- interaction reversed-phase high-performance liquid chromatography. Application to lagoon water, J. Chromatogr. 509, 147,1990.

2. Gennaro M.C., Bertolo P.L., Marengo E., Determination of aromatic amines at trace levels by ion interaction reagent reversed-phase high-performance liquid chromatography . Analysis in hair dyes and other water-soluble dyes, J. Chromatogr., 518, 149,1990.

3. Gennaro M.C., Abrigo C., Marengo E., The use of chiral interaction reagents in the separation of D(-)- and L(+)- ascorbic acids by ion interaction reversed-phase , Chromatographia, 30,311,1990.

4. Gennaro M.C., Bertolo P.L., Baldo M.A., Daniele S., Mazzocchin G.A., The ion interaction reversed phase high-performance liquid chromatography and the voltammetry with microelectrodes in the analysis of milk, J.Liq.Chromatogr., 14, 115,1991.

5. Gennaro M.C., Abrigo C., Separation of food-related biogenic amines by ion-interaction reversed-phase high-performance liquid chromatography. Tyramine, histamine, 2-phenylethylamine , tryptamine and precursor aminoacids, Chromatographia, 31,381,1991.

6. Bidlingmeyer B.A., Separation of Ionic Compounds by Reversed-Phase Liquid Chromatography: An Update of Ion-Pairing Techniques, J.Chromatogr.Sci., 18,525,1980.

7. Levin S.,Grushka E., System Peaks in Liquid Chromatography: their Origin, Formation, and Importance, Anal.Chem.,58,1602,1986.

8. Stahlberg J., The Gouy-Chapman Theory in Combination with a Modified Langmuir Isotherm as a Theoretical Model for Ion-Pair Chromathography., J.Chromatogr.,356,99,1986.

9. Jackson P.E., Haddad P.R., The Occurrence and Origin of System peaks in Non-Suppressed Ion Chromatography, J.Chromatogr., 346,125,1985.

10. Gennaro M.C., Role of the alkyl chain length of the ion interaction reagent, flow-rate,column packing and detection in ion interaction Reversed-Phase High-Performance liquid chromatography in separations of anions using amine salicylates,J.Chromatogr. 449, 103, 1988.

11. Gennaro M.C., Bertolo P.L., A survey of applications of the ion interaction reagent reversed-phase HPLC technique in the analysis of anions in a variety of different real samples, Ann.Chim. (Rome) ,80, 13 ,1990.

12. Miura Y., Fritz J.S., Benzenepolycarboxylic acid salts as eluents in anion chromatography, J.Chromatogr. 482,155,1989.

13. Chauret N., Hubert J., Characterization of indirect photometry for the determination of inorganic anions in natural water by ion chromatography, J.Chromatogr. 469,329,1989.

14. Crommen J., Schill G., Westerlund D., Hackzell L., Indirect Detection in Liquid Chromatography. I.Response Models for Reversed-Phase Ion Pairing Systems, Chromatographia 24,252,1987.

15. Bidlingmeyer B.A., Santasania C.T., Warren Jr.F.V., Ion-Pair Chromatographic Determination of Anions Using an Ultraviolet-Absorbing Co-Ion in the Mobile Phase, Anal.Chem., 59,1843, 1987.

16. Barber W.E.,Carr P.W., UV visualization of inorganic anions by reversed-phase ion-interaction chromatography: factors that control sensitivity and detection, J. Chromatogr.,316,211,1984.

17. Barber W.E., Carr P.W., Ultra-violet visualization of inorganic anions by reversed-phase ion-interaction chromatography; factors that control retention and selectivity. J.Chromatogr. 301,25,1984.

18. Barber W.E., Carr P.W., Ultraviolet visualization of inorganic ions by reversed-phase ion-interaction chromatography, J.Chromatogr. 260,89,1983.

19. Dreux M., Lafosse M., Agbo-Hazoume P., Chaabane-Doumandji B., Gibert M., Levi Y., Determimation of inorganic anions by ion-pair chromatography. Hetaeron adsorption characteristics on some alkyl-bonded silica stationary phases, J.Chromatogr. 354,119,1986.

20. Methra M.C., Pelletier C., Ion chromatographic determination of hypophosphite, phoshite and orthophosphate ions along with chloride, nitrate and sulfate ions using indirect ultraviolet detection, Anal.Sci. 6,431,1990.

21. Dorland P., Tod M., Postaire E., Pradeau D., Indirect detection of inorganic anions by high-performance liquid chromatography : use of papaveraldinium as an ultraviolet absorbing agent. J.Chromatogr. 478,131,1989.

22. M.C.Gennaro, Ghost,vacancy,dip or system peaks? A contribution in investigations about "injection" and "system" peaks in liquid chromatography ,J.Liq.Chromatogr. 10, 3347,1987.