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J. IVASK, J. PENTCHUK

APPLICATION OF PHENYLALANINE AND TYROSINE AS ELUENTS IN ION CHROMATOGRAPHY. THE EFFECT OF ELUENT CONCENTRATION AND pH ON THE RETENTION TIME OF INORGANIC ANIONS

(Presented by J. Kann)

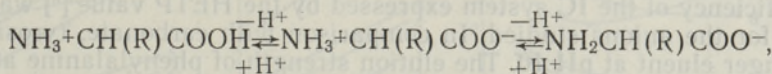
Ion chromatography (IC) [1] is a selective and sensitive method in analytical chemistry for the determination of several ions in a wide range of objects [2].

Suppressed [1] and unsuppressed [3, 4] IC systems are developed, the former, in general, displaying higher sensitivity and reliability.

The eluents utilized in suppressed IC for the analysis of anions have usually been limited to conventional carbonate and bicarbonate solutions or their mixtures. By using these eluents a large number of analytical problems can be solved, but in many cases, however, they do not allow achievement of high sensitivity because sample anions are detected in the background of the carbonic acid solution.

Recently there have been published some papers, in which application of amino acids as eluents to the analysis of anions in suppressed IC has been studied [5, 6].

Amino acids are bifunctional compounds and in aqueous solutions they exist in different ionic (cationic, anionic or zwitterionic) forms whose charge is conditioned by the pH of the solution:



where R is a substituent. In the alkaline medium of the eluent (achieved, for example, by adding NaOH) the amino acid molecule mainly exists as an anion and eluates the sample anions out of the separator column, packed with a low-capacity anion-exchange resin [6]. The application of amino acid eluents permitted accurate separation of F^- , Cl^- , NO_3^- , HPO_4^{2-} and SO_4^{2-} whereas the amino acids used (glycine, β -alanine et al.) were weak eluents and *p*-aminobenzoic acid was of a medium strength [6].

The aim of the present paper is to investigate the application of phenylalanine (Phe) $\text{C}_6\text{H}_5\text{—CH}_2\text{—CH}(\text{NH}_2)\text{—COOH}$ and tyrosine (Tyr) $\text{HO—C}_6\text{H}_4\text{—CH}_2\text{—CH}(\text{NH}_2)\text{—COOH}$ as eluents to suppressed IC of anions and to compare them with the carbonate eluent. The effect of amino acid concentration in the eluent and pH of the latter on the efficiency and selectivity of separation of halogenide, nitrite, nitrate, phosphate and sulphate ions has been studied.

Experimental

The IC system consisted of the following elements: a pump at flow rate 1.4 ml/min; injector with 0.1 ml sample loop; separator column (55×4 mm I. D.), packed with a low-capacity anionite [7]; suppressor column (300×4 mm I. D.), packed with cationite KY-2; conductivity detector with a cell constant 3 cm⁻¹ (293 K) and recorder at chart speed 0.4 cm/min.

All solutions were prepared in carbon dioxide free bidistilled water using analytical grade reagents (Reakhim). The eluents were prepared by dissolving amino acid in carbon dioxide free bidistilled water and by adding 0.5 M NaOH until the required pH value was achieved. The 0.1, 0.2, 0.5 and 1.0 mM Tyr as well as 1.0, 1.25, 1.5, 2.0 and 4.0 mM Phe eluents, both at pH 10 and 1.0 mM Tyr eluents with the pH values of 9.5, 10.0, 10.5 and 11.0 have been tested. To study the efficiency and selectivity of separation of anions, three test solutions were prepared:

1. chloride (4 ppm) + nitrate (20 ppm) + sulphate (25 ppm);
2. nitrite (5 ppm) + phosphate (50 ppm);
3. fluoride (2 ppm) + bromide (15 ppm) + iodide (50 ppm).

Results and discussion

Preliminary experiments were made using the above amino acids as eluents. By the beginning of the experiment the anion-exchange resin had been in contact with the tyrosine eluent for about 12 hours, whereas the eluent was coloured pink. The results obtained showed that separation of anions decreased sharply. The reason for this may be the contamination of anionite with the oxidation products of amino acid which reduces the anion-exchange capacity of resin. To clear up possible alterations to the quality of anionite, the following experiment was carried out using at first 1 mM Na₂CO₃ (1), then 1 mM Phe (2) and 1 mM Tyr (3), both at pH 10, and then again 1 mM Na₂CO₃ (4) as eluents. The adjusted retention times and resolution of eight anions were measured according to [8] (Tables 1, 2). To keep the quality of the anion-exchange resin, after each experiment the amino acid residues were washed out of the IC system with 50 ml 5 mM Na₂CO₃ solution.

The resolution values, listed in Table 2 show that the separation of anions is insufficient for their simultaneous determination. Especially low is separation for chloride/nitrite, nitrate/bromide and sulphate/iodide ions. The efficiency of the IC system expressed by the HETP value [8] was about 1 mm for the anions studied. In comparison with carbonate, tyrosine was a stronger eluent at pH 10. The elution strength of phenylalanine at pH 10 was weaker in comparison with carbonate and much weaker than that of tyrosine. After removing the amino acid residues from the IC system at the end of each experiment, no remarkable changes in the adjusted retention times of anions using eluents 1 and 4 were observed.

The effect of eluent concentration on the adjusted retention time for anions was determined by plotting \ln of the adjusted retention time ($\ln t'$) *v.s.* that of eluent concentration ($\ln C$). The plots were straight lines as could be expected on the basis of an earlier paper [4] (Figs 1, 2).

Table 1

Adjusted retention times (t') of anions on a 0.026 mmol/g anion-exchange resin, fraction 40–63 μm , $t_0=1.58$ min

Eluent N	t' (min)							
	F ⁻	Cl ⁻	NO ₂ ⁻	Br ⁻	NO ₃ ⁻	HPO ₄ ²⁻	I ⁻	SO ₄ ²⁻
1	0.17	0.71	1.13	2.17	2.71	4.62	11.21	10.17
2	0.30	1.33	1.83	3.80	4.58	18.17	>20	>20
3	0.17	0.54	0.75	1.46	1.71	3.33	6.67	6.75
4	0.17	0.67	1.08	2.00	2.38	4.63	9.71	9.21

Resolution (R) of anions (according to [8]). Conditions as in Table 1

Eluent N	R						
	Cl ⁻ /F ⁻	NO ₂ ⁻ /Cl ⁻	Br ⁻ /NO ₂ ⁻	NO ₃ ⁻ /Br ⁻	HPO ₄ ²⁻ /NO ₃ ⁻	I ⁻ /HPO ₄ ²⁻	SO ₄ ²⁻ /I ⁻
1	1.24	0.16	0.95	0.37	0.89	1.34	0.16
2	1.63	0.48	1.18	0.34	2.63	—	—
3	0.80	0.36	0.90	0.25	1.05	1.11	0.02
4	1.09	0.58	0.92	0.29	1.04	1.12	0.09

At pH 10 Phe mostly exists as single-charged anion, the ratio anion: zwitterion (calculated by the Henderson-Hasselbach equation [6]) is 0.88:0.12. So the theoretical slope of $\ln t'$ v.s. $\ln C$ plots would be about -1.0 for the single-charged anions and about -2.0 for the double-charged ones. The results (Fig. 1) showed that the slopes of all the anions were smaller theoretically. For the single-charged anions an average slope was -0.59 (std. dev. = 0.12), for phosphate -1.11 and for sulphate -1.20 . As the slopes of phosphate and sulphate plots were twice the single-charged anions, at pH 10 they mainly exist as double-charged anions.

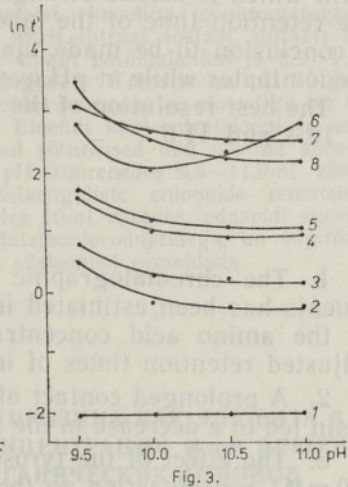
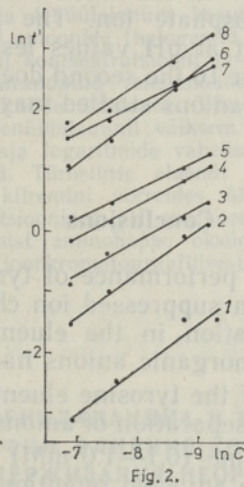
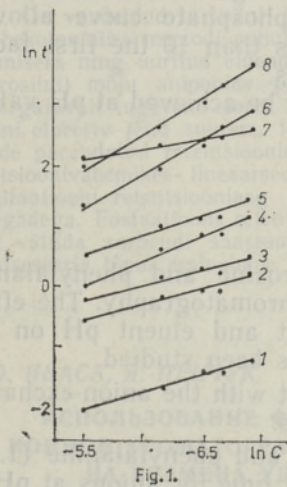


Fig. 1. The effect of the \ln of the phenylalanine concentration ($\ln C$) on the \ln of the adjusted retention times of anions ($\ln t'$), using 0.026 mmol/g anion-exchange resin, fraction 40–63 μm . 1 — fluoride, 2 — chloride, 3 — nitrite, 4 — bromide, 5 — nitrate, 6 — phosphate, 7 — iodide, 8 — sulphate.

Fig. 2. The effect of the \ln of the tyrosine concentration ($\ln C$) on the adjusted retention time of anions ($\ln t'$), using 0.026 mmol/g anion-exchange resin, fraction 40–63 μm . The numeration of anions as in Fig. 1.

Fig. 3. The effect of the pH of the tyrosine eluent on the \ln of the adjusted retention times ($\ln t'$) for anions, using 0.050 mmol/g anion-exchange resin, fraction 25–40 μm (two 55 \times 4 I. D. columns in succession). The numeration of anions as in Fig. 1.

To elucidate the effect of Tyr concentration on the adjusted retention times of anions, dissociation of the phenolic $-OH$ group should be taken into account. At pH 10 the ratio double-charged anion:single-charged anion:zwitterion is 0.43:0.50:0.07 (by the Henderson-Hasselbach equation [6]). So the theoretical slopes would be within -0.5 and -1.0 for each single-charged anion and within -1.0 and -2.0 for each double-charged anion. In Fig. 2 the slopes for the single-charged anions were close to theoretical (an average slope was -0.60 ; std. dev.= 0.10), but the slopes of double-charged anions were lower than the predicted ones (-0.75 for phosphate and -0.68 for sulphate).

The best resolution of the anions studied at pH 10 may be achieved with the 0.5 mM Tyr or 2 mM Phe eluent. Using Phe concentrations lower than 2 mM, the retention times of slowly eluting anions (I^- , SO_4^{2-}) increased too much.

To achieve acceptable retention times of anions the Phe concentration had to be quite high which shortens the working time of the suppressor column. So the following experiment was carried out by using Tyr eluent only.

The effect of the eluent pH on the adjusted retention time for an anion was determined by plotting \ln of the adjusted retention time ($\ln t'$) *v.s.* eluent pH (Fig. 3). The results showed that for the single-charged anions the curves were almost parallel. For sulphate ion the adjusted retention time shortened more considerably in comparison with the single-charged anions. For the phosphate ion two factors had to be taken into consideration. First, an increase in the eluting strength of eluent with increasing pH. Secondly, an increase in the concentration of the triple-charged ionic form which is sorbed stronger on the anion-exchange resin and increases the retention time of the phosphate ion. The phosphate curve allowed a conclusion to be made that at pH values less than 10 the first factor predominates while at pH over 10 the second does.

The best resolution of the anions studied may be achieved at pH values of 10.3 and 11.0.

Conclusions

1. The chromatographic performance of tyrosine and phenylalanine eluents has been estimated in suppressed ion chromatography. The effect of the amino acid concentration in the eluent and eluent pH on the adjusted retention times of inorganic anions has been studied.

2. A prolonged contact of the tyrosine eluent with the anion-exchange resin led to a decrease in the separation of anions.

3. The effect of the tyrosine (0.1–1.0 mM) and phenylalanine (1.0–4.0 mM) concentration on the adjusted retention time for anions at pH 10 was determined. It has been found that tyrosine is a stronger eluent in comparison with phenylalanine because it partially exists as a double-charged anion. At the same concentration tyrosine is a stronger eluent and phenylalanine weaker in comparison with the carbonate eluent.

4. The effect of the pH of the tyrosine eluent (9.5–11.0) on the adjusted retention time for anions has been determined. Increasing the eluent's pH, the adjusted retention time of the sulphate ion decreased faster in comparison with the single-charged anions. For the phosphate ion the adjusted retention time decreased in the pH range of 9.5–10.0. At the pH values over 10 the adjusted retention time of the phosphate ion increased.

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*Academy of Sciences of the Estonian SSR,
Institute of Geology*

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Tartu Sanitation and Hygiene Station

J. IVASK, J. PENTSUK

FENÜÜLALANIINI JA TÜROSIINI KASUTAMINE ELUENDINA IOONKROMATOGRAAFIAS. ELUENDI KONTSETRATSIOONI JA pH MÕJU ANORGAANILISTE ANIOONIDE RETENTSIOONIAJALE

On vaadeldud türosiini ja fenüülalaniini kasutamist eluendinaioonkromatograafia kahekolonnilise meetodi puhul anioonide (halogeeniid, nitrit, nitraat, fosfaat, sulfaat) lahutamiseks ning uuritud eluendi kontsentratsiooni (1—4 mM fenüülalaniini ja 0,1—1 mM türosiini) mõju anioonide parandatud retentsiooniaegadele. Türosiin on pH 10 juures märgatavalt tugevam eluent kui fenüülalaniin. Võrreldes karbonaatse eluendiga on türosiini elueeriv jõud suurem, fenüülalaniinil väiksem. Eluendi kontsentratsiooni ja anioonide parandatud retentsiooniaja logaritmid vahelised sõltuvused olid uuritud kontsentratsioonivahemikes lineaarsed. Türosiini eluendi pH suurenedes 9,5—11,0-ni vähenes sulfaatiooni retentsiooniaeg kiiremini võrreldes ühelaenguliste anioonide retentsiooniaegadega. Fosfaatiooni retentsiooniaeg pH suurenedes 10-ni vähenes, edaspidi suurenes. Et vältida sorbendi saastumist aminohappe oksüdatsiooniproduktidega, on vajalik iga katseseeria lõpul aminohapeioonkromatograafilisest süsteemist eemaldada.

Ю. ИВАСК, Я. ПЕНЧУК

ИСПОЛЬЗОВАНИЕ ФЕНИЛАЛАНИНА И ТИРОЗИНА КАК ЭЛЮЕНТА В ИОННОЙ ХРОМАТОГРАФИИ. ВЛИЯНИЕ КОНЦЕНТРАЦИИ И pH ЭЛЮЕНТА НА ВРЕМЕНА УДЕРЖИВАНИЯ НЕОРГАНИЧЕСКИХ АНИОНОВ

Исследована возможность разделения анионов (галогенидов, нитрита, нитрата, фосфата, сульфата) в двухколоночной ионной хроматографии, используя растворы тирозина и фенилаланина в качестве элюента. Изучено влияние концентраций элюента (1—4 mM фенилаланина и 0,1—1 mM тирозина) на исправленные времена удерживания анионов. Выявлено, что при pH 10 тирозин является более сильным элюентом, чем фенилаланин. По сравнению с карбонатным элюентом элюирующая сила тирозина сильнее, а у фенилаланина слабее. Зависимость между логарифмами концентраций элюента и исправленным временем удерживания анионов линейна в изученном диапазоне концентраций. При увеличении pH тирозинного элюента с 9,5 до 11,0 исправленное время удерживания сульфатона уменьшалось быстрее, чем у однозарядных анионов. Для фосфатона исправленное время удерживания уменьшалось в интервале pH с 9,5 до 10,0 и начинало возрастать при pH выше 10. Найдено, что для предотвращения загрязнения разделяющего сорбента продуктами окисления аминокислоты необходимо аминокислотный элюент удалить из хроматографической системы после проведения эксперимента.