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# Anion separation by high-performance ion chromatography coupled with indirect photometric detection and 1,2-dihydroxybenzene-3,5-disulfonate or sodium sulfosalicylate as the eluent

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## Abstract

Some common inorganic and organic anions were separated by high-performance ion chromatography coupled with indirect photometric detection and 1,2-dihydroxybenzene-3,5-disulfonate and sodium sulfosalicylate, respectively, as single-column single-component eluents. In respect of detection sensitivity, eluent concentration, eluent acidity and chromatographic separation efficiency, 1,2-dihydroxybenzene-3,5-disulfonate or sodium sulfosalicylate were excellent ultraviolet absorbers, capable of completely resolving many common inorganic and organic anions. © 1997 Elsevier Science B.V.

**Keywords:** Mobile phase composition; Inorganic anions; Organic acids

## 1. Introduction

In previous work, we reported [1,2] that the simultaneous separation and determination of inorganic anions and cations can be carried out on a strong basic anion-exchange column using phthalate-EDTA or a sodium sulfosalicylate-EDTA binary eluent. The behaviour of the column has also been studied. In the present paper, we will discuss the behaviour of a single eluent on a basic anion-exchange column and investigate if the separation and determination of some inorganic ions is possible.

In indirect photometry, if the eluent consists of more than two UV-active components, a multicomponent mixed eluent can achieve higher detection sensitivity than a single-component eluent. However, if all components of the eluent do not absorb

radiation in the given wavelength region, then using the UV absorber as eluent gives higher sensitivity than using a multicomponent eluent [3]. Hence, single-component elution is commonly employed unless anions and cations are to be detected simultaneously.

1,2-Dihydroxybenzene-3,5-disulfonate (tiron) and sulfosalicylic acid ( $H_3SS$ ) are common organic reagents. For tiron, the dissociation constants of the hydroxyl groups are  $pK_{a_1} = 8.31$  and  $pK_{a_2} = 13.07$ , respectively, therefore, two -OH groups do not dissociate under neutral conditions and the dominant species is  $H_2L^{2-}$ . For sulfosalicylic acid,  $pK_{a_1} = 2.50$ , and  $pK_{a_2} = 11.70$ , so when the  $pH > 2.50$ , the main species is  $HSS^{2-}$ . Besides, -Ar of these two ligands are strong UV-absorbers. Hence, tiron and sulfosalicylic acid may serve as potential indirect photometric eluents for separating anions. Pacakova et al. [4] tested the isolation efficiency of five kinds of inorganic anions with sulfosalicylic acid ( $pH =$

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7.6) as the eluent by ion-exchange indirect photometry. Hisakuni [5] reported the detection of  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{Br}^-$  with tiron as the eluent using electric conductance and indirect UV detectors. Their experiments proved that tiron and sulfosalicylic acid were excellent indirect UV eluents for inorganic anions. However, using these two eluents to separate inorganic and organic anions simultaneously was not involved.

Therefore, tiron and sodium sulfosalicylate were tested as complexing agents to separate organic and inorganic anions. The influence of eluent concentration and eluent acidity on retention behaviour was studied. The chromatographic separation effects of tiron and sodium sulfosalicylate were better than that of phthalate [1] when these two reagents were applied to the isolation of  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{I}^-$ , tartrate and acetate.

## 2. Experimental

### 2.1. Reagents

Tiron (chemically pure grade) was purified prior to use (Shanghai Chemical Factory) and sodium sulfosalicylate (analytical grade) was obtained from the Beijing Chemical Factory. All of the anion stock solutions were prepared using their corresponding sodium salts. Tiron was used to prepare mobile phases with the required pH values, which were adjusted using  $1.0 \text{ mol l}^{-1}$  NaOH. Before use, the eluent was filtered and degassed.

### 2.2. Apparatus

An LC-6A high-performance liquid chromatograph with a SPD-6AV UV-Vis absorbance detector and a CR-3A data processing system (Shimadzu, Japan) was employed. The analytical column used was an anion-exchange column ( $50 \times 4.6 \text{ mm I.D.}$ ), which was packed by the Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. Absorption spectra was recorded using an UV-300 UV-Vis spectrophotometer (Shimadzu, Japan) and pH values were measured using a PHS-10A digital acidity meter (Xiaoshan, China).

## 3. Results and discussion

### 3.1. Detection wavelength selection

Absorption spectra of tiron in the range of 260–340 nm were measured with  $\lambda_{\text{max}} = 291 \text{ nm}$  and  $\epsilon_{\text{max}} = 3721 \text{ l mol}^{-1} \text{ cm}^{-1}$ , therefore, 291 nm was selected as the detection wavelength in further experiments. For the sulfosalicylate system,  $\lambda_{\text{max}} = 298 \text{ nm}$  was employed as the detection wavelength, where  $\epsilon_{\text{max}} = 2769 \text{ l mol}^{-1} \text{ cm}^{-1}$ .

Compared with phthalate [1] and sulfosalicylate, the absorptivity of tiron was the highest, so, when the separation efficiency is not considered, tiron has the lowest detection limit.

### 3.2. Effect of the pH value of the eluent on the capacity factor

With sulfosalicylic acid as the eluent, the dependence of the capacity factors of eight types of organic and inorganic anion on the pH values of the eluent was investigated. The results are shown in Fig. 1.

For the inorganic anions studied, capacity factors hardly varied as the pH was increased. Because the  $\text{p}K_{\text{a}2}$  and  $\text{p}K_{\text{a}3}$  values of sulfosalicylic acid are 2.50 and 11.70, respectively, and a strongly basic anion-exchange column was used, pH values in the range 3.75–6.50 did not influence the exchange capacity of

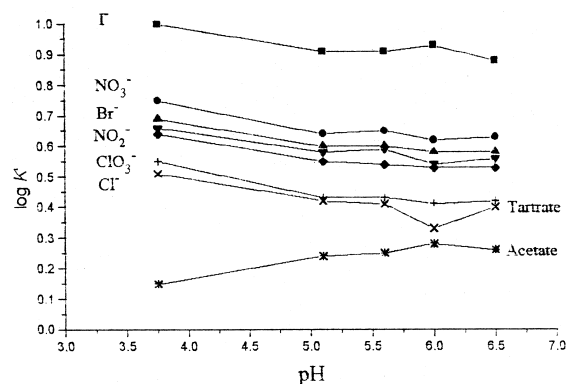


Fig. 1. Plot of  $\log k'$  versus pH of the eluent. Eluent,  $2 \cdot 10^{-4} \text{ mol l}^{-1}$  HSS $^{2-}$ ; flow-rate, 0.60 ml/min; column temperature, 25°C; detection wavelength, 298 nm; injection volume, 3  $\mu\text{l}$ .

the column and dissociation of eluent. However, under these conditions, acetic acid (HAc) and tartaric acid would dissociate ( $pK_a$  of HAc=4.76 and  $pK_{a_1}$  and  $pK_{a_2}$  of tartaric acid=3.04 and 4.37, respectively), giving rise to the abnormal appearance of these two ions in Fig. 1.

### 3.3. Effect of the concentration of the eluent on the capacity factor

According to the literature [6], the higher the absorbance of the matrix, the higher the response error, therefore, matrix absorbance is normally maintained within 0.2–0.8 [2], where the response error ( $\Delta A_s$ ) is negligible. It was experimentally proven that when the concentration of tiron in the eluent was maintained between  $1 \cdot 10^{-4}$  and  $5 \cdot 10^{-4}$  mol  $l^{-1}$ , not only the eluting power, but also the response error were satisfactory, whereas for the sulfosalicylic acid system, appropriate concentration range was between  $1 \cdot 10^{-4}$  and  $8 \cdot 10^{-4}$  mol  $l^{-1}$ .

It was found that the logarithm of the capacity factor,  $k'$ , was proportional to the concentration of the eluent.

For the tiron system, the slopes and correlation coefficients of  $\log k'$  against  $\log [E^{y-}]_0$  curves are given in Table 1.

From Table 1, the difference between the observed slopes and the theoretical ones ( $-0.5$ ) for different anions were  $-0.06$ – $-0.18$ . The experimental value of the  $I^-$  ion with the longest retention time was near the theoretical value. It can be seen that the effective charge is less than two, that is, not every  $-SO_3^-$  from tiron takes part in ion exchange.

According to reference [7], resolution is also a

Table 1  
Slopes and correlation coefficients of  $\log k'$  versus  $\log [tiron]$  curves for different anions

Ion	Slope	Correlation coefficient
$Ac^-$	-0.61	-0.9632
$Cl^-$	-0.68	-0.9548
$NO_2^-$	-0.63	-0.9545
$NO_3^-$	-0.63	-0.9619
$I^-$	-0.56	-0.9590

Conditions: pH=5.7; flow-rate, 0.6 ml/min; detection wavelength, 291 nm; column temperature, 25°C.

function of the concentration of the eluent. As the concentration of the eluent increased, the eluting power increased but the resolution decreased. Generally, when the resolution is less than 0.8, chromatographic separation is worthless. For a  $Cl^-$ ,  $NO_2^-$ ,  $NO_3^-$ ,  $I^-$  and  $Ac^-$  five-component mixture, when  $[tiron]_0$  reached  $4.0 \cdot 10^{-4}$  mol  $l^{-1}$ , the resolution became so low that the components could no longer be isolated completely. Fig. 2 depicts the chromatogram of those five anions with  $[tiron]_0 = 4.0 \cdot 10^{-4}$  mol  $l^{-1}$ , with the chromatographic peaks of  $Cl^-$  and  $Ac^-$  overlapping with each other.

With sodium sulfosalicylate as the eluent, an analogous linear relation for  $\log k'$  against  $\log [HSS^{2-}]_0$  was obtained. Table 2 gives the slopes and correlation coefficients of these curves. The results indicated that ion exchange of sulfosalicylate  $HSS^{2-}$  was not complete either.

Figs. 3 and 4 show the chromatograms of  $Ac^-$ ,  $Cl^-$ ,  $NO_2^-$ ,  $NO_3^-$ ,  $I^-$  and of  $BrO_3^-$ ,  $ClO_3^-$  and  $Br^-$ , respectively. It is evident that these ions have been completely separated.

### 3.4. Influence of the amount of methanol in the eluent

Figs. 5 and 6 are chromatograms of a  $Ac^-$ ,  $Cl^-$ ,

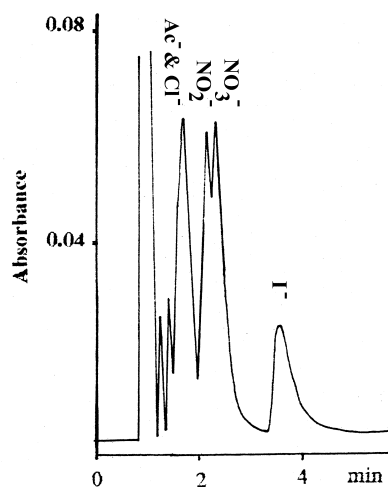


Fig. 2. Chromatogram of  $Cl^-$ ,  $NO_2^-$ ,  $NO_3^-$ ,  $I^-$  and  $Ac^-$ . Eluent,  $4 \cdot 10^{-4}$  mol  $l^{-1}$  tiron (pH=5.7); flow-rate, 0.60 ml/min; detection wavelength, 291 nm; column temperature, 25°C. All of the sample peaks are negative.

Table 2

Slopes and correlation coefficients for the curves of  $\log k'$  versus  $\log [\text{HSS}^{2-}]_0$  for some common anions

Ion	Slope	Correlation coefficient
$\text{Ac}^-$	-0.60	-0.9980
$\text{Cl}^-$	-0.63	-0.9981
$\text{NO}_2^-$	-0.60	-0.9983
$\text{NO}_3^-$	-0.60	-0.9981
$\text{I}^-$	-0.55	-0.9959
$\text{BrO}_3^-$	-0.63	-0.9996
$\text{ClO}_3^-$	-0.60	-0.9997
$\text{Br}^-$	-0.59	-0.9999

Eluent,  $2 \cdot 10^{-4}$  mol  $\text{l}^{-1}$  sodium sulfosalicylate (pH=5.85); detection wavelength, 289 nm. Other conditions as in Table 1.

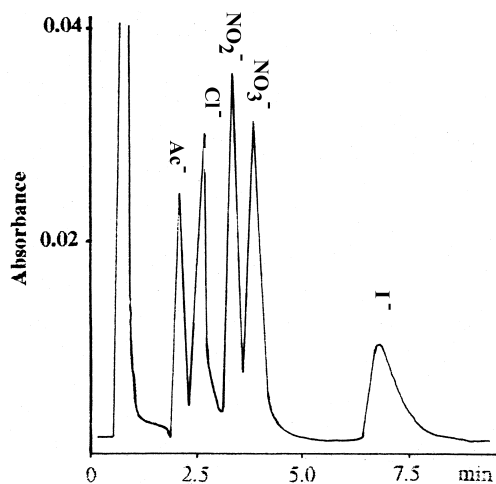


Fig. 3. Chromatogram of  $\text{Ac}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{I}^-$ . Eluent,  $2 \cdot 10^{-4}$  mol  $\text{l}^{-1}$  sodium sulfosalicylate (pH=4.42); other conditions as in Fig. 1. All of the sample peaks are negative.

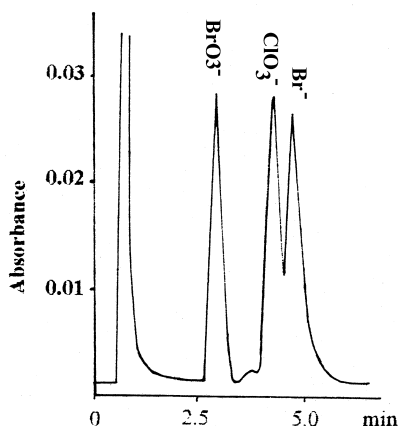


Fig. 4. Chromatogram of  $\text{BrO}_3^-$ ,  $\text{ClO}_3^-$  and  $\text{Br}^-$ . Eluent,  $1 \cdot 10^{-4}$  mol  $\text{l}^{-1}$  sodium sulfosalicylate (pH=5.85); other conditions as in Fig. 1. All of the sample peaks are negative.

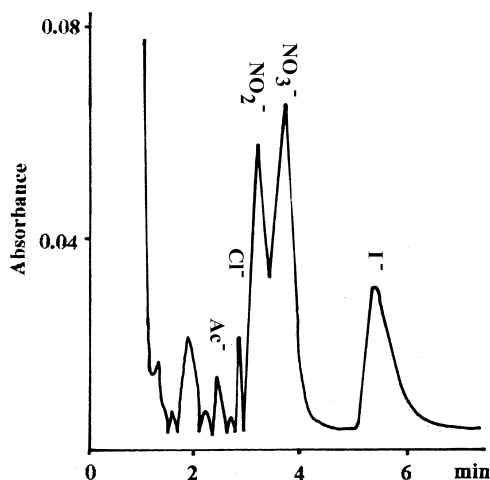


Fig. 5. Separation of  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{I}^-$  and  $\text{Ac}^-$  by indirect photometric detection. Eluent,  $5 \cdot 10^{-4}$  mol  $\text{l}^{-1}$  tiron (10% methanol, pH=6.56), other conditions as in Fig. 2. All of the sample peaks are negative.

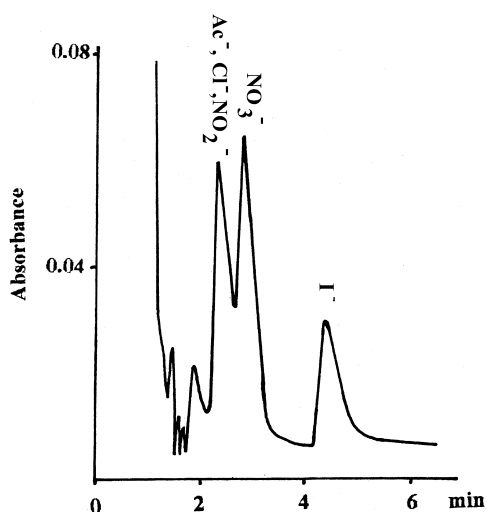


Fig. 6. Separation of  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{I}^-$  and  $\text{Ac}^-$  by indirect photometric detection. Eluent,  $5 \cdot 10^{-4}$  mol  $\text{l}^{-1}$  tiron (pH=6.56); other conditions as in Fig. 2. All of the sample peaks are negative.

$\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{I}^-$  sample in the presence or absence of 10% (v/v) methanol in the eluent.

It is obvious that, as methanol was added, the capacity factor of every ion increases, which is due to the addition of organic solvent changing ion activity.

In this paper, the chromatographic retention be-

Table 3  
Absolute retention times for some common anions

Ion	Absolute retention time (min)
F <sup>-</sup>	5.02
Br <sup>-</sup>	3.36
ClO <sub>3</sub> <sup>-</sup>	3.07
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	2.59
SCN <sup>-</sup>	2.46
BrO <sub>3</sub> <sup>-</sup>	2.16
HCOO <sup>-</sup>	2.54
CH <sub>3</sub> CH <sub>2</sub> COO <sup>-</sup>	2.31
Malate	2.58

Eluent,  $2 \cdot 10^{-4}$  mol l<sup>-1</sup> tiron (pH=5.70). Other conditions as in Table 1.

havior of other common anions was also tested with tiron as the eluent and the results are summarized in Table 3.

To sum up, tiron and sodium sulfosalicylate can serve as better indirect photometric single-column single-component eluents than phthalate. Firstly, they have higher detection sensitivity. Secondly, lower concentrations of eluent result in stronger eluting power for ion-exchange chromatographic columns with different capacities, to avoid column overload. Finally, under normal pH conditions, i.e. pH values

of 2.5 to 7.5 for bonded-phase chromatographic columns, the properties of the mobile phase are kept constant so that the retention of sample is almost independent of the pH value of the eluent.

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