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ISOTACHOPHORESIS

THE QUALITATIVE SEPARATION OF ANIONS

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SUMMARY

Separations of anions by isotachophoresis can be carried out in different ways. Differences in mobilities and/or differences in pK values can be used for the simultaneous separation of anions. The separations of anions, using both the differences in mobilities and/or the differences in $\overline{p}K$ values, were studied in this work. Experimental values for about one hundred anions are given. Equations are derived for the separation of anions in buffered systems and the experimental results are compared with calculated values in order to check the theory.

INTRODUCTION

In a previous paper¹ the theory for the use of buffered systems for separations of cations was described. Some operational systems that are useful for the separations of cations in both water and methanol were given.

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A comparison of the results obtained by calculations and. by experiments showed that corrections have to be made; the influence of relaxation and electrophoretic effects are important, while the influence of the temperature of the zones is negligible for relative calculations.

In this paper we discuss the equations derived for buffered systems and useful for the separation of anions. Some artefacts of the experiments carried out are given.

The results of the experiments are divided into two groups. The experimental results for the first group of ,anions are compared with calculated values, 'For the $\rm{second\ group},$ only the experimental results are given.

THEORETICAL

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In a similar manner and with the same assumptions as described by BECKERS AND EVERAERTS¹, who investigated the separations of cations, the following equations can be set up'.

Equilibrium equations

 $K_{A,i} = c_{H} c_{A,i}/c_{A,i-1}$

' Here the sample ions are anions, and the counter-ions are cations with buffering capacity.

J. Chromatogr., 69 (1972) 165-179

 $\left(1\right)$

$$
K_{B,i} = c_{H} \cdot c_{B,i-1}/c_{B,i}
$$

\n
$$
c_{A,i} = K_{A,i} \cdot c_{A,i-1}/c_{H}
$$
\n(2)

$$
c_{B,i} = c_{B,i-1} \cdot c_{H}/K_{B,i} \tag{4}
$$

Subsituting in eqns. 3 and 4 for $c_{A, i-1}$ and $c_{B, i-1}$, etc.:

$$
c_{A,i} = c_{A,0} \prod_{j=1}^{l} K_{A,j}/c_H^i
$$
 (5)

$$
c_{B,i} = c_{B,0} \cdot c_H^i / \prod_{j=1}^i K_{B,j}
$$
 (6)

For

$$
M_{i} = \prod_{j=1}^{i} K_{A,j}/c_H^{i}
$$
 (7)

$$
N_i = c_H^i / \prod_{j=1}^i K_{B,j}
$$
 (8)

eqns. 5 and 6 will be

$$
c_{\mathbf{A},i} = M_i \cdot c_{\mathbf{A},0} \tag{9}
$$

\n
$$
c_{\mathbf{B},i} = N_i \cdot c_{\mathbf{B},0} \tag{10}
$$

The total concentrations of the buffer and the anion present are

$$
c_{\mathbf{A}}^{\mathbf{r}} = c_{\mathbf{A},0} \cdot \left(1 + \sum_{i=1}^{n_{\mathbf{A}}} M_i \right) \tag{11}
$$

$$
c_{\mathbf{B}}^t = c_{\mathbf{B},0} \cdot \left(1 + \sum_{j=1}^{B} N_j \right) \tag{12}
$$

Isotachophoretic conditions

$$
E_{\rm U} \cdot m_{\rm AU} = E_{\rm V} \cdot m_{\rm AV} \tag{13}
$$

with

 \sim

$$
m_{\text{AU}} = \sum_{i=1}^{n_{\text{A}}} M_{\text{U}i} \cdot m_{\text{AU}i} / \left(1 + \sum_{i=1}^{n_{\text{A}}} M_{\text{U}i} \right)
$$
 (14)

Mass balance

$$
c_{\rm BU}^t \cdot (I + m_{\rm BU}/m_{\rm AU}) = c_{\rm BV}^t \cdot (I + m_{\rm BV}/m_{\rm AV}) \tag{15}
$$

with

$$
m_{\rm BU} = \sum_{i=1}^{n_{\rm B}} N_{\rm UI} \cdot m_{\rm BUI} / \left(1 + \sum_{i=1}^{n_{\rm B}} N_{\rm UI} \right) \tag{16}
$$

Electroneutrality

$$
c_{\text{OHU}} + c_{\text{AU}}^t \cdot \sum_{i=1}^{n_{\text{A}}} i \cdot M_{\text{U}i} / \left(1 + \sum_{i=1}^{n_{\text{A}}} M_{\text{U}i} \right) = c_{\text{HU}} + c_{\text{BU}}^t \cdot \sum_{i=1}^{n_{\text{B}}} i \cdot N_{\text{U}i} / \left(1 + \sum_{i=1}^{n_{\text{B}}} N_{\text{U}i} \right) \tag{17}
$$

J. Chromatogr., 69 (1972) 165-179

Modified Ohm's law

$$
I/K' = \text{constant} = E_{\text{U}} \cdot \left(c_{\text{OHU}} \cdot m_{\text{OHU}} + c_{\text{HU}} \cdot m_{\text{HU}} + \sum_{i=1}^{n_{\text{B}}} i \cdot c_{\text{AU}} \cdot m_{\text{AU}} + \sum_{i=1}^{n_{\text{B}}} i \cdot c_{\text{BU}} \cdot m_{\text{BU}} \right)
$$
(18)

Assuming the right-hand terms of eqn. 18 are Q_1 and Q_U for the first and the Uth zone, respectively, then the function

$$
QU = Q_1/Q_U - I \tag{19}
$$

 \bullet

must be zero,

With these equations, a similar computer program can be written, as described earlierl.

APPARATUS

The experiments are carried out with the apparatus described by **BECKERS** AND EVERAERTS². Instead of a differential thermocouple, a differentiator³ is used.

EXPERIMENTAL

In isotachophoresis, ions can be separated if their effective mobilities differ sufficiently.

The effective mobility can be defined as

$$
m_{\rm eff} = \Sigma \alpha_i \cdot m_i \cdot \gamma_i
$$

The degree of dissociation, α_i , depends mainly on the values of the pK, the temperature of the zone and the pH chosen. The value of y_i , a correction factor used in the Onsager relation, depends mainly on the ionic concentrations. The value of m_t depends on several effects, such as solvation, dielectric constant, viscosity of the solvent, and the radius and charge of the ion. All parameters influence the effective mobility and a well considered choice of the system to be used, makes a good separation possible.

The separation of anions can be carried out in different ways, as follows:

(1) The differences in absolute mobilities can be used for the separations of anions. A particular pH of the buffered system is chosen such that all anions are almost completely dissociated.

 (2) The differences in the pK values of the anions can also be used for the separation. A particular pH is chosen such that most anions are not completely **dissociated,** especially if many anions have about the same absolute mobilities. According to eqn. 20, a pH is chosen such that maximum differences in effective mobilities are obtained.

Se\$avations **t.iccording** *to mobilities*

To demonstrate separations according to mobilities, the pHs of the buffered systems are chosen as 6 and 7. Many acids have pK values up to 5.5, so that most acids will be almost completely ionised. **The set of the set of the**

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 (20)

TABLE I

SOME EXPERIMENTAL AND CALCULATED VALUES FOR ANIONS FOR THE SYSTEM HIST/HCI

a Concentration of the monovalent anion.

b Concentration of the divalent anion.

The system Hist/HCl. The leading electrolyte used was a mixture of histidine and hydrochloric acid at pH 6.02. The concentration of the leading ion, Cl⁻, was o.or N. The current was stabilised at 70 μ A.

The step-heights measured for all the ionic species, for which the experimental results were compared with calculated values, are given in Table I. The step-heights measured for the other ionic species are given in Table II. The step-heights refer to $\alpha \mu A.$

Many anions have the same or almost the same step-heights because their effective mobilities are almost equal, *i.e.*, they cannot be separated. From our experiments, we can state that anions can be separated if they differ by about 10% in stepheight, by using a thermometric detector.

TABLE II

SOME EXPERIMENTAL VALUES FOR ANIONS FOR THE SYSTEMS HISt/HCI AND Imid/HCI

sl. means that an indefinite step-height was obtained. The signal slides slowly to an end-point. t.sl. means too slow. The mobility of the anion was too low to finish the experiment, because of the limited potential delivered by the power supply.

Fig. 1 shows the electropherogram for the separation of a mixture of nitric, oxalic, tartronic, formic, citric, maleic, adipic, iodic, trichloroacetic and mandelic acids. Vitamin C was used as the terminator.

In Fig. 2, the electropherogram is given for the separation of sulphate, chlorate, chromate, malonate, pyrazole $3,5$ -dicarboxylate, adipate, acetate, β -chloropropionate and phenyl acetate. The terminator used was vitamin C. The concentrations of all the above anions in the samples were about 0.001 N . The sample tap volume was about 20 ul.

The system I mid HCl. The leading electrolyte used was a mixture of imidazole and hydrochloric acid at pH 7.05 . The concentration of the leading ion, Cl⁻, was o.or N. The current was stabilised at 70 μ A.

The absolute mobility of the counter-ion is larger than the mobility of the counter-ion applied in the system Hist/HCl. This means that all the zone resistances

J. Chromatogr., 69 (1972) 165-179

Fig. 2. Electropherogram for the separation of anions in the system Hist/HCl at $pH_L = 6.02$.

are smaller; consequently, all the step-heights decrease. However, their step-heights correlate correctly with the calculated zone resistances.

Some step-height shifts are remarkable. This can be ascribed to a dissociation, which is more complete in the system Imid/HCl.

Some examples of shifts are citric acid ($pK_3 = 6.4$), orthophosphoric acid $(pK_2 = 7.21)$ and chromic acid $(pK_2 = 6.49)$. All step-heights are given in Tables II and III.

In Fig. 3, the electropherogram is shown for the separation of a mixture of sulphate, oxalate, chlorate, formate, pyrazole $3,5$ -dicarboxylate, adipate, iodate, β chloropropionate and nicotinate. The terminator used was vitamin C. In this system,

TABLE III

SOME EXPERIMENTAL AND CALCULATED VALUES FOR ANIONS FOR THE SYSTEM Imid/HCI

a Concentration of the monovalent anion.

^b Concentration of the divalent anion.

Fig. 3. Electropherogram for the separation of anions in the system Imid/HCl at $pH_L = 7.05$.

ions can be separated simultaneously if the differences in their step-heights are about 10%.

Sefiarations according **to** *\$I< values*

As mentioned above, separations of anions can be carried out by using the differences between pK values, especially if the anions have about the same absolute mobilities. A pH is chosen such that different effective mobilities are obtained according to eqn. 20..

The choice of the pH of the leading electrolyte is limited. A minimum effective mobility of the ion species is required. If the pH of a zone is more than one unit lower than the pK value of the ionic species, such a low effective mobility is obtained that the potential required rises above the maximum potential of the stabilised directcurrent power supply. Another limitation is due to the buffering capacity of the counter-ion. A maximum buffering effect is obtained if $pK_B + r > pH_{\text{zone}} > pK_B - r$. If the pH of the leading electrolyte is chosen, the pHs of the following zones can be such that the buffering capacity of the counter-ion will be at a minimum in these zones. The relationship pK_{ionic species} and pH_{zone} is shown in Fig. 4 for a pK_B value of 6 and, a pH value of the leading electrolyte (pH_L) of 5.75.

Fig. 4 shows clearly that a buffer will have a minimum buffering effect if its pK value is more than one unit lower than the pK of the ionic species.

QUALITATIVE SEPARATION OF ANIONS 173

To demonstrate the possibility of separation according to pK values, eleven anions that cannot be separated in the system Hist/HCl ($pH_L = 6.02$) were selected. With a computer program, the effective mobilities were computed for five systems at lower pHs and the step-heights for the different systems where measured for the eleven anions. Table IV shows the conditions for the different systems. In Table V, the calculated effective mobilities and the measured step-heights are given.

Fig. 4. Relationship between pH_{zone} and the pK value of an ionic species.

In Fig. 5, the step-heights are plotted for the different systems. Fig. 5 shows that the differences in step-heights are much greater, to obtain a good separation, at lower pH values of the leading electrolyte.

Some separations were carried out. Fig. 6 shows the electropherogram for the separation of a mixture of trichloroacetate, β -chloropropionate, benzoate, crotonate, p -aminobenzoate and trimethylacetate at a pH_L value of 6.02. The terminator was glutamate and the leading ion was chloride. No complete separation could be achieved.

In Fig. 7, the separation is shown for the same mixture in system E (Table V) at a pH_L value of 4. Trimethylacetate was used as the terminator. A complete separa
tion could be obtained easily.

J. Chromatogr., 69 (1972) 165-179

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TABLE V

CALCULATIONS

With the computer program used, the concentrations, pHs and zone resistances of the zones, following the leading electrolyte were computed* for some anions in the systems Hist/HCl and Imid/HCl. The results are given in Table I for the system Hist/ HCl and in Table III for the system Imid/HCl.

Fig. 5. Graphical representation of the step-heights measured in the various systems.

The computations were first made without applying corrections for the influence of the temperature and the relaxation and electrophoretic effects.

Figs. 8a and ga show the relationships between the measured step-heights and calculated zone resistances for the systems Hist/HCl and Imid/HCl, respectively. Two distinguishable curves are obtained for the mono- and divalent anions.

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^{*} Data required for the computations were taken from refs. $4-6$.

Fig. 7. Electropherogram for the separation of an 2.8 of the same sample mixture as used for the separation in Fig. 6, in system E at pH_L = 4.0.
Complete separation has been obtained.

176

Fig. 8 (a). Relationship between the measured step-heights and the calculated zone resistances for some anions in the system Hist/HCl. No corrections were made in the computation. (b) The same relationship as shown in Fig. (a) but with corrections made in the computations.

Fig. 9 (a). Relationship between the measured step-heights and the calculated zone resistances for some anions in the system Imid/HCl. No corrections were made in the computation. (b) The same relationship as shown in Fig. (a), but with corrections made in the computations.

This can be understood easily. If the zone resistance is computed without applying corrections for the Onsager relation, deviations with the resistances actually present will occur. The zone resistances calculated will be smaller than the resistances actually present because the relaxation and electrophoretic effects are neglected. These effects decrease the mobility. Consequently, the resistance of the zone increases. Because these effects are greater for divalent anions, two distinguishable curves can be expected, as shown in Figs. Sa and ga. The influence of temperature is about the same for both mono- and divalent anions. Taking into account the influences of all these effects, one curve can be constructed.

After applying corrections for the influences of temperature and the Onsager effects, one curve is really obtained for both the mono- and divalent anions. Figs. 8b and gb show these curves for the system Hist/HCl and Imid/HCl, respectively,

curve is really obtained for both the mono- and divalent anions. Pigs. 8b and gb show these curves for the system Hist/HCl and Imid/HCl, respectively.

In all instances, the pH values before and after applying the corrections do not differ appreciably. For most ionic species, they differ by not more than about 0.1 pH unit. For this reason, no pH measurements were used as a check on the theory. How**ever, reasonable values were obtained by EVERAERTS AND ROUTS'.**

CONCLUSION

In this paper, we have used only the differences in pK values and mobilities for the separations of anions.

Changing the conditions as well as the combination of systems may be a help in solving certain problems.

a A very important part of the analyses, the quantitative aspect of the separations, is being studied at present and will be discussed in a later paper.

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LIST OF SYMBOLS

- c **Concentration, mole/l**
- **Ct Total concentration, mole/l**
- **23 Potential gradient, V/cm**
- **I** Electric current intensity, μ A
- **K Equilibrium constant**
- K' **Constant**
- *m* Mobility, cm²/V · sec
- m_{eff} Effective mobility, cm²/V · sec
- Mi **Computation constant**
- *n* Valence
- Ni **Computation constant**

J. Chromatogv., Gg (1972) r65-179

- Degree of dissociation α
- Correction factor \mathbf{v}

· Indices

- \mathbf{A} Anion A
- \bf{B} Buffer ion B
- ith step of dissociation, *i.e.*, for an anion *i* negative charges and for a cation i i positive charges
- H^+ ion $\mathbf H$
- OH OH-ion
- Undissociated part \mathbf{o}

 \mathbf{r}

- \mathbf{U} Uth zone
- \mathbf{v} Vth zone
- L Leading zone

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J. Chromatogr., 69 (1972) 165-179