

Short Communication

Isotachophoretic separation of polyols in boric acid solutions

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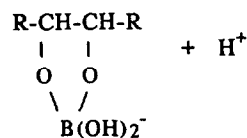
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ABSTRACT

This work deals with a new approach to fractionation of polyhydroxyl compounds and amphoteric ions. The new technique is based on isotachopheresis of borated polyols leading to pH gradient formation. The Theoretical section contains a number of equations to determine pH values for different borated polyol ion zones on the basis of their absolute electrophoretic mobilities or concentrations. The Experimental section gives a description of tentative experiments demonstrating the possibility of isotachopoietic separation of polyhydroxyl compounds.

INTRODUCTION

Boric acid can react with substances of polyhydroxyl nature to form borated polyols (BPs). This reaction is exploited to detect polyols and boric acid as well as to carry out electrophoresis of polyols [1]. Structurally, such complexes of boric acid and polyhydroxyl compounds are commonly presented as dissociable acid:



Compared with free boric acid, the complexes formed are stronger and more mobile in electric field acids.

It has previously been demonstrated that a borate–polyol system (BPS) can be used to produce pH gradients that can be applied to isoelectric focusing of proteins [2–4] and even cells [5]. BPS has some advantages over the widely used standard ampholine gradients, a disadvantage being their comparatively low temporal stability at alkaline pH. This is caused by appreciable BP electrophoresis in the alkaline region and has for a long time prevented BPS from becoming popular in research.

We have attempted to change BP electrophoresis from a destructive factor into the stabilizing one for both BP pH gradients and the electrophoretic system as a whole.

It has been reported [6] that boric acid may act as a complexing agent in the separation of substances by isotachopheresis. Provided ITP of complexes of boric acid and polyhydroxyl compounds is achieved, a discontinuous pH gradient will appear.

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THEORETICAL

From the molecular structure of BPs it can be concluded that observed differences in the electrophoretic mobilities of various complexes must be explained by differences in the composition or space configuration of the polyols used. For example, mannitol and sorbitol, which are stereoisomers, under identical conditions move at different velocities in an electric field. It seems natural to expect Kohlrausch boundary formation in an electrophoretic system into which polyols have been placed according to their mobilities. As voltage steps are formed on the boundaries of different polyol zones, mixing in the electrophoresis is likely to disappear. In other words, if the order of placing polyols into electric field in the presence of boric acid is correct, ITP is sure to take place.

Equilibrium in an ITP system is generally described by the widely used Kohlrausch equation:

$$\frac{C_1}{C_2} = \frac{m_1}{m_1 + m_s} \cdot \frac{m_2 + m_s}{m_2} \quad (1)$$

where C_1 and C_2 are ion concentrations, m_1 and m_2 are their absolute electrophoretic mobilities and m_s is absolute mobility of the common positive ion. The same equation is appropriate for the determination of polyol concentrations by ITP. BP absolute mobilities are given in ref. 3. On the other hand, net mobility, U , in boric acid solution is dependent on polyol concentration, C , according to the equation cited in refs. 2 and 3:

$$U = m \frac{(1-g)C_s + 10^{-\text{pH}}}{C} \quad (2)$$

where m is the absolute mobility of the BP ion, C_s is borate salt concentration and g is its degree of hydrolysis.

The fraction on the right side of the equation is the degree of BP complex dissociation. Since the dissociation of free boric acid and its salts is negligible in comparison with BP complex dissociation, practically all hydrogen ions and cations of the salt not participating in hydrolysis will act as counterions towards BP anions.

We have expressed C by means of eqn. 2 and

substituted C_1 and C_2 in the Kohlrausch equation for the formula obtained. Taking into account the ITP condition, *i.e.* $U_1/U_2 = 1$, we get:

$$\frac{10^{-\text{pH}_1} + (1-g)C_s}{10^{-\text{pH}_2} + (1-g)C_s} = \frac{m_2 + m_s}{m_1 + m_s} \quad (3)$$

Such a complicated expression of pH zone correlation is not very convenient for practical application, although it allows pH values to be related to the ion mobilities given in refs. 2 and 3. That is why we have obtained another expression including empirical, easily determined constants.

According to refs. 2 and 3, boric acid solution pH is known to depend on polyol concentration C owing to:

$$\text{pH} = e + f \log C \quad (4)$$

where e and f are constants characteristic of a certain polyol in boric acid of the given concentration.

Many reports in the literature (especially ref. 1) contain data on the boric acid solution pH *vs.* polyol electrophoretic mobility dependence. This relation is known to be of a typically sigmoid nature. We attempted to deduce empirically an equation that would express satisfactorily the pH *vs.* net mobility sigmoid curve. Sufficient accuracy here, as it happens, may be obtained from:

$$\text{pH} = g + \log \frac{U}{h - U} \quad (5)$$

where g and h are polyol-characterizing constants (Fig. 1).

Provided that ITP of two different polyols (with concentrations C_1 and C_2 and acidities pH_1 and pH_2) is attained, *i.e.* $U_1 = U_2$, it becomes possible to describe the correlation between all parameters of the two zones by means of empirically obtained eqns. 4 and 5 with the help of simple transformations.

Because of this we have to take the anti-logarithm of both sides of eqn. 5 and express U through the obtained equality. We then apply this expression of velocity to polyol zones 1 and 2, equalize the right sides of velocity equations for the two zones as is conditioned by ITP, express $10^{\text{pH}_1}/10^{\text{pH}_2}$ and logarithmically obtain an expression for pH difference between the two

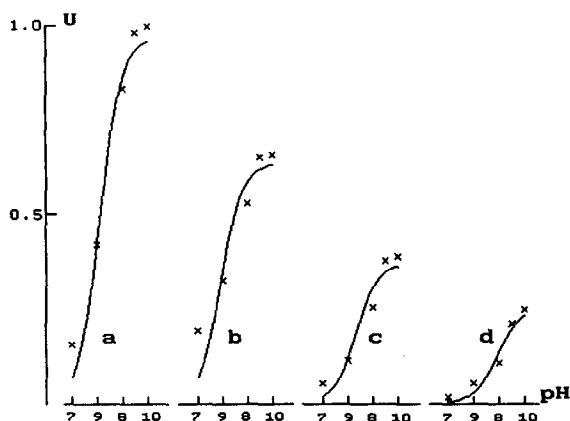


Fig. 1. Relative ionophoretic mobility (U) of various carbohydrates vs. pH (in the alkaline region) [1]. Glucose mobility (<math>pH < 10</math>) was chosen as a unit. Experimental data (\times) are approximated with the curves drawn on the basis of eqn. 5: (a) glucose, $g = 8.1$, $h = 14.6$; (b) mannose, $g = 7.9$, $h = 9.5$; (c) rhamnose, $g = 8.3$, $h = 5.5$; (d) cellobiose, $g = 8.9$, $h = 3.7$.

zones. Then we substitute 10^{pH} for $C \cdot 10^e$ in the right side of the equality for each of the zones. Such a substitution is possible as soon as the antilogarithm of eqn. 4 has been taken.

Since the "g" constant of different polyols varies very little and its value is close to 8.5, it could be considered to be a common constant for all compounds of this type. Under the condition of stable borate concentration, "e" values for different polyols are obviously also identical.

If 10^e , 10^g and $\log(h_2/h_1)$ are thus designated K_e , K_g and K_h , the expression for ITP of two polyol zones is as follows:

$$pH_1 - pH_2 = K_h + \log \frac{K_g + K_e C_1^{f_1}}{K_g + K_e C_2^{f_2}} \quad (6)$$

With this expression being applied, it becomes easy to describe the pH gradient that occurs when a polyol set is used in ITP.

EXPERIMENTAL

Chemicals

Polyhydroxyl compounds used included dulcitol, mannitol, sorbitol, galactose, glycerol, suc-

rose, maltose (in decreasing order of electrophoretic mobility in boric acid solution). Sodium hydroxide or tris(hydroxymethyl)aminomethane was added to obtain the required pH value of buffer solution with boric acid. All the chemicals were of analytical grade and were purchased from Serva (Heidelberg, Germany), including reagents for obtaining polyacrylamide gel. Of these, only galactose and dulcitol were from Sigma (St. Louis, MO, USA). The water used was twice distilled.

Instruments

The instruments used for vertical electrophoresis in tubes and horizontal electrophoresis in layers of polyacrylamide gel were manufactured by Hiju Kalur (Tallinn, Estonia). Ultrathin gel layers were obtained with a set of appliances from Serva. Voltage gradient in gels was measured with a platinum electrode, positioned with high precision by a device of our own design.

Preparation of solutions and gel

Below is given a concrete and simple example of a system in which one could observe Kohlrausch zone formation and ITP of polyols and proteins.

The basic buffer was 0.05 M boric acid and 0.0125 M tris(hydroxymethyl)aminomethane, pH 8.1. The anode solution was prepared by dissolving 0.05 M dulcitol in the buffer; the cathode solution was obtained by dissolving 0.05 M maltose in the buffer.

The following solution was prepared: 5 g of acrylamide, 140 mg of bisacrylamide and the anode solution mentioned above to a final volume of 50 ml. To 5 ml of that solution after deaeration was added 0.1 ml of mixture containing 200 mg of ammonium persulphate and 50 μ g of N,N,N',N'-tetramethylethylenediamine (TEMED) in 1 ml of distilled water. Slab gel, 125 mm \times 50 mm \times 0.3 mm, was formed using the solution described. Electrode wicks were soaked with anode or cathode solution. The electric current density in cooled gel layers was about 0.12 mA/m².

RESULTS AND DISCUSSION

BP compounds are known to be unsteady complexes that exist in aqueous solutions. It is because of their unsteadiness that it has been impossible to isolate them. This explains the importance of showing the possibility of ITP separation of unsteady-in-time BP ions.

ITP of polyols was observed both in a free solution without a supporting medium and in tubes and layers of polyacrylamide gel. In the first case (ITP in free solution), one should take into account the phenomenon of the solution density change on the Kohlrausch zone boundaries as well as the possibility of a change of polyol concentration during ITP. The resultant solution density gradient may have the opposite direction to the one of the gravitation field, which brings about a mixture of zones.

Boundaries of different polyol zones can be registered by measuring the electric potential or heat emission gradient, a striking change in optical refraction or pH indicator colour, etc.

The anode solution used in our experiments contained a high-mobility polyol, such as mannitol or dulcitol. The cathode solution contained, as a rule, such low-mobility polyols as glycerol or sucrose.

A mixture of polyols to be separated was placed directly onto gel, if the separation process was to be carried out in vertical tubes, or by sample applicator strips onto a horizontal gel layer.

After the electric field is applied, one can observe even visually the process of Kohlrausch boundary formation between the separating polyols as well as their motion as sharp steps of optical refraction. As in any ITP, the total electrical resistance of the system increases with time, which makes it necessary that the voltage is increased to maintain constant current.

Zone quantity and dimensions depended on the proportional composition of the sample mixture. If the mixture contained any proteins with distinct isoelectric points, their narrow bands divided by polyols were determined with Coomassie staining. BP bands were detected by means of voltage gradient measurement. One of the simplest cases is shown in Fig. 2.

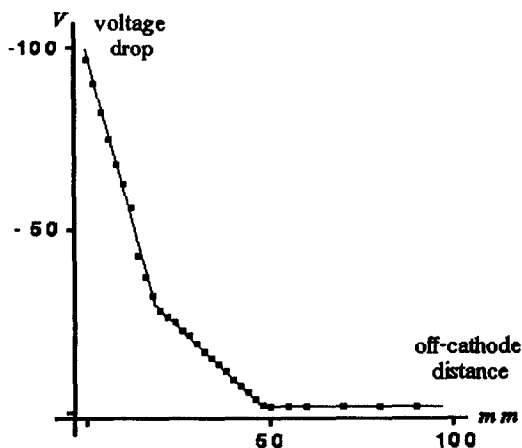


Fig. 2. Voltage gradient formed in a dulcitol-glycerol-maltose ITP system. The steepest part is associated with maltose, which is a carbohydrate of the lowest mobility; the almost horizontal part is associated with dulcitol, a polyol of the highest mobility and lowest specific electric resistance in this system.

In this very case a gel layer was prepared in the way described above, *i.e.* it contained dulcitol. A cathode paper wick was soaked with cathode solution containing 0.025 M maltose and 0.025 M glycerol. Fig. 2 shows that separation of maltose (the steepest part of the gradient) and glycerol (the mid-part of the gradient) occurred. This is evidence of how easily BP ITP in pH alkaline region can be performed.

CONCLUSIONS

The mobility of borated polyols is known to be inversely proportional to their dissociation constant in most cases [2,3]. Consequently, if polyols are gleaned correctly, the natural discrete pH gradient appears and becomes stable in the course of isotachopheresis. The acid part of this gradient belongs to the area of more mobile zones. There appears to be the possibility of using borate-polyol pH gradients not only for the traditional acid range but for the whole pH range. Such gradients can be applied to separation of amphoteric ion mixtures, in particular proteins. Moreover, this is an inexpensive method of purifying and separating substances of polyhydroxyl nature and non-amphoteric ions.

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