

## Phytic Acid can Greatly Enhance Resolution in Capillary Electrophoresis

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The addition of phytic acid to an electrolyte buffer has been found to improve the resolution of analytes in capillary electrophoresis; this improvement is thought to be mainly due to a lowering of electroosmotic flow.

Phytic acid [*myo*-inositol hexakis-(dihydrogen phosphate) **1**] is one of the various phosphate esters of *myo*-inositol. Salts of this hexaphosphate are most abundant in nature<sup>1</sup> and are found in appreciable quantities in extracellular material in higher-plant tissues.<sup>2</sup> For commercial use **1** is commonly extracted from grains of corn and sold in a variety of salt forms.

**1** is almost invisible to ultraviolet radiation (molar absorptivity  $<10 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) at wavelengths  $>ca. 200 \text{ nm}$ . The sodium salt ( $\text{C}_6\text{N}_9\text{Na}_{12}\text{O}_{24}\text{P}_6$ ), **2**, is soluble in water and the  $pK_a$  values<sup>3</sup> of the phosphoric acid groups range from 1.9 to 9.5 so that this molecule will maintain its polyanionic character over a wide range of pH. In fact at a pH of about 9, **2** has at least 11 negative charges. Thus at a concentration as low as  $2 \times 10^{-2} \text{ mol dm}^{-3}$  it will increase the ionic strength of an electrolyte buffer solution to at least  $1.3 \text{ mol dm}^{-3}$ . In contrast, a sodium chloride solution of the same molarity has an ionic strength of  $0.02 \text{ mol dm}^{-3}$ .

It is difficult to predict accurately the electrophoretic mobility of **2** in comparison to that of chloride ion [a commonly used ionic strength adjuster in capillary electrophoresis (CE)]. At  $\text{pH} > 9$  the polyanionic character of **2** is expected to enhance its electrophoretic mobility in comparison to that of chloride anions. However, the ionic radius of **2** in aqueous solution (including hydration) is roughly calculated† to be at least five times larger than that of chloride. In this way

the increase in current due to the addition of **2** to an electrolyte will be lower than that predicted solely from its anionic character.

Control of the ionic strength in CE is important for achieving enhanced resolution.<sup>4,5</sup> In general, resolution increases as the ratio of the concentration of the carrier electrolyte to that of the analyte is increased.<sup>6</sup> However, when attempts to achieve this are made either by the use of a simple inorganic salt such as sodium chloride<sup>7</sup> or by increasing the pH to values close to 12, a marked increase in current for a given applied voltage occurs. The resulting Joule heating is detrimental to analysis because of baseline instability and poor resolution. In this communication we demonstrate that the addition of small amounts of **2** ( $1 \times 10^{-2}$ – $3 \times 10^{-2} \text{ mol dm}^{-3}$ ) to an electrolyte solution in CE does not lead to a marked increase in current and can substantially improve peak shape and resolution. The effect of this relatively inert salt is demonstrated by the improved separation of both simple and more complex molecules.

CE measurements were carried out on a Beckman P/ACE system. Fig. 1(a) shows an electropherogram of a mixture of eight organic acids at pH 9.2. Most of the components are either incompletely (not baseline) resolved or not resolved at all. In contrast the addition of **2** at a concentration of  $3 \times 10^{-2} \text{ mol dm}^{-3}$  leads to much enhanced resolution of the eight compounds [Fig. 1(b)]. The order of elution has not changed. Moreover, the time of analysis is only about 2 min longer than in the absence of **2**. The effect of the addition of **2** on resolution is again clearly demonstrated in Fig. 2(a) and (b). These again show the considerably improved resolution of a mixture of oligodeoxyadenylic acids  $d(\text{dA})_{12-18}$  by the addition of **2** to the electrolyte buffer (pH 9.2). In this experiment,  $0.3 \text{ mol dm}^{-3}$  sodium borate alone as the electrolyte buffer gave a current of  $90 \mu\text{A}$ . The addition of  $1.5 \times 10^{-2} \text{ mol dm}^{-3}$  of **2** increased the current to  $125 \mu\text{A}$ . Another example of the effect of phytic acid on resolution is shown in Fig. 3. Under the separation conditions given the enantiomers of warfarin can

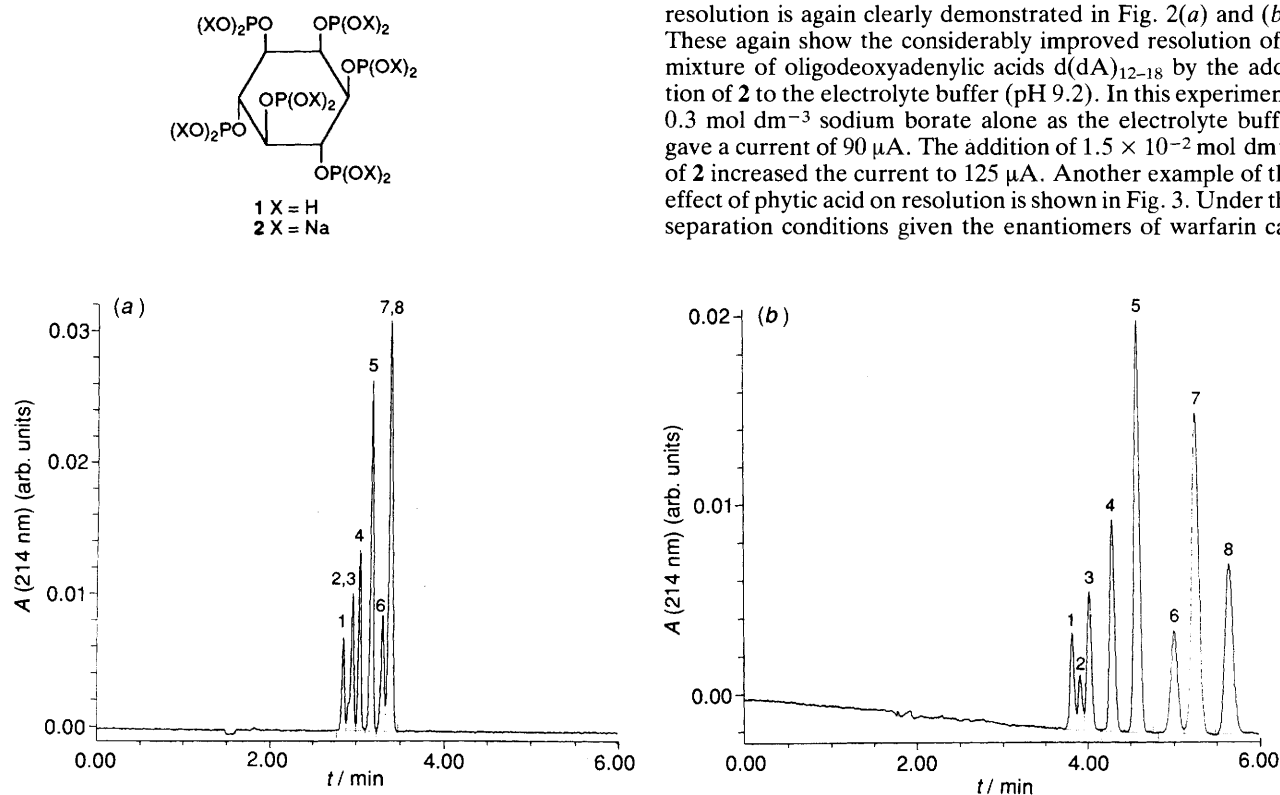


Fig. 1 Electropherograms of a mixture of eight organic acids in the absence (a) and presence (b) of phytic acid ( $3 \times 10^{-2} \text{ mol dm}^{-3}$ ). Capillary, fused silica,  $L = 27 \text{ cm}$ ,  $\text{id} = 50 \mu\text{m}$ ; carrier,  $0.3 \text{ mol dm}^{-3}$  borate buffer (pH 9.2); temperature,  $25 \text{ }^\circ\text{C}$ ; voltage,  $12 \text{ kV}$ . Numbers refer to the following compounds: **1**, *p*-chlorophenoxyacetic acid; **2**, 4-acetylbenzoic acid; **3**, anisic acid; **4**, *p*-bromobenzoic acid; **5**, *p*-nitrobenzoic acid; **6**, *p*-fluorobenzoic acid; **7**, benzoic acid; **8**, salicylic acid.

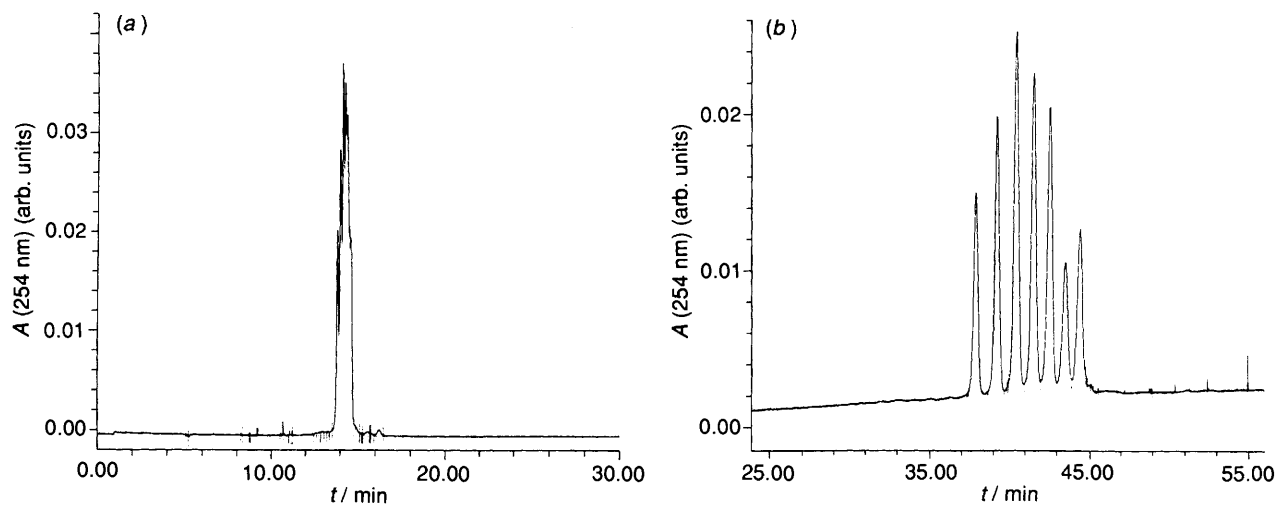


Fig. 2 Electropherograms of oligodeoxydeylic acids  $d(A)_{12-18}$  in the absence (a) and presence (b) of phytic acid ( $1.5 \times 10^{-2}$  mol  $dm^{-3}$ ). Capillary, fused silica,  $L = 57$  mm, id  $75 \mu m$ ; carrier,  $0.3$  mol  $dm^{-3}$  borate buffer (pH 9.2); temperature,  $25^\circ C$ , voltage,  $10$  kV.

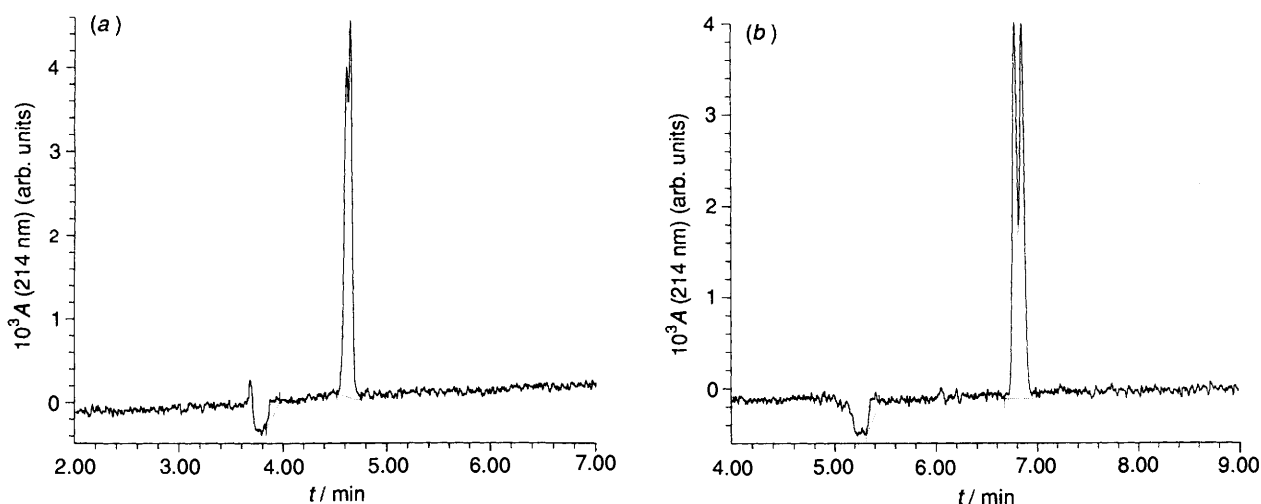


Fig. 3 Electropherograms showing the resolution of the enantiomers of warfarin in the absence (a) and presence (b) of **2**. Heptakisdimethylcyclomaltoheptaose ( $8 \times 10^{-3}$  mol  $dm^{-3}$ ) was added to the buffer solution. Other conditions as in Fig. 1.

only be clearly distinguished in the presence of **2**. The increase in migration time in the presence of **2** for all the examples studied is most probably due to a lowering of electrosmotic flow.

The manipulation of electrosmotic flow by an increase in ionic strength is well known.<sup>4,5,7,8</sup> However, in all cases this has been achieved by the addition of inorganic salts such as sodium chloride or sodium phosphate. The addition of these compounds has been found to limit the range of ionic strength that could be reached in practice owing to the occurrence of Joule heating at values  $>ca. 0.1$  mol  $dm^{-3}$ . We have now shown that the addition of polyanions can allow ionic strengths in excess of unity to be reached with ease and can considerably improve resolution in CE. We are presently investigating the mechanism by which **2** and a number of related polyanions effects electrosmotic flow. It is possible that the addition of these highly charged anionic species can reduce the zeta-potential by altering the thickness of the electrical double layer (predominantly made up of cations) in the vicinity of the negatively charged capillary wall.<sup>9</sup>

At present, we are carrying out studies to investigate the mechanism by which **2** improves resolution in capillary electrophoresis. We are especially interested in the wide applicability of the method and any relationship between resolution and the ionic state of analytes. A preliminary

investigation on the separation of peptides and proteins has given encouraging results.

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### Footnote

† The approximate ionic radius of **2** was calculated from the molecular volume of cyclohexane and phosphate in aqueous solution.

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