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Separation of plant growth regulators by capillary electrophoresis

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ABSTRACT

Capillary electrophoresis (CE) was used for the separation of nine plant growth regulators. Cyclodextrins and cholic acid were used as modifiers in the electrophoretic buffer to enhance selectivity. Satisfactory separation was obtained using a phosphate–borate buffer containing α-, β- and γ-cyclodextrin. The effects of pH and applied voltage on the migration behaviour in this system were studied.

INTRODUCTION

Plant growth regulators have been intensively studied by techniques such as high-performance liquid chromatography (HPLC) [1], gas chromatography [2] and bioassay [3]. However, their separation and migration behaviour in capillary electrophoresis (CE) have not been investigated. CE has recently developed into a powerful analytical technique which offers highly efficient separation. Instead of employing columns packed with stationary phase materials as is typically done in HPLC, blank fused-silica tubing can be used as the separation column. Enhancement of selectivity can be obtained by the addition of modifiers to the elctrophoretic medium [4,5]. The pseudo-stationary phase formed in this way can be easily changed by simply flushing the column with a new electrophoretic medium. Various types of modifiers have been proposed for CE separations [4–6]. In this investigation, the use of cyclodextrins and cholic acid as modifiers in the capillary electrophoretic separation of plant growth regulators was investigated. In addition, the effects of pH and applied voltage on the separation efficiency of the nine plant growth regulators were studied.

EXPERIMENTAL

The experiments were carried out on a laboratory-built CE system. A 30-kV laboratory-built power supply was used. A fused-silica capillary tube 50 cm effective length $\times$ 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) was used as the separation column. The peaks were detected using an on-column UV–VIS photodiode-array detector (Model SPDM6A, Shimadzu, Kyoto, Japan). The detector cell was modified according to the procedure described by Kobayashi *et al.* [7].

The nine plant growth regulators used were purchased from Sigma (St. Louis, MO, USA). Their structures and abbreviations are shown in Fig. 1. Other chemicals were obtained from Fluka (Buchs, Switzerland). The buffer solutions were prepared from sodium dihydrogenphosphate dihydrate and anhydrous sodium tetraborate. Hydrostatic injection was used. The samples were injected at a height 6 cm above the level of the reservoir and the injection time was 8 s.

RESULTS AND DISCUSSION

Fig. 2 shows an electropherogram obtained when no modifiers were added. The migration times in-

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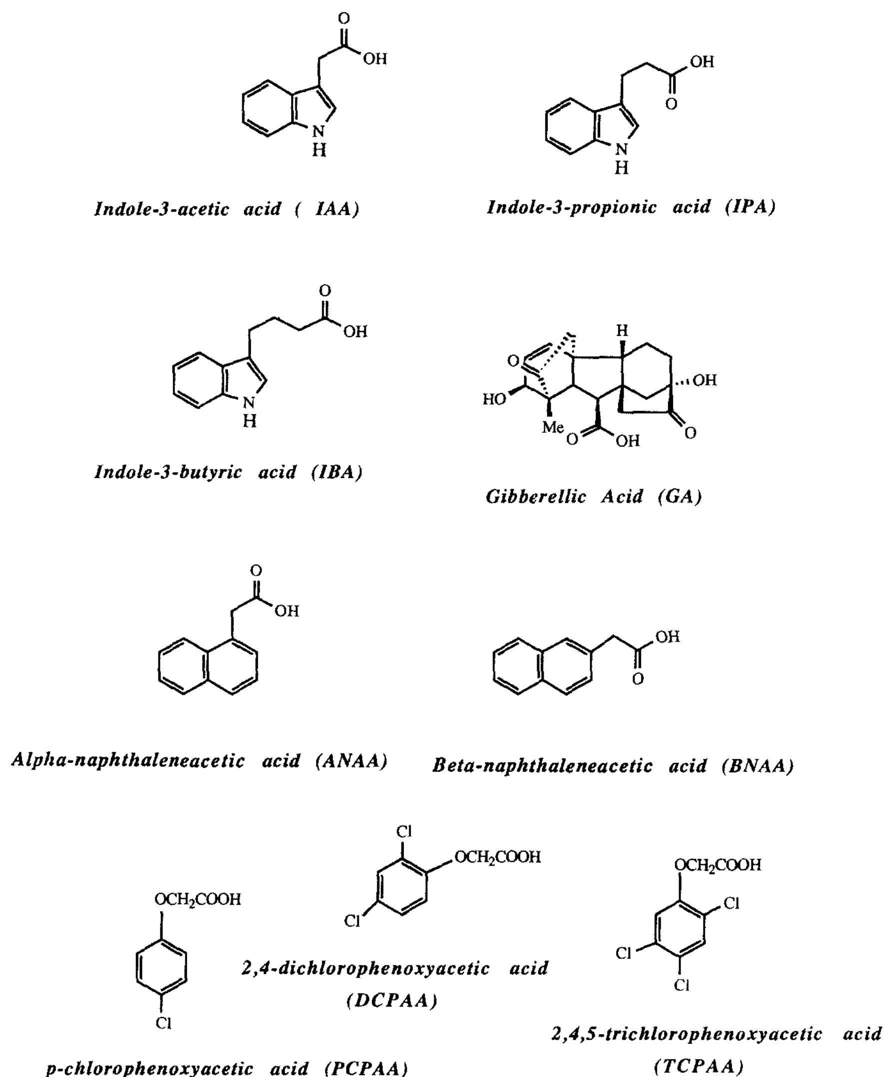

Fig. 1. Structures and abbreviations of plant growth regulators.

creased in the order GA < IBA < TCPAA < DCPAA, IPA < IAA, BNAA < ANAA < PCPAA.

In CE, separation is based on the differences in electrophoretic mobilities of the solutes in the presence of an electric field which is dependent on the size and charge of the solutes. In this investigation, the electroosmotic flow is towards the cathode, which is the low potential end. The direction of electrophoretic flow would depend on the charge of the solutes. For the positively charged solutes, both the electrophoretic and electroosmotic flows are in the same direction. However, for the negatively charged solutes, the electrophoretic and electroosmotic flows are in the opposite directions. Amongst the compounds investigated GA has the shortest migration time, mainly because it is the most hydrophilic among the nine plant growth regulators and its negative charge is least concentrated. Comparing the three indole acids, IBA is the first to migrate

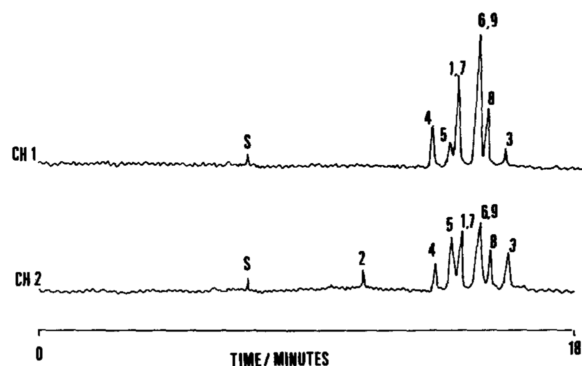

Fig. 2. Electropherogram of the nine plant growth regulators obtained with free solution capillary electrophoresis. S = methanol; 1 = DCPAA; 2 = GA; 3 = PCPAA; 4 = IBA; 5 = TCPAA; 6 = BNAA; 7 = IPA; 8 = ANAA; 9 = IAA. Electrophoretic conditions: 0.05 *M* phosphate–0.1 *M* borate buffer (pH 8.09); voltage, 15 kV; current, 36 μA; detection wavelength range, channel 1 220–230 nm, channel 2 196–210 nm; amount injected, 2 nl.

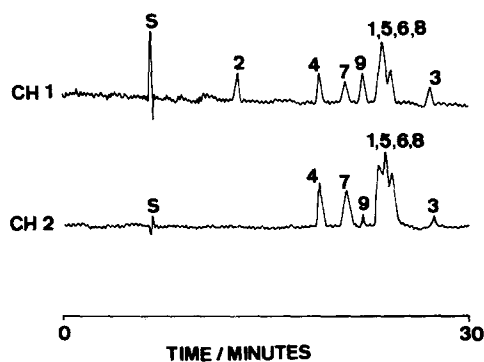

Fig. 3. Electropherogram obtained using cholic acid as modifier. S = methanol; 1 = DCPAA; 2 = GA; 3 = PCPAA; 4 = IBA; 5 = TCPAA; 6 = BNAA; 7 = IPA; 8 = ANAA; 9 = IAA. Electrophoretic conditions: 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.54)–20 m*M* cholic acid; voltage, 21 kV; current, 55 μA; detection wavelength range, channel 1 196–210 nm, channel 2 220–230 nm; amount injected, 2 nl.

out, followed by IPA and finally IAA. This could be explained by an increase in the chain length of the side-chain from acetic acid to butyric acid. An increase in chain length would lead to a decrease in the negative induction ($-I$) effect. Hence the negative charge on IAA is the most stable whereas that on IBA is the least stable. Among the three chlorophenoxyacetic acids, TCPAA has the shortest migration time followed by DCPAA and then PCPAA. This trend can be explained by the increase in the number of chloro substituents from PCPAA to TCPAA. As the number of chloro groups increased, the negative charge on the anion would be less concentrated. As TCPAA has the largest number of chloro groups, it would be expected to be the first to migrate out among the three. As BNAA is found to have a shorter migration time than ANAA, this would imply that it is either less negatively charged than ANAA or it is larger. By constructing the Stuart–Briegleb model, it is found that BNAA is indeed slightly larger than ANAA, hence it would be expected to have smaller electrophoretic mobility, resulting in a shorter migration time.

Bile salts have previously been used as modifiers in the CE separation of corticosteroids [6]. With cholic acid, compounds that are more hydrophobic would be expected to interact more with cholic acid micelles, thus leading to an increase in migration time for the more hydrophobic compounds. Hence cholic acid was investigated as a pseudo-stationary phase for the separation of plant growth regulators. The electropherogram for the plant growth regulators using cholic acid is shown in Fig. 3. In the presence of cholic acid micelles, it was observed that the less hydrophobic compounds such as the indole acids and GA migrated out faster than the more hydrophobic compounds such as the naphthaleneacetic and phenoxyacetic acids. However, DCPAA, TCPAA, BNAA and ANAA were not well resolved.

The presence of modifiers such as cyclodextrins resulted in a change in migration order and separation efficiency. This is due to the fact that the relative stabilities of cyclodextrin inclusion compounds are governed by various factors such as hydrogen bonding, Van der Waals interaction, solvation power and the ability of the molecule to fill the cavity [8,9].

Typical electropherograms obtained using cyclodextrins as modifiers are shown in Fig. 4. In the presence of α-cylodextrin as modifier (see Fig. 4a), the migration times increased in the order DCPAA $<$ GA, PCPAA $<$ IBA $<$ TCPAA, IPA $<$ BNAA, ANAA $<$ IAA. By using the Stuart–Briegleb atomic model, one can deduce that

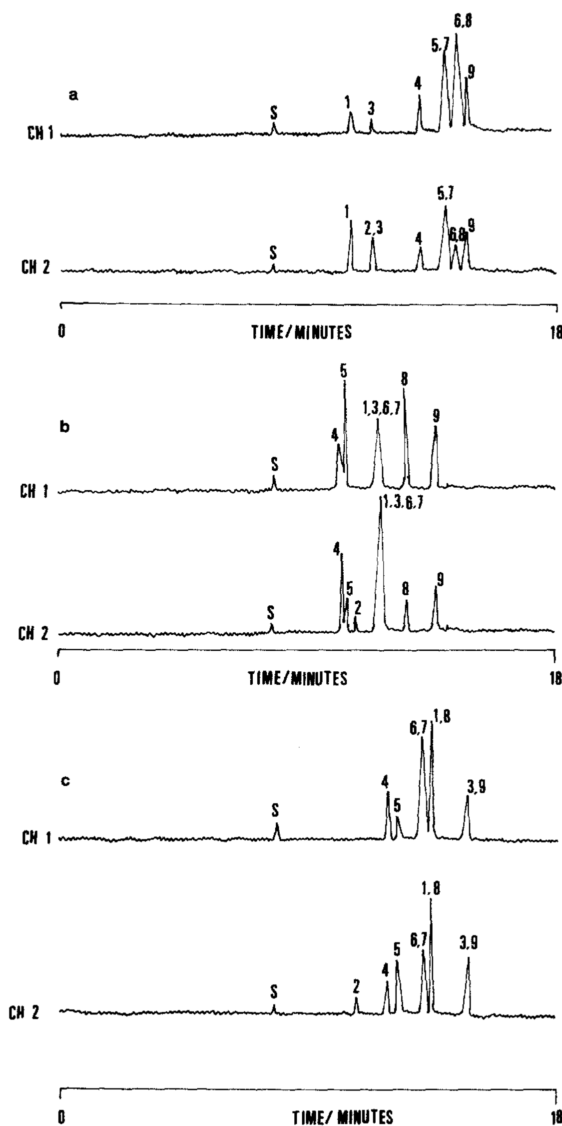

Fig. 4. Elecropherograms of the nine plant growth regulators obtained under the following electrophoretic conditions: (a) 0.05 M phosphate–0.1 M borate buffer (pH 8.09)–10 mM α-cyclodextrin; (b) 0.05 M phosphate–0.1 M borate buffer (pH 8.09)–10 mM β-cyclodextrin; (c) 0.05 M phosphate–0.1 M borate buffer (pH 8.09)–10 mM γ-cyclodextrin. S = methanol; 1 = DCPAA; 2 = GA; 3 = PCPAA; 4 = IBA; 5 = TCPAA; 6 = BNAA; 7 = IPA; 8 = ANAA; 9 = IAA. Voltage, 15 kV; current, 36 μA; wavelength range, channel 1 220–230– nm, channel 2 196–210 nm; amount injected, 2 nl.

DCPAA, PCPAA, TCPAA, GA, IBA and IPA cannot fit into the cavity of α-cyclodextrin completely. Solutes that can enter the cavity of α-cyclodextrin are found to have relatively longer migration times than those which are unable to enter the cavity. This observation leads one to conclude that, despite the fact that an increase in size would normally result in a decrease in electrophoretic mobility, thus resulting in a decrease in migration time [10], the bulky cyclodextrin structure results in it having a smaller overall mobility compared with the unsolubilized solutes which are much smaller. When only β-cyclodextrin was used, the migration order was (see Fig. 4b) IBA $<$ TCPAA $<$ GA $<$ DCPAA, PCPAA, IPA, BNAA $<$ ANAA $<$ IAA. When γ-cyclodextrin was utilized, the migration order again altered (see Fig. 4c), to GA $<$ IBA $<$ TCPAA $<$ BNAA, IPA $<$ DCPAA, ANAA $<$ PCPAA $<$ IAA.

As the use of the three different cyclodextrins gives rise to different migration orders, a decision was made to incorporate all three cyclodextrins in order to obtain optimum conditions for the separation. The optimum conditions were obtained by the use of the overlapping resolution mapping procedure (ORM), which is a commonly used systematic optimization technique for HPLC [11–15]. Modification of the ORM procedure for use in the optimization of CE separations has been described [15]. The electropherogram for the optimum separation is shown in Fig. 5. After the optimum conditions had been obtained, the effects of applied

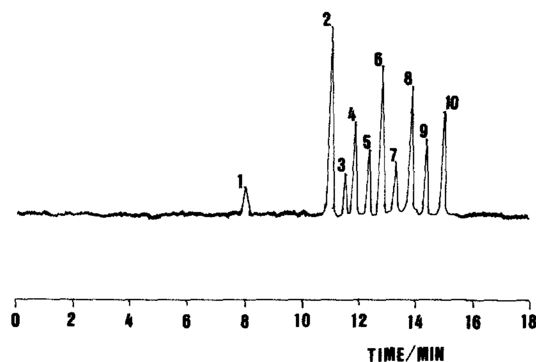

Fig. 5. Electropherogram of the nine plant growth regulators obtained under optimum conditions. S = methanol; 1 = DCPAA; 2 = GA; 3 = PCPAA; 4 = IBA; 5 = TCPAA; 6 = BNAA; 7 = IPA, 8 = ANAA; 9 = IAA. Electrophoretic conditions: 0.05 M phosphate–0.1 M borate buffer (pH 7.54)–7.5 mM α-cyclodextrin–1.5 mM β-cyclodextrin–1.0 mM γ-cyclodextrin; voltage, 15 kV; current, 35 μA; detection wavelength range, 196–210 nm; amount injected, 2 nl.

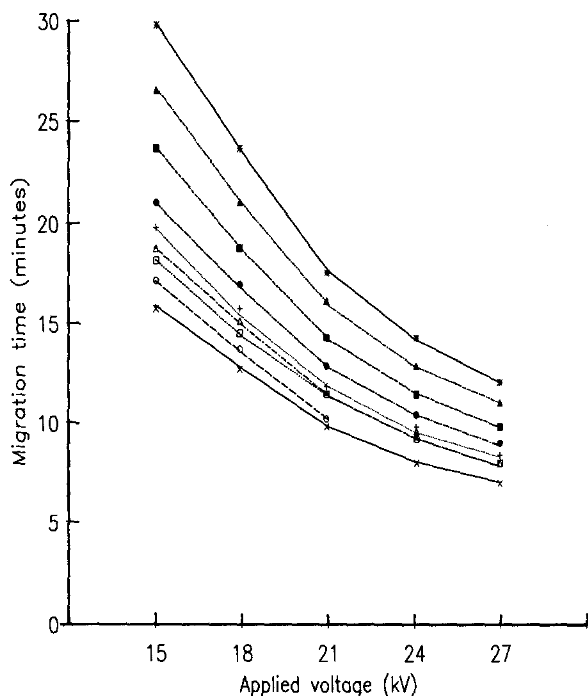

Fig. 6 Plot of migration time (min) against voltage (kV). × = DCPAA; ○ = GA; □ = PCPAA; △ = IBA; + = TCPAA; ● = BNAA; ■ = IPA; ▲ = ANAA; * = IAA.

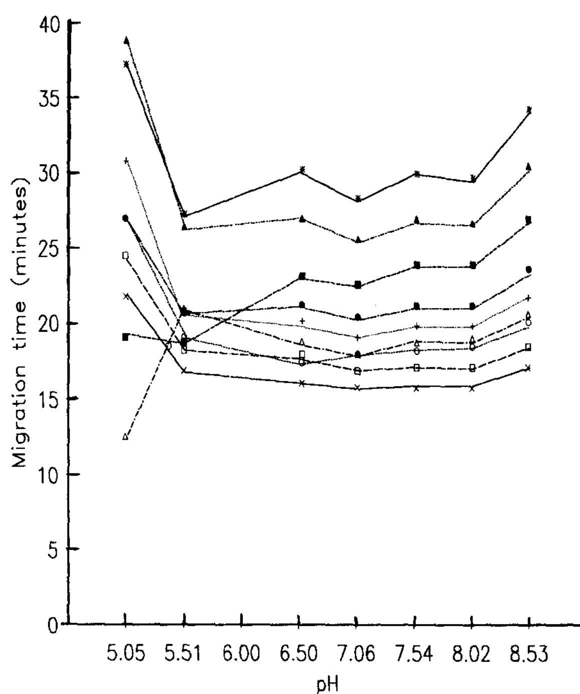

Fig. 7. Plot of migration time (min) against pH. Symbols as in Fig. 6.

voltage and pH on the separation efficiency were investigated.

Fig. 6 shows that as the applied voltage increased, the migration time decreased. This is to be expected, as the electroosmotic flow increased with increasing voltage. However, gibberellic acid was not detected at all at voltages higher than 24 kV. A possible explanation could be that it decomposes at high voltage owing to larger amounts of Joule heating.

Fig. 7 shows in general that the migration times tend to increase with increase in pH from pH 7.06 to 8.53. However, there is a general decrease in migration time from pH 5.05 to 7.06. This could be explained by an increase in electroosmotic flow when there is an increase in pH. The increase in migration time at higher pH (>7.50) could be explained by the dissociation of the acid group in the plant growth regulators resulting in the production of anions, thus increasing the migration time. At pH 4.47 (results not shown), the migration times for all the samples were found to be more than 2 h, because at low pH, the ionization of the surface silanol groups is suppressed and the electroosmotic flow approaches zero [16]. For IBA, an increase in migration time from pH 5.05 to 5.51 was observed. This could be due to fact the pK_a of IBA is more than 5.05, hence it dissociates above pH 5.05, resulting in an increase in migration time.

A point to note is that the migration times obtained when the effects of pH and applied voltage were studied were longer than those obtained in the earlier part of the experiment. This is probably due to the fact the latter were investigated at a much later stage of the experiment and the increase in migration times could be due either to adsorption of a compound on the capillary wall or to the depletion of hydroxyl groups on the wall. In this instance, it is probably due to the depletion of the hydroxyl groups which resulted in the wall being less negatively charged. Consequently, a decrease in the electroosmotic flow and hence an increase in

migration times are observed. It is unlikely that the plant growth regulators are adsorbed on the wall as they would be negatively charged under the pH conditions used, which means that they would be repelled from the wall instead. Nevertheless, it was found that the migration order remained the same, so the discrepancy was not investigated further.

The use of a photodiode-array detector enables one to detect the solutes at more than one wavelength. For example, the ratio of peak heights observed at two different wavelengths was used to confirm the identity of the peaks. With reference to Figs. 2–4, it can be observed that GA was only detected in the wavelength range 196–210 nm. Further, the two naphthaleneacetic acids were found to absorb more strongly at 220–230 nm whereas the chlorophenoxyacetic acids absorb more strongly at 196–210 nm. The indole acids absorb almost identically in both wavelength ranges. Thus, by comparing the ratios of the peaks at different wavelengths and the migration times in an electropherogram, the identity of the peaks could be determined quickly and accurately.

In conclusion, CE separation of plant growth regulators can be achieved by using an electrophoretic medium containing cyclodextrins as modifier. The migration order changes with the type of cyclodextrins used and the size of their cavities. Optimum separation could be achieved when an electrophoretic medium containing α-, β- and γ-cyclodextrin was used. The migration behaviour of the compounds in such an electrophoretic medium at different voltages and pH was studied. The general trends in the migration times could be related to the expected changes in electroosmotic flow.

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