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# Electrophoretic Studies on Cyclodextrins: Separation of Cyclodextrins on Polyacrylamide Gels

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Separation of cyclodextrins by gel electrophoresis has been carried out. Conditions for a favourable separation of cyclodextrins on polyacrylamide gels have been standardised. The method has been subsequently used for a semiquantitative estimation of the individual cyclodextrins in the starch hydrolysate obtained during biochemical synthesis of cyclodextrins and also for checking the effect of inclusion of guests on mobility of cyclodextrins.

Cyclodextrins are a series of oligosaccharides obtained by the action of amylase on starch[1]. Treatment of starch with *B. macerans* amylase gives starch digest containing α-, β- and γ-cyclodextrins as the major products[2]. The method of obtaining a mixture of cyclodextrins is simple; however, their consequent detection and hence separation is difficult due to their closely related properties[3]. The separation of cyclodextrins has been routinely done by taking advantage of the different dimensions of their cavities. Using appropriate guest structures, the cyclodextrins are selectively precipitated[4]. Techniques like adsorption chromatography[5] and high temperature cellulose column chromatography[6] are used along with thin layer chromatography[7], circular paper chromatography[8] and HPLC[9] for their detection in mixtures.

Gel electrophoresis has been used as a routine separation technique for the analysis of proteins[10]. Cyclodextrins form a bridge between the conventional organic molecules and proteins, considering their molecular dimensions and molecular weights. Separation of cyclodextrins using gel electrophoresis method was therefore anticipated, after a proper adjustment in the pore size of gel.

In continuation of our studies on cyclodextrins[11], we report in this paper, a method for the separation of individual cyclodextrins from their mixtures as well as from the starch hydrolysate, on polyacrylamide gels by electrophoresis. The effect of parameters like length and pore size of gel, time of electrophoresis, buffer used for electrophoresis, concentrations of cyclodextrins, etc., has been studied and the optimum conditions for effective separation are described. Cyclodextrins are known for their property of forming 'inclusion compounds' both in solid as well as solution state[12]. The effect of inclusion on mobility of cyclodextrins on gel has been also studied.

## Materials and Methods

Cyclodextrins were obtained commercially and were further purified by crystallisation. Acrylamide, N, N'-methylene diacrylamide (Bis), Temed, Amidoschwartz, tris (hydroxymethyl) methyl amine, ammonium persulphate, sucrose, bromophenol blue, etc., were obtained commercially and used as such.

*Apparatus for electrophoresis*—The study was carried out using an electrode vessel fabricated in our laboratory. A Toshniwal powerpack was used for adjustments of electrical variables.

The procedure of electrophoresis employed is the original disc gel electrophoresis system of Ornstein and Davis[13,14] modified for the separation of cyclodextrins, as indicated below:

(a) Gel composition was altered by using different concentrations of total acrylamide from 7 to 18% keeping the bisacrylamide concentration fixed at 5% of the total acrylamide. The gels were polymerised and used immediately.

(b) The cyclodextrins were loaded in the form of their aqueous solutions and the hydrolysate was concentrated to the maximum possible extent before loading.

(c) The tris-glycine (*p*H 8.3) and barbitone buffer (*p*H 8.6) were used. The gels were however prepared in tris-HCl buffer.

(d) The electrical current was maintained for 2 hr at 50 mA, after keeping it at 20 mA for first 10 min.

(e) The gels were removed from the tubes using a solution of 1:1 glycerol and water.

(f) Amidoschwartz (1%) in acetic acid was used as a stainer dye for 1 hr. The destaining was done by repetitive washings with 7% acetic acid.

(g) Gels were preserved in 7% acetic acid.

## Results and Discussion

The conditions of electrophoresis and variables affecting it had to be standardised so as to get an

effective separation of cyclodextrins. The parameters whose effects were studied are listed below.

*Constitution of gel*—Since the sizes of cyclodextrins are smaller as compared to those of proteins, a gel with a pore size matching with the molecular size of cyclodextrins is needed. The pore size can be reduced by keeping the concentration of N, N'-methylene diacrylamide at 5% and changing the total acrylamide concentration. The effect on mobility of 7 to 18% gel was studied and 16% gel was found to be the most suitable. A gel of a concentration greater than 16% is too brittle and it becomes difficult to remove it from the tubes while that of a lower concentration does not yield a good separation.

*Dimensions of gel*—Though the dimensions of gel are not very important, considering the low mobility of cyclodextrins, a gel of 6 cm length and 6 mm internal diameter was employed. Too long a length of gel reduces the electrical field effect on its mobility while a large gel breadth demands an increase in the cyclodextrin for effective visual, photographic or densitometric detection.

*Buffer*—The selection of buffer is important as it affects the mobility considerably. The interaction of buffer with cyclodextrins leads to a better separation of the latter e.g. the remarkable effect of borate buffer in the separation of cyclodextrins is reported[15] in literature. Considering the different inclusion behaviour of α- and β-cyclodextrins towards barbiturates, a considerable change in mobility and a better separation is expected with barbitone buffer, and this is observed experimentally. Furthermore, the mobility of α-cyclodextrin on a gel prepared with tris-HCl buffer is found to be more than that on the gel prepared in barbitone buffer, as revealed by their relative mobilities (0.29 and 0.18 respectively).

The gels were, therefore, prepared in a tris-HCl buffer, thus employing a discontinuous buffer system.

*Time of electrophoresis*—The low mobility of cyclodextrins requires a larger time of electrophoresis for a satisfactory separation. A total electrophoresis period of 120 min was found to be optimum. A time period of less than 45 min did not yield any separation of cyclodextrins, and a time period above 120 min heated the buffer and electrophoretic assembly causing disturbances.

*Electrical parameters*—The low mobility of cyclodextrins necessitates a higher potential difference between the electrodes and a high current flow. Thus, a steady current of 5 mA per tube was maintained at 450 V.

*Concentration of cyclodextrins*—A concentration lower than 10 mg/ml shows a faint band and thus a minimum of 10 mg/ml for α-and 12 mg/ml for β-cyclodextrin is required. The lower detection limit poses a problem for detection of cyclodextrins from the starch hydrolysate. The hydrolysate was therefore, concentrated before analysis. Addition of authentic cyclodextrins was used to identify them. However, the higher cyclodextrins were not detected probably because of their low concentrations which are below the limit of detection.

Table 1 —Effect of Major Parameters on Migration of Cyclodextrins

| Composition (%) (mobility) | Inclusion of Guest (mobility) |
|---|---|
| 7 (0.57)* | 2, 6-Dichlorophenol-indophenol (0.68*, 0.18†) |
| 14 (0.36)* | |
| 16 (0.29)* | |
| 18 (0.25)* | Bromophenol blue (0.46*, 0.40†) |

* Mobility of α-cyclodextrin † Mobility of β-cyclodextrin

*Effect of inclusion*—A change in mobility of cyclodextrins is observed on inclusion of guests. Inclusion is expected to change the molecular weight and charge and hence the mobility on gel under the influence of electrical forces. As the inclusion behaviour is different for α- and β-cyclodextrins, a further change in their relative mobilities is expected. A guest molecule getting included only in one of the cyclodextrins would lead to an even better separation. The effect of bromophenol blue, a dye which is also used here as a marker, has been studied. A marked change on the mobility of α- and β-cyclodextrins is observed on its addition. Addition of 2, 6-dichlorophenolindophenol to cyclodextrins also changes their mobilities leading to better separation. Table 1 shows effect of these guests on mobilities. A striking observation, which could not be explained, is that the mobility of α-cyclodextrin is much more in the absence of β-cyclodextrin than in its presence.

*Marker and stainer dye*—Amidoschwartz is used as a staining dye for carbohydrates[16] and serves well for cyclodextrins too. The marker dye, bromophenol blue, used as a reference for mobility studies with proteins, was used for cyclodextrins also. However, considering the inclusion properties of cyclodextrins, the mobility of marker dye was checked on a gel separately and not along with cyclodextrins. The separation of cyclodextrins obtained on the polyacrylamide gels by following the above method, was then interpreted by densitometric scanning of these gels. The identification of bands was performed by comparison with standard cyclodextrin samples. Fig. 1 is a representative set of the densitometric scans illustrating these separations.

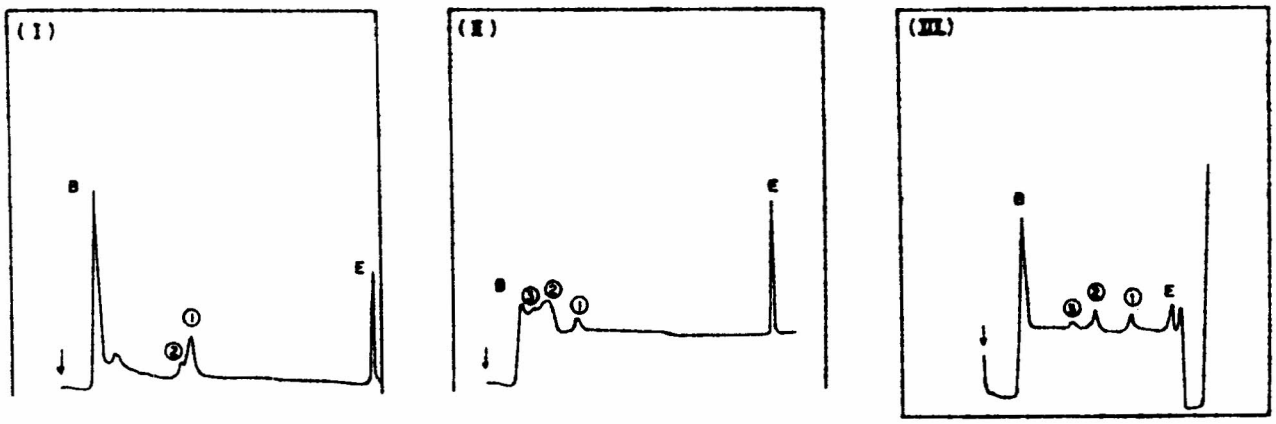


Fig. 1—Densitometric scan showing the separation of cyclodextrins on polyacrylamide gels [I, mixture of α- and β-cyclodextrins; II, hydrolysate; III, concentrated hydrolysate+cyclodextrins; B, beginning of gel; E, and of gel]

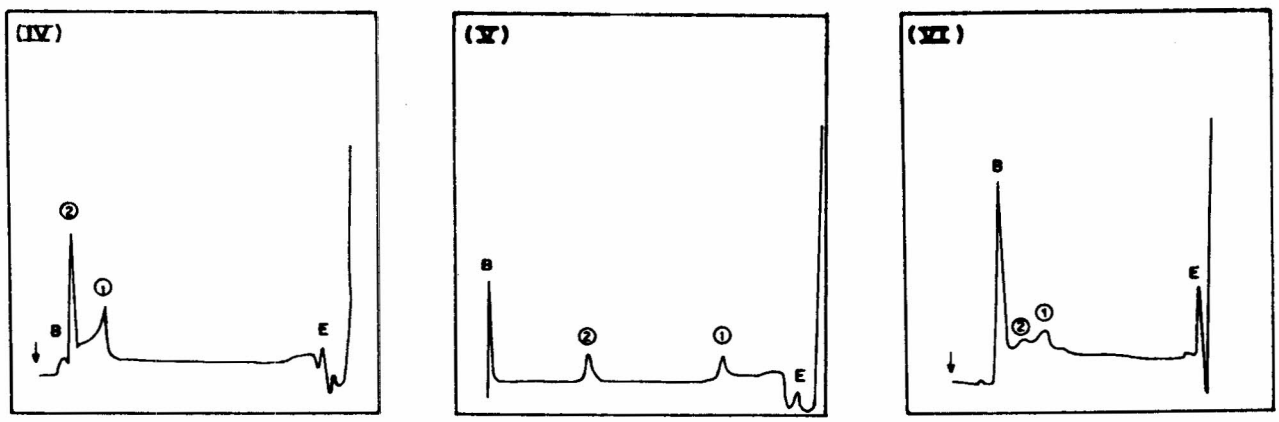


Fig. 2—Densitometric scan showing the effect of buffer and inclusion on cyclodextrin separation [IV, effect of buffer on mobility; V, effect of inclusion of 2, 6-dichlorophenolindophenol; VI, effect of inclusion of bromophenol blue; B, beginning of gel; E, and of gel]

Table 2—Identification and Relative Compositions of Cyclodextrins

| Peak No | Cyclodextrin | Mean rel. mobility ($r$ m)* | Composition(%) in Physical mixture Calc. (prepared)† | Composition(%) in starch hydrolysate |
|---|---|---|---|---|
| 1 | α-Cyclodextrin | 0.22 | 54.12 (50) | 16.66 |
| 2 | β-Cyclodextrin | 0.09 | 45.88 (50) | 80.00 |
| 3 | Higher cyclodextrin (Unidentified) | 0.044 | — | 3.33 |

* S.D. = 0.04; relative mobility with reference to bromophenol blue. † Composition by calculation of peak areas of bands as obtained by densitometric scanning

Effect of some variables on these separations is shown in Fig. 2 and Table 1. The cyclodextrins in hydrolysate were identified by addition of standard samples and also by comparison with authentic cyclodextrins. The densitometric scans were measured by triangulation and planimetry for a semiquantitative estimation of cyclodextrins in their mixtures and hydrolysate (Table 2).

The above investigation successfully correlates the mobility of cyclodextrins with many parameters affecting it. The method developed provides a simple way for the detection and determination of cyclodextrins in a mixture obtained from the starch hydrolysate or otherwise. A preparative aspect of the method i.e. a large scale isolation of cyclodextrins from their mixtures would be studied soon.

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