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Polarographic detection of beta-cyclodextrin inclusion complexes

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Summary

The effect of beta-cyclodextrin (β-CD) on the peak height and voltage of the polarographic reduction of several esters of hydroxybenzoic acid has been investigated. Complexation with β-CD decreased both the amount of current conducted upon reduction and the half-wave potential of that reduction for each of the esters of hydroxybenzoic acid. This fall in conducted current resulted from the decreased diffusion rate of the ester when complexed with β-CD as compared to the ester alone. The half-wave potentials of the reductions became more negative in the presence of β-CD. The change in potential was in the rank order ethyl > propyl > butyl. This was a result of the electron redistribution occurring in the presence of β-CD due to the formation of inclusion complexes, and reflected the tendency of these esters to complex with β-CD. These results suggest that polarography is suitable for studying the inclusion complexation phenomenon of β-CD with electroactive molecules in aqueous solution and may prove to be a powerful technique in further elucidating the nature of the inclusion complex.

Introduction

Beta-cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of seven α-1,4-linked glucopyranose units. This ring shaped molecule encloses a central cavity which can act as a 'host' into which 'guest' molecules may fit. β-CD is therefore capable of forming inclusion complexes with a wide variety of drug molecules and this can confer advantageous properties upon these molecules. Inclusion complexation for many drugs has been widely reported (Szejtli, 1982) and recently reviewed (Jones et al., 1984a and b).

Methods of detection of inclusion complexes in the solid state are well documented. In aqueous solution however, there is a lack of definitive techniques to determine whether complexation occurs.

For an electroactive molecule, polarography would seem to represent a possible technique for studying inclusion complexation. If complexed, the electron distribution of a guest molecule would be different from that in its uncomplexed state, and this should be polarographically detectable. There are many examples of organic drug molecules that are electrochemically active (see Volke, 1983), hence the potential application of polarography.

p-Hydroxybenzoic acid and its esters were chosen as model compounds. Cohen and Lach (1963) have reported on the interaction of the hydroxy-

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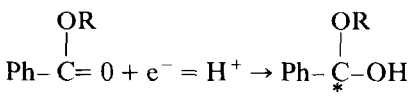
benzoates in aqueous solution using a solubility technique. The parent acid is electroreducible since its dissociated protons are available for reduction at the dropping mercury electrode (DME).

The carbonyl group of the esters of *p*-hydroxybenzoic acid would be predicted to be electroreducible according to the general mechanism:



R = methyl, ethyl, propyl or butyl; * = free radical (according to Volke, 1983). The free radical may go on to form many different reaction products, depending upon the reaction conditions and other reduction products present. The polarographic response of the hydroxybenzoate should follow a pattern as the alkyl groups increase in size and electron donating ability.

Although unconnected with the polarographic analysis of the hydroxybenzoates per se, the response of copper and zinc ions in the presence of β-CD was also examined. β-CD has been reported as being adsorbed onto the mercury drop of a DME (Matsui et al., 1975, Daruhazi et al., 1981 and 1982). Adsorption, if it were to occur, would only be of concern if the reduction processes at the DME were altered. Copper and zinc were chosen to investigate this contention because they do not form complexes with β-CD and are reduced at approximately the same potentials as the organic molecules to be studied.

Materials and Methods

Materials

β-CD (Chinoin Chemical and Pharmaceutical Works, Budapest), *p*-hydroxybenzoic acid (Sigma Chemicals, Poole, Dorset), methyl and ethyl hydroxybenzoate (Thornton and Ross, Huddersfield), propyl and butyl hydroxybenzoate (Sigma Chemicals, Poole, Dorset), D-glucose, potassium chloride, anhydrous sodium acetate, copper acetate (East Anglia Chemicals, Ipswich), zinc acetate (May and Baker, Dagenham), glacial acetic acid (Fisons PLC, Loughborough) and absolute ethanol (Burroughs Ltd., London) were all of analytical quality. Polarographic mercury was trebly distilled and also of analytical quality (Elgar Phosphors and Chemicals Ltd., Enfield).

Methods

The differential pulse polarograms of each test solution were recorded using a Princeton Applied Research Model 384 Polarograph in conjunction with a Model 303 DME. Prior to testing, each solution was purged with oxygen-free nitrogen for 3 min and then scanned across a pre-set voltage range (in the region of 0 to −1.8 V). The scan increment for this range was 50 mV and each concentration of electrochemical species was scanned across the entire voltage range twice. The polarograph averaged the currents conducted, subtracted the current of the support electrolyte (blank) and plotted the resultant polarogram. Stock solutions of β-CD, copper acetate and zinc acetate were prepared in pH 5.0 Walpoles acetate buffer (the support electrolyte).

Stock solutions of *p*-hydroxybenzoic acid and each of its esters were prepared in absolute ethanol. This was to avoid solubility problems with the higher molecular weight esters. The support electrolyte (blank) for these analyses was 0.1 M potassium chloride. Stock solutions of β-CD and glucose were also therefore prepared in 0.1 M potassium chloride.

After scanning 10 ml of the appropriate electrolyte blank, successive 100 μl aliquots of stock solution were added, producing 100-fold dilutions. After each addition, the solution was scanned across a pre-set voltage range and the presence of any reducible molecules resulted in a peak on the polarogram. The peak heights were plotted against the total amount of electroactive species present, if appropriate.

Results and Discussion

Polarographic detection of copper and zinc

A strong reduction peak for copper was obtained at −0.016 V and for zinc at −1.012 V. The height of these peaks increased linearly with an increase in concentration of reducing species. The

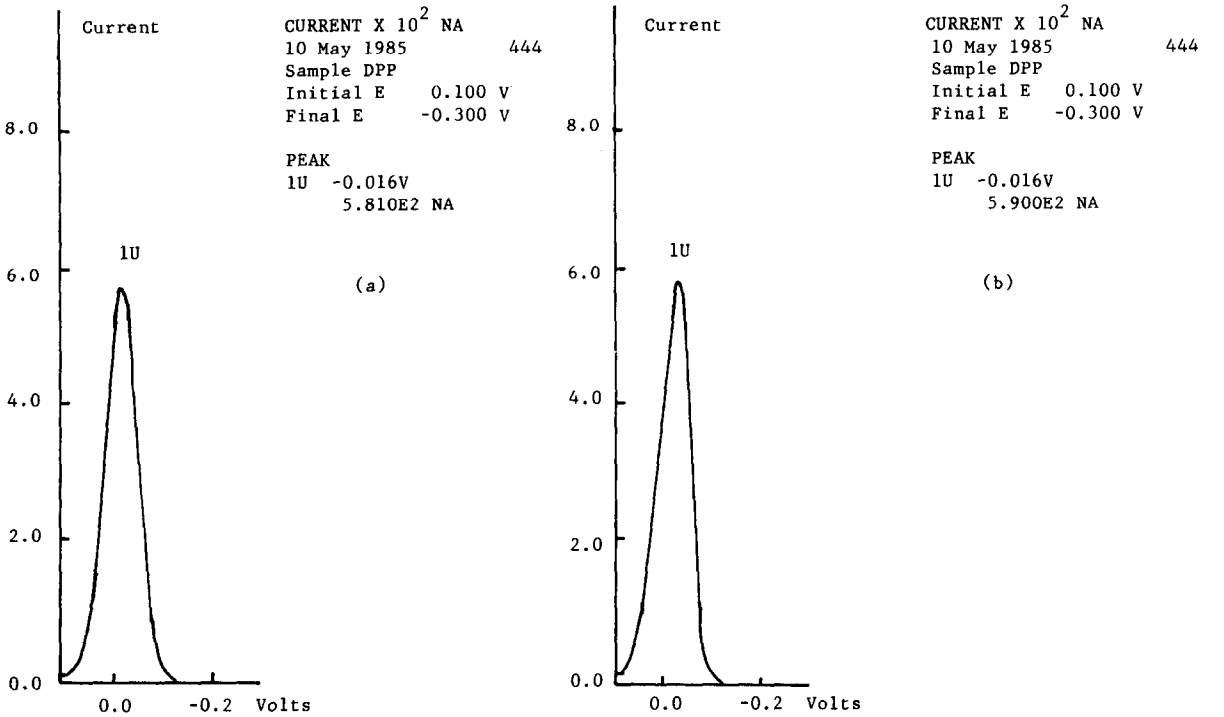


Fig. 1. Polarograms of Cu^{2+} (1.6×10^{-5} M) alone (a) and with 10^{-3} M β-CD (b) in pH 5.0 acetate buffer.

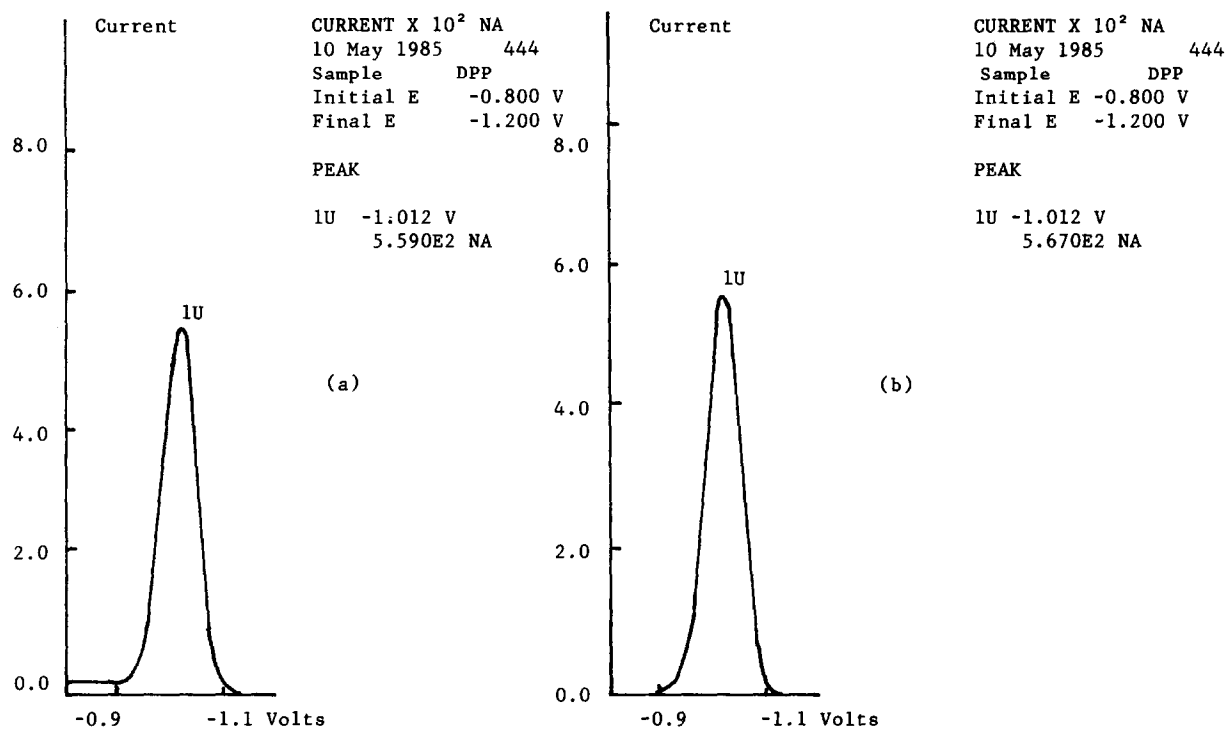


Fig. 2. Polarograms of Zn^{2+} (1.36×10^{-5} M) alone (a) and with 10^{-3} M. β-CD (b) in pH 5.0 acetate buffer.

presence of 10^{-3} M β-CD had no effect on the height or reduction potential of either of these metals. Fig. 1 shows a copper reduction peak in buffer and in the presence of β-CD. Similar polarographic responses for zinc are shown in Fig. 2. If β-CD is being adsorbed onto the mercury drop, then it is not in any way affecting the polarographic responses (and hence reduction processes) of copper and zinc, neither of which form complexes with β-CD.

Reduction of β-CD

Concentrations of β-CD ranged from 10^{-5} to 10^{-3} M. A peak was just noticeable for the reduction of β-CD, at approximately −0.17 V, although the height of this peak did not increase linearly with increasing concentration. The potential of the peak also became less negative as the concentration increased. Although electroactive, the size and voltage of the reduction was such that the polarographic response of β-CD could be ignored.

Reduction of p-hydroxybenzoic acid and its esters

p-Hydroxybenzoic acid reduction produces a strong, quantitative peak with a half-wave potential of approximately −1.54 V. The polarogram can be seen in Fig. 3a. Analysis at less negative potentials did not reveal any peaks. This half-wave potential is very close to the literature value of −1.56 V and is due to the reduction of hydrogen ions of the dissociated acid (Kolthoff and Lingane, 1952). The presence of β-CD did not affect the current produced by reduction of the acid or the potential at which this occurred (see Fig. 3b), providing further proof that even if β-CD is adsorbed by the mercury drop, this does not hinder the reduction processes.

If hydroxybenzoic acid does form inclusion complexes with β-CD, this is not detectable polarographically, due to the mechanism of reduction of the acid.

Methyl hydroxybenzoate did not have a quantitative electroactive species under the conditions of test. Although several small peaks were evident (see Fig. 4), none of these linearly increased in height with increasing concentration.

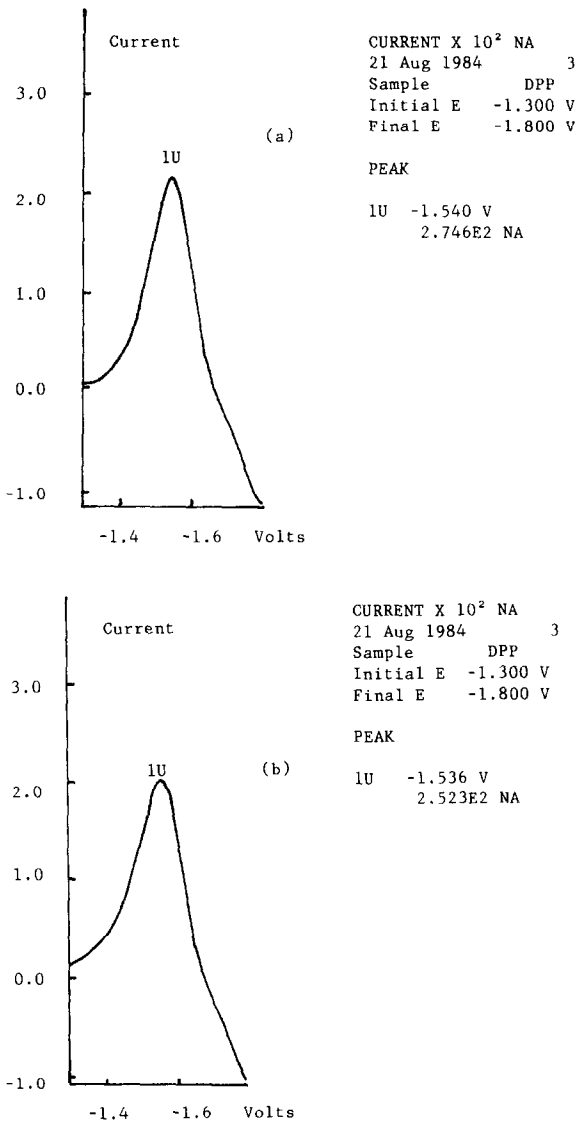


Fig. 3. Polarograms of *p*-hydroxybenzoic acid (3×10^{-5} M) alone (a) and with 1.76×10^{-4} M β-CD (b) in 0.1 M KCl.

Ethyl, propyl and butyl hydroxybenzoates all gave responses of a similar pattern. The reduction current of each ester increased linearly with concentration across the concentration range $0-5\times10^{-5}$ M. All studies with β-CD were conducted within this concentration range.

The amount of current conducted for the reduction of a constant amount of hydroxybenzoate increased as the alkyl chain-length of the hydroxy-

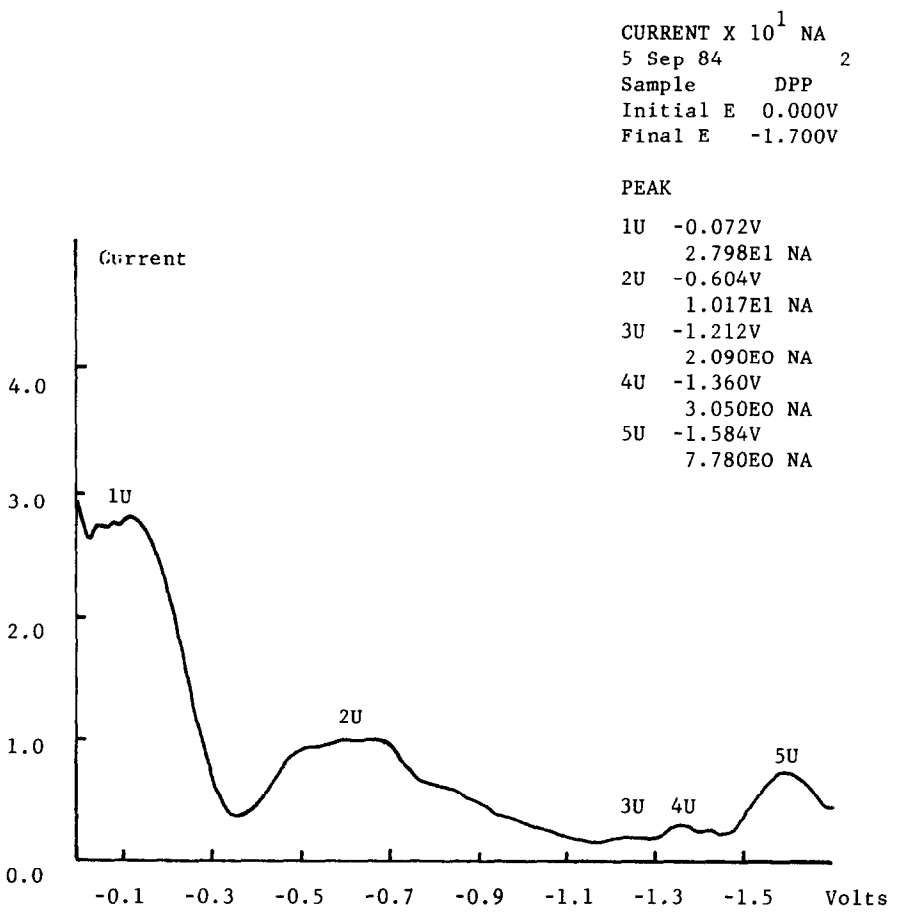


Fig. 4. Polarogram of methyl hydroxybenzoate (6.56×10^{-5} M) in 0.1 M KCl.

benzoate increased. Also, the half-wave potential of these reductions became more negative as the alkyl chain-length increased. For the ethyl ester, the half-wave potential was -0.82 V (see Fig. 5a), -0.92 V for propyl (Fig. 6a), and -0.97 V for butyl hydroxybenzoate (Fig. 7a). The alkyl chain, with its electron releasing tendency, obviously has an effect on the ease of reduction of the carbonyl group of the molecule.

The presence of β-CD markedly diminishes the peak height of each of these molecules (see Figs. 5b, 6b and 7b) i.e. the presence of β-CD is resulting in less conductance by each ester upon reduction. This is illustrated in Fig. 8. The possibility of interference from β-CD being adsorbed onto the mercury drop has already been ruled out, and hence interaction of β-CD and hydroxybenzoate must be responsible for the fall in current conducted by the reduced species.

Fig. 8 therefore depicts the decrease in current conducted for reduction of the esters in the presence of β-CD due to the decreased rate of diffusion of the complexed ester as compared with the ester alone. The electroactive species cannot reach the electrode at the same rate, and this results in a decrease in the amount of (diffusion) current conducted. The half-wave potential of hydroxybenzoic acid was unaltered in the presence of β-CD, since the reducible species of the acid (the protons) do not interact with β-CD.

The half-wave potentials at which these reductions occur become more negative in the presence

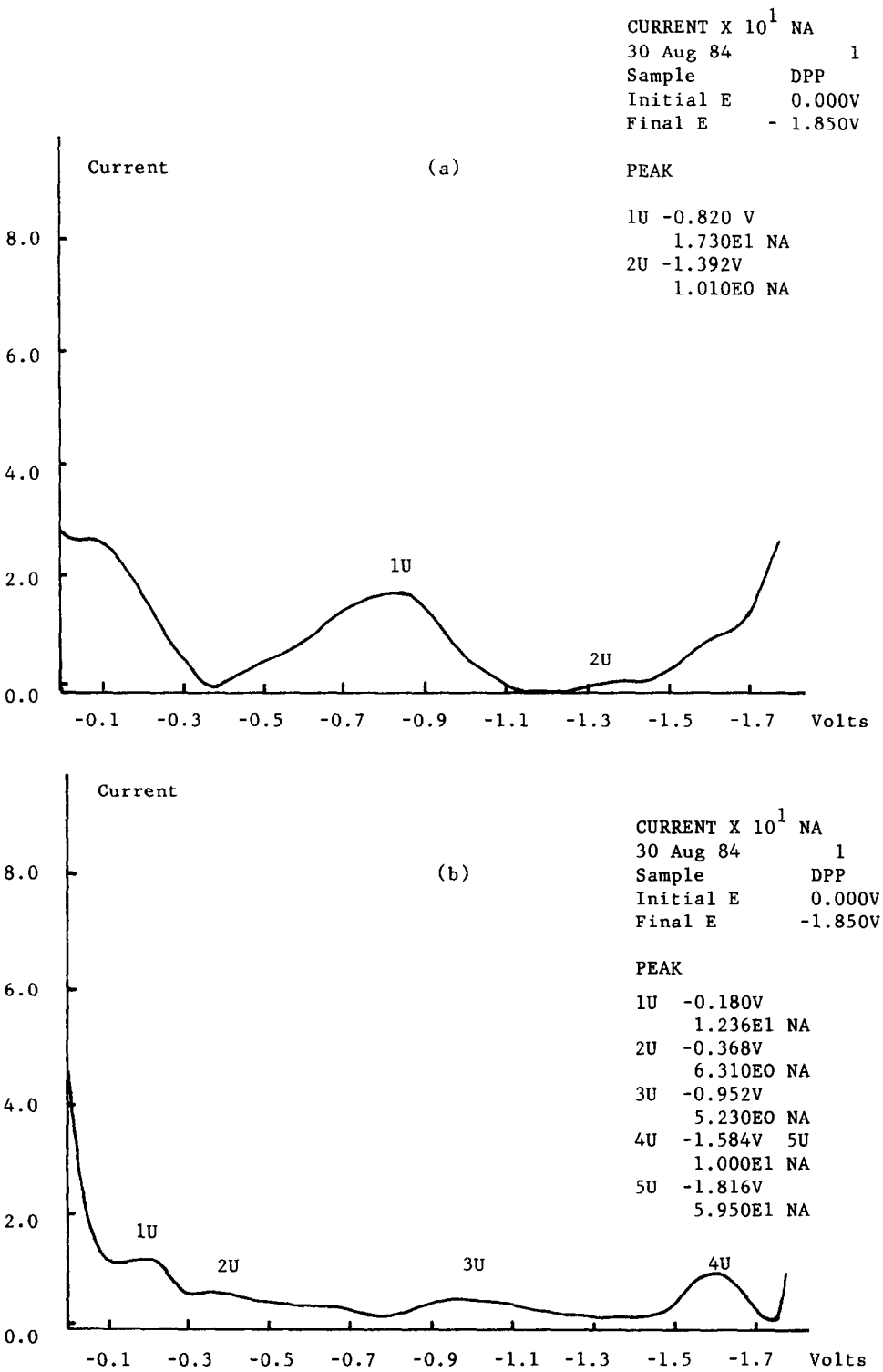
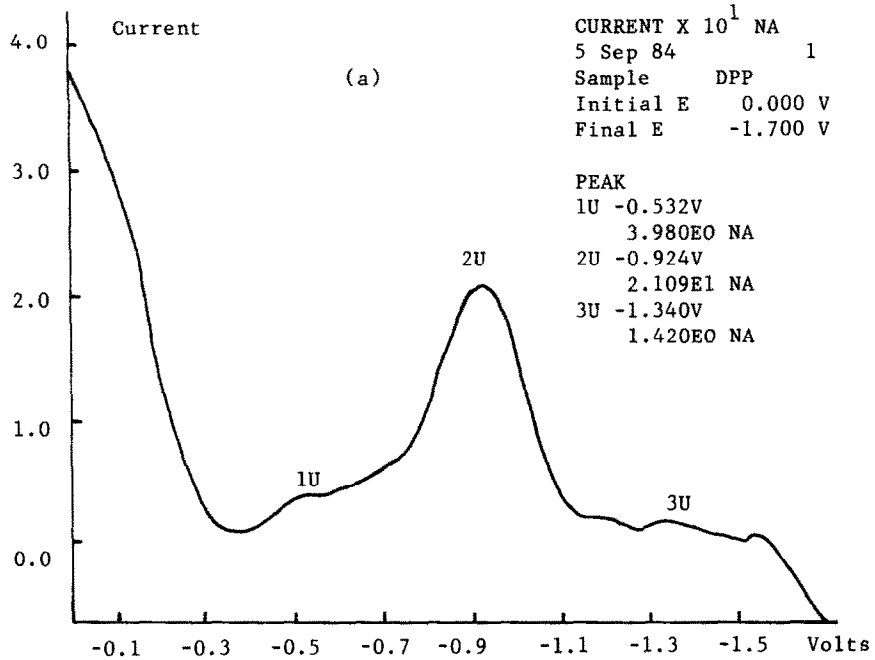


Fig. 5. Polarograms of ethyl hydroxybenzoate (3.0×10^{-5} M) alone (a) and with 2.2×10^{-4} M β-CD (b) in 0.1 M KCl.



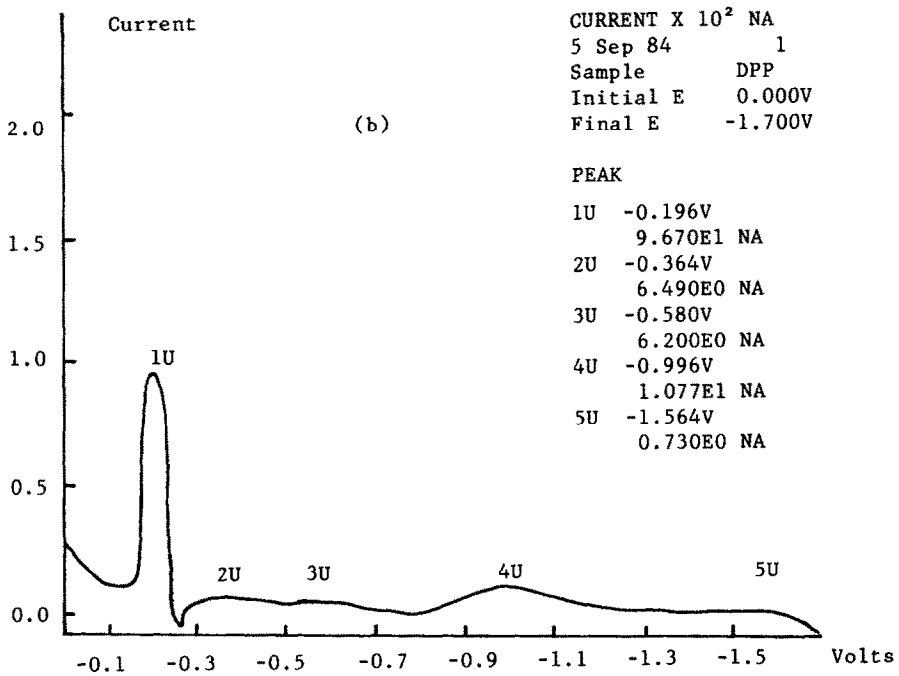
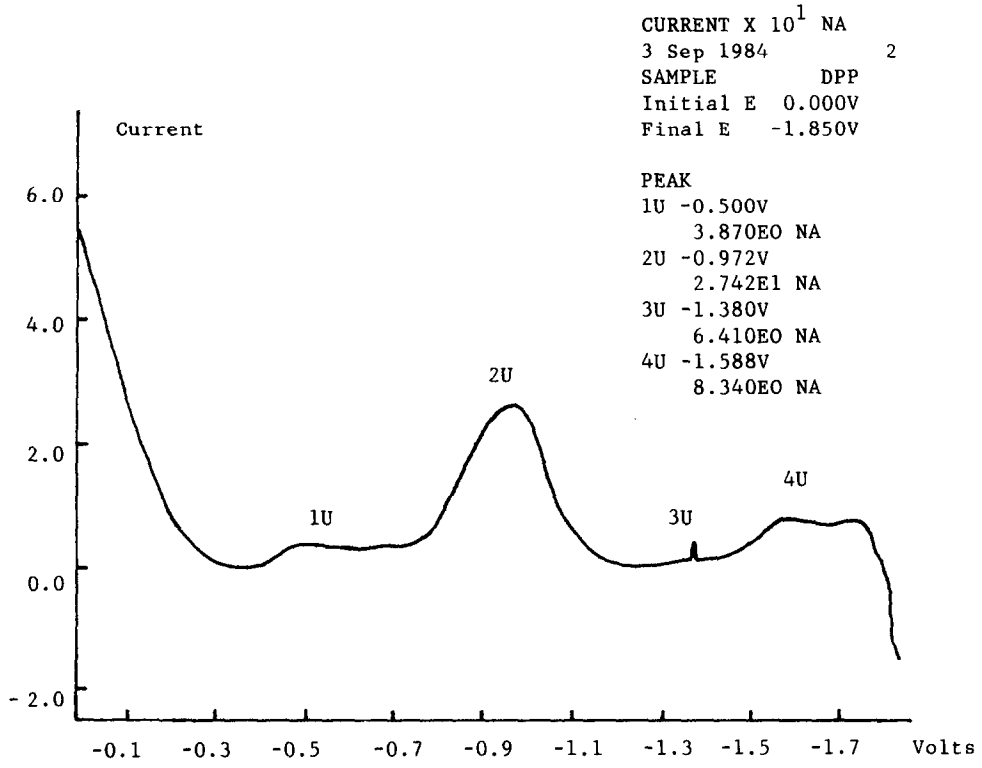


Fig. 6. Polarograms of propyl hydroxybenzoate (2.78×10^{-5} M) alone (a) and with 2.2×10^{-4} M β-CD (b) in 0.01 M KCl.



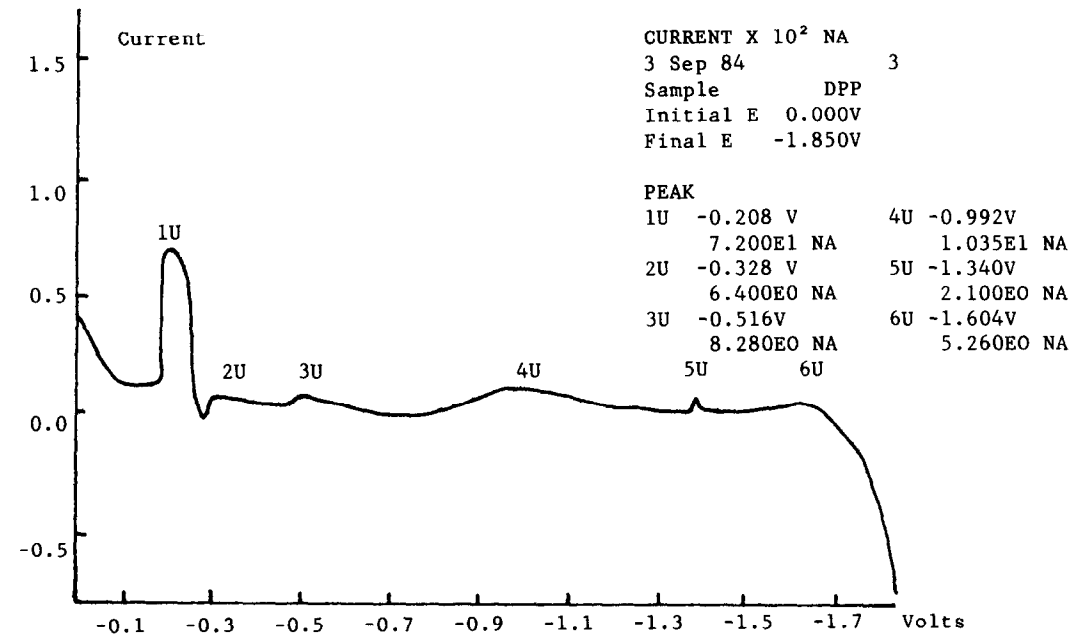


Fig. 7. Polarograms of butyl hydroxybenzoate (2.58×10^{-5} M) alone (a) and with 1.76×10^{-4} M β-CD (b) in 0.1 M KCl.

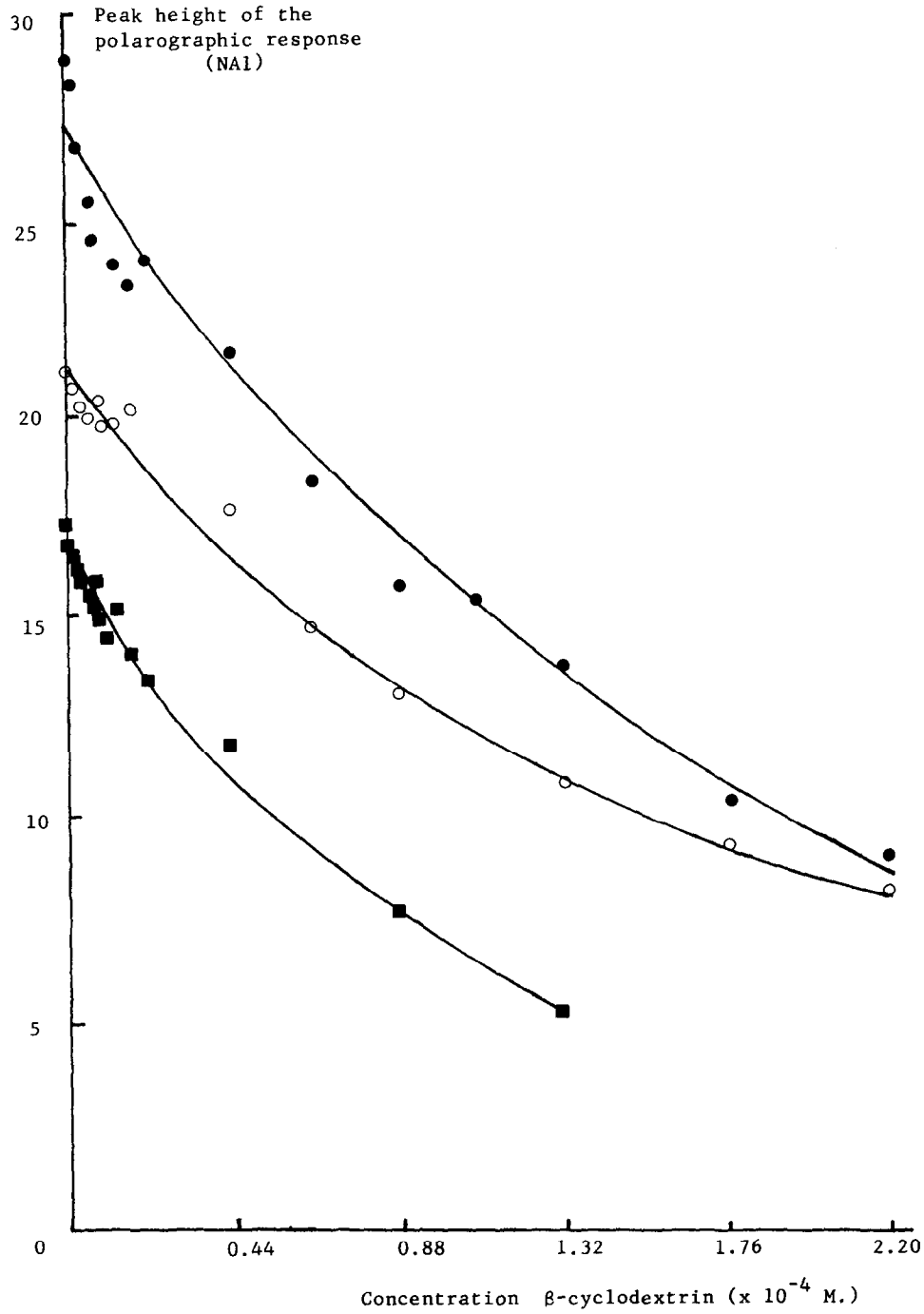


Fig. 8. Polarographic response of butyl (●——●), propyl (○——○) and ethyl (■——■) hydroxybenzoates (2.6, 2.8 and 3.0×10^{-5} M, respectively) to increasing concentrations of β-CD (in 0.1 M KCl).

of β-CD, i.e. the species is more difficult to reduce. This change in potential is in the order ethyl > propyl > butyl, and is most likely to be due to the electron redistribution that is occurring in the presence of β-CD. It is almost certainly only inclusion complexation that can be responsible for this redistribution. Hence, ethyl hydroxybenzoate is more likely to form complexes than propyl, which in turn is more likely to form complexes than butyl hydroxybenzoate. The change in half-wave potential of the reduction of the esters may therefore be regarded as an indication of their complexing ability. The carbonyl group of the esters must be held close to the CD molecule during complexation for these electronic effects to become detectable.

Each of the esters was reduced in the presence of glucose at a concentration 10 times higher than the maximum concentration of β-CD. Polarograms were identical to those obtained for the esters alone. Glucose and the hydroxybenzoates do not form complexes therefore and glucose does not interfere with the electrode processes.

Inclusion complexation with β-CD has been shown to be responsible for the decrease in current conducted upon reduction of the hydroxybenzoate esters. Complexation also made reduction of the hydroxybenzoates at the DME more difficult, as seen by the more negative half-wave potentials of the reduced molecules. The hydroxybenzoate molecule must reside within the β-CD cavity to such a degree that the presence of the β-CD ring causes an electron redistribution of the carbonyl group, which results in the change of reduction potential. The degree to which the half-wave potential is shifted in the presence of β-CD is an indication of the complexing ability of an electroreductive guest molecule with a β-CD host.

Polarography is a useful technique, not only for studying the inclusion complexation phenomenon, but also for investigating the mode of interaction of the host and guest molecules.

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