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Trace analysis of γ-cyclodextrin in a sample of β-cyclodextrin by capillary electrophoresis

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Abstract

A new capillary electrophoretic method for trace analysis of γ-cyclodextrin, γ-CD, in a sample of β-CD has been developed, building on our recent work in which the tetraphenylborate ion, Ph_4B^-, was found to bind to γ-CD three orders of magnitude more strongly than to β-CD. The method involves measurement of the change of net electrophoretic mobility of Ph_4B^- and its CD complexes in a background electrolyte containing a fixed concentration of β-CD. Good linearity was found between $1/\Delta\mu$ and $1/C_\gamma$, where $\Delta\mu$ is the difference in the mobility of Ph_4B^- in the β-CD solution at a given and at zero concentration of γ-CD, and C_γ the γ-CD concentration. The limit of detection for γ-CD in a β-CD sample was found to be 0.020% (w/w), and high precision and accuracy were obtained.

Keywords: Cyclodextrins; Tetraphenylborate; Carbohydrates

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides built of six, seven, or eight glucose units termed α, β and γ, respectively. They have the ability to selectively include a variety of guest molecules. CDs are widely used for the study of CD inclusion complexes [1,2], and as selectors in both high-performance liquid chromatography (HPLC) [3,4] and capillary electrophoresis (CE) [4–8], particularly for separations of enantiomers and positional isomers. In addition, CDs have been recognised as useful pharmaceutical excipients to increase water solubility and solution stability of drugs [9].

Quantitative analysis of trace levels of CDs is difficult because CDs have no appreciable UV–Vis absorbance or fluoresence. Furthermore, they are difficult to label with reagents that would allow visualisation or electrochemical detection [10]. Previous techniques used for separation and analysis of CD mixtures include thin-layer chromatography [11,12], HPLC [13–21] and CE [10,22–25]. Detection methods used in HPLC have been either direct detection, e.g., refractive index [13], polarimetry [14], evaporative light scattering [15] and amperometry [16], or indirect detection using a visualising reagent added to the mobile phase to form complexes with the CDs and UV absorbance [17–19] or fluorescence [20,21]. As CDs are uncharged except at very high pH, their separations in CE are normally carried out by adding a charged chromophoric reagent to the background electrolyte (BGE) to form charged CD inclusion complexes, and then using indirect detection. Using indirect UV

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detection, for example with benzoate [22], benzylamine and 1-naphthylacetic acid [23], concentration limits of detection (LODs) were 0.1–1.0 m*M*. Improvement of sensitivity for CE analysis of CDs has been achieved using 2-anilinonaphthalene-6-sulfonate (2,6-ANS) [10] and 8-anilinonaphthalene-1-sulfonate (8,1-ANS) [24] for indirect fluorescence detection; LODs for α-, β- and γ-CDs were in the range 2–60 μ*M*. Binding constant differences are responsible for 8,1-ANS providing the best LOD for γ-CD, whilst 2,6-ANS is the best for β-CD. However, indirect detection methods in both HPLC and CE suffer from fluctuations in the fluorescence or absorbance background signal, making detection at low concentration of CDs difficult [10]. Recently, a CE method for separation and analysis of α-, β- and γ-CDs using direct amperometric detection at high pH has been reported [25]; LODs were between 1 and 2 μ*M*.

The aim of this paper is to develop a new method for quantitation of trace levels of γ-CD in a sample of β-CD, taking advantage of a three-order of magnitude difference in the binding constants of the chromophoric ionic analyte tetraphenylborate, Ph_4B^-, to γ-CD and β-CD [26].

2. Experimental

2.1. Materials and reagents

β-CD and γ-CD were gifts from Wacker (Egham, UK). A sample of β-CD was obtained from Sigma (Dorset, UK). All samples were used as received. Measurement of optical rotation (sodium D line) showed that the sample of β-CD from Sigma had a slightly lower specific rotation and therefore a slightly lower β-CD content than the sample from Wacker $\{[\alpha]_D$ (Sigma)/$[\alpha]_D$ (Wacker)$=0.991\pm0.005\}$. Sodium tetraphenylborate ($NaPh_4B$) and 4-methylbenzoic acid were obtained from Aldrich (Gillingham, UK). Disodium hydrogenphosphate (Na_2HPO_4), sodium dihydrogenphosphate (NaH_2PO_4) and sodium hydroxide (analytical grade) were supplied by Merck (Leicester, UK). A pH 7 phosphate buffer was prepared by titrating a mixture of 12.5 m*M* Na_2HPO_4 and 12.5 m*M* NaH_2PO_4 with 1.0 *M* NaOH to pH 7.0.

A standard solution of 1.0 m*M* γ-CD was prepared by weighing an appropriate amount of γ-CD from Wacker and then dissolving this in a solution of 8.0 m*M* β-CD in pH 7 phosphate buffer. Solutions of γ-CD in the concentration range 0.005 to 0.05 m*M* were prepared by diluting appropriate amounts of the standard solution of 1.0 m*M* γ-CD in 8.0 m*M* β-CD with the solution of 8.0 m*M* β-CD. All solutions were prepared using ultra-pure water (Elgastat UHQII) and filtered through 0.45-μm filters prior to analysis.

2.2. Capillary electrophoresis

CE experiments were carried out on an automated instrument (Beckman PACE 2100). The capillary used was 47 cm (40 cm to detector)×75 μm I.D., thermostatted at 25°C. Voltage was set at 20 kV and UV detection at 200 nm. The capillary was rinsed with 0.1 *M* NaOH for 3 min and then running buffer for 3 min prior to each injection. Samples containing a mixture of 0.5 μ*M* sodium tetraphenylborate and 5 μ*M* 4-methylbenzoate, made up in BGE diluted 10 times into water to provide sample stacking [27], were introduced with 4 s pressure injection at 0.5 p.s.i. (1 p.s.i.=6894.76 Pa). Each experiment was run in triplicate.

3. Results and discussion

3.1. Principle of the method

In the presence of two types of CDs, β- and γ-CD, in the BGE, binding equilibria for the analyte are represented by:

$$A + \beta \overset{K_\beta}{\rightleftharpoons} \beta A \quad (1)$$

$$A + \gamma \overset{K_\gamma}{\rightleftharpoons} \gamma A \quad (2)$$

where A is the analyte, βA and γA the complexes of β-CD and γ-CD with A, respectively, and K_β and K_γ the binding constants. Equations defining the binding constants are:

$$K_\beta = \frac{C_{\beta A}}{C_A C_\beta} \quad (3)$$

$$K_\gamma = \frac{C_{\gamma A}}{C_A C_\gamma} \quad (4)$$

where C_i is the concentration of species *i* at equilibrium. The mass balance equations are:

$$S_\gamma = C_\gamma + C_{\gamma A} \quad (5)$$

$$S_\beta = C_\beta + C_{\beta A} \quad (6)$$

$$S_A = C_A + C_{\beta A} + C_{\gamma A} \quad (7)$$

with S_i the total concentration of species *i*. The net electrophoretic mobility, μ, is given by:

$$\mu = x_A \mu_A + x_{\beta A} \mu_{\beta A} + x_{\gamma A} \mu_{\gamma A} \quad (8)$$

where x_i is the mole fraction and μ_i the electrophoretic mobility of species *i*. It follows that [28]:

$$\mu = \frac{\mu_A + K_\beta C_\beta \mu_{\beta A} + K_\gamma C_\gamma \mu_{\gamma A}}{1 + K_\beta C_\beta + K_\gamma C_\gamma} \quad (9)$$

When the concentration of the CDs present in the BGE are much greater than the concentration of the analyte, C_γ is assumed to be equal to S_γ, and C_β to S_β.

From Eq. (9), by keeping a constant β-CD concentration in the BGE, the change of electrophoretic mobility depends only on the γ-CD concentration. The difference in mobility, $\Delta\mu$, of analyte in two solutions with total concentrations of γ-CD zero and S_γ is given by the following equation:

$$\Delta\mu = \frac{[\mu_A - \mu_{\gamma A} + K_\beta C_\beta(\mu_{\beta A} - \mu_{\gamma A})]K_\gamma C_\gamma}{(1 + K_\beta C_\beta)(1 + K_\beta C_\beta + K_\gamma C_\gamma)} \quad (10)$$

Inversion of Eq. (10) gives:

$$\frac{1}{\Delta\mu} = \frac{a}{C_\gamma} + b \quad (11)$$

where

$$a = \frac{(1 + K_\beta C_\beta)b}{K_\gamma} \quad (12)$$

$$b = \frac{(1 + K_\beta C_\beta)}{[\mu_A - \mu_{\gamma A} + K_\beta C_\beta(\mu_{\beta A} - \mu_{\gamma A})]} \quad (13)$$

suggesting a linear relationship between $1/\Delta\mu$ and $1/C_\gamma$ which should allow determination of the concentration of γ-CD by measurement of the mobility difference. In using equations throughout this paper, mobility is used as an unsigned quantity, i.e, the modulus of μ.

In our recent paper [26], tetraphenylborate was found to have $K_\gamma = (1.08 \pm 0.06) \cdot 10^5\ M^{-1}$, approximately 1000-times higher than $K_\beta = 77 \pm 7\ M^{-1}$. Using these data plus values for the moduli of mobilities μ_A, $\mu_{\beta A}$ and $\mu_{\gamma A}$ equal to $1.67 \cdot 10^{-8}$, $0.69 \cdot 10^{-8}$ and $0.77 \cdot 10^{-8}$ $m^2\ V^{-1}\ s^{-1}$, respectively [26], it follows using Eq. (10) that it should be practicable to determine very small amounts of γ-CD in β-CD, at a level less than 0.1% (mol/mol).

3.2. Choice of optimum β-CD concentration

We first calculate the optimum β-CD concentration, the concentration which gives maximum mobility difference. Defining the mole ratio of γ-CD to β-CD, C_γ/C_β, equal to *m*, C_γ in Eq. (10) may be set equal to mC_β, giving:

$$\Delta\mu = \frac{[\mu_A - \mu_{\gamma A} + K_\beta C_\beta(\mu_{\beta A} - \mu_{\gamma A})]mK_\gamma C_\beta}{(1 + K_\beta C_\beta)(1 + K_\beta C_\beta + mK_\gamma C_\beta)} \quad (14)$$

Fig. 1 shows plots of $\Delta\mu$ as a function of C_β for

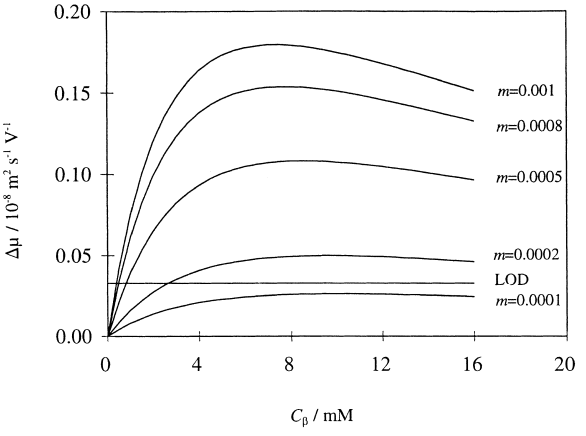

Fig. 1. Mobility difference as a function of β-CD concentration for a range of γ-CD/β-CD concentration ratios, *m*. The horizontal line shows the limit of detection.

five values of m in the range 0.0001 to 0.001. When m is constant, $\Delta\mu$ increases with increase of C_β up to a maximum, then falls off gradually. The β-CD concentration at maximum mobility difference, $C_{\beta,opt}$ can be determined from Eq. (14) by using differential calculus. When $d(\Delta\mu)/dC_\beta = 0$:

$$C_{\beta,opt} = \frac{K_3 + \sqrt{K_3^2 + K_2K_\beta - K_3(K_2 + K_\beta)}}{K_2K_\beta - K_3(K_2 + K_\beta)} \quad (15)$$

where

$$K_2 = K_\beta + mK_\gamma$$

$$K_3 = K_\beta \cdot \frac{\mu_{\beta A} - \mu_{\gamma A}}{\mu_A - \mu_{\gamma A}}$$

By giving values to parameters in Eq. (15) using data from our previous work [26], it follows that $C_{\beta,opt}$ = 10.4, 9.9, 8.7, 7.9 and 7.5 m*M* for m = 0.0001, 0.0002, 0.0005, 0.0008 and 0.001, respectively. From Fig. 1, when m is constant, there are only slight differences of $\Delta\mu$ over the C_β range 6 to 16 m*M*.

3.3. Theoretical LOD

We now consider the LOD. The accepted definition for LOD in separation science and spectroscopy is the analyte concentration at a signal-to-noise ratio of 3 [29]. In our method, the LOD is taken to be the γ-CD concentration which gives $\Delta\mu$ three times the standard deviation of the measured electrophoretic mobility. The standard deviation of μ was measured to be $0.011 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} in a set of 10 replicates of the electrophoretic mobility of Ph_4B^- in a BGE containing 0.01 m*M* γ-CD and 8.0 m*M* β-CD. The horizontal line drawn at $\Delta\mu = 0.033 \cdot 10^{-8}$ m^2 V^{-1} in Fig. 1 sets the LOD. It is evident from Fig. 1 that a sample with a γ/β mole ratio m = 0.0001 lies below the LOD for all values of C_β, whereas one with m = 0.0002 is above the LOD when $C_\beta > 3$ m*M*. LOD values as a function of β-CD concentration are given in Table 1, and range from 0.8 μ*M* at C_β = 6 m*M* to 2.1 μ*M* at C_β = 16 m*M*. In practice, it is more helpful to consider for detection purposes the ratio by mass of γ-CD in a sample of β-CD.

Table 1
Predicted limit of detection for γ-CD as a function of β-CD concentration

C_β (m*M*)	LOD (μ*M*)	LOD (%, w/w)
6	0.8	0.015
8	1.0	0.014
10	1.2	0.014
16	2.1	0.015

The value in percentage (w/w), *P*, is related to the mole ratio, *m*, as follows:

$$P = 100m\frac{M_\gamma}{M_\beta} \quad (16)$$

where *M* is the molar mass. As is evident from Table 1, predicted LODs on a w/w basis do not vary significantly over the C_β range 6 to 16 m*M*, and the value calculated is P = 0.014–0.015% (w/w).

From calculated $C_{\beta,opt}$ and LOD values, suitable concentrations of β-CD for determination of trace levels of γ-CD in a sample of β-CD, m = 0.0001 to 0.0005, are in the range of 8 to 10 m*M*. The 8.0 m*M* β-CD solution was chosen for our experiment.

3.4. Calibration

The electrophoretic mobilities of Ph_4B^- were measured in the standard solutions at various concentrations of γ-CD in 8.0 m*M* β-CD. Fig. 2 shows an example of an electropherogram of Ph_4B^- in the 8.0 m*M* β-CD solution containing 0.01 m*M* γ-CD.

Observed electrophoretic mobilities, μ_{obs}, were calculated using the equation:

$$\mu_{obs} = \frac{lL}{V} \cdot \left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (17)$$

where l and L are the length of the capillary to the detector and the total length of the capillary, respectively, and t_m and t_{eo} the migration times of the analyte and the electroosmotic flow, EOF.

The EOF migration time was determined in the electropherograms from the minimum of the absorbance dip. The 4-methylbenzoate ion, 4-MB^-, was used as an internal standard for correction of changes in electrophoretic mobility of Ph_4B^- due to any change in temperature and viscosity. 4-MB^- has

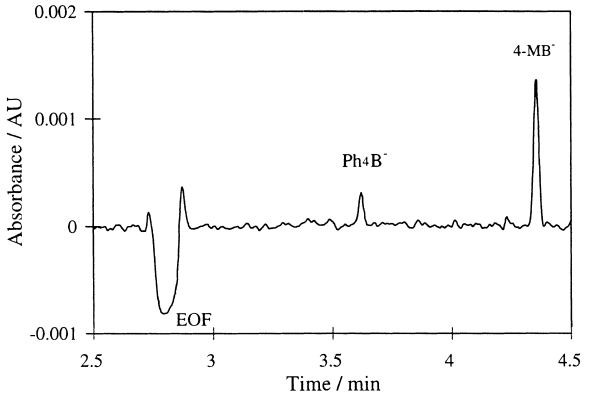

Fig. 2. Electropherogram of tetraphenylborate and 4-methylbenzoate (internal standard) in the BGE containing 8.0 m*M* β-CD and 0.01 m*M* γ-CD in 25 m*M* phosphate buffer, pH 7.0. Capillary 47 cm (40 cm to detector)× 75 μm I.D.; voltage 20 kV; temperature 25°C; UV detection at 200 nm; sample 0.5 μm $NaPh_4B$ and 5 μm 4-methylbenzoic acid in BGE diluted ×10 with deionised water; pressure injection 4 s at 0.5 p.s.i.

a binding constant to β-CD of 100 M^{-1} [30,31], which is very similar to that of Ph_4B^- (77 M^{-1}). Because 4-MB^- binds only weakly to γ-CD, $K_\gamma <$ for 30 M^{-1} [31], there is a negligible amount of its complex with γ-CD at concentrations of the latter less than 0.05 m*M*. Thus the presence of γ-CD has negligible effect on the electrophoretic mobility of 4-MB^-; we calculate $\Delta\mu < 0.005 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}.

Mobility values are given in Table 2. For 4-MB^-, the mean of all tabulated value is $(1.893 \pm 0.015) \cdot 10^{-8}$ m^2 V^{-1} s^{-1}. The standard deviation of the mobility may be acceptable in typical CE analysis, but is not in our case where accuracy and precision of $\Delta\mu$ are required for quantitative analysis. Values for any one γ-CD concentration have a lower standard deviation, and there is no correlation between μ_{obs} and the concentration of γ-CD. There are two possible reasons for the rather high relative standard deviation, RSD, of 0.8% for the complete set of data for 4-MB^-. Firstly, fluctuations in ambient temperature, which affect the non-thermostatted sections of the capillary; secondly, uncertainties in measurement of the EOF. An increase in temperature results in a mobility increase by ~2% per °C [32]. Furthermore, a change in temperature will affect the binding constant [33], leading to a mobility change (cf. Eq. (9)). As seen in Fig. 2 the EOF peak is quite broad. This is in part due to the length of the injection plug (injection time = 4 s), which is needed to observe 0.5 μ*M* Ph_4B^-.

The objective of the use of 4-MB^- as internal

Table 2
Mobility of Ph_4B^- in 8 m*M* β-CD at various concentrations of γ-CD

C_γ (m*M*)	μ_{obs} (Ph_4B^-) (10^{-8} m^2 V^{-1} s^{-1})	μ_{obs} (4-MB^-) (10^{-8} m^2 V^{-1} s^{-1})	*f*	μ (10^{-8} m^2 V^{-1} s^{-1})
0	1.326	1.907	0.997	1.350
	1.327	1.908	0.997	1.351
	1.315	1.889	1.007	1.352
	1.324	1.904	0.999	1.351
0.005	1.197	1.868	1.018	1.245
	1.203	1.870	1.017	1.250
	1.202	1.872	1.016	1.248
0.010	1.143	1.909	0.996	1.163
	1.153	1.903	1.000	1.177
	1.149	1.900	1.001	1.175
0.020	1.047	1.881	1.011	1.081
	1.052	1.882	1.010	1.085
0.050	0.934	1.903	0.999	0.953
	0.933	1.901	1.001	0.954
	0.942	1.901	1.001	0.963

standard is to compensate for these uncertainties. In using results for the internal standard to correct observed mobilities of Ph_4B^-, the internal standard mobility correction factor, *f*, is defined as the ratio $\mu_{obs}(4\text{-}MB^-)/\overline{\mu}_{obs}(4\text{-}MB^-, C_\gamma = 0)$. The term in the denominator is the average electrophoretic mobility of 4-MB^- at $C_\gamma = 0$, and is equal to $1.902 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}.

Since observed electrophoretic mobility also depends on the viscosity of the BGE containing CDs [6,34], an additional viscosity correction factor, η_c/η_0, is applied, where η_c/η_0 is the viscosity of the CD solution relative to that of the aqueous BGE without any added CD. This was found to have a value of 1.021 for the 8.0 m*M* β-CD solution, using the method reported in [26]; due to the small amount of γ-CD in the 8.0 m*M* β-CD solution, the relative viscosities were assumed to be equal for all CD solutions. Corrected electrophoretic mobilities of Ph_4B^-, μ, were calculated using the equation:

$$\mu = f\mu_{obs} \cdot \frac{\eta_c}{\eta_0} \quad (18)$$

Fig. 3 shows a plot of the reciprocal of the difference in the electrophoretic mobility of Ph_4B^- in 8.0 m*M* β-CD solution at zero and at a given γ-CD concentration against the reciprocal of γ-CD concentration. The relationship was found to be linear, as predicted from Eq. (11), with a high correlation coefficient of $r^2 = 0.9997$.

Table 3 shows a comparison of observed and predicted values of slope, *a*, and intercept, *b*. The predicted *a* and *b* values were calculated from Eqs. (11)–(13) using K_β, K_γ, μ_A, $\mu_{\beta A}$ and $\mu_{\gamma A}$ from our previous paper [26]. The observed and predicted values of *b* are found to agree within experimental error, whilst there are differences in the observed and predicted values of *a*. This could be due to a limitation of the assumption that $S_\gamma = C_\gamma$, since the concentration of analyte in the sample (0.5 μ*M*) is 10% of the γ-CD concentration at the lowest value used in the calibration (5 μ*M*), and could be even higher after stacking during application of the voltage following injection.

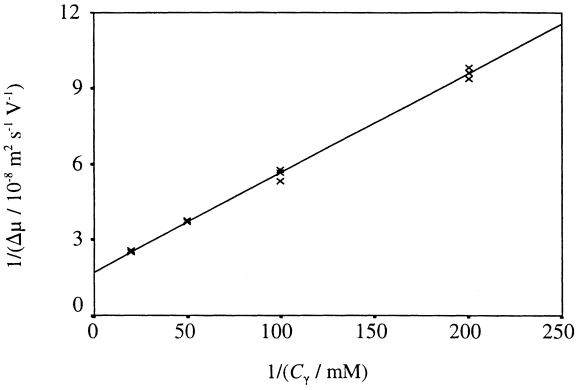

Fig. 3. Relationship between $1/\Delta\mu$ and $1/C_\gamma$. CE conditions as in Fig. 2 and $C_\beta = 8.0$ m*M*.

Table 3
Observed and predicted slope and intercept for plot of $1/\Delta\mu$ versus $1/C_\gamma$

	Observed	Predicted
a (slope) (m^{-2} V s *M*)	$(3.93 \pm 0.06) \cdot 10^3$	$(2.84 \pm 0.50) \cdot 10^3$
b (intercept) (m^{-2} V s)	$(1.73 \pm 0.08) \cdot 10^8$	$(1.90 \pm 0.14) \cdot 10^8$

3.5. Accuracy and limit of detection

Two samples were prepared spiked with known amounts of γ-CD at γ/β levels 0.06 and 0.11% (w/w), and the calibration established in Section 3.4 was used to determine the accuracy and precision of the method. Table 4 shows a comparison of actual and observed amounts of γ-CD in β-CD. The observed precision is high, ≤0.003% (w/w), and the accuracy is good; both values are in agreement to within 0.007% (w/w).

As previously discussed, the LOD is taken as the γ-CD concentration which gives $\Delta\mu$ three times the standard deviation of the measured electrophoretic mobility. The standard deviation value of $0.011 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} corresponds to 0.006% (w/w) γ in β, and the measured precision and accuracy are comparable to this. The LOD is 0.020% (w/w), marginally worse than the theoretical value of

Table 4
Observed and actual percentage of γ-CD in a spiked sample of β-CD

Spiked (%, w/w)	Observed (%, w/w)
0.057	0.061±0.001
0.114	0.107±0.003

0.014% (w/w) (Table 1). The difference is due to the requirement to use analyte at concentration comparable to that of γ-CD.

In comparing LODs in this and previous work, the LOD for γ-CD in the present study is 1.4 μ*M*. For the reason given in the previous paragraph, this is marginally worse than the theoretical value of 1.0 μ*M* predicted from Table 1 with a β-CD concentration of 8 m*M*. However, the observed LOD is significantly better than values from CE with fluorescence detection, 24 μ*M* [10] and 7 μ*M* [24]. A CE separation at high pH coupled with amperometric detection [25] provides a comparable LOD, 1 μ*M*, but has a longer analysis time (22 min) than our method (5 min). Whilst resolution was achieved for γ-CD and β-CD when these were present at comparable concentrations [25], the resolution between the CE peaks is insufficient to allow trace levels of γ-CD to be quantified in the presence of β-CD.

3.6. Application to determine the amount of γ-CD in a sample of β-CD

Three 0.228 g samples from a batch of β-CD obtained from Sigma were weighed and dissolved in 25 ml phosphate buffer to give concentrations of 8.0 m*M*. The electrophoretic mobilities of Ph_4B^- in each CD solution were measured in triplicate. Table 5 shows corrected electrophoretic mobilities of Ph_4B^- and the percentages of γ-CD in β-CD, calculated using the calibration line in Fig. 3. The average corrected electrophoretic mobility of Ph_4B^- in the sample solution was found to be $(0.062\pm0.005)\cdot 10^{-8}$ m^2 V^{-1} s^{-1} less than that in the standard solution of 8.0 m*M* β-CD without γ-CD. This indicates that there was a trace amount of γ-CD in the sample of β-CD. From Table 5, the average percentage of γ-CD in the sample of β-CD was found to be 0.039±0.004% (w/w).

Table 5
Percentage of γ-CD in a sample of β-CD from Sigma

Sample	$\Delta\mu$ (10^{-8} m^2 V^{-1} s^{-1})	*P* (%, w/w)
1	0.069, 0.069, 0.068	0.044, 0.043, 0.043
2	0.058, 0.058, 0.058	0.036, 0.036, 0.036
3	0.059, 0.061, 0.060	0.037, 0.038, 0.037
Average	0.062±0.005	0.039±0.004

The possible effect of a systematic error in β-CD concentration due to the measured slightly lower β-CD content in the Sigma than the Wacker (standard) sample was investigated. A 0.9% decrease in β-CD content would give rise to a change $\Delta\mu = +0.002\cdot 10^{-8}$ m^2 V^{-1} s^{-1}, which is less than the standard deviation of the measured $\Delta\mu$.

4. Conclusion

We have reported a new CE method for quantitation of trace levels of γ-CD in a sample of β-CD by measurement of the difference between the electrophoretic mobility of the tetraphenylborate ion in solutions of the sample and a reference β-CD. The method is specific for trace analysis of γ-CD in the presence of other cyclodextrins, and relies on the binding constant of Ph_4B^- to γ-CD being a factor 10^3 higher than to other cyclodextrins. Benefits of the method include a good LOD (0.020%, w/w, γ-CD in β-CD), fast analysis time, high accuracy and precision. In addition, the technique is ideal for determining the level of γ-CD as a minor contaminant in β-CD: other methods are inapplicable when the β-CD concentration is very much greater than that of γ-CD.

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