Short communication

Determination of cyclodextrin content using periodate oxidation by capillary electrophoresis

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Abstract

A simple and general method suitable for the determination of cyclodextrin content in various matrices is described. The proposed method involves selective cleavage of C–C bonds with vicinal hydroxyl groups by means of periodate (Malaprade's reaction). The amount of produced iodate is monitored by capillary electrophoresis. Optimized electrophoretic conditions (20 mM disodium tetraborate with 1 mM tetradecyltrimethylammonium bromide, direct UV detection λ=200 nm) ensure complete separation of periodate and iodate ions and sufficient sensitivity towards iodate. Under optimized reaction conditions (2-fold excess of periodate, temperature 70°C) reproducible quantitative results were obtained for α-, β- and γ-cyclodextrins as model samples. The proposed method was tested on a real sample of acrylamide — 2^I-O-allyl-β-CD copolymer. The values of β-cyclodextrin content were compared with those obtained by reference NMR measurement and were found to be identical.   © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Chiral analysis has well recognized importance at present. The need of reliable analytical methods distinguishing between enantiomers stimulated fast development, namely in the field of separation methods. Numerous types of chiral additives suitable for the use in gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been tested and practically utilized. Cyclodextrins (CDs) have estab-lished themselves as highly perspective structural and chiral selectors [1–4]. Besides natural cyclo-dextrins the growing number of semi-synthetic de-rivatives and co-polymers has been prepared and is already commercially available. Many of them found their use as structural and chiral selectors with new properties given by the type and number of sub-stituents [5–7].

Specific problems arise with the analytical evalua-tion of CDs and co-polymers. Mostly the lack of suitable spectral and electrochemical properties re-stricts the number of sufficiently sensitive methods of detection ensuring reliable results of the analysis. Assays based on the utilization of thin-layer chroma-

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tography (TLC), HPLC [8] and CE with indirect UV detection [9] have been described in the literature. Even more complex problem represents the analytical evaluation of CD co-polymers. Although the assay of CDs in a polymeric matrix is essential for the characterization corresponding co-polymer, no analytical method specifically designated for this purpose has been described in the literature.

The aim of this paper is to present a general method of the determination of CDs, their derivatives and CD co-polymers based on the monitoring of the products of Malaprade's reaction [10]. In the course of this reaction the specific oxidative cleavage of the C–C bond with vicinal hydroxyl groups by means of periodate occurs. The reaction products are the corresponding fragments with aldehydic groups and iodate. Monitoring of either the decay of periodate or the increment of iodate in the reaction mixture may provide useful information about the structure and the assay of various carbohydrates [11–14]. Although widely used in carbohydrate research the Malaprade's reaction has not been utilized for the analysis of CDs.

The spectrum of analytical methods which have been used for the assay of periodate and iodate in reaction mixture comprises titrations [13,15,16], photometry [12,17], potentiometry [11], ion-exchange chromatography [18,19] and CE [20–22]. On the basis of published data and our previous experience with the analysis of inorganic ions using CE, we decided to utilize this method for the selective quantification of iodate.

2. Experimental

2.1. Materials

The substances of α-, β- and γ-CDs were of the highest grade commercially available obtained from Sigma (St. Louis, MO, USA). Acrylamide–2^1-O-allyl-β-CD copolymer was synthesized at the Department of Organic Chemistry (Charles University, Prague, Czech Republic) by copolymerization of acrylamide and 2^1-O-allyl-β-CD [24]. Intrinsic viscosity of the copolymer in water at 25°C was 62.5 cm^3/g, from which the molecular mass 72 000 was estimated using parameters of the Mark–Houwink

equation for poly(acryl amide) [25]. Disodium tetraborate, sodium iodate and sodium periodate were of analytical purity (Lachema Brno, Czech Republic); tetradecyltrimethylammonium bromide (TTAB) was purchased from Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The aqueous solutions of sodium periodate and sodium iodate used for calibration were prepared in the concentration range 3–20 mmol/l. Stock solutions of sodium periodate (20 and 200 mmol/l) and α-, β- and γ-CDs (10 mmol/l) were prepared by dissolving the corresponding amount of substances in deionized water.

2.2. Instruments

Capillary electrophoresis was performed on a Crystal CE system (ATI Unicam, Great Britain) with a UV detector Unicam 4225. The reaction mixture during Malaprade's reaction was thermostated in U-15 water thermostat (Veb MLW Frufgeratewerk, Medingen, Germany).

2.3. CE determination of iodate and periodate

Electrophoretic experiments were performed in a fused-silica capillary tube 67.1 cm×75 μm I.D. (Composite Metal Services, UK). The distance to the detector was 54.5 cm.

UV spectrophotometric detection was carried out at a wavelength of 200 nm. The running buffer solution was 20 mM disodium tetraborate with 1 mM TTAB. Sample solutions were injected at a pressure 1000 Pa for 6 s. The separation voltage was −15 kV, which typically generated a capillary current of about −39.1 μA. All measurements were done at the temperature 30°C.

2.4. Oxidation by periodate

2.4.1. Procedure 1

Fourteen ml of 20 mM NaIO$_4$ was added to 2 ml of 10 mM stock solution of α-, β- or γ-CD. The mixture was thermostated at 25°C and shielded from the daylight. At specific intervals 0.2 ml aliquots of the solution were transferred into 0.5 ml sample vial and analyzed by CE.

2.4.2. Procedure 2

Fourteen ml of 200 mM NaIO$_4$ was added to 2 ml of 10 mM stock solution of β-CD. The mixture was thermostated at 25°C and shielded from the daylight. At specific intervals 0.2 ml aliquots of the solution were transferred into 0.5 ml sample vial and analyzed by CE.

2.4.3. Procedure 3

Fourteen ml of 20 mM NaIO$_4$ was added to 2 ml of 10 mM stock solution of α-, β- or γ-CD. The mixture was thermostated at 70°C and shielded from the daylight. At specific intervals 0.2 ml aliquots of the solution were transferred into 0.5 ml sample vial and analyzed by CE.

2.4.4. Procedure 4

Fourty five mg of acrylamide–2^1-O-allyl-β-cyclo-dextrin copolymer was dissolved in 200 μl of deionized water. Then 1.4 ml of 20 mM NaIO$_4$ was added and the mixture was thermostated at 70°C and shielded from the daylight. The amount of cyclo-dextrin was determined after 30 min when 0.2 ml aliquot of the solution was transferred into 0.5 ml sample vial and analyzed by CE.

3. Results and discussion

Based on our experience, the most appropriate buffer for the separation and determination of iodate and periodate ions proved to be 20 mM disodium tetraborate with 1 mM TTAB added as an electro-osmotic flow reverser. Honda et al. [20] reported the use of 100 mM acetate buffer pH 4.5 for the determination of the same analytes. However, the borate buffer applied in this study exhibited a lower absorbance at the wavelength of 200 nm, at which both periodate and iodate strongly absorb, and allowed a good resolution of both analytes and faster, more sensitive and more accurate determi-nation of both analytes compared to the acetate buffer. As a consequence, small amounts of iodate could be determined in the samples containing excess of periodate in 6 min using the borate buffer. Moreover, preparation of the borate buffer from disodium tetraborate and TTAB (i.e., solid com-pounds) weighing of them is a more repeatable and

reproducible procedure than the preparation of the acetate buffer from acetic acid solution and sodium hydroxide pellets [20]. These buffer properties are very important if it should be used for routine determination of analytes.

In the borate buffer periodate and iodate ions are completely separated as is documented in Fig. 1. Calibration curves for both ions are linear in the concentration range of 3–20 mmol/l and their parameters are reported in Table 1. Intercept values, statistically insignificant from zero at the 5% risk, indicate that no systematic error appears in proposed analytical procedure. The RSDs for seventeen con-secutive determinations are 0.77% for iodate and 0.91% for periodate analyzed at a concentration level of 9 mmol/l.

The suitability of the periodate oxidation reaction for monitoring of the content of the cyclodextrin was tested on model solutions of α, β and γ-CDs. The results obtained under the experimental conditions of procedure 1 are shown in Fig. 2. The value of the fractional conversion, α, is defined as a ratio of the experimental to the theoretical amount of iodate released during Malaprade's reaction. The amount of iodate increased rapidly during the initial 3 (α-CD), 3.5 (β-CD) and 4 (γ-CD) h, respectively. The initial part of the reaction, related to the selective oxidative
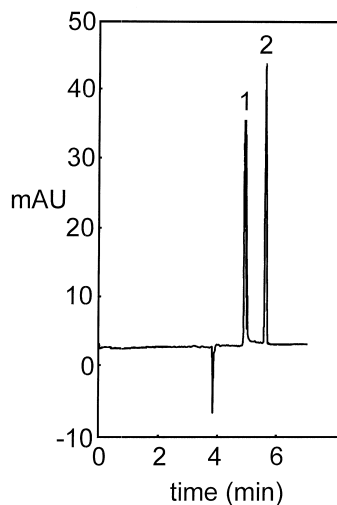

Fig. 1. Electrophoregram of the separation of a mixture of 10 mM periodate (1) and 10 mM iodate (2). Conditions of measurement are described in Section 2.3.

Table 1
Parameters of the calibration curves of iodate and periodate

Substance	Slope (mAU s mol^{-1} l)[a]	Intercept (mAU s)[a]	Correlation coefficient	Determination coefficient
NaIO$_3$	6615	3.18	0.9958	0.9916
NaIO$_4$	5826	−1.17	0.9916	0.9833

[a] AU = absorption unit.

cleavage of D(+)-glucose units to dialdehyde fragments on the C2–C3 bond [23], can be described by second order formal kinetics. The following slower reaction rate, where non-selective oxidation predominates, can be described by first-order formal kinetics. As it is evident from the shown kinetic curves, even after 16 h the value of fractional conversion, α, is still lower than one, which means that the amount of released iodate does not reach the theoretical value of 6, 7 and 8 mol per mol of α-, β- and γ-CDs, respectively. However, an additional kinetic study proved that non-selective oxidation leads to complete destruction of cyclodextrin skeleton and the value of α significantly exceeds unity. Simultaneous analysis of aldehydic compounds should give more reliable information on the Malaprade's oxidation. We plan to examine aldehydic products by micellar electrokinetic chromatography in the future.

In order to accelerate the studied reaction, the amount of periodate was increased from 2-fold (procedure 1) to 20-fold excess (procedure 2) over the stoichiometric value. The kinetic curve for the oxidative degradation of β-CD measured under the conditions of procedure 2 is shown in Fig. 3. From presented data it is evident that the degradation of β-CD is substantially accelerated. The amount of produced iodate reaches the theoretical value predicted for Malaprade's reaction, $\alpha = 1$, in 2.5 h. Unlike the previous example, the break in the kinetic curve is favorably shifted towards $\alpha = 1$, thus indicating fast and stoichiometric progress of Malaprade's reaction followed by slow non-specific oxidation. However, the large access of periodate makes the CE determination of iodate less precise, mostly due to integration errors caused by periodate and iodate peak overlapping.

Another possibility of how to accelerate the oxidation of CDs is to increase the temperature of the reaction mixture. Based on the preliminary experiments the optimum temperature 70°C was found. The results obtained under the experimental conditions of procedure 3 are shown in Fig. 4. As is
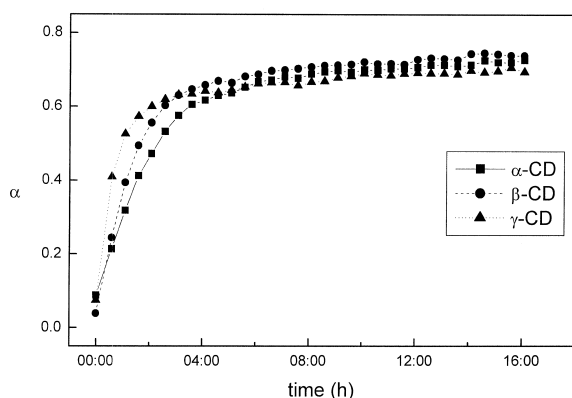

Fig. 2. Courses of periodate oxidation of α-, β-, γ-CD; 2-fold excess of periodate, temperature 25°C. $\alpha = 1$ for 6 mol of iodate/1 mol of α-CD, 7 mol of iodate/1 mol of β-CD and 8 mol of iodate/1 mol of γ-CD.
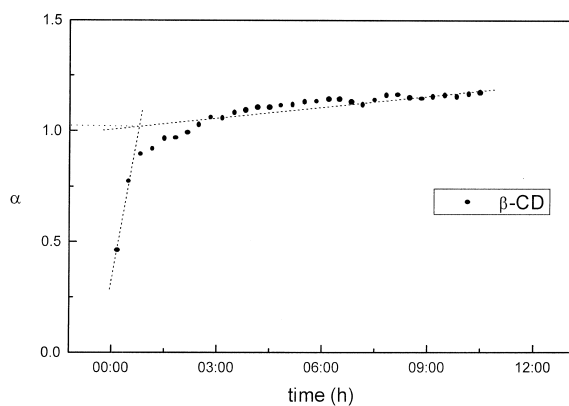

Fig. 3. Courses of periodate oxidation of β-CD; 20-fold excess of periodate, temperature 25°C. $\alpha = 1$ for 7 mol of iodate/1 mol of β-CD.
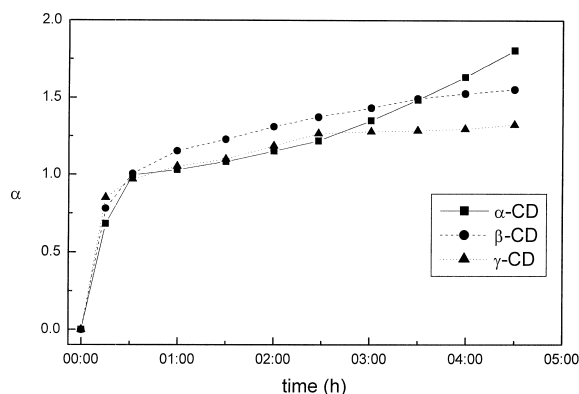
Fig. 4. Courses of periodate oxidation of α-, β-, γ-CD; 2-fold excess of periodate, temperature 70°C. $\alpha=1$ for 6 mol of iodate/1 mol of α-CD, 7 mol of iodate/1 mol of β-CD and 8 mol of iodate/1 mol of γ-CD.

evident from the course of the kinetic curves the value of fractional conversion, α, reaches unity in 30 min for α-, β- and γ-CDs. Sharp breaks in the kinetic curves lie close to $\alpha=1$, indicating fast stoichiometric progress of Malaprade's reaction followed by much slower non-specific oxidation for all studied CDs. Procedure 3 provides the most accurate and highly repeatable results. For five consecutive measurements of the contents of α-, β- and γ-CDs in model solutions the RSD 1.7%, 1.2% and 2.0%, respectively, were found. Different concentrations of β-CD were investigated to examine the effect of overoxidation on the accuracy of the results (Table 2). As it follows from the experimental data, non-specific overoxidation is a very slow process. When suitable sampling interval is chosen it has negligible effect on the accuracy and reproducibility of β-CD

Table 2
Accuracy and reproducibility of the assay at different concentrations of β-CD

$c_{(\beta\text{-CD})}$ (mmol 1^{-1})	$t^{a}_{(\alpha=1)}$ (min)	$\alpha^{b}_{(t=30\ min)}$	$RDS_{(t=30\ min,\ n=5)}$ (%)
5	20	1.05	1.3
10	30	1.01	1.2
20	35	0.94	1.5

[a] t for fractional conversion $\alpha=1$.
[b] Fractional conversion, α, in $t=30$ min.

assay. On the basis of previous experiments, procedure 4, which is suitable for the evaluation of the content of β-CD in acrylamide–2^{I}-O-allyl-β-CD copolymer, was proposed and tested. Results obtained under procedure 4 were compared with NMR measurements. The content of β-CD in copolymer was determined as 21.0±0.7% (w/w; $n=6$) of β-CD in copolymer while independent NMR measurements provided the value 21.5±3.8% (w/w; $n=6$). The content of β-CD in copolymer using ^{1}H-NMR was determined by comparing integrals of signals of H1 (of cyclodextrin glucose units, δ~5.05–5.09) and CH (of acrylamide residues, δ~2.1–2.4). The variances of the corresponding sets of results were compared using the two-tailed F-test and were found to be statistically insignificant at the 5% risk.

4. Conclusion

The presented study proved the suitability of Malaprade's reaction for the purposes of analytical evaluation of free CDs and CDs bonded in a polymeric matrix. The model experiments with α-, β- and γ-CDs revealed the complexity of the oxidation process. Malaprade's reaction is always accompanied with non-specific oxidation reactions that contribute to a lower accuracy of analytical results. The key factor making it possible to minimize the extent of non-specific oxidation of cyclodextrin skeleton is the optimization of the excess of periodate, reaction temperature and reaction time. It was found that a temperature of 70°C and a 2-fold excess of periodate ensure fast progress of Malaprade's reaction, while minimizing the extent of non-specific oxidation reactions. A reaction time 30 min ensured quantitativeness of Malaprade's reaction for all studied CDs.

The developed method was applied to the determination of β-CD content in the acrylamide–2^{I}-O-allyl-β-CD copolymer. The results were compared with those obtained by quantitative NMR measurement. Both methods provided identical results with statistically insignificant variances of the measurement at the 5% risk. However, calculated values of the confidental intervals proved a higher accuracy of the method proposed in this study.

Acknowledgements

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