Carbohydrate Research, 114 (1983) 303–305
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Colorimetric analysis of cyclomalto-octaose (γ-cyclodextrin)

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(Received July 12th, 1982; accepted for publication, September 14th, 1982)

The inclusion of a compound in a cyclodextrin may cause changes in the absorption spectrum of the compound comparable to those observed on dissolution in a non-polar solvent. Such spectral changes have been described for the inclusion complexes of simple phenols and dye molecules with cyclomalto-hexaose, -heptaose, and -octaose[1,2] (α-, β-, and γ-cyclodextrin; α-, β-, and γ-CD) and have been applied in the determination of the activity of cyclodextrin glycosyltransferase (EC 2.4.1.19) using Methyl Orange[3], and in the analysis of β-CD using phenolphthalein[4]. Likewise, the large increase in u.v. absorption of iodine by inclusion in α-CD has been used[5] in a photometric assay of the activity of cyclodextrin glycosyltransferase.

The analysis of cyclodextrins by chromatography on a silica derivative containing amino groups (elution with acetonitrile–water) and on a cation-exchange

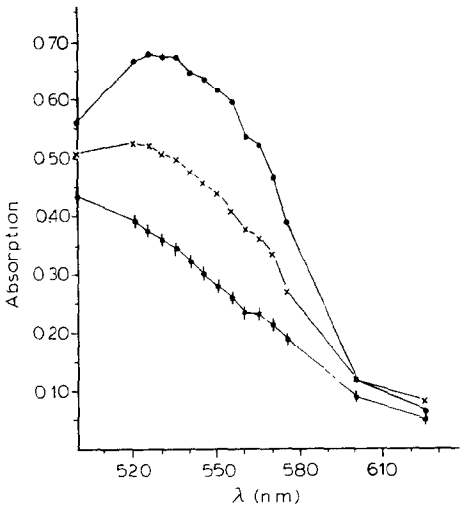

Fig. 1. Spectra of calmagite (—●—) and calmagite in the presence of 1 mg of γ-CD (—x—) and 10 mg of γ-CD (—✦—) at low pH; total volume, 10 mL.

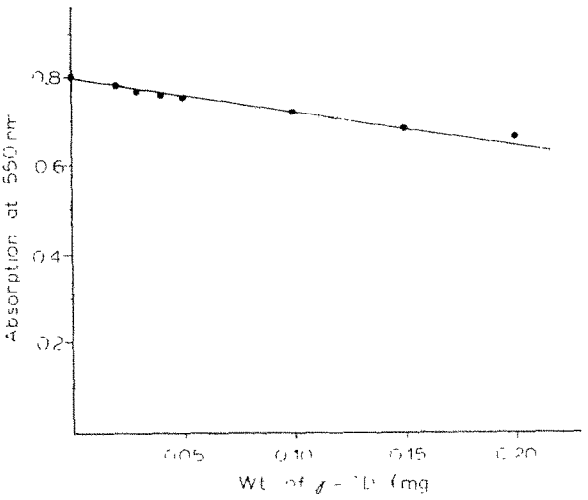

Fig. 2. Absorption at 550 nm of γ-CD–calmagite complexes with different concentrations of γ-CD; total volume, 10 mL.

resin (elution with water) has been described[6–8]. The colorimetric assays may be used as alternatives to chromatography, especially for low concentrations of cyclodextrin and also where the presence of linear oligosaccharides interferes. For γ-CD, a specific colorimetric analysis method was not available hitherto.

Whereas changes in absorption spectra occur[2] on the inclusion of such dyes as Congo Red, Methyl Orange, and Crystal Violet in different cyclodextrins, the metal indicator calmagite [1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid] appeared to form complexes with γ-CD only. A calmagite solution shows[9] a bright red colour at low pH. Inclusion in γ-CD causes the spectral changes shown in Fig. 1; γ-CD does not absorb in this region. A wavelength of 550 nm was chosen for subsequent colorimetric measurements.

The effect of the addition of γ-CD on the absorption of a calmagite solution at 550 nm is shown in Fig. 2. The decrease in absorbance approached linearity up to 0.115 μmol (0.15 mg) of γ-CD. Since the range of linearity is relatively small, it is important to determine the correct dilution ratio.

The interaction of calmagite and γ-CD was not disturbed by the presence of starch, linear gluco-oligosaccharides, and α-CD in concentrations 20–50 times that of γ-CD (0.1 mg/10 mL). β-CD did not cause a detectable spectral change at 550 nm when present in equal amounts. A ten-fold concentration of β-CD showed ~10% of the change in absorbance caused by γ-CD. The calmagite molecule is probably too large to be included in the smaller cyclodextrins or the helices of starch. The purity of the calmagite used was not known. However, no attempts were made to purify it or to determine an equilibrium constant for complex formation, since, for the analysis of γ-CD, using a standard solution of γ-CD, the determination of such a constant is not essential.

Calmagite is normally used[9] as an indicator in the titration of calcium or magnesium with EDTA, where such heavy metals as copper and iron interfere. They also interfere seriously with the analysis of γ-CD at concentrations as low as 0.4 μmol/mL. However, addition of EDTA (5mM) to the diluting citrate buffer or to the γ-CD solution before mixing with calmagite completely suppressed the interference, without causing any spectral effects.

A series of samples containing unknown quantities of γ-CD was analysed by the calmagite method and by chromatography using a cation-exchange resin[7,8]. The results were in good agreement (correlation coefficient, $r = 0.99$), showing a linear relationship ($y = 0.996\ x + 0.30$, in which y gives the calmagite results), with a slope close to 1 and passing approximately through the origin. The calmagite method therefore provides a fast and reliable way for the analysis of γ-CD.

EXPERIMENTAL

Cyclodextrins (α-CD, β-CD, and γ-CD) were obtained from Sigma, and calmagite was a product of unknown purity from BDH.

An aqueous stock solution (50 mg/100 mL) of calmagite, prepared at room temperature and filtered, was completely stable when stored in the dark[9]. This solution was diluted, as required, with 0.1M citrate (pH 3).

Spectra of calmagite and calmagite–cyclodextrin complexes were recorded with a Pye–Unicam SP 600 spectrophotometer. Further colorimetric measurements at 550 nm were performed with a Vitatron DCP filtercolorimeter, using 1-mL samples (Brand-Multilutor automatic dilution apparatus) of cyclodextrin solutions, transferred with 4 mL of water into 5 mL of calmagite solution (diluted from stock, 15→100 mL).

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