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Microcalorimetric Method of Analytical Determination of Cyclodextrins

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Instrument: *2277 BioActivity Monitor*

Field of Application: *Biochemistry*

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INTRODUCTION

Several methods are used to determine cyclodextrins (CD) in solutions. For individual compounds, spectrophotometric and polarimetric methods can be used to find the content of CD or to control the purity of a compound. For the mixtures containing two or more CDs the chromatographic methods are available but in some cases they are not convenient to use.

A simultaneous determination of α- and β-CD is important, e.g. for the optimization of enzymatic synthesis of CDs from starch. In certain conditions some enzymes yield mainly α- and β-CD and only small quantities of γ-CD.

In this study, we propose a new analytical method which is based on microcalorimetric measurements of heat effects due to the formation of molecular complexes between CDs as "hosts" and two substrates, benzoic acid and 5-methylresorcinol (1,3-dihydroxy, 5-methylbenzene), serving as "guests".

Theory

α -and β-CD are cyclic oligomers of glucose containing 6 or 7 glucose residues respectively. In solutions they form molecular inclusion complexes with substrates at a molar ratio of 1:1.

For example, α-CD forms the complex with the first substrate, benzoic acid (S_1):

$$\alpha\text{ - CD} + S_1 \underset{}{\overset{K_1}{\rightleftharpoons}} \alpha\text{ - CD-}S_1$$

where K_1 is the dissociation constant of the complex α - CD-S_1

The analogous equations can be written for the α-CD complex with S_2 orcinol and for the β-CD complexes with S_1 and S_2.

Obviously, the steady concentrations of complexes depends on the dissociation constant values and on the initial (total) concentrations of components in solution.

A significant exothermic effect is typical of the complex formation.

Using the flow-mix cell of Calvet-type microcalorimeter, two calorimetric measurements have to be carried out for the simultaneous determination of α and β-CD in solution.

In the first experiment, a solution containing α- and β-CD is mixed with that of the first substrate S_1 and the heat effect is measured. The second measurement has to be made, mixing the same solution with the second substrate.

Two solutions are pumped into the measuring cell with flow rates q_1 and q_2 (ml/s), a total flow rate being $q = q_1 + q_2$ and the heat flow value is measured after the thermal stabilization of the system. From the measured heat flow values N_1 and N_2, the initial (total) concentrations of α- and β-CD can be calculated considering that the observed heat effects depend linearly on the steady concentrations of complexes in solution.

Choosing two quite different substrates, the values of N_1 and N_2 contain enough information to calculate the CDs content with a sufficient accuracy. For the α - and β-CD system, benzoic acid and orcinol have been found to be suitable.

For calculations the values of dissociation constants (M) and molar heat effects of complex formation (kJ/mol) have to be known. These values have been found in special microcalorimetric experiments and they are presented in **Table 1.**

System	K_d, M	Q, kJ/mol
α-CD + benzoic acid	0.0017	39.4
α-CD + orcinol	0.0028	16.0
β-CD + benzoic acid	0.065	14.0
β-CD + orcinol	0.011	21.0

Table 1. *The values of dissociation constants and molar heat effects of α- and β- CD complexes.*

EXPERIMENTAL

Dried "Chinoin" cyclodextrins, benzoic acid (S_1) and 5-methylresorcinol (orcinol) (S_2) were used. All solutions were prepared in distilled water. The concentrations of the substrates were as follows: benzoic acid 2 - 2.2 g/l and orcinol 5 -10 g/l. The solutions of α- and β-CD contained 0-10 g/l of CD, usually less than 5 g/l of each.

The 2277 BioActivity Monitor was used in the flow-mix mode at 30°C. The solutions were pumped into a flow-mix cell by an LKB 2132 twin channel microperpex peristaltic pump at a total flow rate about 20 ml/h (0.0056 ml/s). The rates on both the channels were precisely measured.

A scheme for thermochemical analysis is given in **Figure 1.**

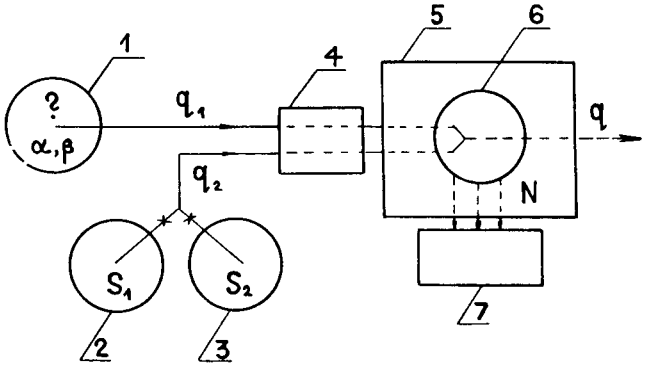

Figure. 1. *Scheme of thermochemical analysis of α - and β - CD solutions. 1) Vessel of CD solution, or reactor for CD synthesis. 2) Benzoic acid solution. 3) Orcinol solution. 4) LKB 2132 microperpex pump. 5) LKB 2277 microcalorimeter. 6) Flow-mix cell of combination measuring cylinder 2277-204. 7) LKB 2210 potentiometric recorder.*

RESULTS AND DISCUSSION

We have prepared water solutions of pure α - and β-cyclodextrins at different concentations using an α-CD/β-CD molar ratio of 1:1 to 8:1. Such solutions imitate the reaction mixtures of CD enzymatic synthesis where the excess of α-CD is usually noticeable and the content of γ-CD is low. The heat flow values were measured, mixing the CD solutions separately with benzoic acid and orcinol solutions. Then the CD concentration values were calculated and compared with the real ones. To illustrate this, some results are given in **Table 2.**

Run	Heat flow (μW)		Concentrations (g/l)			Relative
	N_1	N_2		Actual	Found	error (%)
1.	441	151	α	3.63	3.87	6.6
			β	3.70	3.64	-1.6
2.	302	100.5	α	2.37	2.58	8.9
			β	2.41	2.37	-1.7
3.	229.5	83	α	1.82	1.88	3.3
			β	1.85	2.03	9.7
4.	736	207	α	8.19	7.74	-5.5
			β	4.78	4.83	1.0
5.	339	86.6	α	3.28	3.23	-1.5
			β	1.91	1.92	0.5
6.	174	43.5	α	1.64	1.62	-1.2
			β	0.957	0.949	-0.8
7.	945	186	α	10.93	11.42	4.5
			β	3.19	3.17	-0.6
8.	436	79.1	α	4.37	4.56	4.3
			β	1.27	1.32	3.9
9.	255	35	α	2.62	2.70	3.1
			β	0.381	0.384	0.8

Table 2. *Determination of α- and β-CD. Concentrations of benzoic acid and orcinol before mixing in runs 1-3 2.2 and 5.6 g/l, in runs 4-9 2.0 and 5.0 g/l respectively. Flow rates $q = 0.00566$, $q_1 = 0.0029$, $q_2 = 0.00276$ ml/s. Substrates on the first channel.*

The mean absolute relative error for 18 estimations is equal to 3.3 per cent. It seems that the accuracy of analysis may be estimated as being about 5 per cent.

About 15 minutes is needed for one heat flow measurement, consequently, about 30 minutes for one run. The dilution effects are very weak but it is necessary to measure them periodically to get correct heat flow values corresponding to the complex formation. The method seems to be convenient, simple and quite rapid.

We have used the above method to determine CDs in solutions of their enzymatic synthesis. It has been established that the heat effects of starch conversion are insignificant and the linear products of starch hydrolysis do not disturb the cyclodextrin analysis.

REFERENCES

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