Performances of evaporative light scattering detector for unmodified cyclodextrins analysis by liquid chromatography

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Abstract

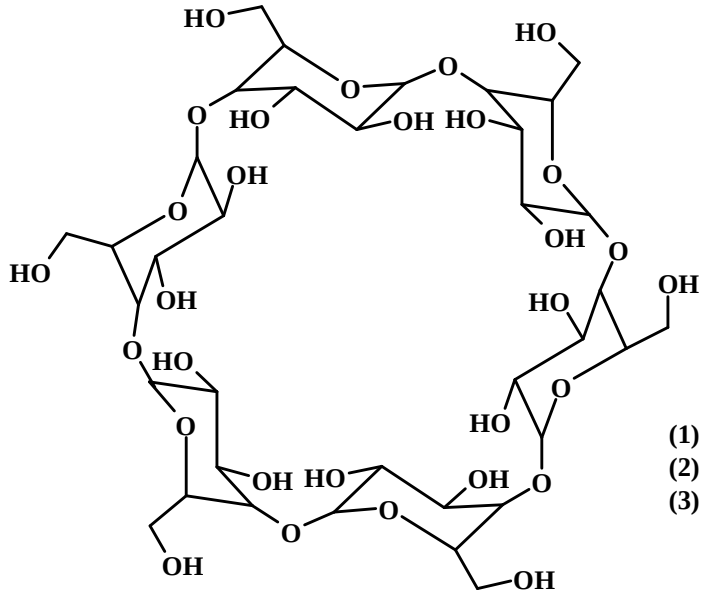
Evaporative light scattering detector (ELSD) was used as detection system for isocratic analysis of α-, β- and γ-cyclodextrins by liquid chromatography (LC) on Zorbax phenyl (250 x 4.6 mm I. D.) column. An evaluation of ELSD response by study of the variation of drift tube temperature was realized. Simultaneous analysis of minoritary compound in presence of a majoritary compound without interferences and saturation is investigated by ELSD offering the possibility of gain variation.

Keywords: Evaporative light scattering detector, drift tube temperature, gain variation, simultaneous analysis, α-, β- and γ-cyclodextrins, liquid chromatography

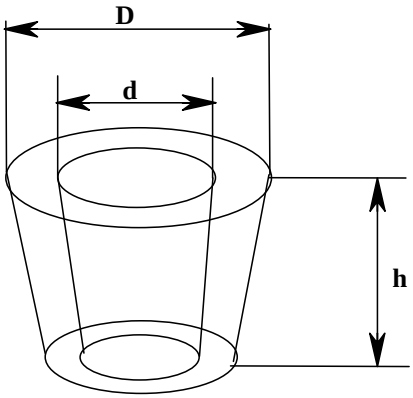
1. Introduction

Cyclodextrins (CDs) are seductive molecules, appealing to investigators in both research and applied technologies. CDs are cyclic oligosaccharides composed of glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity [1]. The cavity of CDs is relatively hydrophobic comared to water, while the external faces are hydrophilic [2].
The most extraordinary characteristic of CD is its ability to form inclusion complexes with a variety of compounds, i.e., caging foreign molecules (guest) in its cavity (host). Generally, hydrophobic molecules or some hydrophobic residues have the highest affinity with the CDs cavity in aqueous solution. It has been well established that the ability of β-CD to enhance the stability and solubility of drugs is mediated through the formation of inclusion complexes [3].
In pharmaceutical product development, β-CD a category of pharmaceutical excipients, have been widely used to improve solubility, chemical stability and bioavailability of a number of poorly soluble compounds [4-6]. Figure 1 shows the chemical structures and molecular dimensions of α-, β- and γ- CDs.
CDs are produced by the action of certain microbial enzymes on starch [7]. The commercially available members of this series are α-, β-, and γ-CDs consisting of 6, 7 and 8 glucose units, respectively. They are known as parent CDs because of their chemically modified products. Both parent and chemically modified CDs are extensively used in food, cosmetic, drug and chemical industry to increase aqueous solubility and stability, and reduce or eliminate the unpleasant taste and smell of many products [8-12]. CDs are water soluble compounds with a hydrophobic cavity capable of dissolving hydrophobic compounds. When a hydrophobic guest molecule such as cholesterol is encapsulated into the hydrophobic cavity of CD molecule, apparent solubility and stability of the guest molecule in aqueous solution are increased [8, 13].
Several reviews have discussed the spectroscopic effects of CDs and how these can be applied in analytical chemistry [14, 15]. Under deoxygenated solvent conditions, CDs can enhance the room temperature phosphorescence of various compounds such as 6-bromo-2-naphtol derivatives [16-19], polynuclear aromatic hydrocarbons [20, 21], or acid-base indicators such as neutral red [22]. In the case of some exceptional, well protected complexes strong room temperature phosphorescence signals can even be observed without deoxygenation [23]. CDs can be used to discriminate between

(+)- and (-)- naphtylethylamine based on differences in fluorescence lifetimes [24]. Recently, we have used the potentialities of CDs to separate enantiomeric compounds by electrophoresis capillary [25].



(1) α - CD : 6 glucopyranose unit
(2) β- CD : 7 glucopyranose unit
(3) γ - CD : 8 glucopyranose unit



	α-CD	β-CD	γ-CD
D (nm)	1.37	1.53	1.69
d (nm)	0.57	0.78	0.96
h (nm)	0.78	0.78	0.78

Figure 1: Chemical structures and molecular dimensions of α-, β- and γ-CDs

CDs can be detected in LC with ELSD system. While ELSD principle appears ideally suited when there is a large difference in volatility between eluent and sample. This principle excludes the analysis of volatile solutes, for which GC and suitable detection were conceived.

The principal steps of ELSD are nebulization of column effluent in a stream of warm gas followed by vaporization of the solvent leaves a cloud of particles consisting on the non-volatile material contained in the eluent. These particles are carried by the warm across a laser beam. Light scattered by the particles is collected and transformed into a current used as the detector signal.

This paper describes the performances of ELSD for the simultaneous analysis of α-, β- and γ-CDs by LC in a single and isocratic run analysis. Realization of a study concerning the effect of drift tube temperature (DTT) on the response of α-, β- and γ-CDs by ELSD was achieved. The study shows also the possibility analysis of a minoritary analyte α-CD in presence of a majoritary analyte β-CD. The result was occurred by realization of a gain variation of peaks signals.

2. Experimental

The pump of the chromatographic system used was a P200 from ThermoQuest (Les Ulis, France). It was connected to the A100 autosampler from TSQ. A Sedex 75 ELSD system from Sedere (Alfortville, France) was used for detection. The usual ELSD settings were as follows: DTT 40°C, nebulizer gas pressure 3.6 bars, gain 8 and 16.

Data were collected with EZChrom Elite software from Scientific Software (Pleasanton, CA, USA) running under Windows NT 4.0.

All experiments were carried out with a Zorbax phenyl (250 x 4.6 mm I. D.) (Rockland Technologies, INC., Newport, DE, USA). The flow rate was 1 mL/min. Experiments were carried out at room temperature.
Acetonitrile (RS for LC) was purchased from Carlo Erba (Milan, Italy) and water from the Elgast UHQ II System from Elga (Antony, France). α-, β- and γ-CDs were obtained from Wacker (Lyon, France).

3. Results and discussion

3.1. Effect of DTT of ELSD on the response of α-, β- and γ-CDs

ELSD performances for CDs analysis have been studied in order to optimize the CDs response, to compare the responses of CDs and to obtain a crude estimation of the linearity of the detection system and an approximation of the detection threshold.
Column nebulization favors the elimination of the solvent constituting the mobile phase while avoiding partial vaporization of the solute. Increasing the DTT causes solutes which possess a moderate or light molar volatility to evaporate and consequently, the scattered light has a lower intensity.
Already, we have discussed the effect of DTT on response of ELSD for inorganic anions and cations. So, the DDT don't affect the response of inorganic cations, sulfate and phosphate but these of nitrate and chloride decreases with increasing DTT [26, 27].
The aim of this study is the determination of the effect of DTT on the response of ELSD in order to evaluate optimum temperature conditions for the analysis of different CDs. This work involves studying the detector response for the CDs at various temperatures. This variable response is limited by area peak of each CD. To facilitate the analysis of the results, we traced the area peak of the signal according the DTT.
Figure 2 reports peaks areas of CDs according DTT. Conditions of these analysis were as follows: Nucleosil Phenyl column, mixture of water and acetonitrile (40/60) as mobile phase at 1 mL/min, ELSD 75 (Gain 8, gas nebuliser pressure 3.6 bar). Concentration of each CD was 250 mg/l. The retention times of α-, β- and γ-CDs are respectively 2.5 min, 7.9 min and 5.8 min.

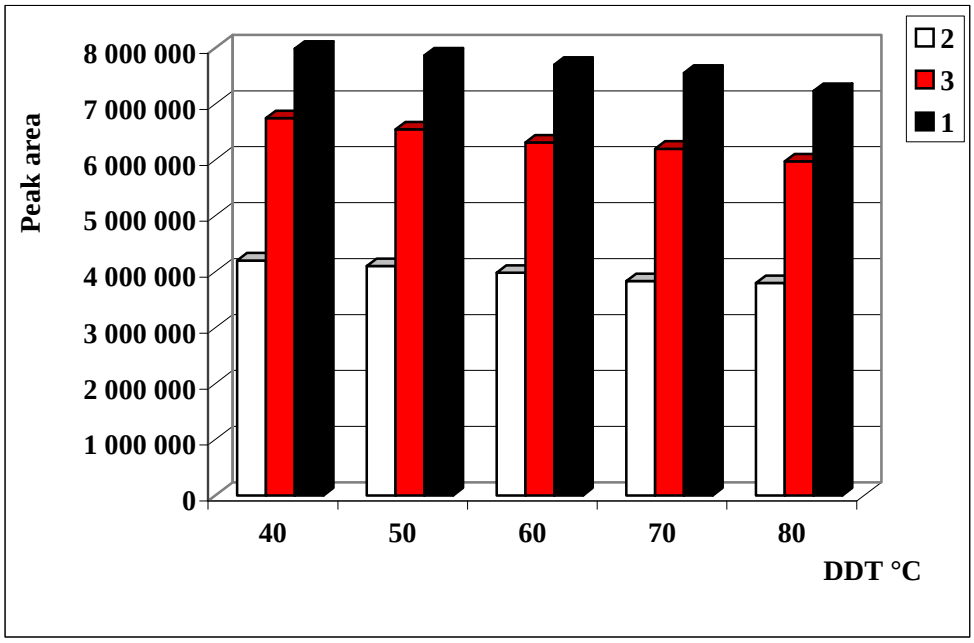


Figure 2: Effect of DTT of ELSD on the response of α-CD (1), β-CD (2) and γ-CD (3)

The variations of DTT studied don't cause a significant background noise with mobile phase containing water and acetonitrile (40/60).
At the same DTT, the order of response of ELSD for CDs is: β-CD < γ-CD < α-CD.
These results of CDs analysis showed that the detector is especially dependent on DTT. The ELSD response for α-, β- and γ-CDs decreases when increasing DTT from 40°C to 80°C. The variation response of α-, β- and γ-CDs passing from 40°C to 80°C was respectively 10%, 11% and 12%. Thus, for all studied CDs the best response of ELSD is with a DTT equal to 40°C. This result is in good agreement with that already published for sugars [28], i.e. a low evaporator tube temperature is better than a high temperature. An increasing of acetonitrile proportion in mobile phase, the times retention of CDs decrease but the values of there areas peaks are unchanged. So, the variation response of ELSD system does not depend of the mobile phase composition. These results are in good agreement with those commonly obtained with series of homologous compounds [29, 30].

3.2. Gain variation of ELSD for the simultaneous analysis of α-, β- and γ-CDs

The gain controls the detector signal amplification to ensure the detection of small peaks. It can take values 1, 2, 4, 8 or 16. A gain of 1 produces an unamplified signal and each other value *n* produces a *n*-fold amplification compared to the original signal [31].

In this study, we realized instead of we hope to realize the simultaneous analysis by LC of a minoritary analyte in presence with a majoritary analyte. We use the ELSD performances that permit the analysis with gain variation. The study was realized at two different values of gain such 8 and 16. Figure 3 offers the variation of baseline with variation of gain. We measured the background noise at these two values. For gain 8 the background corresponds to 0.2 mV and to 1 mV for a gain equal to 16. Application of this technique is realized of a mixture of native CDs such as α-CD, β-CD and γ-CD with concentrations respectively as 5 mg/l, 2500 mg/l and 2.5 mg/l. Ratios of (β-CD / γ-CD) and of (β-CD / α-CD) were respectively 1000 and 500.

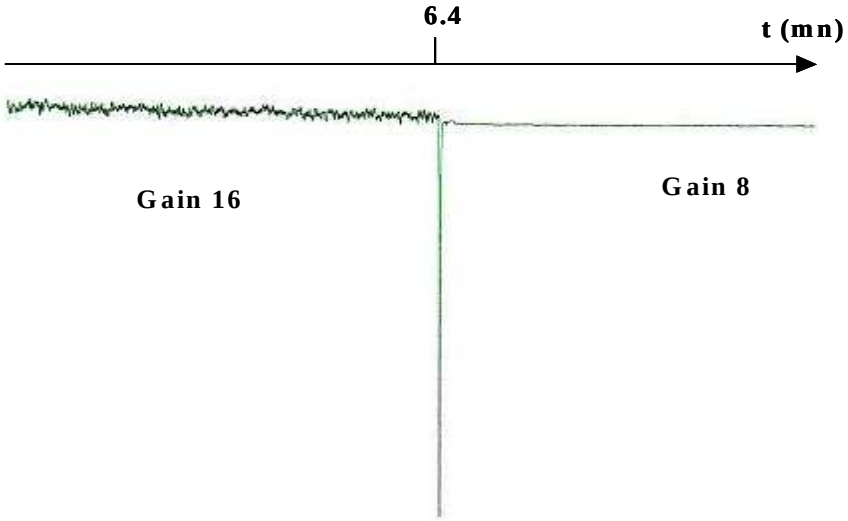


Figure 3: Background variation at two different values of gain

With a gain equal to 8, peaks of α-CD and γ-CD dont appears in chromatogram. So, then of β-CD appears as an unsaturated peak form. Figure 4a represents the separation profile. Under a gain equal to 16, peaks of α-CD and γ-CD appears simultaneously with of then of β-CD. The latter is appeared as saturated peak. Figure 4b reports the profile of the simultaneous separation of CDs.

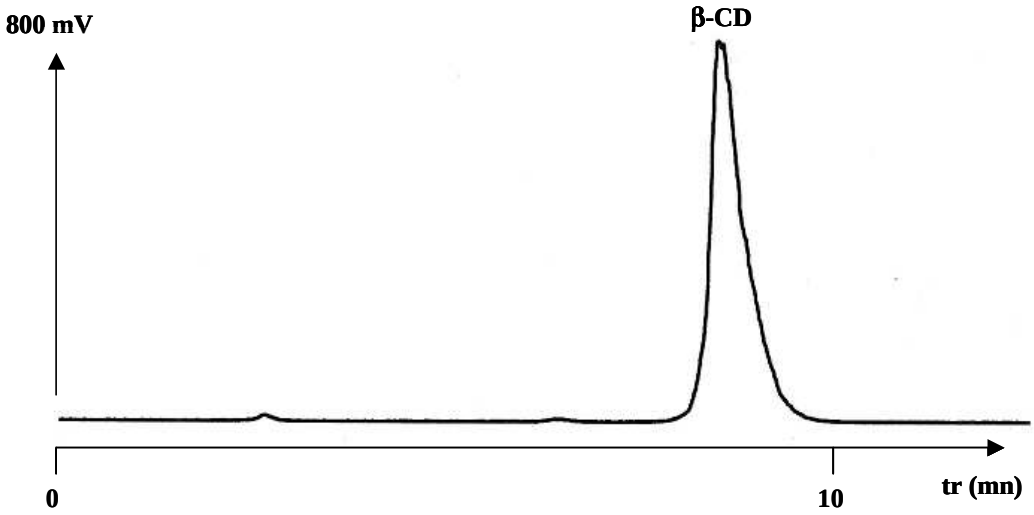


Figure 4a: Isocratic analysis of α-, β- and γ-CDs with gain 8

In order to achieve efficient analysis without any saturated peak and the appearance of all peaks, we have used ELSD program software to realize such analysis with variation of gain.

Peaks of α-CD and γ-CD appear very quickly than that of β-CD. Thus, analysis was occurred with a gain equal to 16 before 6.4 minutes of run time analysis. After 6.4 minutes analysis was achieved with a gain equal to 8. The separation was realized successfully with good selectivity and is presented in Figure 4c. Thus, we can develop this knowledge to carry out the analysis of other matrix containing minoritary analyte in presence with majoritary analyte.

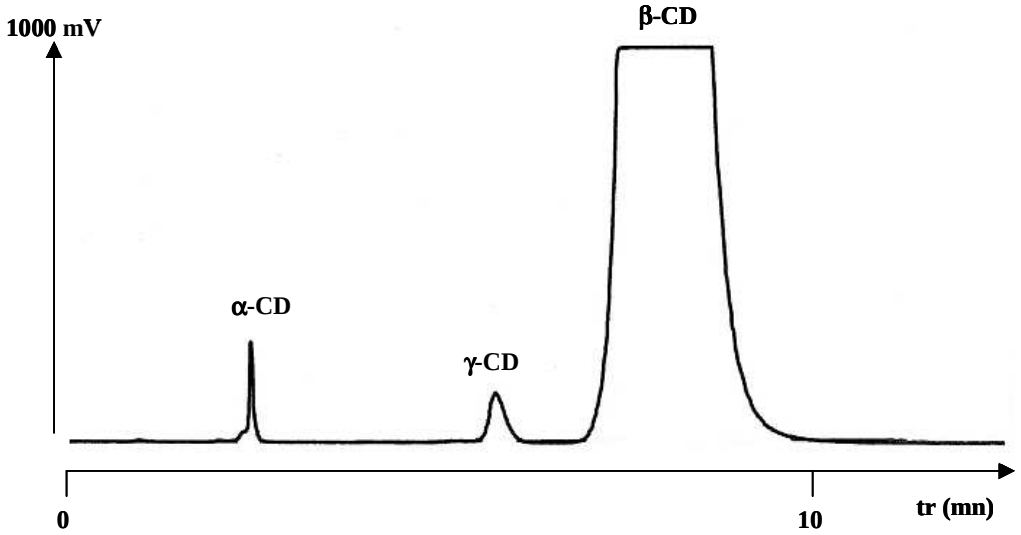


Figure 4b: Isocratic analysis of α-, β- and γ-CDs with gain 16

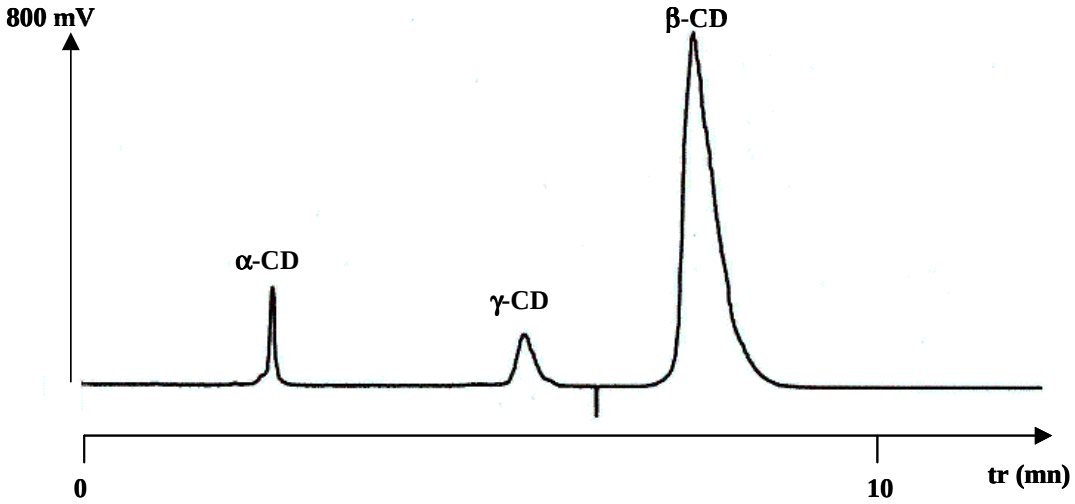


Figure 4c: Isocratic analysis of α-, β- and γ-CDs with gain 8 and 16

3.3. Analytical performance data

Although the variation of the ELSD response is very complex, it was assumed that in a large range of sample size the measured peak area could be related to sample size by the following relationship: $A = aC^b$

Where b is the slope of the response line, C is the solute concentration, and a is the response factor. As a result, the linearity between peak area response, A, and concentration is obtained in double logarithmic coordinates [32, 33] according to: Log A = b Log C + Log a

Calibration curves have been carried out with the same chromatographic conditions cited below. For α- and γ-CDs studied (Concentrations vary from 2.5 to 1000 mg/l), curves calibration were linear with an acceptable correlation coefficient (r^2 = 0,998). Calibration curve of β-CD (Concentrations varied from 500 to 3000 mg/l) is linear. Curves parameters are reported in Table 1.

Table 1: Calibration curves (log A = b log C + log a) for α-, β- and γ-CD

Analyte	Slope (b)	log a	Correlation coefficient (r^2)
α-CD	1.40	6.87	0.998
β-CD	1.42	6.98	0.999
γ-CD	1.47	6.37	0.998

Slope b mentioned in the literature has generally comprised between 1 and 1.6 with 1.3 being the most representative value [32, 33]. Slopes obtained for α-, β- and γ-CDs are contained in the expected interval and are close to the most

representative value 1.3 and also to those of sugars, for which the slope is 1.24 [34] (average slope for α-, β- and γ-CDs is 1.40).
It has been observed by different authors that within a group of homologous compounds, the detector response is the same. Considering the low value of relative standard deviation of slope b (RSD = 1.41%) and the intercept Log a (RSD = 4.76%), the detector response can be considered as equal for α-, β- and γ-CDs.
We concluded that, in order to achieve a more accurate determination of quantitative analysis, it's necessary to make one calibration curve in gamme concentrations for α- and γ-CDs. This technique offer the possibility to realize a quantitative analysis of a mixture constituted of a majoritary and minoritary analytes in one run analysis.

4. Conclusion

Separation of α-, β- and γ-CDs by LC on Zorbax phenyl stationary phase with mobile phase constituted by a mixture of water and acetonitrile (40/60) with ELSD as system detection was realized under isocratic run analysis with good selectivity between all analytes. Study concerning variation of DTT on the response of ELSD for α-, β- and γ-CDs shows that the increasing DTT causes a decrease on the response of CDs by ELSD. The variation is very close to 12% form DTT varying of 40°C to 80°C. The best sensitivity is obtained with DTT equal to 40°C. Realization of a gain gradient in ELSD offers the possibility to make up a good separation of a mixture of high concentration of β-CD at 2500 mg/l and α- and γ-CDs with 5 and 2.5 mg/l.

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