Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, Nos 10–12, pp. 809–812, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Fluorescence enhancement of two terpenes commonly present in essential oils*

R.J. OCHOCKA,†‡ A. MARZEC§ and H. LAMPARCZYK†

‡*Medical Academy, Faculty of Pharmacy, Hallera 107, PL-80416 Gdańsk, Poland*
§*Medical Center of Postgraduate Education, Dept. of Biopharmaceutics, Dębowa 3, PL-85626 Bydgoszcz, Poland*

Abstract: The fluorescence spectra of anethole and eugenol dissolved in methanol–aqueous binary systems with the addition of α- and β-cyclodextrin were studied. Observed enhancement of the fluorescence intensity is possibly due to the higher quantum yield of the cyclodextrin–hydrocarbon inclusion complexes. The measured fluorescence intensities for eugenol and anethole in the presence of α- and β-cyclodextrin were processed using principal component analysis. The results obtained suggest 1:1 and 2:1 complexation, as confirmed by a double reciprocal plot for terpenes complexed to cyclodextrins. In both cases (anethole and eugenol) detection limits were improved after addition of cyclodextrins. This phenomenon can be applied for improvement of direct fluorescence and HPLC-fluorescence assays.

Keywords: *Terpenes; cyclodextrin inclusion complexes; fluorescence enhancement.*

Introduction

Cyclodextrins (CD) are a group of oligosaccharides that contribute to several guest-associated phenomena in solution [1]. They were found to enhance the fluorescence intensity of hallucinogenic drugs [2], pyrene [3], naphthalene and its derivatives [4], anilinonaphthalenesulphonates [5], and methyl salicylate [6]. The primary factors in CD-guest complexes are Van der Waals forces. However, other factors such as shape of the guest molecules may also be important [7].

Terpenes are an important group of compounds found in large amounts in plants. Many complex drugs derived from plant material and also most of the essential oils are mixtures of various terpenes. Moreover, many terpenes are chiral compounds and hence may be present in natural mixtures in homochiral or racemic mixture form [8]. From the chemical point of view terpenes represent a group of relatively simple compounds with low molecular weight and low water solubilities. On the other hand, they display great variability of physical properties and have found many practical applications.

As the analytical limits of conventional fluorescence instrumentation are approached, chemical manipulation of analytes may be attempted in order to improve sensitivity and/or selectivity of the analysis. One method involves complexation of the fluorescent analyte with a host in solution. Two cyclodextrin homologues, namely α- and β-cyclodextrin are used here as host molecules; they have internal diameters of approximately 6 and 7.5 Å, respectively and can produce inclusion complexes of various composition. For naphthalene and β-cyclodextrin, for instance, 1:1, 1:2 and 2:2 inclusion compounds have been proposed; each of these complexes have different fluorescence characteristics [4]. Therefore, in order to resolve this multivariate problem principal component analysis (PCA) [9] has been applied.

Materials and Methods

Eugenol (Merck) and *trans*-anethole (Janssen-Chimica) were used as received. α-Cyclodextrin and β-cyclodextrin (Chinoin, Budapest) were recrystallized once from boiling water. All solvents used were triply-distilled and water was also deionized.

Steady-state fluorescence excitation and emission spectra were obtained with an Aminco–Bowman model Ratio II spectro-

*Presented at the "Fourth International Symposium on Drug Analysis", May 1992, Liège, Belgium.
†Author to whom correspondence should be addressed.

fluorimeter equipped with double excitation and emission monochromators and a 450 W continuous xenon light source. Hard copies of spectra were generated with an Aminco Multi-Range *x–y* recorder. Conventional cuvettes with Teflon stoppers were used.

Methanol–water (40:60, v/v) stock solutions of eugenol and anethole were prepared in concentrations of 1.02 and 1.01 × 10^{-3} M, respectively. α-Cyclodextrin and β-cyclodextrin were dissolved in the same binary phase in concentrations of 2.5 and 1 × 10^{-2} M, respectively. Working solutions were obtained by appropriate mixing of stock solutions. The relative fluorescence intensities (I_f) were measured using concentrations of terpenes 0.5, 1 and 2 × 10^{-5} M. The concentrations of α-CD and β-CD differed because the solubility of α-CD in water is 10 times that of β-CD. Therefore for α-CD the working concentrations were 0.25, 0.75, 1, 1.5, 2 and 2.25 × 10^{-2} M, whereas for β-CD they were 1, 3, 5, 7, 8 and 9 × 10^{-3} M.

The absorbance spectrum for each solution was obtained in order to select a suitable wavelength for recording emission. The emission maximum was used in recording the fluorescence excitation spectra.

Results and Discussion

Figure 1 shows the fluorescence spectra of anethole in the absence and presence of CDs in methanol–water solution. Similarily, Fig. 2 shows the effect of CDs addition upon the corresponding spectrum of eugenol. The fluorescence band induced by excitation near 260 nm is enhanced by addition of the CDs. The enhancement of the emission bands seen in Figs 1–2 can be interpreted as due to the formation of a terpene–cyclodextrin complex that has a higher fluorescence yield than the guest compound.

Application of fluorimetry to the study of inclusion complexes produces a multivariate problem which can be resolved by principal component analysis. In this method the starting point for further calculations is a data matrix X, consisting of $K = 1,2 \ldots k$ variables and $N = 1,2, \ldots n$ objects. PCA provides an approximation of a data matrix (X) in terms of two small matrices T and P. These matrices capture the essential data patterns of X. Plotting the columns of T gives a picture of the dominant object patterns of X. Similarly,

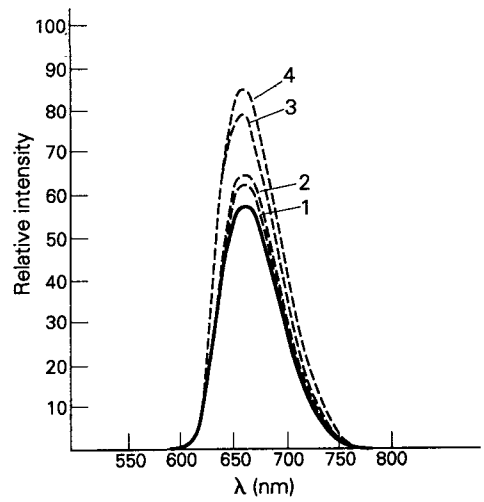

Figure 1
Fluorescence spectra of anethole (1.0 × 10^{-5} M) in a methanol–water (40:60, v/v) system in the presence of α- and β-CD. Without CD ———, with CD ------. Initial concentrations of β-CD: 1 = 3.0 × 10^{-3} M; 2 = 9 × 10^{-3} M and α-CD: 3 = 2.0 × 10^{-2} M; 4 = 2.25 × 10^{-2} M. λ_{exc} = 264 nm.

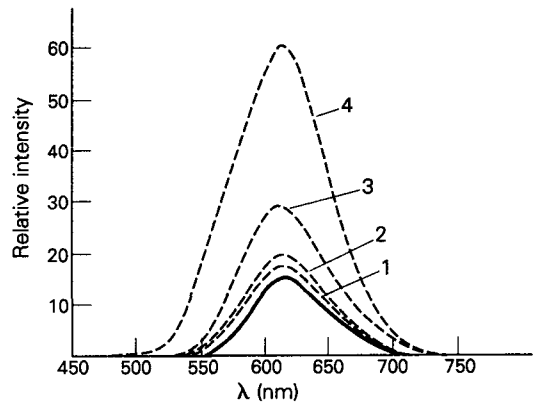

Figure 2
Fluorescence spectra of eugenol (1.0 × 10^{-5} M) in a methanol–water (40:60, v/v) system in the presence of α- and β-CD. Without CD ———, with CD ------. Initial concentrations of β-CD: 1 = 3.0 × 10^{-3} M; 2 = 9 × 10^{-3} M and α-CD: 3 = 7.5 × 10^{-3} M; 4 = 2.25 × 10^{-2} M. λ_{exc} = 264 nm.

plotting the rows of P shows the complementary variable patterns.

In order to elucidate whether anethole and eugenol in various concentrations display similar or different fluorescence behaviour in the presence of α- and β-CD a data matrix X of $N = 6$ objects (anethole and eugenol at concentrations; 0.5, 1 and 2 × 10^{-5} M) and $K = 12$ variables (α- and β-CD at concentrations listed in the Experimental section) was con-

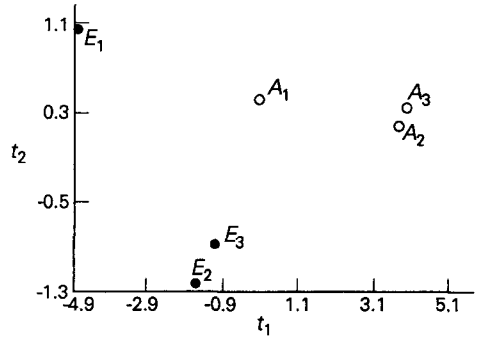

Figure 3
Principal component score plot derived from fluorescence intensities. Terpenes were considered as objects. A, anethole; E, eugenol. Subscripts denote concentration of terpene: $1 = 0.5 \times 10^{-5}$; $2 = 1.0 \times 10^{-5}$ and $3 = 2.0 \times 10^{-2}$ M. Anethole and eugenol are well separated along the t_1 axis.

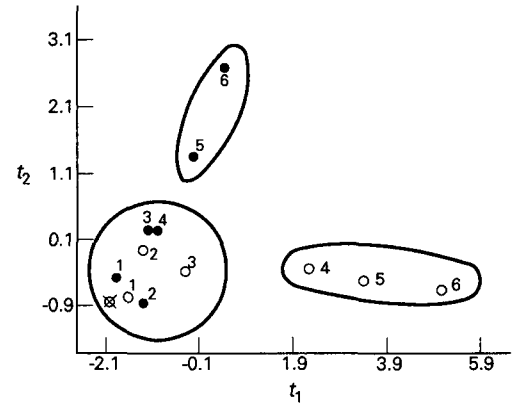

Figure 4
Principal component score plot derived from fluorescence intensities. Cyclodextrins at various concentrations were considered as objects. ○, α-CD; ●, β-CD; ⊗ without CD. Numbers denote concentrations of α-CD: 1 = 0.25; 2 = 0.75; 3 = 1; 4 = 1.5; 5 = 2 and $6 = 2.25 \times 10^{-2}$ M, whereas for β-CD: 1 = 1; 2 = 3; 3 = 5; 4 = 7; 5 = 8 and $6 = 9 \times 10^{-3}$ M. Objects cluster into three groups (circled).

sidered. The following sequence of positive eigenvalues was calculated; 11.2, 0.72, and 10 values less than 0.05. The first two PC score values explain over 99% of the total variance. Figure 3 shows the PC score plot. As can be seen, anethole samples are clearly distinguished from eugenol solutions. The enhancement differences between the two terpenes can be rationalized by possible differences in quantum yields of the respective terpenes. The differences in cavity size between the two cyclodextrins seem to be less important.

In order to elucidate whether α- and β-CD in various concentrations display similar or different inclusion behaviour in the presence of eugenol and anethole a data matrix X of $N = 13$ (α and β-CD at concentrations listed in the Experimental section including 0) and $K = 6$ objects (anethole and eugenol at concentration 0.5, 1 and 2×10^{-5} M) was considered. The following sequence of positive eigenvalues was calculated; 4.76, 1.03, 0.13 and three values less than 0.05. The first two t values describes 96% of the total variance. Figure 4 shows the t_1 vs t_2 plot. Along the t_1 axis the samples fall into three clear classes: α-CD at concentrations from 1.5 to 2.25×10^{-2} M, β-CD at concentrations 8 and 9×10^{-3} M and α-, β-CDs in lower concentrations including samples without addition of cyclodextrins 'O'. Result obtained indicate that the inclusion complex formation of terpenes with cyclodextrins depends mainly on concentration of an appropriate cyclodextrin. This suggests formation of complexes of different host–guest stoichiometry.

The observed enhancement differences between the different cyclodextrins for the same terpene can be explained by considering the differences in physical cavity size of the respective cyclodextrins. However, as stated previously, this effect seems to be less important than that of concentration.

According to Catena *et al.* [5] the host–guest binding constant, K, can be obtained from a plot of $1/I$ vs $1/c_{CD}$, where I denotes the fluorescence intensity and c_{CD} the concentration of cyclodextrin. Plots of this type are called double reciprocal plots. Figure 5 shows a double reciprocal plot for anethole complexed to β-CD. In the same way Fig. 6 shows a plot for eugenol complexed to α-CD. These plots are best described by two linear segments. The initial linear portion represents the formation constant for a 2:1 2(β-CD)–terpene complex, while the final linear portion represents that for a 1:1 β-CD–terpene complex. Similar plots were observed over all ranges of terpene concentrations investigated (not shown). Moreover, the two stage inclusion behaviour of both cyclodextrins investigated is confirmed by results from the PCA method.

The fluorescence intensity enhancements of terpenes seen in the cyclodextrin solutions, resulting in ppb limits of detection, are significant because such analytes are often found to be too weakly fluorescent for quantitative spectrofluorimetry. Although the detection levels in the $\mu g\ l^{-1}$ range are not outstanding,

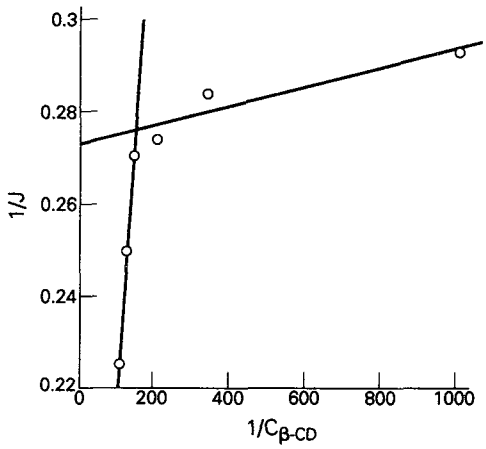

Figure 5
Double reciprocal plot for anethole (0.5×10^{-5} M) complexed to β-CD. The plot indicates 2:1, as well as 1:1 complexation.

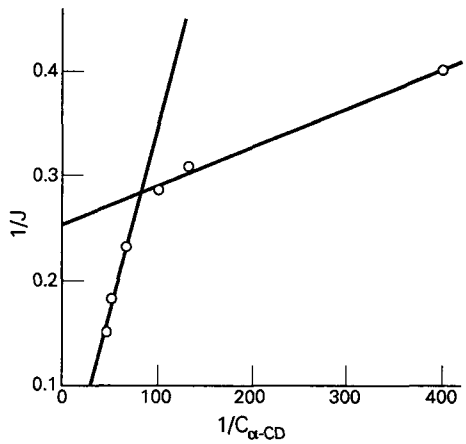

Figure 6
Double reciprocal plot for eugenol (2.0×10^{-5} M) complexed to α-CD. The plot indicates 2:1, as well as 1:1 complexation.

they do represent a considerable improvement in the detectability of these weakly fluorescent compounds which have quantum yields in other media that are too low to allow quantification. This conclusion may be particularly useful in the high-performance liquid chromatographic analysis of terpenes. The detection limit using UV absorption is often too low for quantification. Hence gas–liquid chromatography is assumed to be the method of choice [8], a method, however, which carries the risk of thermal decomposition and/or isomerization [10]. Moreover, HPLC is more adaptable to the determination of optical isomers than GLC. The results presented here suggest that application of cyclodextrins in HPLC with fluorimetric detection to the analysis of terpenes might improve selectivity as well as the detection limit.

Acknowledgement — This work was supported in part by State Committee for Scientific Research grant 00242 9101.

References

[1] D. Sybilska and J. Żukowski, in *Chiral Separations by HPLC*, pp. 147–172. Ellis Horwood, Chichester, UK (1989).
[2] O. Jules, S. Scypiński and L.J. Cline Love, *Anal. Chim. Acta* **169**, 355–360 (1985).
[3] A. Nakajima, *Spectrochim. Acta Part A* **39**, 913–915 (1983).
[4] S. Hamai, *Bull. Chem. Soc. Jpn* **55**, 2721–2729 (1982).
[5] G.C. Catena and F.V. Bright, *Anal. Chem.* **61**, 905–909 (1989).
[6] G.S. Cox and N.J. Turro, *Photochem. Photobiol.* **40**, 185–188 (1984).
[7] H. Lamparczyk, P. Zarzycki, R.J. Ochocka, M. Asztemborska and D. Sybilska, *Chromatographia* **31**, 157–162 (1991).
[8] R.J. Ochocka, D. Sybilska, M. Asztemborska, J. Kowalczyk and J. Goronowicz, *J. Chromatogr.* **543**, 171–177 (1991).
[9] S. Wold, *Pattern Recognition* **8**, 127–139 (1976).
[10] A. Kraus and F.J. Hammerschmidt, *Dragoco Report* **27**, 31–40 (1980).

[*Received for review 6 May 1992*]