Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 55 (2003) 247–251
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Phase solubility studies of pure (−)-α-bisabolol and camomile essential oil with β-cyclodextrin

K.J. Waleczek[a], H.M. Cabral Marques[b], B. Hempel[c], P.C. Schmidt[a,*]

[a]*Department of Pharmaceutical Technology, Eberhard-Karls-University Tuebingen, Tuebingen, Germany*
[b]*UCTF, Faculty of Pharmacy, University of Lisboa, Lisbon, Portugal*
[c]*Robugen GmbH, Esslingen, Germany*

Received 18 March 2002; accepted in revised form 22 November 2002

Abstract

(−)-α-bisabolol was found to form an inclusion complex with β-cyclodextrin (β-CD) in solution as well as in the solid state. To investigate molecular associations of β-CD with pure (−)-α-bisabolol or (−)-α-bisabolol as a component of camomile essential oil, phase solubility studies were undertaken. A B_s type solubility with an apparent complex constant of 273 M^{-1} for the pure (−)-α-bisabolol and 304 M^{-1} for (−)-α-bisabolol as a constituent of the essential oil were obtained. The two curves in the phase solubility diagram reach their plateau at different concentrations of (−)-α-bisabolol, 7.04×10^{-4} M for the pure substance and 2.88×10^{-4} M for the substance as a component of the essential oil. Although the shapes of the curves are almost similar, the intrinsic solubility's of pure (−)-α-bisabolol (4.85×10^{-4} M) and (−)-α-bisabolol as a component of the essential oil (1.82×10^{-4} M) differ significantly. An inclusion complex having a stoichiometric composition of 2:1 (β-CD: drug) was obtained. A mechanism of complexation has been proposed on the basis of the stability constant calculated from phase solubility data and the stoichiometric ratio of the solid state complexation.

Keywords: Phase solubility study; β-Cyclodextrin; (−)-α-Bisabolol; Camomile oil; Inclusion complex; Stability constant

1. Introduction

Cyclodextrins are cyclic oligosaccharides of α-(1,4)-linked D-glucopyranose units in a ring formation containing a relatively hydrophobic central cavity and a hydrophilic outer surface. The most common cyclodextrins are α-, β- and γ-cyclodextrin (CD), which are formed by six, seven, and eight glucose units, respectively. Among the cyclodextrins, the β-CD is widely used since its cavity size is suitable for common pharmaceutical drugs with molecular weights between 200 and 800 g/mol and due to its reasonable price. The most interesting characteristic of cyclodextrins is their ability to form inclusion complexes with a wide variety of guest molecules [1,2]. This phenomenon has received extensive attention in the pharmaceutical field to improve the aqueous solubility, chemical stability, dissolution and release rates of various drug molecules [2–4]. In addition, the complexation may also suppress the volatility and unpleasant odours or tastes associated with the drug [5,6].

The essential oil of *Chamomilla recutita* (L.) RAUSCHERT syn. *Matricaria recutita* L. contains up to 50% (−)-α-bisabolol (Fig. 1). (−)-α-Bisabolol contributes to the anti-inflammatory properties of camomile oil. In 1951, Isaac et al. [7] isolated this monocyclic sesquiterpene from the blossoms of the camomile plant. Bisabolol is a very lipophilic substance, with a tendency to oxidise. The products of oxidation are mainly bisabolol-oxide A and B whose anti-inflammatory activity is about 50% lower [8]. An increase in the oxidation stability of the camomile oils or extracts, including the bisabolol, under the form of a CD complex, is extensively reported in the literature [9,10]. The aim of this study is to characterise the bisabolol/β-CD complex with regard to the positive effects of complexation.

* Corresponding author. Department of Pharmaceutical Technology, Eberhard-Karls-University Tuebingen, Auf der Morgenstelle 8, D-72076 Tuebingen, Germany. Tel.: +49-7071-2972-462; fax: +49-7071-295-531.
E-mail address: peter-christian.schmidt@uni-tuebingen.de (P.C. Schmidt).

0939-6411/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0939-6411(02)00166-2

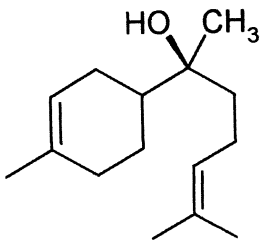

Fig. 1. Structural formula of (−)-α-bisabolol.

2. Materials and methods

2.1. Materials

β-CD was purchased from Wacker Chemie GmbH (Muenchen, Germany), camomile oil and (−)-α-bisabolol were supplied by Robugen (Esslingen, Germany). Other chemicals and solvents were of analytical reagent grade and deionised double distilled water was used throughout the study.

2.2. Methods

2.2.1. Phase solubility studies

The solubility studies were carried out according to the method of Higuchi and Connors [11]. In this, an excess amount of (−)-α-bisabolol or camomile oil (1000 or 500 mg, respectively) was added to screw capped vials containing a β-CD solution in ethanol/water (25/75 V/V) mixture (5.0 ml) at various concentrations ranging from 0.1×10^{-2} to 1.2×10^{-2} M. The vials were shaken at 32°C until equilibrium was reached, i.e. for 48 h, on an Infors shaker (Basel, Switzerland). The shaker was thermostated by a temperature box (WTB Binder, Tuttlingen, Germany). The samples were centrifuged using Megafuge 1.0 R (Heraeus Instruments, Osterode, Germany) at 3000 rpm for 10 min. The resulting solution was then filtered through a cellulose acetate membrane filter (Sartorius, Goettingen, Germany), with 0.8 μm pore size and analysed for (−)-α-bisabolol by gas chromatography as described below.

2.2.2. Analysis of (−)-α-bisabolol with gas chromatography

Analysis of (−)-α-bisabolol was done with gas chromatography using a HP 5890 Series II gas chromatography system and FI detector, equipped with an auto injector HP 7673A and an integrator HP 3392A (Hewlett-Packard, Waldbronn, Germany). A capillary column type Optima®δ 3 (Macherey-Nagel GmbH & Co. KG, Dueren, Germany) was used. The temperature gradient programme was as follows: 120–170°C at 7°C/min, 170–194°C at 3°C/min, 194–300°C at 5°C/min and 300°C for 10 min [12].

2.2.3. Surface tension measurements

The following solutions were measured: purified water, ethanol/water (25/75), (−)-α-bisabolol and camomile oil, both in the ethanol/water mixture. An excess amount of next? (−)-α-bisabolol or camomile oil were added to an ethanol/water (25/75) mixture and shaken for 2 days. The samples were then centrifuged and filtered as described above for the in phase solubility studies. The surface tension measurements of the resulting solutions were performed with a Kruess K12 torsion balance tensiometer (Hamburg, Germany) thermostated at 20°C, using a platinum Wilhelmy plate. The Wilhelmy plate was fixed to the tensiometer prior to starting the measurement. The lifting table was elevated until the plate and the solution to be measured were in contact. Data acquisition began when an augmentation of weight of 0.0005 g occurred. Force values were recorded every 10 s during 3600 s and the surface tension curve was plotted. Data were recorded and analysed periodically using a computer (M2 4/66, software K121, version 2.04a). Mean values, standard deviations and 95% confidence intervals of three samples were calculated.

2.2.4. Preparation of the complexes

Forty-four mg (2×10^{-2} M), 22 mg (1×10^{-2} M) or 16 mg (7.5×10^{-3} M) (−)-α-bisabolol were added to 10.0 ml β-CD solutions (1×10^{-2} or 1.5×10^{-2} M) and shaken for 48 h. The mixtures of (−)-α-bisabolol and β-CD were prepared in triplicate to provide molar ratios of 1:1, 1:2 and 2:1. After equilibrium was reached, the mixture was centrifuged and the supernatant was decanted to provide the complex as a microcrystalline precipitate.

2.2.5. Characterisation of the complexes

The stability constants of the complexes were calculated from the phase solubility diagrams while the stoichiometric ratios were determined by analysing the pure solid complexes (three studies were performed). True complex formation was confirmed by the *n*-hexane-washing procedure and subsequent determination of the non-complexed drug [13,14]. In this, the precipitate was washed with 4 ml *n*-hexane, dissolved in DMSO and extracted with *n*-hexane.

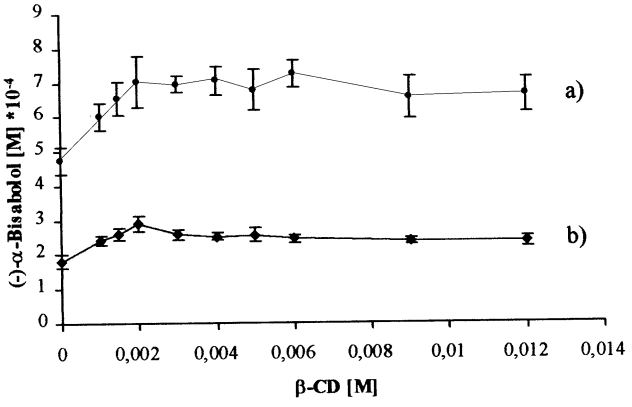

Fig. 2. Phase solubility diagram of β-CD-system of: (a) pure (−)-α-bisabolol (–•–); and (b) (−)-α-bisabolol in camomile oil (–◆–). Error bars represent the 95% confidence intervals.

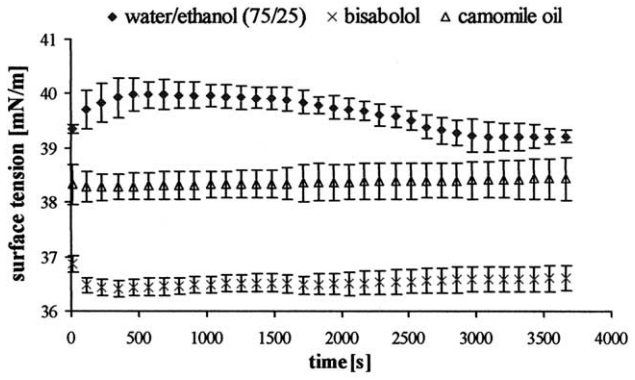

Fig. 3. Surface tension of (◆) water/ethanol mixture, (△) saturated camomile oil water/ethanol solution and (×) saturated (−)-α-bisabolol water/ethanol solution. Error bars represent the 95% confidence intervals.

The total concentration of (−)-α-bisabolol in the wash-hexane, in the precipitate and in the supernatant were analysed using the gas chromatographic techniques described above.

Complex formation was also proved by Leitz hot-stage microscopy (Wetzler, Germany). Thus, about 1 mg of β-cyclodextrin: (−)-α-bisabolol (2:1 complex) or one drop of (−)-α-bisabolol were placed on a microscope slide and covered either with the appropriate cover (conventional method) or with another microscope slide placed 2 mm above the sample (non-conventional method) in order to allow evaporation of the partially volatile (−)-α-bisabolol. Samples were heated between 25 and 360°C and observed during all heating processes.

2.2.6. Molecular modelling

Molecular modelling was done using the Visualiser module of the Material Studio 2.0 Software (Accelrys Inc., San Diego, CA, USA), running on a standard WIN 2000 PC platform. Energy minimising was achieved using the Compass force field of the Discover module.

3. Results and discussion

3.1. Inclusion complexation in solution

The phase solubility diagram of (−)-α-bisabolol and β-CD was obtained by plotting the changes in guest solubility as a function of β-CD concentration. As shown in Fig. 2, the solubility curves can be classified as the B_s type according to Higuchi and Connors [11]. The complex exhibits higher solubility than the guest molecule, but its limit is reached within the tested CD concentration range. Increasing the amount of available CD-molecules does not lead to a rise in solubility, indicating that all guest molecules have been converted into a less soluble inclusion complex [15]. Although the solubility constants [16] are possibly reduced by solvents like ethanol, a mixture of ethanol and water was used to improve the solubility of the lipophilic bisabolol in water. The value of the complex stability constant K_s, for a 1:1 complex can be calculated from the slope of the initial straight line as follows:

$$K_s = \frac{[\beta - \mathrm{CD} \times G]}{[\beta - \mathrm{CD}] \times [G]}\ (\mathrm{M}^{-1}) \tag{1}$$

and

$$K_s = \frac{(S_t - S_o)}{S_o\{[\beta - \mathrm{CD}]_t - (S_t - S_o)\}} = \frac{\tan\alpha}{S_o(1 - \tan\alpha)} = \frac{\text{slope}}{S_o(1 - \text{slope})} \tag{2}$$

Where S_o = solubility of the guest (G) in the absence of β-CD; S_t = concentration of dissolved guest; $[\beta\text{-CD}]_t$ = concentration of dissolved β-CD [15].

At higher β-CD concentrations, a solid microcrystalline complex precipitates, which is supposed to have a higher order than 1:1. This can be derived from the descending part

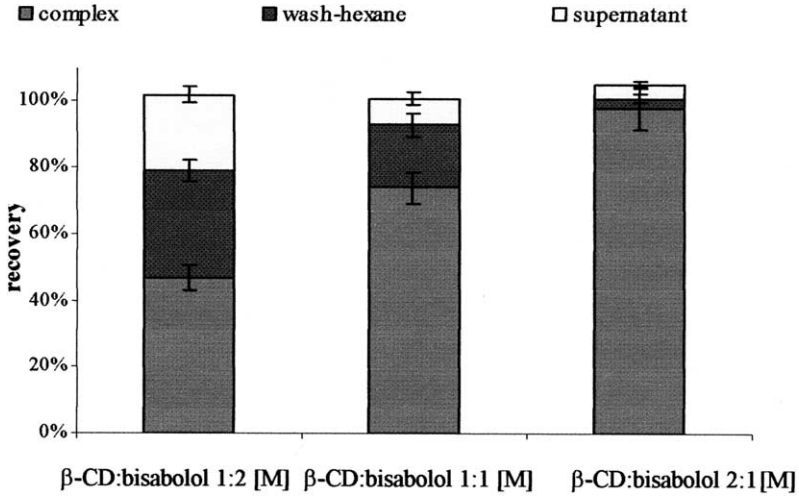

Fig. 4. Recovery of complexed and non-complexed bisabolol after 48 h equilibration time from three different stoichiometric compositions of β-CD-bisabolol inclusion complexes. Error bars represent the 95% confidence intervals.

of the solubility curve, which is described below under inclusion complexation in the solid state.

A stability constant of 273 M^{-1} was calculated for pure (−)-α-bisabolol (a); and 304 M^{-1} for the same substance as a component of camomile oil (b). Williams et al. [17] have described the phase solubility studies of lipophilic natural substances like limonene and cineol. They reported the apparent stability constants for D-limonene and 1.8-cineole with β-CD to be 272 and 193 M^{-1}, respectively, which are similar to the results obtained in this study.

Ono et al. [18] studied binary (CD + guest) and ternary (CD + guest + competitor) systems and found that the stability constant remains unchanged in both cases. In the current study, the shape of both curves (Fig. 2) are similar except that there is only an offset because of the difference in solubility of the pure (−)-α-bisabolol and (−)-α-bisabolol as a component of the camomile essential oil. This indicates that the pure (−)-α-bisabolol shows higher solubility than bisabolol as a constituent of the essential oil. However, this difference did not influence the stability constant in a negative way. Thus, the presence of other camomile compounds does not impair the complexation.

To investigate the influence of the accompanying substances, the surface tensions of solutions of (−)-α-bisabolol and camomile oil in ethanol/water were measured and the results are shown in Fig. 3. The surface tension/time curve of a 75/25% (V/V) water/ethanol mixture is in the range of 39.3–40 mN/m. The saturated camomile oil water/ethanol solution shows a constant value of 38.5 mN/m, whereas for the (−)-α-bisabolol water/ethanol solution after a small initial drop a constant value of 36.5 mN/m is observed, indicating a slightly higher surface activity of the pure (−)-α-bisabolol as compared to the essential oil. Thus, the surface tension measurements support the results of the phase solubility studies. The reduced surface tension observed with the solutions of pure (−)-α-bisabolol could be attributed to the higher intrinsic solubility of (−)-α-bisabolol. The shift in the phase solubility diagram (Fig. 2) is therefore due to the higher surface activity of (−)-α-bisabolol which in turn leads to increased solubility.

3.2. Inclusion complexation in the solid state

Since the cyclodextrin complexes presented above resulted in a B_s-type phase solubility diagram, it was assumed that a solid complex could easily be prepared. In order to determine the stoichiometric composition of the complex, three mixtures of β-CD and (−)-α-bisabolol (1:2, 1:1, 2:1) were prepared and examined. To dissolve non-complexed (−)-α-bisabolol, the solid complex was washed with *n*-hexane. The hexane wash together with the supernatant contains the non-complexed portion. The *n*-hexane-wash method is based on the fact that the β-CDs, as well as their complexes, are insoluble in *n*-hexane. Since only the pure drug is soluble in *n*-hexane, the amount of dissolved drug should provide an estimation of the non-complexed drug fraction in the powder. Fig. 4 shows that the free drug fraction recovered from the 1:1 (β-CD: (−)-α-bisabolol) complex amounts to 18%, 34% from the 1:2 complex and 4% from the 2:1 complex. The low value for free drug in the 2:1 complex suggests optimum inclusion complexation between (−)-α-bisabolol and β-CD, compared to the higher percentages of free (−)-α-bisabolol in the 1:1 and 1:2 complexes. Accordingly, the amount of complexed (−)-α-bisabolol (in the precipitate) is 94% in

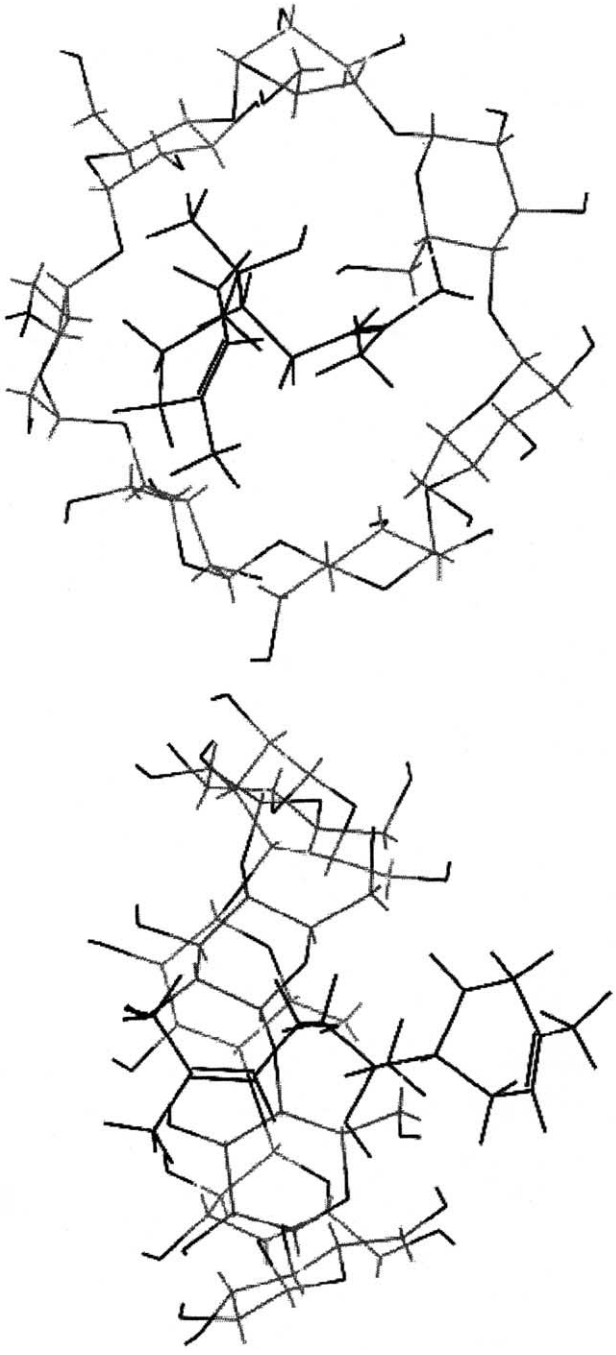

Fig. 5. Molecular model of a 1:1 β-CD: (−)-α-bisabolol complex depicting energy-minimised structure of the complex.

the 2:1 complex, 77% in the 1:1 and 48% in the 1:2 complex.

Thermomicroscopic observations were also carried out. This technique revealed to be very interesting as the included compound has a very low melting point and shows volatility. (−)-α-bisabolol became less viscous? with heating and started to carbonise after about 275°C when studied by the conventional method. When studied by the non-conventional method, the drop showed thinner walls after 95°C, being imperceptible at about 120°C. Slight vapour started to condense at the upper slide at 100°C, being more intense between 110 and 120°C and the oil drop of bisabolol remained on the microscope slide. Between 130 and 135°C, very irregular drops started to be noticeable at the upper slide and at about 140°C the oil drop of (−)-α-bisabolol on the microscope slide was smaller, irregular and by 150°C disappeared giving place to transparent crystals.

β-cyclodextrin: (−)-α-bisabolol (2:1 complex) showed completely different behaviour towards thermal treatment: during heating no change of particle aspect was observed when submitted to the non-conventional method. These observations led us to conclude that bisabolol is protected against heat changes due to the formation of an inclusion complex.

Finally, molecular modelling has been carried out by computer simulation in order to visualise the composition of the complexes. In Fig. 5 a 1:1-complex is presented. The complex-formation in this ratio can also be derived from the phase solubility study (see Section 3.1). The modelling image also reveals that the (−)-α-bisabolol molecule is not completely included in the β-CD. This is evidenced by the insufficient stability to *n*-hexane washing of the 1:1 and 1:2 complexes. Thus, it may be concluded that an excess amount of β-CD improves the stability of the complexes leading to a higher inclusion rate in a 2:1 complex.

Acknowledgements

We gratefully acknowledge Robugen GmbH for financial and material support and Dr K.G. Wagner for the molecular modelling.

References

[1] G. Le Bas, N. Rysanek, Structural aspects of cyclodextrins, in: D. Duchêne (Ed.), Cyclodextrins and their Industrial Uses, Editions de Santé, Paris, 1987, pp. 107–130.
[2] W. Saenger, Cyclodextrin-Einschluβverbindungen in Forschung und Industrie, Angew. Chem. 92 (1980) 343–361.
[3] K. Uekama, F. Hirayama, S. Nasu, N. Matsuo, T. Irie, Determination of the stability constants for inclusion complexes of cyclodextrins with various drug molecules by high performance liquid chromatography, Chem. Pharm. Bull. 26 (1978) 3477–3484.
[4] H.M. Cabral Marques, Structure and properties of cyclodextrins. Inclusion complex formation, Rev. Port. Farm. 64 (1994) 77–84.
[5] J. Buschmann, D. Knittel, E. Schollmeyer, Eigenschaften von Cyclodextrin Komplexen mit Duftstoffen, Seifen-Öle-Fette-Wachse 117 (1991) 585–588.
[6] L. Szente, M. Gal-Füzy, J. Szejtli, Stabilization of camomile oil with β-cyclodextrin, I. Int. Symp. on Cyclodextrins, Budapest, 1981, pp. 431–442.
[7] O. Isaac, H. Schneider, H. Eggenschwiller, Beitrag zur Kenntnis des Kamillen-Bisabolols, Dtsch. Apoth. Ztg. 108 (1968) 293–298.
[8] H. Schilcher, Pharmakologie und Toxikologie in: Die Kamille, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany, 1987, p. 66.
[9] J. Szejtli, L. Szente, G. Kormoczy, P. Tetenyl, A. David, T. Zilahy, A. Kelemen, J. Harshegyi Cyclodextrin-Kamilien-inklusionskomplex. Verfahren zur Herstellung der Komplexe und die Komplexe enthaltenden Praeparate, German patent 2944350, 1980.
[10] M. Thoss, L. Schwabe, K.H. Froemming, Storage and photostability of cyclodextrin inclusion compounds of lemon, orange, hop and chamomile oil, Pharmazie 49 (1994) 252–257.
[11] T. Higuchi, K.A. Connors, Phase-solubility techniques, Adv. Anal. Chem. Instr. 4 (1965) 117–212.
[12] A. Ness, P.C. Schmidt, Kamillenpräparate – Bespiel für die Stabilisierung von Phytopharmaka, Dtsch. Apoth. Ztg. 39 (1995) 3598–3610.
[13] F. Veiga, C. Fernandes, Ph. Maincent, Influence of the preparation method on the physicochemical properties of tolbutamide/cyclodextrin binary systems, Drug Dev. Ind. Pharm. 27 (2001) 523–532.
[14] K.J. Waleczek, B. Hempel, P.C. Schmidt, Preparation and stability of a chamomile ointment containing a supercritical carbon dioxide chamomile extract/b-cyclodextrin inclusion complex, S.T.P. Pharma 12 (2002) 329–333.
[15] K.-H. Frömming, J. Szejtli, Cyclodextrin inclusion complexes, Cyclodextrins in Pharmacy, Kluwer Academic Publishers, Dodrecht/Boston/London, 1994, pp. 50–57.
[16] T. Loftsson, M. Masson, J.F. Sigurjonsdottir, Methods to enhance the complexation efficiency of cyclodextrins, S.T.P. Pharma 9 (1999) 237–242.
[17] A.C. Williams, S.R.S. Shatri, B.W. Barry, Transdermal permeation modulation by cyclodextrins: a mechanistic study, Pharm. Dev. Technol. 3 (1998) 283–296.
[18] N. Ono, F. Hirayama, H. Arima, K. Uekama, Analysis of the phase solubility diagram of a phenacetin/competitor/β-cyclodextrin ternary system, involving competitive inclusion complexation, Chem. Pharm. Bull. 49 (2001) 78–81.