Drug Development and Industrial Pharmacy, 27(5), 457–464 (2001)

RESEARCH PAPER

Evaluation of Stereoselective Dissolution of Racemic Salbutamol Matrices Prepared with Commonly Used Excipients and ^{1}H-NMR Study

T. Srichana[1] and R. Suedee[2,*]

[1] *Department of Pharmaceutical Technology*
[2] *Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-yai, Songkla 90112, Thailand*

ABSTRACT

The purpose of this work was to examine the in vitro enantioselective dissolution of salbutamol from matrix tablets containing various chiral excipients, such as γ-cyclodextrin (γ-CD), heptakis (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), sulfobutyl-β-cyclodextrin (SBE-β-CD), hydroxypropylmethylcellulose (HPMC), and egg albumin. In this study, two types of tablets were prepared; the coated tablet contained the complex of racemic salbutamol and cyclodextrin (γ-CD, DM-β-CD, and SBE-β-CD), and the uncoated tablet was composed of the drug with either HPMC or egg albumin. Subsequently, these formulations were evaluated for enantioselective release. The results revealed that the formulations containing either SBE-β-CD, HPMC, or egg albumin had no enantioselective release, while the formulation with DM-β-CD gave slightly different release of the two enantiomers at the end of the dissolution profile. The formulation containing γ-CD provided significant stereoselectivity throughout the dissolution profile. The release of the eutomer R-salbutamol was higher than that of the distomer S-salbutamol from the γ-CD tablet. In addition, the enantioselective interaction for the γ-CD inclusion complex was investigated by ^{1}H-NMR (nuclear magnetic resonance) spectroscopy and gave evidence to support the enantioselectivity obtained on dissolution.

Key Words: *Chiral excipients; Enantiomer; ^{1}H-NMR; Salbutamol; Stereoselective dissolution.*

* Corresponding author.

INTRODUCTION

One of the key aspects in the formulation of a drug product is the nature and quality of the excipients. The choice of excipient can have an impact on the site of release, the release rate, and the bioavailability of the drug substance. The excipients often interact with the drug substance, forming complexes, which can change the physicochemical properties of the particular compound. Numerous publications reported on investigations of stereoselectivity in the formulation of chiral drugs with chiral excipients (1–6).

Salbutamol is a β_2-agonist agent widely used in the treatment of bronchial asthma and to relieve bronchospasm; it is usually administered as a racemic mixture despite the fact that therapeutic activity resides dominantly in the R-isomer, with no activity attributed to the S-isomer (7). In the formulations of racemic salbutamol, cyclodextrins (CDs), cellulose derivative, or egg albumin are commonly employed as diluents.

These materials could possibly provide enantioselectivity for the release of salbutamol. This is based on the fact that they have been used as chiral selectors for enantioselective separations. For example, CD and its derivatives were found to exhibit excellent chiral recognition as a stationary phase for liquid chromatography, and some of the chiral phases are commercially available and extensively utilized for the purpose of analysis and laboratory chiral separation (8). Moreover, egg white protein has been developed as chiral stationary phases to resolve enantiomers in high-performance liquid chromatography (HPLC) (9,10). Cellulose derivatives such as hydroxypropylmethylcellulose (HPMC) have been used for different functions in pharmaceutical formulations, including use as binders, fillers, disintegrants, and dissolution rate modifiers; many also possess intrinsic chirality.

The aim of this work was to study in vitro enantioselective dissolution of salbutamol from matrix tablets composed of racemic salbutamol and various chiral excipients (γ-CD, heptakis (2,6-di-*O*-methyl)-β-cyclodextrin [DM-β-CD], sulfobutyl-β-cyclodextrin [SBE-β-CD], HPMC, and egg albumin). In addition, ^{1}H-NMR was employed to investigate the enantioselective interaction between salbutamol enantiomer and the chiral excipient that gave the greatest stereoselectivity in dissolution.

EXPERIMENTAL

Materials

Racemic salbutamol was obtained from Allchem International Limited (London, England). R-Salbutamol and S-salbutamol were kindly donated by Prof. Clive Page (King's College, London). Egg albumin was purchased from Sigma (St. Louis, MO). γ-CD and DM-β-CD were obtained from Wacker Chemie Limited (Burghausen, Germany). SBE-β-cyclodextrin (Captisol^{tm}) was obtained from Cydex (Overland Park, KS). HPMC (K4M grade) was supplied by Colorcon (MA). The other reagents were analytical grade and were used without further purification.

Preparation of the Cyclodextrin-Drug Complexes

The complexes of salbutamol and the CDs were obtained by adding equimolar concentrations of either the racemate or the individual enantiomer of salbutamol to each CD in distilled water. The clear solution of a mixture was then freezed-dried for 48 h until it yielded a solid cake. The product was stored in an environmental chamber at 25°C and 45% relative humidity (RH) in a well-closed container.

Preparation of Tablets

In the preparation of the matrix tablets, two types of tablets, coated and uncoated, were prepared. The uncoated tablets included formulations of HPMC and egg albumin, and the coated tablets included formulations of all types of CD-drug complexes. Every tablet was prepared by the direct compression method using 4-kN forces (Manesty E, England) and had a total weight of 80 mg, containing 4 mg racemic salbutamol, 75 mg chiral excipient, and 1% magnesium stearate. To prepare the CD tablets, each CD-drug complex was mixed with 1% magnesium stearate, and the mixture was compressed as the tablet was finally coated with 20% Eudragit E-100 in acetone : isopropanol 1 : 1 w/v (film thickness 2 mm).

Dissolution Studies

The dissolution tests (six replicates) were carried out according to the USP 23 (1995) apparatus II paddle method for all formulations. The medium was 50 mM phosphate buffer pH 7.4 (1 L) stirred at 50 rpm at 37°C. At proper time intervals, 3 ml was automatically withdrawn from each dissolution vessel and replaced with fresh dissolution medium. The samples were immediately assayed for the dissolved concentration for each enantiomer by enantiospecific HPLC following solid-phase extraction (SPE).

Analysis Methods

To extract the drug from aqueous dissolution medium, the samples (1 ml) were loaded onto the silica-packed cartridges further washed with acetonitrile (1 ml). Then, the cartridges were left for 5 min before the drug was eluted from the cartridges with 2% ammonia in methanol (1 ml). The dried extracts were reconstituted with the mobile phase, and 100 µl was injected into the HPLC. For HPLC analysis, the system was composed of a Waters 600 HPLC system (Milford, MA) with a 486 variable wavelength ultraviolet (UV) detector set at 276 nm. A 250 mm by 4 mm Chirex 3022 column (Phenomenex, Torrance, CA) was employed. The mobile phase consisted of hexane, 1,2-dichloromethane, methanol, and trifluoroacetic acid (60:35:5:0.1) pumped at a flow rate of 1.5 ml/min. The retention times of S- and R-salbutamol were 8.5 and 14.5 min, respectively.

Data Analysis

The percentage of cumulative salbutamol enantiomer released was plotted versus time for all formulations. The results are presented as mean ± standard deviation (SD). The significance of the difference between the enantiomers released at each time point was tested using the paired *t* test ($\alpha = .05$).

^{1}H Nuclear Magnetic Resonance Studies

The ^{1}H-NMR spectrum of the required samples was obtained with a 500-MHz spectrometer (Varian, CA) using D_2O as the solvent. The peak at 4.7 ppm assigned to DHO and H_2O as impurities was considered an internal standard in the measurement of the chemical shifts of the peaks of the compounds.

RESULTS AND DISCUSSION

In this study, the matrix tablet of all formulations was made via a direct compression technique; a similar procedure for tablet preparation was previously reported for egg albumin (11). A high content (95%) of all excipients was used as this was expected to provide enantioselectivity.

Release Experiments

Formulations Prepared from the Solid Complexes of Cyclodextrin Derivatives

Preliminary dissolution experiments of the tablets without CD coating indicated dissolution of the whole matrices within 10–20 min. Therefore, the CD-coated tablets were evaluated for stereoselectivity of release. The tablets containing DM-β-CD provided completed release of enantiomers in 6 h, while the tablets containing γ-CD or SBE-β-CD sustained the release of salbutamol enantiomer longer than 10 h. Visually, at the end of dissolution, the matrix of all types of CD entirely dissolved.

The release profiles for the formulations of DM-β-CD, γ-CD, and SBE-β-CD are shown in Figs. 1A, 1B, and 1C, respectively. In the case of DM-β-CD tablets, the release of the two enantiomers of salbutamol was fast, and no stereoselective release was noticed during the first 3 h. After 3 h, the release of enantiomers was slow and close to 100% in 6 h; there was an enantioselective trend in that slightly more of the S-enantiomer was released than the R-enantiomer. However, the level of selectivity demonstrated by this type of tablet was quite small, and the result was significant ($P < .05$) at some time points of the last phase of the dissolution profile (3–6 h). The nonenantioselective release observed at the initial phase might be due to the rapidity of dissolution release, such that it was difficult to determine a difference for the enantiomeric release rates. However, the coating film of the tablet preserved the matrix and extended the release of salbutamol enantiomers longer than should be obtained from the uncoated tablet. In a previous study (12), it was evident that a DM-β-CD tablet prepared without coating did not provide any stereoselective release of salbutamol enantiomers.

The γ-CD matrices showed fast release in the first half period (0–6 h) and slow release in the second half period (6–12 h) (see Fig. 1B). The statistically significant difference of release of enantiomers ($P < .05$) was found throughout the dissolution profile. On the stereoselectivity observed, this matrix formulation released R-enantiomer more than S-enantiomer. As shown in Fig. 2, the R/S ratio of release for γ-CD matrices was time independent with a consistent value at 1.13.

The SBE-β-CD tablets showed biphasic release curves similar to those obtained with γ-CD tablets. Nevertheless, the SBE-β-CD tablet failed to demonstrate enantioselectivity during the dissolution run, although this formulation could prolong the release of salbutamol enantiomers from the matrix up to 10 h. It is possible that the SBE-β-CD dose not virtually have the enantioselective interaction with salbutamol enantiomers.

Among the matrices of CDs, the γ-CD matrix demonstrates the most significant stereoselectivity when compared to the matrix of DM-β-CD or SBE-β-CD. The complex formation between host and guest could be responsible for the enantioselectivity of release. The apparent enantioselectivity obtained in the case of γ-CD im-

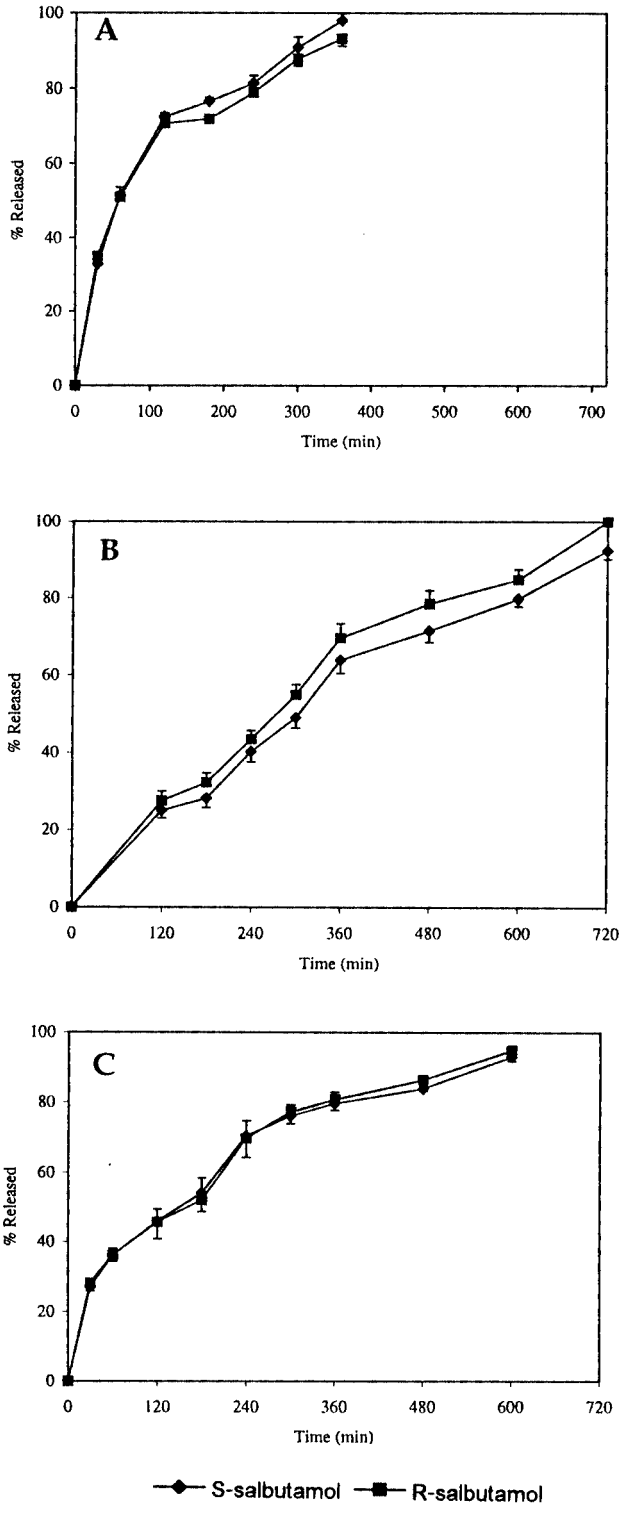


Figure 1. Dissolution profiles: (A) DM-β-CD tablets; (B) γ-CD tablets; (C) SBE-β-CD tablets (mean ± SD, $n = 6$).

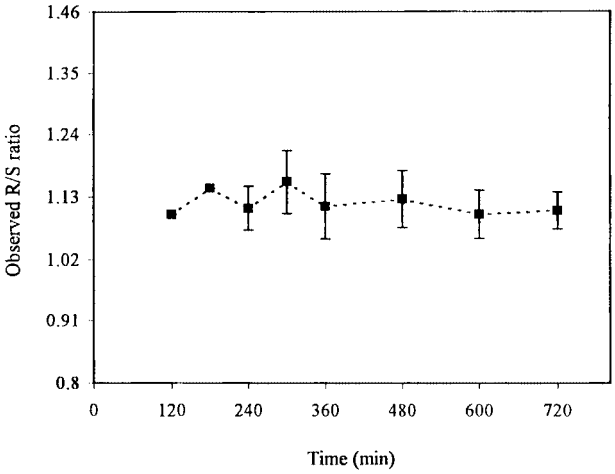


Figure 2. R/S ratio of salbutamol released from γ-CD tablets (mean ± SD, $n = 6$).

plies that the S-enantiomer more strongly interacts with γ-CD than the R-enantiomer. However, the indirect evidence for selectivity is provided by dissolution investigations. Information regarding the host-guest structure would also be of great interest. For this case, ^{1}H-NMR analysis was conducted in a further experiment.

Formulations Prepared from Hydroxypropylmethylcellulose and Egg Albumin

When the HPMC was combined in the tablet, good tablet appearance was achieved. The mean dissolution profile for HPMC tablets is depicted in Fig. 3A. Over 80% of both enantiomers was released from this matrix in 4 h, and dissolution was complete within 8 h with no significant difference between the release of the enantiomers. This observation is in accordance with that in previous work (12,13) using a different polymer:drug ratio and a different tablet preparation method. Visually, the matrix swelled and subsequently dissolved; at the end of the dissolution, all matrices disappeared. The erosion could be a major mechanism of release. As the tablet erodes, aqueous test medium penetrates into the matrix structure and dissolves water-soluble filler. As a result, the release of salbutamol enantiomers was fast. Thus, the HPMC matrix could not modulate the release of enantiomers and hence lacked stereoselectivity.

Likewise, when the stereoselective dissolution of the matrices prepared from egg albumin was evaluated, the egg albumin tablets were intact throughout the dissolution test. The release of enantiomers from the matrix of this

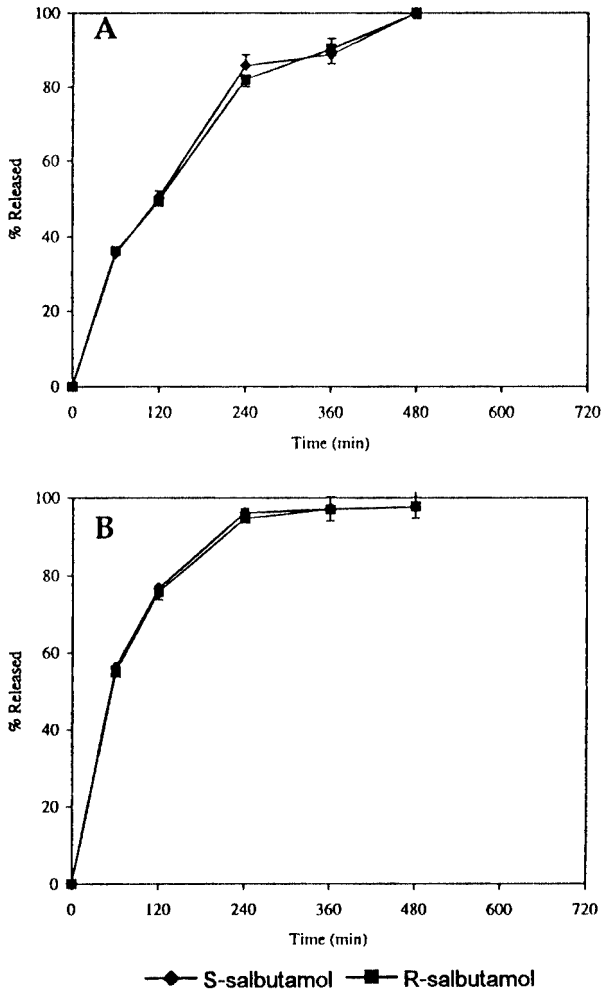


Figure 3. Dissolution profiles: (A) HPMC tablets; (B) egg albumin tablets.

formulation was faster compared other formulations. As shown in Fig. 3B, over 90% of salbutamol enantiomers was released from egg albumin tablets in 4 h. The release pattern seen is also analogous to that obtained with the formulation reported in previous work (11). No significant difference of release was evident for this formulation. Although several studies reported the release of salbutamol from the matrices containing egg albumin, the present study provides information with respect to stereoselective dissolution that differs from the previous studies (9–11)

^{1}H Nuclear Magnetic Resonance Studies

Growing studies by the ^{1}H-NMR technique of the host-guest interaction include studies for the complex of racemic salbutamol with β-CD or DM-β-CD (15). Kim and Park (16) studied the interaction between hydroxypropyl-β-CD and terbutaline enantiomers structurally related to salbutamol using ^{1}H-NMR spectroscopy. Similarly, ^{1}H-NMR spectroscopy was employed here as a probe for the study in the mode of inclusion of salbutamol enantiomers into the CD host to confirm the stereoselectivity obtained from dissolution of the γ-CD tablet (with coating). The technique is based on the shielding of the CD and drug protons. If inclusion does occur, protons located within or near the CD cavity should be strongly shielded.

The ^{1}H-NMR spectra (D_2O) of salbutamol, free γ-CD, and the complexes of either the individual enantiomers or the racemate of salbutamol with γ-CD are illustrated in Fig. 4. As shown in Table 1, the upfield shifts for all the protons of γ-CD were induced by salbutamol in the inclusion complex, suggesting that salbutamol may interact on the exterior surface of γ-CD, as well as in the interior surface. It can be seen that the magnitudes of the shift changes of the corresponding proton signals in the presence of racemic salbutamol were greater than in the presence of salbutamol enantiomers. The effects of S-salbutamol on the resonances of the H-3′ and H-5′ (located within the cavity of γ-CD) are more significant than the effect of R-salbutamol (S-R = 1.0 Hz for all), suggesting that S-salbutamol included deeper into the inner cavity than the R-isomer. Similarly, the effect of S-salbutamol on the resonance of the H-4′ (located outside the cavity of γ-CD) is more significant than the effect of R-salbutamol (S-R = 1.0 Hz).

The effects of γ-CD on the ^{1}H-NMR spectra of salbutamol were also observed (see Table 1). It was evident that, for all the complexes, the salbutamol protons were shifted to lower fields, among which the shift of the phenyl protons and side-chain protons nearest the phenyl moiety was larger than that observed for the *t*-butyl protons, indicating complexation at the phenyl end. The Δδ values in terms of the inclusion mode of the racemate were similar to those reported for an analogous molecule using β-CD as the host (15). In comparison, the shift for the aromatic H-6 of S-salbutamol was larger than that of R-salbutamol, with S-R = 4.5 Hz. It is supposed that the phenyl moiety of salbutamol entered in the cavity in such a way that the H-6 of S-salbutamol is closer to the wall of the cavity of γ-CD than that of the R-enantiomer.

There was also the significant effect of γ-CD on the resonance of the aliphatic side-chain protons of racemic salbutamol and both R- and S-salbutamol enantiomers. Nevertheless, the shift changes of the methine and methylene protons of R-salbutamol were similar to those of S-salbutamol, with the variations within 0.5 Hz. This means

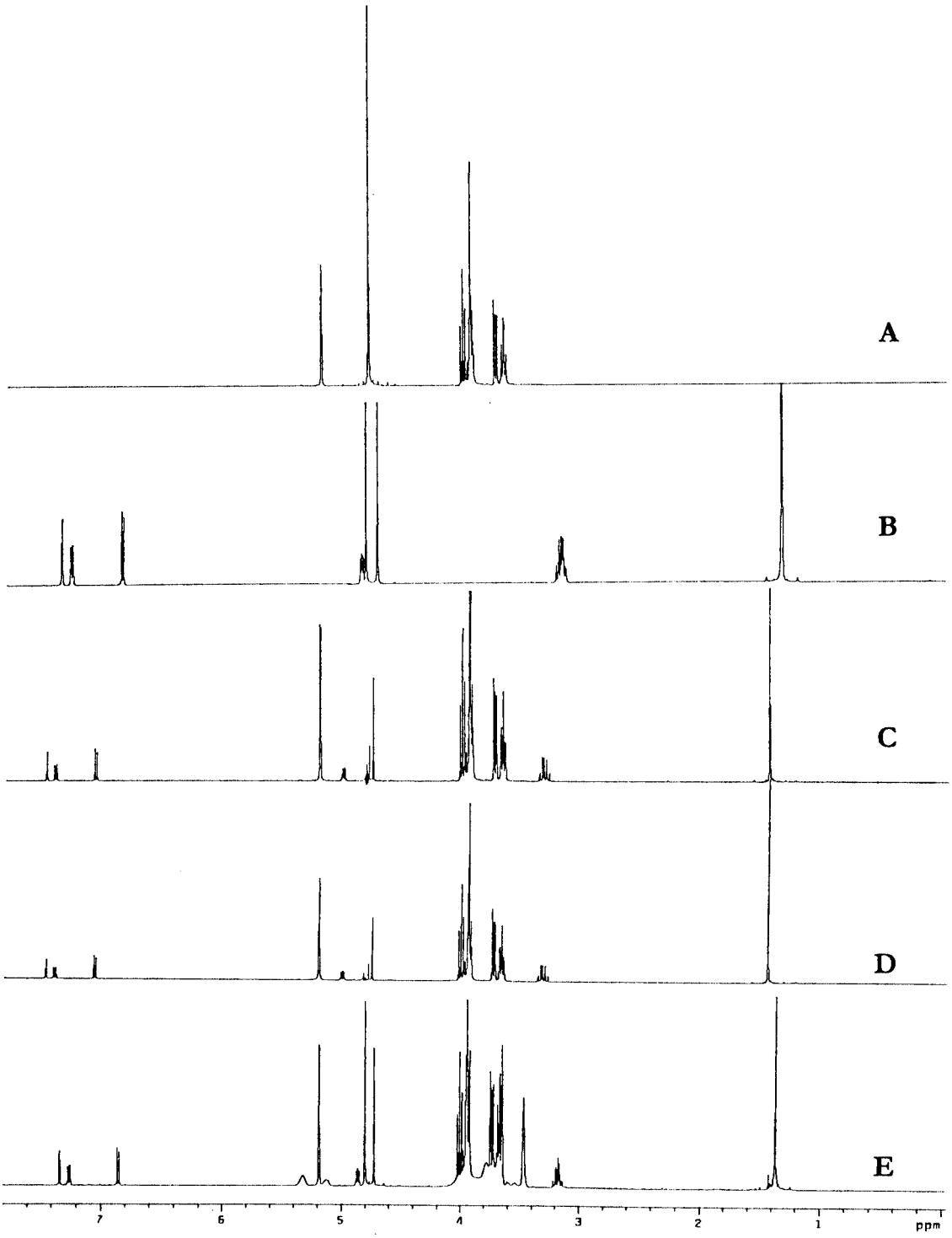
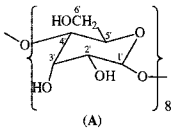


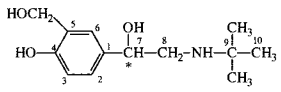
Figure 4. ^{1}H-NMR spectra (D_2O): (A) salbutamol alone; (B) free γ-CD; (C) complex of S-salbutamol; (D) complex of R-salbutamol; (E) complex of racemic salbutamol.

Table 1

Change in Chemical Shifts δ of Some Protons in the Complex of Salbutamol and γ-Cyclodextrin



(A)



(B)

	$\Delta\delta_{\gamma\text{-CD}}$ (Hz)[a]						$\Delta\delta_{\text{salb.}}$ (Hz)[b]						
Complex (1:1)	$\delta_{5.197}$ H-1′	$\delta_{3.740}$ H-2′	$\delta_{4.997}$ H-3′	$\delta_{3.672}$ H-4′	$\delta_{3.934}$ H-5′	$\delta_{3.971}$ H-6′	$\delta_{7.331}$ H-6	$\delta_{7.251}$ H-3	$\delta_{6.843}$ H-2	$\delta_{4.605}$ H-7	$\delta_{3.155}$ H-8	$\delta_{3.121}$ H-8	$\delta_{1.240}$ H-10
R-Salbutamol/γ-CD	−2.0	−2.0	−2.5	−1.5	−2.5	−2.0	+65.5	+70.0	+116.5	+84.0	+88.5	+82.0	+5.0
S-Salbutamol/γ-CD	−2.0	−2.0	−3.5	−2.5	−3.5	−2.0	+70.0	+70.0	+116.5	+83.5	+88.0	+82.0	+5.0
RS-Salbutamol/γ-CD	−5.0	−4.0	−6.5	−4.0	−7.0	−4.5	+13.0	+15.0	+24.5	+15.0	+6.5	+6.5	+2.0

A, γ-CD; B, salbutamol.

[a] $\Delta\delta_{\gamma\text{-CD}} = \delta_{\text{salbutamol/γ-CD}} - \delta_{\gamma\text{-CD}}$

[b] $\Delta\delta_{\text{salb.}} = \delta_{\text{salbutamol/γ-CD}} - \delta_{\text{salbutamol}}$

that the difference in effect of γ-CD on the side chains of R- and S-salbutamol is insignificant. The formation of an inclusion complex is an essential requirement for the chiral separation, but to be sufficient for chiral recognition also needs the interactions of other functional groups around the chiral center with CD to form a secondary inclusion complex (17). For this test, further molecular modeling studies may provide greater insight into the precise mechanism of the chiral separation process.

CONCLUSION

This article is the first report of stereospecific ^{1}H-NMR determination of a complex of γ-CD with salbutamol enantiomer. The ^{1}H-NMR results obtained reveal that the S-salbutamol, particularly the aromatic moiety, is more tightly bound to the γ-CD host than the R-salbutamol. So, the release of S-salbutamol was more retarded by γ-CD than that of R-enantiomer. The results of ^{1}H-NMR support the release result obtained for the γ-CD tablets.

In conclusion, the present study showed that the enantiomers of salbutamol have slightly different dissolution profiles with DM-β-CD. The difference on dissolution for the different enantiomers is very minor. However, the more significant differences of the dissolution studies were demonstrated in the case of the formulation with γ-CD. The results proved that there was the possibility in enantioselective interaction between the drug and excipient in the formulation of racemic salbutamol containing the chiral excipient, which affected the difference in release rates of stereoisomers of salbutamol.

ACKNOWLEDGMENT

Financial support from Prince of Songkla University is gratefully acknowledged.

REFERENCES

1. Aubry, A.; Wainer, I.W. An In Vitro Study of the Stereoselective Dissolution of (rac)-Verapamil from Two Sustained Release Formulations. Chirality **1993**, *5*, 584.
2. Vakily, M.; Jamali, F. Human Pharmaceutics of Tiaprofenic Acid After Regular and Sustained Release Formulations: Lack of Chiral Inversion and Stereoselective Release. J. Pharm. Sci. **1994**, *83*, 495.
3. Carr, R.A.; Pasutto, F.M.; Longstreth, J.A.; Foster, R.T. Stereospecific Determination of the In Vitro Dissolution of Modified Release Formulations of (±)-Verapamil. Chirality **1993**, *5*, 443.
4. Duddu, S.P.; Vakilynejad, M.; Jamali, F.; Grant, D.J.W. Stereoselective Dissolution of Propranolol Hydrochloride from Hydroxypropyl Methyl Cellulose Matrices. Pharm. Res. **1993**, *101*, 648.
5. Maggi, L.; Massolini, G.; deLorenzi, E.; Conte, U.; Caccaialanza, G. Evaluation of Stereoselective Dissolution of Verapamil Hydrochloride from Matrix Tablets Press-Coated with Chiral Excipients. Int. J. Pharm. **1996**, *13*, 943.
6. Suedee, R.; Brain, K.R.; Heard, C.M. Enantioselective Retardation of rac-Propranolol from Matrices Containing Cellulose Derivatives. Chirality **1997**, *9*, 307.
7. Pesola, G.R.; Walle, T. Enantiomeric Interaction in the Sulfate Conjugation of the β_2-Agonist Drug Albuterol by the Human Liver. Res. Commun. Chem. Pathol. Pharmacol. **1992**, *75*, 125.

8. Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademai Kaido: Budapest, 1982.
9. Miwa, T.; Miyakawa, T.; Kawayano, M.; Miyake, Y. Application of an Ovomucoid-conjugated Column for the Optical Resolution of Some Pharmaceutically Important Compounds. J. Chromatogr. **1987**, *408*, 316.
10. Oda, Y.; Asakawa, N.; Kajima, T.; Yoshida, Y.; Sato, T. Online Determination and Resolution of Verapamil Enantiomers by High Performance Liquid-Chromatography with Column Switching. J. Chromatogr. **1991**, *541*, 411.
11. Al-Meshal, M.A.; Bayomi, A. Evaluation of Egg Albumin as a Filler for Prolonged Release Direct Compressed Tablets. Drug Dev. Ind. Pharm. **1995**, *21*, 739.
12. Solinis, M.A.; Lugara, S.; Calvo, B.; Hernanndez, R.M.; Gascon, A.R.; Pedraz, J.L. Release of Salbutamol Sulfate Enantiomers from Hydroxypropyl Methylcellulose Matrices. Int. J. Pharm. **1998**, *161*, 37.
13. Esquisabel, A.; Hernandez, R.M.; Gacon, A.R.; Igartua, M.; Pedraz, J.T. Determination of Salbutamol Enantiomers by High-Performance Capillary Electrophoresis and Its Application to Dissolution Assays. J. Pharm. Biomed. Anal. **1997**, *16*, 357.
14. Dong, Z.C.; Jiang, J.T. Mechanism of Soluble Drug Release from Hydrophilic Matrices, the Effect of Hydroxypropyl Methylcellulose (HPMC) Content. Acta Pharm. Sinica. **1996**, *31*, 43.
15. Cabral Marques, H.M.; Hadgraft, J.; Kellaway, I.W.; Pugh, W.J. Studies of Cyclodextrin Inclusion Complexes. II Molecular Modelling and ^{1}H-NMR Evidence for the Salbutamol-β-Cyclodextrin Complex. Int. J. Pharm. **1990**, *63*, 267.
16. Kim, Y.H.; Park, K.H. Enantioselective Inclusion Between Terbutaline Enantiomers and Hydroxypropyl-β-Cyclodextrin. Int. J. Pharm. **1998**, *175*, 247.
17. Li, S.; Purdy, W.C. Circular Dichroism, Ultraviolet, and Proton Nuclear Magnetic Resonance Spectroscopic Studies of the Chiral Recognition Mechanism of β-Cyclodextrin. Anal. Chem. **1992**, *641*, 1405.