3. Results and Discussion

3.1 Preparation and characteristics of the cyclodextrin inclusion complexes of racemic salbutamol

Intermolecular interactions such as the hydrophobic interaction, van der Waal's force, H-bonding and other physical forces are involved in the inclusion complexation process. In this study, the sensitive structure analytical techniques such as FT-IR spectroscopy, DSC and XRD were used to verify complex formation of salbutamol with the three CDs.

3.1.1 FT-IR spectra

The typical IR spectrum of salbutamol with absorption peak at 3320 cm^{-1}, indicating O-H and N-H bonds is shown in Fig. 2A. All types of CDs showed a broad peak characteristic of the hydroxyl group stretching vibration in the 3500-3200 cm^{-1} region in IR spectra (Figs 2B-2D). Besides, the characteristic IR spectra in the C-H stretching vibration of DM-β-CD (methyl group band at 2875 cm^{-1}), and the O=S=O stretching region of SBE-β-CD (SO_2-OR- group at 1370 and 1100 cm^{-1}) appeared, as shown in Fig. 2B and Fig. 2D, respectively. As seen in Figs 2E-2G, the IR spectra of the three complexes remained the peaks due to CDs but not the drug. Therefore, the formation of complexes of drug with the CDs is possible.

3.1.2 DSC thermograms

The thermal behavior of the solid complexes was also determined to confirm in the complexity of salbutamol and CDs. Where guest molecule are

incorporated in the CD cavity or in the crystal lattice, their melting, boiling and sublimation points usually shift to a different temperature or decompose. The DSC curves of the freeze-dried racemic salbutamol (Curve A), the freeze-dried CDs (Curves B-D) and the freeze-dried of inclusion complexes of salbutamol with the CDs (Curves E-G) are shown in Fig. 3. The DSC curve of the freeze-dried salbutamol showed an endothemic peak at 150°C, representing the melting of salbutamol. The DSC curves (Curves E-G) of three freeze-dried complexes showed that the endothermic peak of salbutamol disappeared within the temperature range where the CD hosts were decomposed. This may be attributed to the formation of the inclusion compounds.

3.1.3 *XRD patterns*

The XRD analysis was performed to confirm the formation of complexes of salbutamol with CDs. Fig 4 shows the X-ray patterns of the freeze-dried racemic salbutamol alone, the freeze-dried CDs and the freeze-dried the inclusion complexes of salbutamol with CDs. Characteristic peaks of salbutamol appeared at a diffraction angle of 2θ at 8.46, 16.32, 19.69 and 25.15°. The diffraction pattern of the CDs inclusion complexes (E-G) showed the disappearance of salbutamol peak. These results indicate the production of the amorphous state of salbutamol in the complexes of all CDs as thermal analysis does prove to be.

3.2 The tablet preparation

The matrix tablets of all test formulations were prepared by direct compression method. The preparation of egg albumin tablets in this present work was similar to the direct compression method reported by Al-Meshal and Bayami (1995). The amount of the test excipients in formulation should be concerned because they may affect to the release of salbutamol (Al-Meshal and Bayami, 1995; Dong and Jiang, 1996). In this study the high excipient content (95%) was used but this was supposed to be seen for enantioselectivity.

3.3 In vitro release studies

3.3.1 The release studies with the formulations prepared from the solid complexes of cyclodextrin derivatives

Preliminary in vitro dissolution experiments of the tablets without coating of CDs indicated dissolving of the whole matrices within 10 –20 min. Therefore, the coated CD tablets were used to investigate for the stereoselectivity of release. The tablets containing DM-β-CD provided the completed release of enantiomers 100% drug release in 6 h while the tablets consisting of γ-CD or SBE-β-CD sustained the release of salbutamol enantiomer up to 12 h.

The release profiles for the formulations of DM-β-CD, γ-CD and SBE-β-CD are shown in Figs. 5A, 5B and 5C, respectively. In the case of DM-β-CD tablets, the release of the two enantiomers of salbutamol was rapid and no

stereoselective release was noticed during first 3 h but beyond that time there was a clear enantioselective trend in that slightly more of the R-enantiomer was releasing than the S-enantiomer. Although the level of selectivity demonstrated in this case but it was quite small, the result was of significant ($P< 0.05$), particularly at the end of dissolution run. At early stage of release, the matrix rapidly dissolved and released into dissolution medium hence the enantiospecificity was not exhibited.

For the γ-CD tablets (see Fig. 5B), the release profiles comprised of two phases namely the fast release in first half (0-6 h) and the slow release in second half (6-12). This formulation provided the statistically significant difference of release of enantiomers ($P<0.05$). Upon the enantiospecificity of this formulation, it was greater with the R-salbutamol to release than S-salbutamol. As shown in Fig. 6, 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 for release of the two enantiomers of salbutamol similar to that obtained from γ-CD tablets. Nevertheless, this formulation failed to demonstrate the enantioselectivity during dissolution assay. The reason for this is that the solubility of the complex may be influenced more by the interaction of water molecules with SBE-β-CD than by their interaction with enantiomers.

The results suggest that the complex formation between host and guest is responsible to enantioselectivity of release. Among the test formulations of

CD, the formulation of γ-CD exhibits the significant stereospecificity when compared to DM-β-CD or SBE-β-C. The enantioselectivity in the case of γ-CD formulation indicates that γ-CD preferentially interacts to S-enantiomer than R-enantiomer. However, the indirect evidence for selectivity is provided by dissolution investigations. Information regarding to host-guest structure would also be of great interest. For this case ^{1}H-NMR analysis was conducted in further experiment.

3.3.2 The release studies with sustained release formulations prepared from HPMC and egg albumin

When the HPMC is included in tablet it was found that good appearance of tablets were achieved. The mean dissolution profile for HPMC tablets is depicted in Fig. 7A. Over 80% of both enantiomers released from this matrix in 4 h and completed within 8 h with no significant difference between the release of the enantiomers. This observation is in accordance with that in the previous work (Esquisabel et al. 1997), using a different formulation of the drug and HPMC. Visually, the matrix swelled and subsequently dissolved and at the end of the dissolution all matrices disappeared. The erosion could be a major mechanism. As the tablet erodes, aqueous test medium penetrates into the matrix structure and dissolves water-soluble filler. The dissolved filler moves off the matrix leaving the void space and increasing tablet porosity. This effect results the increase of salbutamol release from matrix. The lack of stereoselectivity of HPMC matrix may be due to the rapidity of dissolution, such that it was difficult to determine difference of enantiomeric release rates.

Further, the stereoselective dissolution of the matrices prepared from egg albumin was determined. The egg albumin matrix tablets were intact throughout the dissolution test and could sustain the release of drug shorter time than other formulations. As seen in Fig. 7B, over 90% of salbutamol enantiomers released from egg albumin tablets in 4 h. The release pattern seen is also analogous to that obtained with the formulation reported by Al-Meshal and Bayomi, 1995. No significant difference of the release was evident for this formulation. Although, there are several reports on the development of the use of egg albumin as an excipient in the controlled-release formulations of salbutamol [Jun and Lai (1983); Torrado, Torrado and Cadorniga (1992); Mora and Pato (1993); Al-Meshal and Bayomi, (1995)]. The present report gives information with respect to stereoselective dissolution of salbutamol formulated with this excipient that alters from those reports.

3.3.3 The release studies with commercial product (Volmax®).

Further, the commercial tablet Volmax® was determined for the stereoselective release of salbutamol. The dissolution profile for commercial tablets Volmax® is shown in Fig. 7c. The release of up to 70% of both enantiomers of salbutamol was during first 6 h and close to 90% after 12 h. The dissolution curves show no difference between the release of enantiomers at first 2 h beyond which a little difference appeared with release of R-isomer being higher than that of S-isomer. However, this difference is not statistically significant ($p > 0.05$).

3.4 The investigation in the enantioselective interaction for the cyclodextrin complex.

^{1}H-NMR spectroscopy was employed as a probe for the study in the interaction of enantiomer of salbutamol with CD host that confirms in the stereoselectivity obtained from in vitro dissolution of γ-CD matrix tablet (with coating). The growing studies in the host-guest interaction by ^{1}H-NMR technique including that for the complex of racemic salbutamol with β-CD or DM-β-CD (Cebral Marques et al.,1990) has been done. The use of ^{1}H-NMR to study the interaction between hydroxypropyl-β-CD and terbutaline enantiomers stucturally related to salbutamol was reported [Kim and Park 1998]. However, this present study first presented in stereospecific ^{1}H-NMR determination for the complexes of salbutamol with γ-CD host. This technique is based on the shielding of the CD and drug protons. If inclusion does occur, protons locates within or near the CD cavity should be strongly shielded. In this experiment, the 500 MHz spectrometer was used to allow the required degree of spectral resolution. The peak at 4.7 ppm assigned to DHO and H_2O as impurities was considered as an internal standard in the measurement of the chemical shifts of the peaks of γ-CD in the presence and absence of salbutamol.

^{1}H-NMR spectra of salbutamol, free γ-CD and the complexes either of the individual enantiomers or racemate of salbutamol with γ-CD are illustrated in Fig. 8. Table 2 shows the upfield shifts for all the protons of γ-CD induced by salbutamol in the inclusion complex, suggesting that salbutamol may interact

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both on the exterior surface of γ-CD as well as in the interior. 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-isomer included deeper into the inner cavity than 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 are also observed (see Table 1). It was evident that all the complexes, the salbutamol protons were shifted to lower fields among which the shift of the phenyl protons and side chain protons nearest to 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 inclusion mode of the racemate were anyway similar to those reported for analogous molecule using β-CD as the host (Cebral Marques et al.,1990). In contrast, the shift for the aromatic H-6 of S-salbutamol was larger than that of R-salbutamol, with S-R = 4.5 Hz. It was 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 R-enantiomer. Downfield proton resonance shifts of a molecule can usually be observed when this molecule binds to another by interactions by dipole-dipole, dipole-induced dipole and van der Waal's by steric perturbation or diamagnetic anisotropy or regions of the host (Suzuki and

Sasaki, 1979; Uekama et al., 1983; Jones and Parr, 1987). In this case the downfield shift of aromatic H-6 may be mainly caused by the van der Waal's interaction with the hydrophobic cavity of γ-CD.

There was also the significant effect of 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 that the difference in effect of 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 the sufficient for chiral recognition also needs the interactions of other functional group around the chiral center with cyclodextrin to form a secondary inclusion complex (Li and Purdy, 1992). For this test, further molecular modelling studies may provide greater into the precise mechanism of the enantiodiscrimination process.

The ^{1}H-NMR result demonstrates that the S-salbutamol, particularly aromatic moiety is more tightly bound to γ-CD host than the R-salbutamol is to its host. It appears the evidence in ^{1}H-NMR to confirm the enantioselective dissolution result obtained with the complex of salbutamol and γ-CD that the matrix of γ-CD preferentially releases R-enantiomer into dissolution medium than S-enantiomer.