2. Materials and Methods

2.1 Materials

RS-(±)-salbutamol was obtained from Altech Chemical, Ltd (USA). R-(+)-salbutamol and S-(-)-salbutamol were kindly donated from Prof. Clive Page (King's College, London). Egg albumin was purchased from Sigma (St. Louis, MO, USA). γ-CD and DM-β-CD were obtained from Wacker Chemie Ltd. (Burghausen, Germany). SBE-β-cyclodextrin (Captisol™) was obtained from Cydex (Overland Park, USA). HPMC (K4M grade) was supplied by Colorcon (MA, USA). The commercial controlled release tablet Volmax® 4 mg was purchased from Glaxo-Wellcome (Bangkok, Thailand). Silica gel 60 was purchased from Fluka Chemie AG (Buchs, Switzerland). All the solvents were HPLC grade purchased from J.T. Baker (Philadelphia, USA). The other reagents were analytical grade and used without further purification.

2.2 Preparation of the cyclodextrin inclusion complexes

The stoichiometry of the CD complexes with salbutamol was determined by UV spectroscopy technique with seven chosen molar ratios of salbutamol and each CD derivative (1:0.5, 0.5:1, 1:1, 1:1.5, 1.5:1, 1:2, 2:1). Job's plots, obtained similarly to the method by Suzuki and Sasaki 1984, indicated the formation of 1:1 complexes in all cases (data not shown). In the case of DM-β-CD complex, the result is also in accordance with that obtained by other authors (Cebral Marques et al.,1990). Thus, the 1:1 of drug-CD complex for

all types of CDs was prepared and further used for the investigations. The method used for the preparation of the solid inclusion complexes was similar to that described by Higuchi and Connors (1965), using lyophilization method. The freeze-dried complexes were obtained by adding equimolar concentrations either of the racemate or the individual enantiomer of salbutamol to each of CD in distilled water. The clear solution of a mixture was then freezed-dried for 48 h until yielding a solid cake. The product was stored in environmental chamber at 25°C and 45%RH in well-closed container. In this experiment, the freeze-dried cyclodextrins and freeze-dried salbutamol alone were also prepared.

2.3 The properties of the cyclodextrin inclusion complexes

In this experiment, the properties of the prepared complexes of salbutamol with CD were determined by using infrared spectroscopy (IR), differential scanning calorimetry (DSC) and X-ray diffraction (XRD).

2.3.1 FTIR spectroscopy studies

IR spectra were recorded in the range 4000-500 cm^{-1} with a Perkin Elmer FT-IR Model 1600 spectrophotometer (Norwalk, CT, USA) on KBr pellets using 16 scan for each sample with a resolution factor of four. The spectra were corrected by subtracting the spectrum of a KBr blank pellet and were presented in transmittance mode.

2.3.2 DSC studies

Thermal analysis using DSC method was performed using a Perkin Elmer DSC 7 (Norwalk, CT, USA) with a Pyris software was used to record the DSC curves. The samples (5-6 mg) were weighed into aluminium pans which was hermetically sealed with a lid. An empty pan sealed in the same way, was used as reference. All the samples were scanned at a speed of 5°C/min under nitrogen purging for the temperature range 50-300°C.

2.3.3 XRD studies

The X-ray diffraction measurments were carried out with a Philips PW 3710 powder diffractometer with the following experimental setting; Ni filtered CuKα radiation (λ = 1.5418 A), tube setting 40 kV, 20 mA, time constant 4 s, angular speed 1°/ min and angular range $5° < 2\theta < 50°$.

2.4 Preparation of matrix tablets

Two types of the test formulations; film-coated tablet and uncoated tablet were prepared. The coating tablets included the formulations of all types of CD complexes while the tablets without coating were the formulations of HPMC and egg albumin. For all test formulation products, the formulation with 80 mg total tablet weight and 4 mg drug content was imitated as the commercial formulation Volmax® (Glaxo-Wellcome). The direct compression technique was employed to prepare the tablets. The tablet was made on a Manesty F3 tableting machine (England, UK) equipped with single flat face punches ¼ inch. The powder blend comprising of salbutamol and a chiral

excipient was prepared in a dry bottle. After adding lubricant (magnesium stearate 1%) the powder mixture (80 mg), was manually filled in the die and followed by compressing at constant force (4 kN). During the tableting, the weight variation of pressed tablets was determined and that presented a variation higher than ± 2.5% of theoretical weight were rejected. Also, the hardness of tablets was evaluated.

To prepare the test formulations of CDs, each CD complexes was mixed with the lubricant (magnesium stearate 1%) and compressed as the tablets in a similar manner to the formulations of HPMC and egg albumin except the presence of tablet coating. The pressed tablets of CD complexes were dip coated with 20% Eudragit E-100 in acetone / isopropanol (1:1, w/v) and dried with cool air for 30 min (film thickness = 2 mm).

2.5 In vitro dissolution studies

All tablet batches were subjected to in vitro dissolution studies using a continuous flow dissolution system consisted of Vankel dissolution tester Model VK 7000 (Vankel Industries, Edison, NJ, USA), peristaltic pump and Vankel VK 8000 autosampler (Vankel Industries, Edison, NJ, USA). The dissolution tests were carried out according to the USP apparatus II paddle method for all formulations. The medium was 50 mM phosphate buffer pH 7.4. The volume of dissolution media was 1 L. The media were stirred at 50 rpm at 37°C. At proper time intervals a 3 ml was automatically withdrawn from each dissolution vessel and replaced with fresh dissolution medium. The

samples were immediately assayed for the dissolved concentration and hence for the dissolved amounts of each enantiomers by solid phase extraction together with enantiospecific HPLC method. Six replicates were performed for each dissolution experiment.

2.6 Drug enantiomeric assay

2.6.1 Solid-phase extraction (SPE)

Solid phase extraction was used to separate salbutamol from aqueous medium. Employed cartridges were made in-house from silica packed in Pasteur pipettes [150 mm (length) x 6 mm (diameter)]. Glass beads (2.5 mm) were placed at the tip of the Pasteur pipette to act as the bed support, with the silica gel (100 mg dry weight) forming a column approximately 2.5 cm in height. The cartridges were conditioned with 1 ml of 2% ammonia in methanol under gravity and followed with 1 ml of water. The samples (1 ml) containing drug were loaded onto the cartridges further washed with 1 ml of acetonitrile. Subsequently, the cartridges were left to be dried for 5 min then the drug was eluted from the cartridges with 1 ml of 2% ammonia in methanol. The extracts were dried in the water bath at 60°C under stream of nitrogen . Finally, the dry mixtures were reconstituted with 1 ml mobile phase and 100 µl was injected into the HPLC.

2.6.2 Enantiospecific chromatography

After extracting, the quantitative analyses of salbutamol were carried out using the enantiospecific HPLC composed of Waters 600 HPLC system

(Milford, USA) with Waters 717 plus autosampler, equipped with a 486 variable wavelength UV detector connected to Waters 746 integrator (Milford). A 250 mm x 4 mm Chirex 3022 column (Phenomenex, Torrance, CA, USA) connected to a Chirex 3022 guard column (30 mm x 4 mm) was employed. The mobile phase consisted of hexane, 1,2-dichloromethane, methanol and trifluoroacetic acid (65:30:5:0.1) pumped at a flow rate of 1.5 ml/min. The typical chromatogram of R- and S-salbutamol is shown in Fig. 1. The concentrations of the two enantiomers were determined by comparison of peak areas with peak areas from the calibration curves of standard solutions containing 1, 2, 3, 4, 5 μg ml^{-1} of racemic salbutamol.

2.6.3 Assay validation

The method for the enantiomeric assay of sulbutamol was verified. Three determinations in the amounts of each enantiomer in racemic standards were made as described above. Subsequently, the peak area ratio was calculated to construct the curve. The validation procedures were carried out under three categories; intraday, interday accuracy and precision and recovery at three levels of concentration being low, medium and high concentration; 0.5, 2 and 5 μg ml^{-1}. It was found that the assay method used was valid as applied to the salbutamol dissolution studies, with the detection limit of drug concentration of 0.1 μg ml^{-1}.

2.7 Data analysis

The percentage of cumulative drug enantiomer released was plotted versus time for all formulations. The results are presented as mean ± SD. The significance of the difference between the enantiomers released in each and every dissolution test was tested. Also, the significance of the difference observed between the two enantiomers was examined by pooling data points from all dissolution experiments. The significance of differences between the enantiomers between the treatments were made using the paired *t-test*, α = 0.05.

2.8 The NMR study of the complex formation

The inclusion mode of salbutamol enantiomer into CD was investigated by ^{1}H-NMR technique. The analysis was performed on a 500 MHz Varian spectrometer (Palo Alto, CA, USA), operating at 499.85 MHz, in D_2O solution. Typical conditions were probe temperature 30°C, 16K data points, sweep width 6 kHz, giving a digitization accuracy of 0.3 Hz per point, pulse width 5.5 μs, acquisition time 2.7 s and 32 accumulations. The chemical shift change of some protons of either CD or salbutamol was compared to acquire structural information on the complex.