CHAPTER III

MATERIALS AND METHODS

Materials

The following materials were purchased from commercial sources except nifedipine which was kindly donated by MOEHS, S.A. Barcelona Spain, Eudragit RS100 and RL100 by Rohm Pharma GmbH (Darmstadt, Germany) and Povidone K30 by BASF. Deionized water was used throughout this study.

Model drug

Nifedipine (batch no. 71/2, MOEHS, S.A., Barcelona Spain)

Carriers

1. Eudragit RS 100 (lot no. 837408031, Rohm Pharma, Germany)
2. Eudragit RL 100 (lot no. 0860106957, Rohm Pharma, Germany)
3. Povidone K30 (lot no. 51-4960, BASF, Germany)

Other substances

1. Absolute ethyl alcohol, analytical grade (lot no. L868107, E. Merck, Germany)

2. Hydrochloric acid 37% (lot no. K 25290117 825, E. Merck, Germany)
3. Dichloromethane (lot no. 4881N18753, Mallinckrodt, France)
4. Sodium chloride (lot no. 47/874, E. Merck, Germany)
5. Sodium hydroxide (lot no. 305c242198, E.Merck, Germany)
6. Potassium dihydrogen phosphate (lot no. A 894271 605, E.Merck, Germany)
7. Potassium bromide (lot no. 378170/1 50398, Fluka, Switzerland)

Apparatus

1. Analytical balance (Sartorius, GMBH, Germany)
2. Spray dryer (B-190, Buchi, Switzerland)
3. UV spectrophotometer (Model 7800, JASCO Corporation, Japan)
4. Dissolution apparatus (Model AT7, Sotax, Switzerland)
5. Fourier transform infrared spectrometer (FT/IR-230, JASCO Corporation, Japan)
6. X-ray diffractometer (Rigaku Denki 2027, Japan)
7. Differential scanning calorimeter (Model TA 9900, Du Pont, USA)
8. Scanning electron microscope (JSM - 5800 LV, Jeol, Japan)
9. Low pressure sodium lamp (SOX - EXWC 121 K, Phillips, UK)
10. Moisture content measurement (SATORIUS MA 40, Germany)
11. Laser Particle Size Analyzer (Mastersizer S long bed Ver.2.11, Malvern Instrument, UK)

Methods

As nifedipine is sensitive to light, all experiments were conducted under yellow sodium light which radiates wavelength region nonabsorbed by nifedipine to prevent any influences to photodegradation (Abrahamsson et al., 1998). In addition, containers used for nifedipine were wrapped with aluminium foil, when needed, throughout the experiment.

1. Preparation of nifedipine spray dried microspheres

Since nifedipine and Eudragit are water insoluble, the nonaqueous solvent system has to be used. To avoid the explosion problem, pure ethanol and other flammable solvents cannot be used. Dichloromethane a nonaqueous solvent was found to be suitable for spray drying. From the preliminary study, 1:1 mixture of ethanol-dichloromethane was the most appropriate system.

Instrumental conditions, for example, inlet air temperature, outlet temperature, aspirator setting, pump setting and spray flow, were optimized to obtaine a dried nonsticky product. In spray drying process, the spray solution was atomized into droplets into a hot dry air. The solvent was vaporized immediately and the powder, granules or agglomerate was obtained within seconds.

Nifedipine was weighed and dissolved in 1:1 mixture of ethanol-dichloromethane containing combined carries, Eudragit RS100 and PVP K30 or Eudragit RL100 and PVP K 30 to obtain the drug:carrier mixing ratio of 1:10 by

weight. The amount of PVP K 30 in the combined carriers was varied as 100, 80, 50, 20 and 0% to make the mixing ratios of nifedipine:Eudragit RS100:PVP K30 as 1:10:0, 1:8:2, 1:5:5, 1:2:8 and 1:0:10 and of nifedipine: Eudragit RL100:PVP K30 as 1:10:0, 1:8:2, 1:5:5, 1:2:8 and 1:0:10. The 1:1 ethanol-dichloromethane mixture was used as the solvent. The spray dried microspheres of nifedipine were prepared by spray drying of nifedipine solution using a Mini Spray Dryer (Buchi, B-190). The experimental parameter setting were as follows :

Inlet temperature	:	55^{o}, 65^{o} and 75^{o}C
Aspirator setting	:	10
Pump setting	:	5 ml/min
Spray - flow	:	600 NL/h
Concentration of spray solution	:	5 and 10% (w/v)

A 0.5 mm nozzle was used throughout the experiments. The inlet temperature and concentration of spray solution were varied while other spray drying parameters were kept constant. The spray dried powders from the process were collected from the chamber and collector of the apparatus. The weight of each portion was recorded and % yield was calculated. The spray dried microspheres were stored in a desiccator at room temperature for further investigations.

2. Quantitative analysis of nifedipine

2.1 Calibration curve of nifedipine

1) Nifedipine of 0.030 g was accurately weighed into a 100 ml volumetric flask and dissoved in absolute ethanol to 100 ml.
2) Transferred 10 ml of nifedipine solution was diluted to 100 ml in a 100 ml volumetric flask and used as a stock solution
3) Appropriate amounts of the stock solution were individually pipetted into separated volumetric flasks and adjusted to volume with simulated gastric fluid without enzyme (pH 1.2) and simulated intestinal fluid without enzyme (pH 7.5), the final concentrations of standard solution were between 1.5-12.0 mcg/ml.
4) Absorbances of nifedipine standard solutions were measured using a UV spectrophotometer (Jasco 7800) at 238 nm. All studies were run in triplicate.

2.2 Nifedipine content of spray dried microspheres

1) An accurately weighed spray dried sample equivalent to 10 mg of nifedipine was dissolved in the solvent mixture of ethanol-dichloromethane (1: 1) in a 100 ml volumetric flask and adjusted to 100 ml.
2) Five ml of the aforementioned solution was diluted to 50 ml with absolute ethanol.
3) One ml of the solution was then transfered into a 10 ml volumetric flask

and was adjusted to volume with simulated gastric fluid without enzyme or simulated intestinal fluid without enzyme.

4) Absorbance of the resulting solutions were measured spectrophotometrically at 238 nm.

5) The content of nifedipine was calculated and averaged from triplicate results.

3.The percentage yield

The amounts of spray dried products that could be collected from the chamber and collector of the spray dryer were recorded. The percentage yield was calculated from the following equation :

$$\% \text{ yield} = \frac{\text{amount of spray dried powders collected (g)}}{\text{total amount of all ingredients (g)}} \times 100 \qquad (10)$$

4. Residual solvent determination

The residual solvent content of spray dried sample were determinate using a moisture balance (SARTORIUS MA 40). An accurate weight of 0.1000 g of sample was exposed to an IR lamp until a constant weight was obtained. The percentage residual solvent content was calculated automatically. Results were obtained from the average of three determinations.

5. Size and particle size distribution

Particle size and size distribution of spray dried samples were determined using a laser particle size analyzer (Mastersizer S long bed Ver.2.11, Malvern Instrument). Approximately 1 g of powder was dispersed in light mineral oil. The geometric mean diameter, 50% cumulative undersize was calculated automatically.

6. Scanning electron microscopic (SEM) study

Electron photomicrographs of samples were taken with a scanning electron microscope (JSM-5800 LV, Jeol). The sample was uniformly adhered to the surface of the stubs using double-sided adhesive tape. A thin layer of gold was then coated on the surface before examination, using ion sputtering technique. Then they were photographed at appropriate magnification scales.

7. Powder X-ray diffraction study

The powder X-ray diffraction (XRD) pattern was investigated using a diffractometer (Rigaku Denki 2027) with Cu target and Ni filter. The measurement conditions were as follows:

Voltage	30 KV
Current	5mA
Scanning speed	4^{o}C/min
Scanning range (2θ)	$5\text{-}40^{o}$

8. Differential scanning calorimetry study

Differential scanning calorimetry (DSC) was investigated using a differential scanning calorimeter (Du Pont, Model TA 9900). The 2 mg samples were accurately weighed and placed in a closed aluminium pan. The measurement conditions were as follows :

Scanning speed	5^{o}C/min
Temperature range	25 - 250^{o}C
Atmosphere	Nitrogen gas, flow rate 60 ml/min

9. Fourier transform infrared spectrophotometry study

Fourier transform infrared (FTIR) spectra were measured by KBr disc method using a Fourier transform infrared spectrometer (FT/IR-230, JASCO) in the wavenumber range of 650-4000 cm^{-1}. The characteristic bands were observed.

10. Dissolution study

1) The dissolution of nifedipine spray dried samples containing equivalent amount to 10 mg nifedipine was performed in triplicate with the dissolution apparatus II (USP XXIII).

2) Each vessel contained 900 ml of either simulated gastric fluid without enzyme or stimulated intestinal fluid without enzyme as the dissolution medium(USP XXIII).

3) The medium was allowed to equilibrate to a temperature of $37 \pm 0.5^{\circ}C$ and using a rotation speed of 150 rpm.

4) A 5 ml of solution was withdrawn through a 10 μm filter at appropriate time interval and replaced with 5 ml of the same medium after each sampling to maintain a constant volume. The dissolution study was operated separately for 2 and 24 hours in simulated gastric fluid without enzyme and simulated intestinal fluid without enzyme, respectively.

5) The withdrawn solution was determined spectrophotometrically at 238 nm.

6) The dissolution profile was obtained from the plot of the percentage dissolved of nifedipine against time.