CHAPTER 5

PREFORMULATION STUDY OF THE DRUG

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5.1 A Preview

Preformulation study[1,2,3] involves the application of biopharmaceutical principles to the physicochemical parameters of a drug with the goal of designing an optimum drug delivery system. This study is the first stage in the rational development of a dosage form with drug substances. In other way, it can be defined as the investigation of physico-chemical properties of the drug alone and/or in combination with excipients. Characterization of drug molecule is a very important step at the preformulation phase of product development. The objective of preformulation studies is to generate sufficient information for the formulation scientists in developing a stable, bioavailable and industrially feasible dosage form of a drug substance. In a single phrase thus preformulation can be defined as "a case of learning before doing". The entire preformulation program can be divided into four basic phases of drug development.

Phases of development of a dosage form:

1. Preliminary- preformulation
2. Preformulation
3. Early-stage development
4. Late-stage development

Following physico-chemical parameters are conducted as basic preformulation studies:

1. Purity of drug
2. Molecular weight and molecular formula
3. Density and Hygroscopicity
4. Particle size and shape
5. Crystalline properties and Polymorphism
6. Surface area
7. Flowability and Compressibility
8. Solubility – Intrinsic and Extrinsic
9. Partition coefficient
10. Ionization constant

11. Dissolution parameters
12. Compatibility with excipients
13. Spectral data
14. Stability
15. Organoleptic properties

Apart from these parameters lot of other parameters are also ascertained as and when required. Special studies are conducted depending on the type of dosage form and the type of drug molecule. For a new molecule thorough investigation is essential. But in case of an established drug, these physicochemical data need not be investigated again but it is customary to use the standard values published in official compendium like Indian or British Pharmacopoeia. In these cases only basic tests like solubility, spectral data, chemical assay, purity and compatibility study with excipients of the established drug are done to see whether the drug sample complies with the standard requirements.

5.2 Analytical study of the drug – Frusemide

5.2.1 Preparation of USP Phosphate buffer[4] - pH 6.8 and pH 7.4

General Method: 50 ml of 0.2 M of Potassium di hydrogen ortho phosphate solution was taken in a 200 ml volumetric flask. Specified volumes, 22.4 ml for pH 6.8 and 39.1 ml for pH 7.4, of 0.2 M sodium hydroxide solutions was added and then distill water was added to make up the volume. The pH of the solution was checked and adjusted in a pH meter. The pH meter used for all the experiments was double electrode calomel pH meter manufactured by ORION, Japan.

Preparation of 1 litre of 0.2 M Potassium di hydrogen ortho phosphate

27.22g of Potassium di hydrogen ortho phosphate was dissolved in distill water and diluted it to 1000ml.

Preparation of 1000ml of 0.2 M Sodium hydroxide solution

8g of Sodium hydroxide was dissolved in distill water to produce 40-60%w/v solution and allowed it to stand. Taking precaution to avoid absorption of carbon dioxide gas, siphoned off the supernatant liquid and diluted with carbon dioxide free distill water to 1000ml.

5.2.2 Determination of λmax of Frusemide in USP Phosphate buffer pH 6.8 and 7.4

10mg of Frusemide was dissolved in 2ml methanol in a 100ml volumetric flask and the volume was made upto the mark with Phosphate buffer (pH 6.8 and pH 7.4). 10ml of this solution was further diluted to 100ml with the buffer media. The resulting solution (0.001%w/v) was scanned in SHIMADZU UV-VIS PharmSpec 1700 double beam spectrophotometer in a range of 200- 400nm within 1.00A – 0.00A, using the same buffer as blank control. Scan speed was maintained at 250nm /min. The scanning spectrum is given in Fig. 5.1 and Fig. 5.2.

5.2.3 Calibration curve for Frusemide in USP Phosphate buffer- pH 6.8 and 7.4

Accurately weighed 30mg of Frusemide was dissolved in 2ml methanol and the volume was made upto 100ml with the Phosphate buffer (pH 6.8 and pH 7.4). This stock solution (0.3mg / ml) was filtered through 0.45μ filter paper and from the filtrate 0.16ml, 0.33ml, 0.5ml, 0.66ml, 0.83ml and 1.0ml were taken to six standard 25ml volumetric flasks so that the concentration of drug varies within a range of 0 to 12 μg / ml. The final volume was adjusted upto the graduation mark using the same buffer. The absorbance of these solutions were measured at 277.5nm, the wavelength of maximum absorption of Frusemide, in SHIMADZU UV-VIS PharmSpec 1700 double beam spectrophotometer, using same buffer media as blank control. The experiment was performed three times and the average values of the absorbance were plotted in Y-axis against the concentration in X-axis. The calibration data and its' corresponding curve are represented in the Table 5.2.

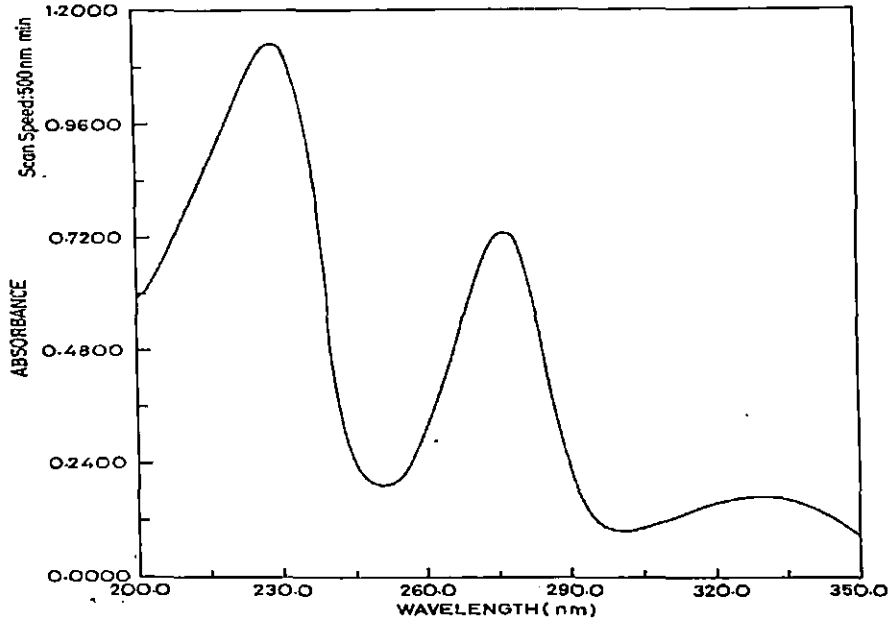

Figure 5.1 Scanning of λ max of Frusemide in PBS at pH 6.8

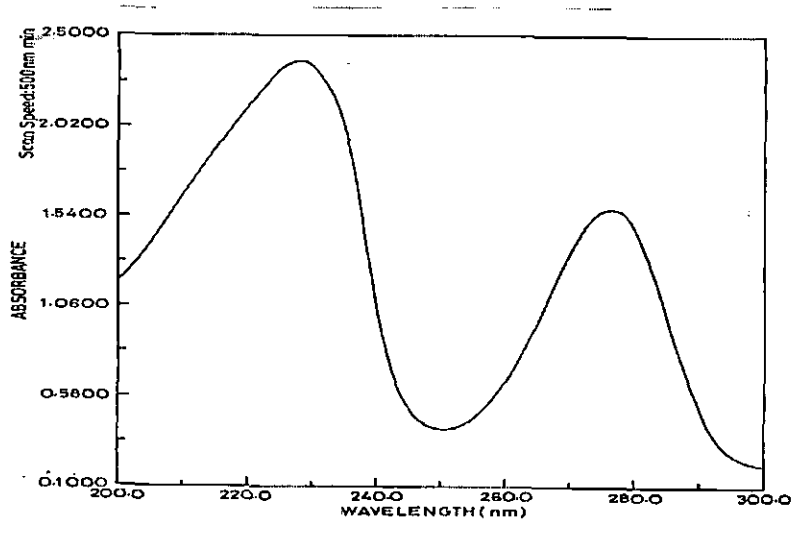

Figure 5.2 Scanning of λ max of Frusemide in PBS at pH 7.4

Table 5.2 Absorbance data for the calibration curve of Frusemide in USP Phosphate buffer of pH 6.8 and 7.4 at 277.5nm

Sl No.	Concentration (µg / ml)	Absorbance at 277.5 nm in PBS, pH 6.8	Absorbance at 277.5 nm in PBS, pH 7.4
1	0	0.000	0.000
2	2	0.254	0.114
3	4	0.330	0.253
4	6	0.405	0.402
5	8	0.532	0.536
6	10	0.624	0.707
7	12	0.683	0.859

* n=3; PBS – Phosphate Buffer System

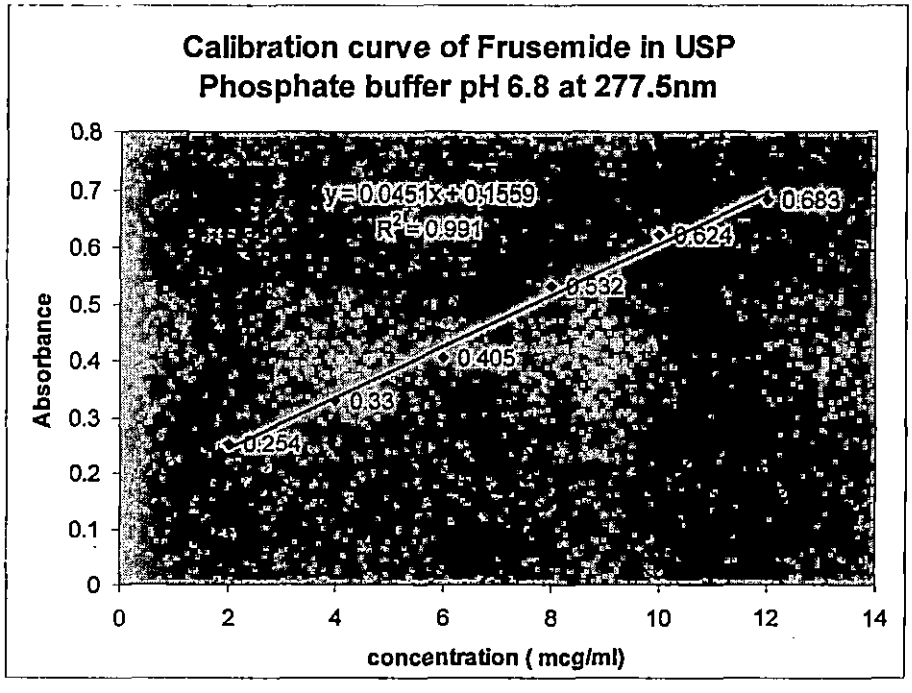

Figure 5.3 Calibration curve of Frusemide in USP PBS at pH 6.8

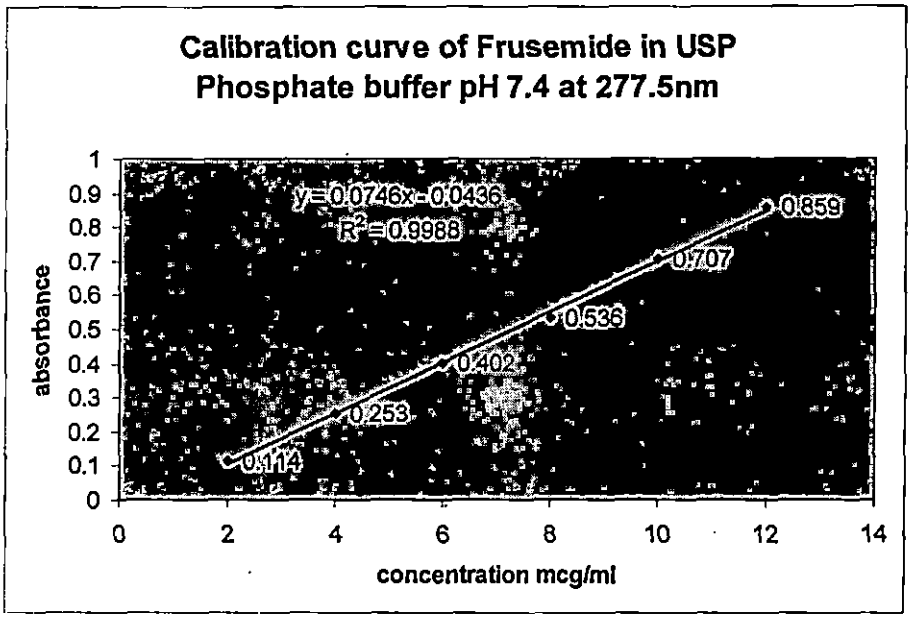

Figure 5.4 Calibration curve of Frusemide in USP PBS at pH 7.4

5.2.4 Assay of Frusemide[5]

The supplied Frusemide powder drug received as a gift sample from Aventis Pharmaceuticals, Ankleshwar, was assayed as per Indian Pharmacopoeia 1996[5]. About 0.5 g of the drug powder was accurately weighed, dissolved in 40 ml of dimethylformamide and titrated with 0.1 M sodium hydroxide using bromothymol blue solution as indicator. A blank determination was performed with any necessary correction. Each ml of 0.2M sodium hydroxide is equivalent to 0.03307 g of $C_{12}H_{11}ClN_2O_5S$.

The assay result of Frusemide was found to be **99.723%.** The result was well within the IP standards of Frusemide powder i.e., Frusemide contains not less than 98.5 percent and not more than 101.0 percent of $C_{12}H_{11}ClN_2O_5S$ calculated with reference to dried substance. Hence the drug sample was used for the entire experimental procedures.

5.2.5 Determination of Solubility of frusemide in USP Phosphate buffer pH 6.8 and pH 7.4 at room temperature (28°C)

Excess quantity of the drug was taken in a 50 ml volumetric flask and to it accurately measured 25 ml of USP Phosphate buffer (pH 6.8 and pH 7.4) was added, ensuring sufficient space in the flask for allowing easy shaking. The flasks were then shaken in a

mechanical shaker for 24 hour at 28° C. The solution was then filtered using Whatman filter paper. From the filtrate 5 ml aliquot was taken and diluted to 25 ml with the same buffer and analyzed in SHIMADZU UV-VIS PharmSpec 1700 double beam spectrophotometer at 277.5nm. The resultant solubility was found to be **27.16mg/100 ml** at **pH 6.8** and **24.89 mg / 100 ml** at **pH 7.4.**

5.2.6 Infrared Spectrum of the Drug – Frusemide[6]

The drug frusemide was dispersed in 1:100 KBr ratios and analyzed in FTIR spectrophotometer (SHIMADZU FTIR - 8400S, JAPAN) over a range of 400- 4000 cm^{-1}. The IR spectrum as displayed in Figure: 5.5 were found to comply with that of official compendium.

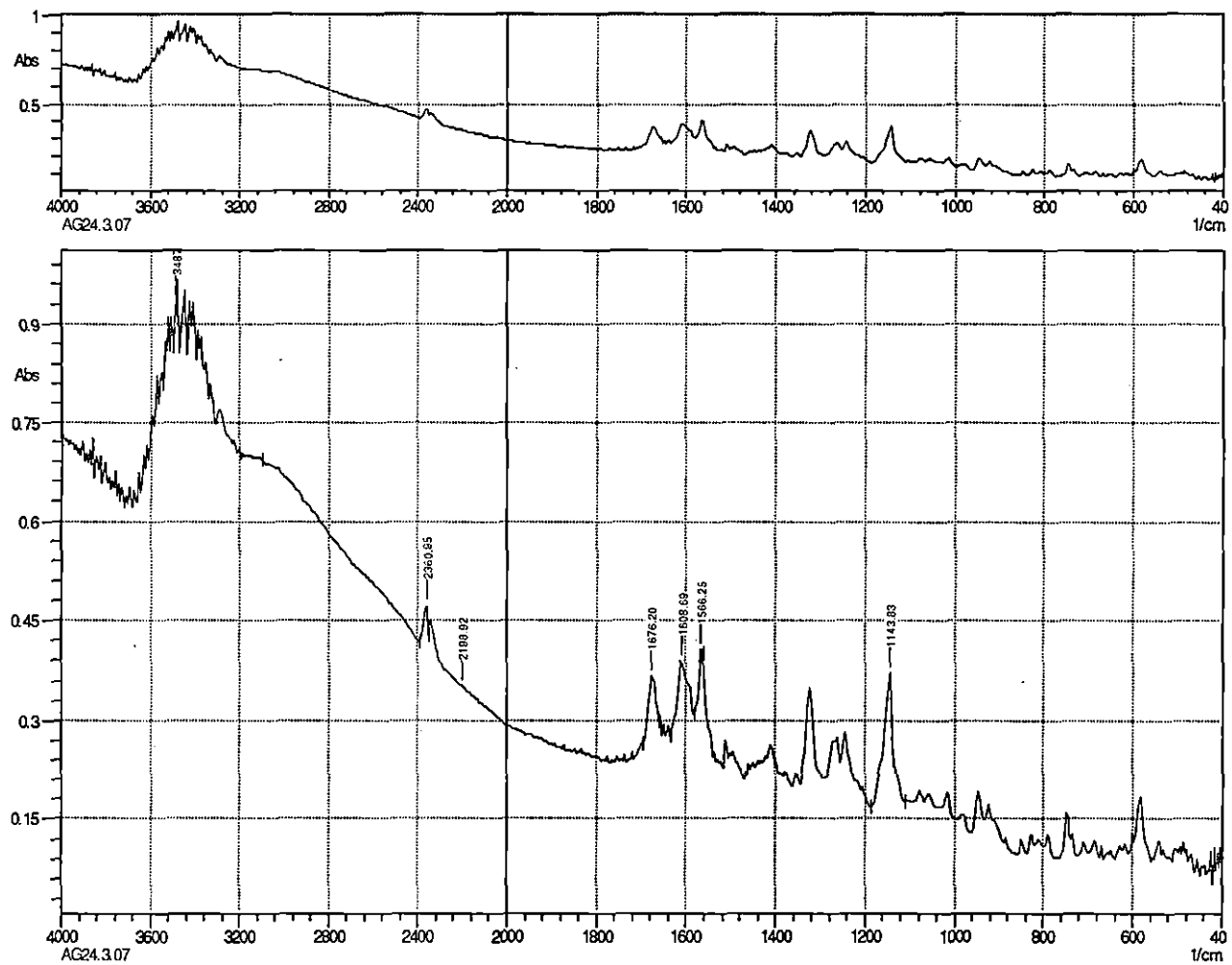

Figure: - 5.5 Infrared Spectrum of Frusemide sample used

5.3 REFERENCES

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5. *Indian Pharmacopoeia,* The Controller of Publication, New Delhi, 1996, **Vol. 1,** 332-334.
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