CHAPTER VI

SUMMARY AND CONCLUSIONS

A pharmaceutical formulation is a drug delivery system designed to provide a mechanism for safe and convenient delivery of accurate dose. It also intends to maintain physical, chemical, therapeutic as well as toxicological specifications of the drug. This research project was undertaken to fabricate, formulate, and evaluate oral controlled release drug delivery systems of some drugs.

Tetracycline hydrochloride and hydralazine hydrochloride were selected for present investigation. Incomplete absorption, peaks and troughs in its blood levels due to high and frequent dosing, incidence of side effects due to high dosing and prolonged therapy, necessitate formulating controlled release drug delivery systems of tetracycline hydrochloride. Although a few prolonged release products of this drug have appeared in the market (Tetrabid Organon - Organon, U.K., Sustamycin capsules - MCP Pharmaceuticals, U.K.), these being proprietary products, the information on the formulation aspects is negligible. The slow release dosage forms of hydralazine hydrochloride will permit large hydralazine dose to be administered less frequently and reduce the fluctuations in blood levels and the incidence of side effects. Thus these two drugs emerge out as potential candidates for the design of controlled release dosage form.

British Pharmacopoeial method of finding dissolution rate of tetracycline hydrochloride was used for in vitro evaluation of its various formulations. Methods reported by Mahgoub et al. and Hall were employed to develop the method for estimation of tetracycline hydrochloride in human urine for present investigation. The method is based on formation of a yellow complex, uranyl-tetracycline, with an absorption maximum at 430 nm. Stability indicating assay method described in USP XX, under determination of 4-epianhydro-tetracycline and anhydrotetracycline in tetracycline hydrochloride dosage forms, was used for stability studies of tetracycline hydrochloride products.

United States Pharmacopeia XX method of estimation of hydralazine hydrochloride in reserpine, hydralazine hydrochloride and chlorthiazide tablets was used for both in vitro and in stability evaluation of hydralazine hydrochloride products. The method is based on formation of a complex of ferrous ions with 1,10-phenanthroline with an absorption maximum at 510 nm. The method reported by Zak et al. was used for estimation of hydralazine hydrochloride in dog plasma. Spectrophotometric technique for estimation of hydralazine hydrochloride is based on reaction with p-methoxybenzaldehyde to form hydrazone with an absorption maximum at 355 nm.

Since a variety of coating materials were used in

the present investigation, interference, if any, of these materials in the estimation of tetracycline hydrochloride and hydralazine hydrochloride was checked separately. No significant interference was detected.

Three types of preparations, matrix granules, coated beads and matrix tablets, were attempted to formulate controlled release drug delivery systems of tetracycline hydrochloride. The loading and maintenance doses were calculated based on reported pharmacokinetic data. Prepared products were subjected to in vitro dissolution studies using a basket stirrer assembly of USP XX and following the method recommended in NF XIV under "Timed Release Tablets and Capsules In Vitro Test Procedure". Products having release pattern close to the desired release pattern; and mixed granules or beads to match the desired release pattern were tested in triplicate for their dissolution behaviour in vitro to find out any batch to batch variation in the release pattern of the drug.

Matrix granules type products consist of matrix formed of tetracycline hydrochloride, shellac-polyvinylpyrrolidone or cellulose acetate phthalate-polyvinylpyrrolidone, succinic acid and excipients. Succinic acid was included in the matrix to prevent the hydrolysis of tetracycline hydrochloride at pH value of 3 or more (as upon leaving the stomach) and resultant precipitation of the drug as

base. This is necessary to make the drug available for absorption throughout the GI tract.

Method of preparation of such matrices was found to be tedious and giving a lower yield. Results of release of the drug from the products tested in triplicate also showed larger variation in the release pattern of the drug. Hence the products of this type were not taken up for further studies.

Beads of tetracycline hydrochloride were prepared using pelleter EXDS—60 and marumerizer Q-230 and coated differentially with ethyl cellulose, cellulose acetate phthalate, glyceryl monostearate-bees wax, glyceryl distearate, eudragit S100, and eudragit RL100 and eudragit RS100 separately and in combination. Products of this type consist of tetracycline hydrochloride, avicel, succinic acid and excipients in core, coated with different coating material.

Results of release of the drug from products tested in triplicate show that ethyl cellulose, cellulose acetate phthalate, glyceryl monostearate-bees wax, and glyceryl distearate coated beads have larger inter-capsule variation in release of the drug as compared to eudragit S100, eudragit RL100 and/or RS100 coated beads. Release pattern of the drug from eudragit RL100 and/or RS100 coated beads

was found to be reproducible. Yield of the products was also relatively higher i.e. 85 to 95%. Coated beads were spherical and uniformly coated. Hence the products prepared with eudragit RL100 and/or RS100 coating were selected for *in vivo* studies.

Effect of alteration in composition of plain beads was evaluated. Incorporation of part of eudragit RS (as eudragit RSPM) inside the beads core by replacing part of avicel and then coating of beads with eudragit RS100, helped in further delaying the release of tetracycline hydrochloride. But larger amount of eudragit RS in total is required to obtain similar results as that for coating of plain beads. Results were found to be reproducible. This procedure can be indicated when larger amount of eudragit RS is to be applied by coating to get desired release pattern and application of that much amount of eudragit RS by coating is not feasible due to problems such as sticking, constraints in use of solvents etc..

Decrease in release of tetracycline hydrochloride was observed with increase in percentage of the drug inside the eudragit RS100 coated beads. This may be attributed to reduction in surface area available for diffusion per unit weight due to an increase in the amount of tetracycline hydrochloride.

The tetracycline hydrochloride matrix tablets were prepared by the two processes - coating of granules and compression of such granules into tablets; and compression of tablets with sustaining materials and then exposing these tablets to acetone vapours. Tablets prepared by the two processes consist of tetracycline hydrochloride, succinic acid, sustaining material and excipients. Succinic acid was included in the matrix for reasons referred to earlier.

Process of glyceryl monostearate-bees wax and glyceryl distearate matrix tablet preparation was tedious, resulted in lower yield and observation on release of the drug from these materices showed significant variation precluding selection of these products for in vivo evaluation.

The procedure adopted to formulate eudragit RLPM and/or RSPM matrices was simple, quick and giving better reproducibility. Release pattern of tetracycline hydrochloride from this type of matrices varies with the change in amount of drug, eudragit, and avicel. Other important factors influencing release of the drug from this type of matrices are dependent on acetone vapour exposure conditions i.e. time, temperature, and vacuum.

It was also noted that the tablet characteristics like hardness, gauge, friability etc. before acetone

exposure do not make significant difference in the final release pattern of drug from this type of matrices i.e. after acetone exposure.

Tablets prepared with combination of eudragit RLPM-RSPM were found most close to the desired release pattern with lesser inter-tablet variation in release of the drug and hence were selected for *in vivo* studies.

Two types of preparations - coated beads and matrix tablets were attempted to formulate controlled release drug delivery system of hydralazine hydrochloride. The loading and maintenance doses were calculated based on reported pharmacokinetic data. Prepared products were subjected to *in vitro* dissolution studies using basket stirrer assembly of USP XX, following the method recommended in NF XIV under "Timed Release Tablets and Capsules *In Vitro* Test Procedure". Products having release pattern close to the desired one and mixed beads prepared to match the desired release pattern were tested in triplicate for their dissolution behaviour *in vitro* to find out batch to batch variation in the release pattern.

Beads of hydralazine hydrochloride were also prepared using pelleter EXDS-60 and marumerizer Q-230. Hydralazine hydrochloride beads were coated differentially with glyceryl monostearate-bees wax, glyceryl distearate,

eudragit S100,and eudragit RL100 and eudragit RS100 separately and in combination. These products consist of the drug, avicel and other excipients in core with different sustaining material constituting the coating.

Results show that glyceryl monostearate-bees wax and glyceryl distearate coated beads have larger inter-capsule variation as compared to eudragit S100, eudragit RL100 and/or RS100 coated beads. Release of the drug from these beads was independent of pH and reproducible. Yield was also fairly good. Coated beads were spherical and uniformly coated. Hence product prepared with eudragit RL100 and/or RS100 coating was selected for in vivo studies.

The hydralazine hydrochloride matrix tablets were prepared by the two processes as in case of tetracycline hydrochloride. Process of glyceryl monostearate-bees wax and glyceryl distearate matrix tablet preparation was tedious, resulted in lesser yield and poor reproducibility as compared to eudragit RLPM and/or RSPM matrices. Tablets made with combination of eudragit RLPM-RSPM were found most close to the desired release pattern with less inter-tablet variation in release of drug and hence were subjected to in vivo studies.

Tetracycline hydrochloride and hydralazine hydrochloride controlled release products, found satisfactory

in *in vitro* studies, were subjected to *in vivo* evaluation in normal human volunteers and dogs respectively.

Four healthy male human volunteers were fed conventional and controlled release capsules and tablets of tetracycline hydrochloride. Urine samples were collected at definite time intervals and assayed. Experiment was carried out in a crossover design allowing one week washout period in between.

The urinary excretion rates of tetracycline hydrochloride at the midpoint of urine collection time after oral administration of each of the products were computed from the data of cumulative amount excreted of tetracycline hydrochloride. Approximate serum levels of tetracycline hydrochloride at the midpoint of urine collection time were predicted based on relationship between urinary excretion rates and serum levels as reported by Barr *et al*.

Studies have shown that controlled release products have significantly higher bioavailability in comparison to conventional product. As an amphoteric substance,tetracycline forms salts with acids as well as with bases. With hydrochloric acid it forms a hydrochloride, the solutions of which are strongly subjected to hydrolysis in a neutral medium. Tetracycline bases which precipitate above pH 3 are very difficultly soluble. Therefore, absorption of tetracycline hydrochloride is limited to small area of GI tract.

In case of controlled release products of present investigation, as soon as they come to a region with a pH value of 3 or more, such as upon leaving the stomach; the medium is influenced by the organic acid (succinic acid of the product) in a way that the pH of the surrounding fluid never exceeds a value which would permit hydrolysis of the tetracycline and thereby precipitation of the free base. This makes tetracycline hydrochloride available throughout the GI tract from controlled release products.

Higher mean tetracycline peak serum levels were achieved with controlled release products as compared to conventional products. Controlled release products studied have shown comparatively much smaller peak to trough ratio i.e. better sustained action.

The areas under the serum level curves were significantly higher for the controlled release products in comparison with those achieved by conventional tetracycline hydrochloride in single dose. This may be due to comparatively faster changes in serum concentrations of the drug following the administration of the conventional capsule.

From the studies, the controlled release products appear to be promising with regard to making medication simpler to the patients and to reduce fluctuation in tetracycline serum levels throughout the therapy. Controlled release

products have shown comparatively better bioavailability. Further, the incidence of side effects should be established through clinical trials.

Four mongrel dogs of either sex were fed conventional and controlled release capsules and tablets of hydralazine hydrochloride. Blood samples were collected at definite time intervals and assayed. The study was carried out in crossover design allowing one week washout period in between.

The controlled release products gave lower but therapeutically acceptable and delayed peak drug concentrations, thus altering the plasma profile of hydralazine in a mode consistent with the sustained release characteristics claimed for these products. Higher and consistent plasma hydralazine concentrations were maintained between 5-12 hrs with controlled release products when compared with the conventional 50 mg capsule. Furthermore, average plasma concentration ($\bar{C}$ i.e. AUC/dosing interval) was found significantly higher for controlled release products. This further proves consistent and higher hydralazine plasma concentration after administration of controlled release products.

The areas under the curves are significantly higher for controlled release products in comparison with those achieved by conventional hydralazine hydrochloride in single dose. From the studies, the new controlled release products

appear promising with regard to making medication simpler for hypertensive patients. However, as the suggestions have been raised that even conventional hydralazine hydrochloride tablets may be prescribed only twice daily, the role for controlled release products in treatment of hypertension can only be settled from clinical trials, with particular emphasis on the incidence of side effects. We would expect products to cause less side effects, but this could not be established in the present study.

Controlled release products of both tetracycline hydrochloride and hydralazine hydrochloride, found satisfactory in *in vivo* studies, were subjected to stability studies at air condition (20±2°C, 45±5% R.H.), room temperature, 37°C-65% R.H. and 50°C for 90 days. Samples were withdrawn at different time intervals and subjected to physicochemical examination and *in vitro* dissolution rate test.

The controlled release capsules of tetracycline showed little change in colour of beads at 37°C-65% R.H., and 50°C in three months. However, the change was not visible as such because the capsule shells were opaque. As mottling of the controlled release tablets increased at 37°C-65% R.H., and 50°C in three months, it will be desirable to sugar or film coat the tablet for keeping the products more elegant. All products of tetracycline hydrochloride have shelf life

of more than 1½ years at room temperature and at other stress conditions also, they have sufficient shelf life. However, there is no need of incorporating excess of the drug to enhance shelf life of product. No significant change was observed in release pattern of tetracycline hydrochloride from products kept on stability at different conditions.

The controlled release capsule as well as tablet of hydralazine hydrochloride showed no significant change in physical characteristics of these products. Both the products have shelf life of over a year at room temperature and for increasing shelf life to two years, it will be necessary to incorporate 5% excess of the drug. At other stress conditions, both the products have satisfactory shelf life. There is no significant change in release pattern of hydralazine hydrochloride from the products kept on stability at different conditions.

CONCLUSIONS

Over the years attempts have been made to control the time course and specificity of drug in the body through a variety of drug modifications and dosage forms. However, a maximization of therapy has not been achieved. The essence of any controlled release drug delivery system is to produce safe and consistent therapeutic blood level of drug in the body for required period of time.

In the present investigation attempt had been made, based on reported pharmacokinetic data, to fabricate, formulate and evaluate controlled release drug delivery system of water soluble drugs taking tetracycline hydrochloride and hydralazine hydrochloride as model drugs.

Basically two types of formulations were successfully worked out for these two drugs. First type of formulation involves preparation of beads using pelletar and marumerizer and finally coating of these beads with eudragit RL100 and/or RS100. The other type of formulation involves preparation of tablet by simple wet granulation using eudragit RLPM and/or RSPM as sustaining material. Finally exposure of these tablets to acetone vapours at controlled conditions of vacuum, temperature, and time causes the formation of matrix. Release of the drug from products prepared by either method is expected to be diffusion controlled and found to be independent of pH of the surrounding fluid. Both of the methods, with some modifications depending upon physico-chemical characteristics of the drug, may be useful in development of controlled release drug delivery system of other water-soluble drugs as well. Especially the procedure adopted to formulate eudragit RLPM and/or RSPM matrix tablets was found to be simpler, quicker, reproducible without requiring any special equipment for preparation.

Controlled release products of tetracycline hydrochloride and hydralazine hydrochloride appear to be promising with regard to making medication simpler to patients and to reduce fluctuation in their blood level throughout the therapy. Controlled release products of tetracycline hydrochloride have shown comparatively better bioavailability as compared to conventional product. In vivo evaluation of controlled release products of hydralazine hydrochloride should be undertaken on human subjects under medical supervision in order to substantiate the results of the in vivo evaluation on dogs. However, the role for controlled release products of the present investigation can only be settled from clinical trails with particular emphasis on the incidence of side effects.

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