7. SUMMARY

7.1. Floating- Bioadhesive Drug Delivery System

Diltiazem hydrochloride (DTZ) is a calcium channel blocker belonging to the benzothiazepine family. It is widely prescribed for the treatment of hypertension and angina. It has an elimination half-life of 3.5 hours and has an absorption zone in the upper intestinal tract. Efficacy of the administered dose may get diminished due to incomplete drug release from the device above the absorption zone. DTZ requires multiple daily drug dosage in order to maintain adequate plasma concentrations (Streubel *et al.*, 2006). The floating bioadhesive drug delivery systems can improve the controlled delivery of the drugs exhibiting an absorption window by continuously releasing the drug for a prolonged period before it reaches the absorption site, thus ensuring its optimal bioavailability (Arora *et al.*, 2005; Gambhire *et al.*, 2007). Various approaches have been proposed to control the gastric residence of drug delivery systems in the upper part of the GIT, include floating systems, swellable and expandable systems, high density systems, bioadhesive systems, altered shape systems, gel forming solution and suspension system (Patel *et al.*, 2006). Among these, the floating dosage forms have been used most commonly. But these systems suffer from a major disadvantage that they are effective only when the fluid level in the stomach is sufficiently high. However, as the stomach empties and the tablet is at the pylorus, the buoyancy of the dosage form may be impeded. This serious limitation can be overcome by making the floating system eventually adhere to the mucous lining of the stomach wall (Chitnis *et al.*, 1991; Chueh *et al.*, 1995). Floating and bioadhesive DDS, thus, offer the advantages of increased gastric residence, leading to improved bioavailability of drugs esp. with narrow absorption window.

The aims and objectives of present work were to develop and systemically optimized floating bioadhesive tablets of diltiazem hydrochloride.

A central composite design (with $\alpha = 1$) using three levels each of the two factors viz., CP 934P and HPMC K4M was adopted to optimize the various responsive variables viz., drug release in 16 hrs, time taken to release 60 % drug, total floating time and bioadhesive strength. The floating bioadhesive tablets of diltiazem hydrochloride were formulated using hydroxyl propylmethylcellulose and carbopol and other ingradients like magnesium stearate, talc and microcrystalline cellulose. The tablets were prepared by physical blending of all ingredients, except magnesium stearate for 20 minute. The magnesium stearate (60-mesh sieved) was added into powder blend as a lubricant and mixed for an additional 3 minutes

before compaction process. Then, 360 mg tablets containing 90 mg diltiazem hydrochloride were prepared by a lab press.

The formulated dosage forms were evaluated for physical characterization, assay, bioadhesive strength, floating time, swelling index and drug release pattern. The *ex- vivo* bioadhesive strength of various formulations was determined using a modified double beam physical balance against goat gastric mucosa. Goat gastric mucosal membrane was excised by removing the underlying connective tissue. After washing the mucosa was tied on the slide and the slide was fixed in the petriplate filled with distilled water and the petriplate was put inside the left arm of physical balance. The tablet was fixed on the left pan of the physical balance by adhesive. The left arm was lowered until a contact of the tablet with the membrane was made. A contact force of 10 g was placed on the left arm for 5 minutes. After 5 minutes the weight was removed from the left pan and the assembly remains undisturbed. Now the weight was slowly added on the right hand side pan till the tablet just detached from the membrane surface. The peak detachment force was recorded as a measure of bioadhesion.

The dissolution studies of all tablet formulations were carried out in triplicate, employing USP XXII paddle method (Apparatus II) at 50 rpm and 37 ± 0.5 °C, using 900 ml simulated gastric fluid (SGF) pH 1.2 without pepsin as the dissolution medium. 5 ml of sample was withdrawn periodically at suitable time intervals and volume replaced with an equivalent volume of fresh dissolution medium. Samples were analyzed by U V Spectrophotometer at λ_{max} 237 nm and total floating time was determined periodically after every 15 min, by careful visual observation during the dissolution run.

The data obtained from *in vitro* dissolution studies were analysed using ZOREL software which had in-built provisions for applying the correction factor for volume and drug losses during sampling. Drug release data were also fitted into Korsemeyer–Peppas model for swellable compressed matrices.

For optimization studies, multiple linear regression analysis (MLRA) method was applied to fit full second-order polynomial equation with added interaction terms to correlate the studied responses with the examined variables using Design expert software version 8.0.5 (Stat-Ease, USA). The polynomial regression results were demonstrated for the studied responses viz, time taken to release 60 % drug (t_{60}%), amount of drug released in 16 hours (Q_{16h}), total floating time (TFT) and bioadhesive strength (BS). Finally, the prognosis of optimum formulation was conducted using a two-stage brute force technique using MS-Excel

spreadsheet software. First, a feasible space was located and second, an exhaustive grid search was conducted to predict the possible solutions.

The validation of Response Surface Methodology was carried out to formulate six formulations as the confirmatory check-points. The tablets were formulated as per the method described earlier using chosen optimal composition and evaluated for physical test, tablet assay, BS, TFT, and dissolution performance in the same manner described earlier. The observed and predicted responses were critically compared. Linear correlation plots were constructed for the chosen six optimized formulations. The residual graphs between predicted and observed responses were also constructed separately, and the percent bias (= prediction error) was calculated with respect to the observed responses (Singh *et al.*, 2010).

The optimized tablets were evaluated for *in vivo* gastric residence time in albino rabbits by X-ray technique and also evaluated in human volunteer using gamma scientigraphy.

In the results it was observed that all the formulated tablets showed acceptable physicochemical properties and complied with the pharmacopoeial specifications. The percentage of drug content ranged from 98.38 % to 101.56 %. The weight of the tablets ranged from 359.5 ± 1.35 to 361.5 ± 1.69 mg. Representative tablets tested from each batch possessed hardness values ranging between 4.5 ± 0.051 to 4.8 ± 0.81 Kg indicating adequate strength to prevent friability losses. The weight of the tablets ranged from 359.5 ± 1.35 to 361.5 ± 1.69 mg. All the tablets tested from each batch exhibited friability values ranging between 0.45 % and 0.69 % w/w.

The bioadhesive strength of the tablets was measured against gastric mucosa as force of detachment and it was observed that the bioadhesive strength was increased in linear manner with increase in the amount of either polymer (Singh *et al*., 2006) and the maximum bioadhesive strength attained at higher level of both the polymers, because increasing the polymer content may provide more adhesive sites and polymer chains for interpenetration with mucin, resulting in augmentation of bioadhesive strength.

The duration of floating was observed during dissolution run and it was found that floating time of the formulation was increased with increased in the HPMC content in the formulation due to hydration of the hydrocolloid particle resulting in an increase in the bulk volume. While with increase content of CP, the floating time decreased in linear manner, this may be due to higher density of CP than HPMC. But the presence of CP could help in retaining the tablets in the stomach after oral ingestion by assisting the adhesion of dosage form with the

gastric wall, which in turn, may prolong the tablets gastric residence time (Nur and Zhang, 2000).

Various dissolution parameters calculated for all the floating bioadhesive tablets showed that the value of *n* varies between 0.4911 and 0.6585, indicating non-fickian release behaviour of all formulations. The values of *n* increased with increase in HPMC content, even at higher levels of CP. The values of the kinetic constant, k followed a decreasing trend with increase in the level of either polymer. Similarly, the values of Q_{16h} decreased with increase in the polymer content. But, the values of $t_{60\%}$ were found to rise markedly from 4.95 hours to 7.78 hours from lowest to highest levels of both the polymers.

In Response surface analysis, the coefficients of the polynomial equation were generated using MLRA for Q_{16h}, TFT, BS and $t_{60\%}$ of formulated floating bioadhesive tablets along with values of r^2. Seven coefficients from B_1 to B_7 were calculated with Bo as intercept. The r^2 values ranged from 0.928 to 0.996, indicating high prognostic ability of RSM polynomials. Since the values of r^2 are quite high for all the four responses, the polynomial equations form excellent fits to the experimental data and highly valid statistically. The 3D response surface plots and 2D contour plots were also constructed to facilitate an understanding of the contribution of the variables and their interactions for all four responses.

The optimized formulation was selected by feasibility and grid searches. The criteria for selecting the suitable feasible reason were primarily based on the highest possible values of $t_{60\%}$, $Q_{16\ hrs,}$ BS and TFT. The optimized formulation was selected by trading off various response variables and adopting the following maximizing criteria: $t_{60\%} > 5.5$ hours, $Q_{16hrs,}>$ 90 %, BS> 13 gm, TFT>8 hours. To validate the response surface methodology six formulations were prepared as check points and evaluated. The observed responses were compared with the anticipated responses and percentage errors were calculated. The plots between the observed and predicted responses, forced through the origin, were also constructed for the four response variables. All the plots were found to be highly linear as the value of r^2 ranged 0.928 to 0.994.

Following ingestion of the optimized formulation prepared by the addition of $BaSO_4$, the durations of tablet stayed in the stomach of rabbit were examined by radiograms and it was estimated that tablet stayed in the stomach for 8 hours.

The radiolabeled optimized formulation stayed in the stomach and duodenum region of human volunteer for 6 hours.

The optimized tablets were evaluated for stability for 3 months and at end of 3 months no significant changes were observed in the physical characteristics and drug release pattern of floating bioadhesive tablets.

7.2 Multiple Units Floating Drug Delivery Systems

In spite of extensive research and development in the area of single unit floating dosage forms, e.g., hydrodynamically balance systems and floating tablets, these systems suffer from an important drawback of high variability of the gastrointestinal transit time, when orally administered, because of their all-or-nothing gastric emptying nature. In order to overcome this, multiple unit floating systems were developed, that has all the advantages of a single-unit forms and also are devoid of disadvantages of single-unit formulations. Reports have described the development of both non-effervescent and effervescent multiple unit systems. In the present investigation a multiple units floating drug delivery system was developed using 3^2 factorial design and evaluated. The multiple unit floating drug delivery system was prepared by emulsion gelation technique. In this method solution of sodium alginate was prepared by adding required amount of sodium alginate in distilled water. Oil was then added to the polymer solution. The mixtures were homogenized at 500 rpm using a homogenizer (Remi-motors, RQ- 122, Vasai, India) for 20 min. Diltiazem hydrochloride was then dispersed in the formed emulsion. The bubble free drug loaded emulsion was extruded, using a 20 gauge syringe needle into 250 ml, 0.45 mol L^{-1} calcium chloride solution maintained under gentle agitation at room temperature. The emulsion gel beads were allowed to stand in the solution for 15 min before being separated and washed with distilled water. The beads were dried at 40^oC temperature and stored.

For systematic optimization, the amount of sodium alginate and oil were taken as independent variables while total floating time, floating lag time and percent drug released were taken as dependent variables. The formed beads were evaluated for physical parameter, duration of floating, floating lag time and drug release pattern.

Duration of floating of formulations was measured simultaneously as a part of dissolution studies and time taken by the dosage form introduction into the medium to its buoyancy to the upper one third of the dissolution vessel termed as floating lag time (Bera *et al.*, 2009). The dissolution studies of all the floating formulations were carried out in triplicate, employing USP XXII paddle method (Apparatus 2) at 50 rpm and 37 ± 0.5 °C, using 900 ml simulated gastric fluid (SGF) pH 1.2 without pepsin as the dissolution medium. 5 ml of

sample was withdrawn periodically at suitable time intervals and volume replaced with an equivalent volume of fresh dissolution medium. Samples were analyzed by U V Spectrophotometer at λ_{max} 237 nm.

For the studied design, the MLRA method was applied to fit full second-order polynomial equation with added interaction terms to correlate the studied responses with the examined variables using Design Expert Software version 8.0.5. The polynomial regression results were demonstrated for the studied responses. Finally, the prognosis of optimum formulation was conducted using a two-stage brute force technique using MS-Excel spreadsheet software. First, a feasible space was located and second, an exhaustive grid search was conducted to predict the possible solutions. To validate RSM six formulations were selected as the confirmatory check-points. The formulation was prepared as the method described earlier using chosen optimal composition and evaluated for physical test, assay, TFT, and dissolution performance in the same manner described earlier. The observed and predicted responses were critically compared. Linear correlation plots were drawn for the chosen six validation check points and the percent error (= prediction error) was calculated with respect to the observed responses (Singh *et al*., 2010).

The results of evaluation of beads revels that the mean diameter of beads ranges between 1.49±0.6 to 2.06±0.17mm. Moreover, beads size was found to increase with an increase in oil concentration. This might be due to an increase in the droplet viscosity caused by higher oil content. Drug entrapment efficiency ranged from 56.5 % to 64.5 % depending on the composition of the nine batches. There was a correlation observed between proportion of oil and drug entrapment efficiency of the beads. A higher proportion of oil in the formulation of beads increased the drug entrapment efficiency in different batches due to partitioning of the drug in the oil phase. Moreover, it was observed that an increase in the amount of alginate increases drug entrapment efficiency due to increased space for drug molecules to be retained throughout a larger cross linked network of calcium alginate.

It was also observed that all floating formulations floated within 2 minutes after being placed into the acidic medium and remained buoyant in the acidic medium throughout the study, irrespective of the proportion of oil in the formulation. Drug release pattern from the all the formulations was affected by polymer concentration and amount of oil used. All the batches showed the initial burst release because some amount of the drug, which might have been dragged to the surface during curing in surrounding medium, was released immediately. The

results indicated that the drug release slows down with increasing polymer concentration as well as increasing in amount of oil.

For Response Surface Analysis**,** the coefficients were generated using MLRA for Q, EE, $T_{b,}$ S and $t_{60\%}$ of the floating formulations along with values of r^2. Seven coefficients from B_1 to B_7 were calculated with Bo as intercept the r^2 value ranged between 0.988 and 0.999, indicating high prognostic ability of RSM polynomials. Since the values of r^2 are quite high for all the three responses, the polynomial equations form excellent fits to the experimental data and highly valid statistically.

The 3D response surface plots and2D contour plots were also constructed to facilitate an understanding of the contribution of the variables and their interactions.

The optimized formulation was selected by feasibility search and grid searches. The optimum formulations were selected by trading off various response variables and adopting the following maximizing criteria: Q> 91 %, T_b< 24 hours, EE> 61.53 %, S> 1.71, $t_{60\%}$ > 4.10.

For the validation of the results, six formulations were prepared as method described earlier and evaluated. Now the observed responses were compared with that of the anticipated responses. The plots between the observed and predicted responses for the three response variables were constructed. All the plots were found to be highly linear as the value of r^2 ranged from 0.968 to 0.996.

Following ingestion of the optimized formulation prepared by the addition of $BaSO_4$, the durations of beads stayed in the stomach of rabbit were examined by radiograms and it was observed that the beads stayed in the stomach for 8 hours.

At the end of storage period for stability study, the optimized formulation showed no significant changes in their physical characteristics and drug release pattern.

7.3 FUTURE SCOPE OF FDDS:

This work has immense scope in that it has demonstrated the successful application of modern “design of experiments” technique, in combining

- Drug delivery
- and bioadhesion

for the diltiazem hydrochloride using the *in vitro* and *in vivo* evaluation methods. However, much needs to be done, in the present work, in the form of:

1. Scale up of the experimental technique to production levels

2. Validation of the results *in vivo* using improved animal models and analytical techniques etc.

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