CHAPTER 3
RESUSLTS AND DISCUSSION

RESULTS AND DISCUSSION

Mucilages or mucopolysaccharides of plant origin have been used widely as demulcent because of their unique properties to bind with the mucus membrane. The selection of the materials for the current investigation was based on their edibility, blandness, availability and economics.

Isolation of water-soluble components from the natural edible sources was carried out by cold/hot aqueous extraction process followed by the organic solvent precipitation. The selection of the process was based on previous literature giving utmost importance to preserve the components against thermal, enzymatic and hydrolytic degradation. The organic solvents used for precipitation can be recovered back by fractional distillation, making the process more economical. The processes used were found to be effective in selective isolation of the material and the yielded material possesses good handling properties.

Table 8 represents the details of the extraction process, respective yield, and their physical properties such as pH, swelling volume, swelling capacity, moisture absorption capacity, loss on drying etc. The yield of DP, DI, CE and ZJ was ≈5.49, 4.91, 3.46, 3.87 % w/w respectively to the initial weight. pH of 1% w/v solutions of DP and DI was found to be 5.67, 6.68 respectively which is very closer to the pH of saliva ≈6.6 suggesting its non-irritability to the buccal mucosa. Swelling is the primary characteristic of any material to be a mucoadhesive substance, but over hydration causes slippery surface. Excessive swelling also causes loss of mechanical strength that is required to maintain the structural integrity of the solid dosage forms [138]. Swelling volumes after 24 hours of hydration was found to be 12.1, 12.4, 13.3, 18.3 indicating their moderate swellability compared to 27.4 of CP 934 P, 25.7 of sodium alginate and 31.2 of guar gum. Swelling was also assessed by the determination of swelling capacity and moisture absorption profile [139]. Study of moisture absorption is also of considerable importance since it reflects the relative physical stability of dosage forms when stored under

humid conditions. In all, this property showed that the CE & ZJ powders are sensitive to atmospheric moisture and should therefore be stored in airtight containers. But it was found that the moisture absorption capacities of DP & DI are very less. The loss on drying of DP, DI, CE & ZJ were less than the official limit of 6% stated in British Pharmacopoeia 2004.

Fig 9 & 10 represents the schematic diagrams of the apparatus used for preliminary screening of mucoadhesive strengths. Fig 11- 16 represents the weight required to detach the blocks/tissues attached together by the mucoadhesive solutions after specified contact times. The results suggest that the isolated mucoadhesive material possessed comparable shear and tensile strengths to the commercially available GRAS (generally regarded as safe) category polymers and higher than the other natural polymers such as sodium alginate and guar gum. Further, these strengths were increased with the increase in concentration but no considerable increase was observed after 15 minutes of contact time, irrespective of polymers studied. Strengthening of bioadhesion may be due to the formation of more number of secondary bonds as time progresses.

Table 9 represents the various formulation factors in the preparation of adhesive cups. The extracted natural materials were mixed with each other at varying proportions to find out the best possible combination that shows ideal qualities of bioadhesive material in respect of mucoadhesive strength, swellability, leaching of drug, *in vitro* residence time, and good flowing and handling properties.

Table 10 represents the physical properties of the granules used for the preparation of adhesive cups. The flow properties such as angle of repose, Hauser Index and Compressibility Index are considered as indirect measurements of powder flowability. Hauser Index is indicative of inter- particular friction; the Compressibilty Index shows the propensity of a material to diminish in volume. As the values of these indices increase, the flow of the powder decreases [140].

Adhesive cups were studied for their bio/mucoadhesive strengths by using the specially fabricated apparatus as shown schematically in Figs 19. Tensile, shear and peel strengths were calculated after five minutes of contact time and represented graphically in Figs 20 -22. These methods differ in direction of force applied to detach the adhesive cups

Among these, peel strength is one of the important measures for bioadhesive buccal preparations. These studies were conducted on excised bovine as well as porcine buccal mucosa in order to assess the mucoadhesion in varied textural properties. Bioadhesive strengths were found to be more in case of bovine mucosa rather than the porcine mucosa irrespective of polymers studied, but was in the bioadhesive range. Five minutes of contact time was found to achieve optimum mucoadhesive strength. Further increase in contact time has not affected the mucoadhesive strength considerably. Mucoadhesion with mucin molecules gradually occurs due to the formation of hydrogen bonding with the mucus, resulting in the formation of a strengthened network between polymer and mucus. Thus, results suggest that these mucoadhesive agents may have high density of available hydrogen bonding groups that would be able to interact more strongly with mucin glycoproteins. It is speculated that the higher mucoadhesive strength of the delivery system may lead to the prolonged retention and increased absorption across mucosal tissues [6].

Fig 23 represents the schematic diagram of the apparatus used to determine the *in vitro* residence of the adhesive cups. Modified disintegration apparatus was used for the study. The results showed that the AC 17, AC 18, AC 19 and AC 22 formulations exhibited residence times of 4.98, 4.82 and 5.08 hours respectively, which are beyond the period required for the complete absorption of the drug.

All the results suggests that the adhesive cups in combination of AMP with other mucoadhesive materials at the ratio of 1 : 3 possessed better qualities than the other studied ratios and are comparable with the adhesive cups similarly prepared with commercially used synthetic polymers. Hence, AC 17, AC 18, AC 19 and AC 22 formulations from the investigated materials were selected for further studies.

Table 10 represents the various fact idered for the formulation of core tablets. Microcrystalline cellulose, one of the most used filler-binders in direct tablet compression due to its excellent binding properties and high dilution potential in direct compression formulations is used in the formulation [141]. Mucoadhesive polymers at varying proportions were used to retard the release of drug to achieve sustained release. Table 12 represents the respective combinations of adhesive cups and core tablets used for the formulation of NBATs.

Table 13 represents the results of various evaluation procedures adopted for the evaluation of physicochemical properties of NBATs. The thickness of NBATs using the isolated material falls between 1.14 to 1.28 mm and weight between 32.8 to 39.8 mm, suggesting its suitability for ease of administration without any discomfort. Weight variation and drug content uniformity studies suggest uniform mixing, validation of manufacturing process and its reproducibility. Results such as percent friability (0.21 to 0.87%) and hardness (3.11 to 4.42 g/cm^2) were found to be within the recommended values of Indian Pharmacopoeia [142].

Figs 24 - 29 represents the histological studies conducted on excised porcine buccal mucosa. The photomicrographs suggest that there was no considerable damage after the administration of NBATs.

Figs 30-33 represent the FTIR Spectra's of mucoadhesive polymers under investigation. Figs 34 – 38 represent the FTIR Spectra's of Diltiazem hydrochloride, NBATs 3, 7, 11, and 15. Results suggest that Diltiazem hydrochloride has not undergone any unacceptable interactions with the mucoadhesive polymers isolated from the natural edible sources.

Figs 39 - 43 represent the DSC thermographs of Diltiazem hydrochloride and NBATs 3, 7, 11, and 15. The thermographs suggest that there is no significant interactions between the Diltiazem hydrochloride with the additives used in the formulation, thus the additives used and the methods adopted are acceptable.

Fig 44 represents the λ_{max} of Diltiazem hydrochloride in Phosphate Buffered Saline (pH 7.4) showed absorption maxima of 236 nm. Fig 45 represents the calibration curve for the Diltiazem hydrochloride. The linear equation was y = 0.087x with the correlation coefficient of 0.9997.

Table 14 represents the kinetic parameters of *in vitro* dissolution studies. The zero order, first order, Higuchi diffusion, Korsmeyer – Peppas, and Hixon Crowell were plots were drawn as represented in Figs 46 – 55 and the respective linearity equations were enlisted in Table 14 and the corresponding correlation coefficients were represented in the Table 15. Results suggest that the NBATs could release the drug following first order in formulations without the inclusion of mucoadhesive material in the core tablets, but followed Higuchi diffusion or Korsmeyer – Peppas patterns after the inclusion of the same. Further the rate of release was sustained as the proportion of mucoadhesive substance is increased irrespective of the materials used.

In vitro dissolution studies with the mucoadhesive layer exposed to the dissolution medium are represented in Table 13. It was found that less than 4% of drug diffused through the backing layer in four hours of study compared to 5.87% in sodium alginate and 6.18% in guar gum. The results suggest that the mucoadhesive material under investigation has not allowed the drug to diffuse through its backing layer

Fig 56 represents the set up of Franz diffusion apparatus used for the *ex vivo* permeation studies. Porcine buccal mucosa was selected for permeation studies because of its permeation characteristics are similar to humans. This is due to the presence of non-keratinized epithelial tissues, which is not common in other experimental animals. The amount of Diltiazem hydrochloride that permeated through the buccal mucosa at defined intervals in a period of four hours was estimated spectrophotometrically. Results were plotted to assess the permeation pattern as given in Figs 57 – 66 and enlisted the respective linearity equations in Table 16 and correlation coefficients in Table 17. All results suggest that the permeation was similar to the *in vitro* dissolution studies in most cases and the amount permeated is slightly less than the actual amount of drug dissolved at the similar conditions.

Pharmacokinetic parameters of Diltiazem were studied for optimized NBAT formulations, i.v. bolus injection and orally administered core tablets of same batch of NBATs on anaesthetized male New Zealand rabbits and were represented in Table 18. During the period of experiment the NBATs remained at the site of application. Prior to the HPLC analysis, a calibration curve was prepared for Diltiazem using Diazepam as internal standard. Under the operated conditions, Diltiazem and Diazepam had retention times of approximately 5.267 and 3.813 minutes, respectively. Results showed linearity related to concentration at the range of 5 ng to 400 ng. The linear equation for the concentration vs the ratio of peak area was $y = 0.0078x - 0.0003$ with correlation coefficient of 0.9994 (n=4) .

Fig 68 – 71 represents the plasma concentration profiles in anesthetized rabbits after the administration of Diltiazem hydrochloride through intravenous, oral and Novel Buccal Adhesive Tablets. Plasma concentration of Diltiazem declined to less than the minimum effective concentration in about 2.5 hours after intravenous administration. Conversely, in oral tablets and NBATs at the same dose MEC was reached after 0.5-1.0 and 1.0-2.0 respectively and remained above the desired level till 2-2.5 and 4-5 hours respectively. Time to reach maximum concentration (T_{max}) for NBAT was 3 - 4 hours whereas it was 1 - 1.5 hour on oral administration. Maximum plasma concentration (C_{max}) for oral (46.9 - 58.9) was found to be less than the NBATs (57.1 - 73.5). The AUC values for after iv administration was 437.53 ± 24.36 (hr)*(ng/ml). On oral administration, the F (bioavailability) values were found to be 0.384±0.36*, 0.367± 0.6**, 0.411±0.1* and 0.353±0.06*respectively for NBAT 3, NBAT 7, NBAT 11 and NBAT 15. Same formulations on buccal administration yielded F values of 0.794±0.09*, 0.766±0.09**, 0.839±0.09** and 0.744± 0.08** respectively. Statistical analysis of all the parameters suggests that the methods and the dosage forms are reliable and highly reproducible.