Chapter – 6

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Natural mucoadhesive agents from plant source have been extensively used due to its unique property to bind with the mucus membrane. The selection of the materials under study was based on their edibility, blandness, abundant availability and economics.

Table No. 5.1 describes all the physical parameters studied for the mucoadhesive substances isolated from the natural origin and compared with the commercially used synthetic polymers. Results suggest that all the mucoadhesive materials except AMP and HC has shown better yield with respect to the initial weight of the raw material. All the material has exhibited good handling properties.

The pH of the natural mucoadhesive agents varied from 5.2 to 6.14 suggesting non irritability to the nasal mucosa as the pH of nasal mucosa falls between 5.5 and 6.5.

Swelling is the primary characteristic of any material to possess mucoadhesive property, however over hydration causes slippery surface. Swollen volumes of natural mucoadhesive agents under investigation after 24 hours of hydration were found to be between 7.4 to 18.3 ml indicating their moderate swellability. Swelling was also assessed by the determination of swelling capacity and moisture sorption profile.
Study of moisture sorption capacity is also of considerable importance since it reflects the relative physical stability of the dosage form when stored at varying moisture conditions. Moisture sorption capacities were also found to be within the pharmacopoeal limits suggesting its suitability as additive and stable at room temperature. It was found that AMP alone was sensitive to atmospheric variations and should therefore be stored in air tight containers.

The loss on drying of all the materials under study ranged from 1.2 to 5.4% w/w. These values are less than the maximum tolerable limit (6% w/w) stated in British Pharmacopoeia.

Fig.No. 5.1 - 5.6 represents the adhesive strengths (0.5%, 1%, and 1.5% w/v) of natural mucoadhesive agents studied by Shear Stress and Park and Robinson’s methods. The results suggest that the adhesive strength of the polymers was found to be increased progressively as the contact time increases in all materials studied irrespective of the concentration. The progressive increase in adhesive strength may probably be due to strengthening of adhesive forces by the formation of more number of secondary bonds as time progresses. Similarly, the mucoadhesive strength was found to be increased as the percentage of polymer concentrations was increased. The results suggest that the isolated mucoadhesive materials possessed comparable shear and tensile strengths to the commercially available GRAS (Generally regarded as safe) category polymers. Among all the natural mucoadhesive materials studied, YMM and HRS exhibited the
highest mucoadhesive strength and the values are almost equivalent to SCMC and HPMCK4M at all the studied concentrations. Hence YMM and HRS were selected for further studies among the natural agents based on superior physical properties like flowability, swellability and bioadhesive strength.

Fig.No. 5.7- 5.27 represents the FTIR spectras of natural mucoadhesive polymers under investigation, carvedilol and their respective formulations. Fig.No. 5.28- 5.38 represents the DSC spectras of natural mucoadhesive polymers under investigation, carvedilol and their respective formulations. Results suggest that carvedilol has not undergone any unacceptable interactions with the mucoadhesive polymers.

Table No. 5.4 represents the gelation transition temperatures of the mucoadhesive polymers by both visual inspection and viscometric methods for all the formulations. Viscosity is one of the important physical parameter for bioadhesive semi solids/liquid dosage forms to increase its bioavailability by prolonging residence time. Thermoreversible nasal mucoadhesive gel formulations with an in situ gelling property delays the clearance of the dosage form from nasal cavity. The studies were intended to measure the gelation transition temperature around the temperature of the nasal cavity. In general, the gelation temperatures have been considered to be suitable if they are in the range of 27°C to 34°C. If the gelation temperature of the formulation is lower than 25°C, a gel might be formed at room
temperature leading to difficulty in manufacturing, handling, and administration through the narrow nozzle. If the gelation temperature is higher than 34°C, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the gel prior to the absorption of drug. As the temperature of the nasal cavity is around 34°C, this study was aimed to prepare the dosage forms that gels around 34°C. Fig. No. 5.39 shows the viscosity versus temperature curves of all the formulations. It is evident from the data that the gelation temperature measured both by visual inspection and viscometric methods have not varied more than ±1.2°C. These formulations exhibited excellent thermoreversibility at the room temperature and at body temperature. Hence these formulations are expected to adhere at applied site, maintain dose uniformity, easy to apply and also minimizes the loss of administered drug caused due to clearance from the site of application.

Fig No. 5.40 represents peel strengths of the formulated mucoadhesive nasal gels. Three minutes of contact time was required for the formulation to reach the optimum bioadhesive strength for the formation of secondary bonds and physical entanglement between polymer chains and mucin molecules. Further increase in contact time did not affect the mucoadhesive strength appreciably. Assessment of the mucoadhesive strength in terms of detachment stress showed that the mucoadhesive preparations possessed peel strength at the bioadhesive range i.e 3000 to 4500 N/m². From the literature it was found that mucilages from natural edible sources
contains a high amount of mono, di, oligo and poly saccharides and abundant free hydrogen bonding sites which are mainly responsible for mucoadhesion with mucin molecules. These agents gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus as well as epithelial membrane. Thus, the mucoadhesive agents having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoprotein. In addition, it may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is also speculated that the optimum mucoadhesive strength of the gel ensures its retention at the applied site until the drug is completely absorbed.

Fig.No. 5.42 represents the calibration curve of carvedilol, which was obtained when concentration (µg/ml) was plotted against absorbance. It resulted in a straight line having the linear equation of \( y = 0.009x - 0.011 \) with a correlation coefficient of 0.998.

*Ex vivo* permeation studies using excised goat nasal mucosa were carried out for a period of 4 hr through Franz diffusion cell. Goat nasal mucosa was selected for these studies because it is a validated *ex vivo* model to study the transport and metabolic pathways. A maximum of 95% of carvedilol was permeated in formulation C1 i.e formulation containing AMP, indicating permeation enhancing effect of
AMP. Hence AMP was included in all other formulations for its permeation enhancing effect. It was observed that a 10-12% of increase in permeation of carvedilol when AMP was combined with YMM and HRS (91%, 92%, 87%, 89% with C7, C8, C10, C11 respectively). From these findings it was evident that a separate absorption enhancer is not required for increasing the permeation of the drug through the nasal mucosa as the AMP exhibited permeation characteristic in addition to mucoadhesive property. It was also observed that the mucoadhesive strength was found to be decreased as the proportion of AMP increased. The formulations having optimum bioadhesive strength and better release through mucosa were selected for further studies.

The samples withdrawn at predetermined time intervals were plotted for zero order, first order, Higuchi diffusion, Hixon-Crowell and Korsmayer-Peppas plots as shown in Fig. No.5.43 - 5.47 and the respective linearity equations were enlisted in Table No. 5.7. Correlation coefficient ($R^2$) values were calculated to identify the exact release mechanism and were represented in Table No. 5.8.

Fig.No. 5.48 represents the calibration curve of carvedilol, which was obtained when concentration ($\mu$g/ml) was plotted against area of the chromatogram. It resulted in a straight line having the linear equation of $y = 87713x - 85109$ with a correlation coefficient of 0.999.

Table 5.9 enlists the means of all the parameters such as $C_{\text{max}}$, $t_{\text{max}}$, $\text{AUC}_{0-t}$, $K_e$, $t_{1/2}$, MRT, CL, $V_d$, etc so calculated for both oral
solution as well as optimized nasal gels (C6, C7, C8, C10, C11) administered to anaesthetized rabbits.

During the period of experiment, it was found that the nasal gel remained intact at the site of application. Prior to the HPLC analysis, a calibration curve was prepared for carvedilol in plasma. Under the operated conditions, carvedilol had retention time of approximately 3.773 min. Results showed linearity related to concentration at the range of 2 to 20 µg. Statistical analysis of all the parameters studied were either very significant or significant suggest that the methods and the dosage form are reliable and highly reproducible.

Three fold increases in bioavailability was observed in nasal gel formulations compared to oral solution. The increased bioavailability may plausibly due to avoidance of hepatic first pass effect. The rate of absorption was found to be rapid compared to oral administration. This may have resulted from the fact that nasal mucosa is continuously profuse with blood, which acts as an infinite sink for carvedilol. In addition, gels applied directly to the nasal mucosa of the rabbit presumably provide closer and more intimate contact with the mucosa. Addition of AMP to the formulations resulted in increased carvedilol permeation appreciably\(^{146}\).

However, nevertheless the fact nasal gel prepared from the extracts of the natural edible source delivered carvedilol progressively at a continuous rate for 1\(^{1/2}\) hr on administration to rabbits and released the drug in sustained fashion till the fifth hour of study. In
addition, no serious histological changes in the applied mucosal area were observed. Thus the results suggest that the nasal mucoadhesive gels prepared using natural mucoadhesive agents and AMP as permeation enhancer released the carvedilol in a quicker fashion and also possessed requisite mucoadhesive strength.

Fig No. 5.50 exhibits representative photomicrographs of nasal mucosa obtained from the nose of rabbits following a typical 5 hour experiment. Carvedilol loaded gels were applied to the nasal mucosa into the one nostril of the rabbit, while the other nostril served as the control tissue. No significant alteration of nasal mucosa was observed to which carvedilol loaded gels were applied. Moreover, photomicrographs of dissected mucosa demonstrated no tissue damage by either placebo or carvedilol loaded gels in comparison to control mucosa.

From Fig No. 5.51 - 5.56, it was evident that all the nasal gels formulated with both natural and synthetic polymers when analyzed at regular intervals for any degradation stored at a temperature 40°C and a relative humidity of 25% for six months did not show any degradation of the drug in the formulations hence it was found to be stable for the entire period of analysis. Moreover interaction among any of the substances that could degrade the drug was also not observed. The United States Food and Drug Administration (FDA) accepts only real-time stability data for protein/peptide pharmaceuticals for the purpose of assessing shelf life, hence
accelerated isothermal stability studies may only serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature\textsuperscript{147}. The drug content results indicated that there was no significant change in the content after 6 months when compared with the initial value. One of the important parameters that have to be considered while formulating a gel formulation is the viscosity. In case of the nasal drug delivery, viscosity is also required to retain the formulation at the point of application. The results indicated that the formulation did not show any change in viscosity when stored under conditions described above. Similarly no change in pH was observed during the stability-testing period. Carvedilol solution available in the market has to be stored at 2°C to 8°C preferably in a refrigerator, but not in or near the freezing compartment. When Carvedilol is exposed to direct sunlight without refrigeration there is a loss in potency with the formation of a cloudy solution. However, results indicated that these gels containing Carvedilol could be stored at room temperature and controlled relative humidity.