13.0 SUMMARY & CONCLUSION

The investigations reported herein were based on the objective of designing and characterizing buccoadhesive formulations of Carvedilol and Pravastatin sodium aimed at reducing drawbacks of conventional formulations. It was envisaged that the proposed delivery systems will overcome first pass metabolism and result in increased bioavailability. It was expected to provide sustained release of Carvedilol and Pravastatin sodium which will reduce the dose and frequency of administration of conventional dosage form and will improve patient compliance.

- **Carvedilol:**

Three formulations were prepared for Carvedilol,

1. Core in cup tablets (CCT)
2. Bilayer patches (CBP)
3. Bilayer tablets formulated with chitosan microspheres (CTM)

- **Pravastatin sodium:**

Two formulations were prepared for Pravastatin sodium,

1. Core in cup tablets (PCT)
2. Bilayer patches (PBP)

The above formulations were prepared with *easy to manufacture* methods and the methods were found suitable. All the formulations were evaluated for critical aspects or parameters required for optimized sustained buccoadhesive drug delivery system. Optimized formulations were selected on the basis of satisfactory in vitro properties.

13.1 CARVEDILOL:

Optimized batches for core in cup tablets, bilayer patches and bilayer tablets formulated with microspheres were CCT7, CBP7 and CTM1 respectively. Detailed composition of which is given in experimental part.

Surface pH exhibited by Carvedilol core in cup tablets, bilayer patches and bilayer tablets with microspheres were 6.15 ± 0.20, 6.01 ± 0.25 and 5.71 ± 0.17. As surface pH of these delivery systems was near to salivary pH of the buccal mucosa, it can be reasonably assumed that it will not cause any irritation to the mucosa because of alteration of pH due to delivery system.

The **swelling** of the core in cup tablets, bilayer patches and bilayer tablets with microspheres in phosphate buffered saline were 4.09 ± 0.39 %, 4.19 ± 0.35 % and 2.95 ± 0.29 % respectively. Maximum swelling was shown by bilayer patches and least by bilayer tablets with microspheres probably due to lower affinity of chitosan to phosphate buffer saline at basic pH. In patches, placebo patches showed less swelling as compared to medicated patches, indicating that the presence of Carvedilol in the patches affected the swelling.

Mechanical properties of bilayer patches were found to be suitable as it demonstrated relatively high TS (7.92 ± 0.34 $kgmm^{-2}$), high E/B (137.36 ± 7.49 % mm^{-2}) and high Strain (2.01 ± 0.34 kg) but a low EM (3.94 ± 0.11 $kgmm^{-2}$) indicating that the patch had both strength as well as elasticity.

In vitro mucoadhesive force shown by core in cup tablets, bilayer patches and bilayer tablets with microspheres were 50 ± 2.45, 53 ± 2.13 and 50 ± 1.84 N respectively. Obtained value of in vitro mucoadhesive force was sufficient to prevent dislodgement of the system from buccal mucosa for at least 6 hr (as proved subsequently by in vivo acceptability testing). Placebo patches showed high mucoadhesive force (58 ± 3.01) as compared to medicated patches. It implies that presence of Carvedilol in the patches had negative effect on in vitro mucoadhesive force.

***In vitro* diffusion** of pure drug was 93.02 ± 2.93 % as expected from its lipophilic characteristics and high partition coefficient. Diffusion of Carvedilol through core in cup tablets, bilayer patches and bilayer tablets with microspheres was 78.23 ± 3.56, 79.86 ± 2.99 and 71.12 ± 2.59 % respectively. Less Carvedilol diffusion was shown by bilayer tablets may be due high concentration of polymer and less swelling of tablets at salivary pH.

In vitro drug dissolution

It was observed that bilayer tablets with microspheres showed less dissolution (72.08 ± 3.05 %) as compared to other dosage forms. Bilayer patches showed highest dissolution may be due higher surface area of patches available for dissolution.

Core in cup tablets showed best fit for zero order. Peppas model also fitted for core in cup tablets, implying non-fickian pattern i.e. drug release is by diffusion mechanism. Bilayer patches showed best fit for zero order model, followed non-fickian diffusion pattern implying diffusion is the dominant release mechanism. Bilayer tablets with chitosan microspheres followed non-fickian release mechanism with super case II transport i.e. dissolution is the combination of diffusion and chain relaxation and showed best fit for zero order kinetics. Comparative in vitro dissolution profile of all formulations showed that bilayer tablets formulated with chitosan microspheres showed better sustained release of Carvedilol.

Pharmacokinetic studies:

The plasma concentration profile for buccal formulations showed sustained release of Carvedilol as compared to oral administration. The Cmax for oral conventional tablets was 58.25 ± 4.26 ng/ml while that of core in cup tablets, bilayer patches, bilayer tablets with microspheres were observed to be 64.14 ± 7.36, 69.18 ± 6.69 and 71.26 ± 3.88 ng/ml respectively, showing increase in peak concentration of Carvedilol through buccal route. Oral conventional tablets showed AUC of 155.24 ± 8.43 ng/ml/hr while core in cup tablets, bilayer patches and bilayer tablets with microspheres showed AUC of 297.53 ± 8.20, 319.44 ± 6.65 and 390.92 ± 5.23 ng/ml/hr respectively. Thus, all buccal formulations were able to provide increase in bioavailability, probably due to bypassing first pass metabolism. Core in cup tablets, bilayer patches and bilayer tablets with microspheres showed 1.92, 2.06 and 2.52 fold increase in bioavailability respectively as compared to oral conventional tablets.

When Carvedilol buccal patches and core in cup tablets were compared for their pharmacokinetic efficacy, it was found that Carvedilol buccal patches (319.44 ± 6.65 ng/ml/hr) showed slightly higher AUC than buccal core in cup tablets (297.53 ± 8.20 ng/ml/hr). This may be because greater surface area and less diffusional path length available for absorption of Carvedilol from the patch (14.00 mm) when compared with core in cup tablets (6.00 mm). Another reason behind higher bioavailability of bilayer patches

could be permeation enhancing activity of propylene glycol used as a plasticizer in the formulation (as observed from in vitro diffusion studies).

Absorption of Carvedilol from the bilayer buccal tablets formulated with microspheres appeared slightly slow as compared to other buccal formulations. The lag time for the release of Carvedilol may be due to its slow rate of swelling of the microspheres present in the tablet matrix. Prolonged plasma levels (10.02 ± 0.95 ng/ml at 10^{th} hr) were exhibited by bilayer buccal tablets formulated with chitosan microspheres while core in cup tablets and bilayer patches showed sustained plasma levels up to 8 hr.

Amongst all formulations, bilayer buccal tablets formulated with chitosan microspheres were effective in sustaining plasma Carvedilol levels for maximum time and also showed highest increase in bioavailability.

The results obtained in these studies prove the justification of administering Carvedilol through the buccal route for avoiding pre-systemic metabolism and improving bioavailability.

Histological study of buccal mucosa:

Light microscopy:

Control buccal mucosa showed all the three distinctive layers of the oral mucosa, the epithelium, basement membrane, and connective tissues.

Buccal mucosa treated with core in cup tablets showed negligible changes in epithelium and basal membrane. The principal components of the permeation barrier appeared same as control at the end of the study. Sections of sample mucosa treated with bilayer patches showed little modification in the epithelial layer. Permeation enhancing effect of propylene glycol might have caused certain disruption of cells of epithelium. It is clear from the results of the permeation experiments that no major alteration in the barrier function of the tissue was seen.

Sections of sample mucosa treated with bilayer tablets formulated with chitosan microspheres showed slight modification to the epithelial layer because of chitosan which is characterized by absorption enhancing effects, as it improves the paracellular transport by opening the tight junctions in the epithelial layer. It can also be assumed that the slight change in epithelial layer was may be due to retention of drug on mucosa.

It was clear from the permeation experiments that no major alterations were found in the mucosa and it can be concluded that the changes may be reversible.

Scanning Electron Microscopy of buccal mucosa:

SEM of the control buccal mucosa showed the presence of the superficial cells of the epithelium and showed that stratified squamous cells have intact cell junctions with microridges.

Sample buccal mucosa treated with core in cup tablets showed that the squamous cells are normal and to some extent similar to those of the control. But, slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue.

SEM of sample buccal mucosa treated with bilayer patches showed shrinkage of superficial cells. Use of dichloromethane in the buccal patches might have contributed to the shrinkage of cells.

Treatment of the buccal mucosa with the bilayer buccal tablets formulated with chitosan microspheres showed that the squamous cells are normal and similar to those of the control. Chitosan is characterized by permeation enhancing effect which opens paracellular junctions and results in shrinkage of superficial layers. From available literature it can be expected that these slight changes may be reversible and not affected overall structure, surface and function of the buccal mucosa.

***In vivo* acceptability testing:**

It can be concluded that the core in cup tablets, bilayer patches and bilayer tablet formulated with microspheres would be comfortable and acceptable by the patients and retained in the buccal cavity long enough for the complete drug release to occur. Comparative results showed that bilayer patches was most acceptable by the volunteers as compared to other formulations.

Pharmacodynamic studies:

These studies were divided into 2 parts,

a. Development of hypertension
b. Treatment with oral and buccoadhesive formulations

Development of hypertension:

Fructose model was used to induce hypertension in rats. The model was found suitable and using this model hypertension was induced in 6 weeks. Parameters like mean arterial pressure (MAP), heart rate (HR/min), body weight (g) and triglyceride levels (mg/dl) were

elevated. These hypertensive rats were then used for studying effect of Carvedilol when administered in the form of conventional oral as well as buccal core in cup tablets, bilayer patches and bilayer tablets formulated with chitosan microspheres.

Treatment with buccoadhesive formulations:

At the end of 2 weeks, reduction in MAP (mm Hg) was found to be 8.72 % by oral conventional tablets while core in cup tablets, bilayer patches and bilayer tablets formulated with microspheres showed 21.47, 23.48 and 25.50 % respectively. Reduction in HR/min was found to be 5.12, 11.46, 14.39 and 17.07 % by oral conventional tablets, core in cup tablets, bilayer patches and bilayer tablets respectively. Body Weight (gm) reduction was found to be 9.61, 18.84, 19.23 and 23.07 % by oral conventional tablets, core in cup tablets, bilayer patches and bilayer tablets respectively. Similarly triglycerides levels were reduced up to 33.80, 53.33, 56.66 and 59.05 % respectively.

This clearly indicated that all the buccoadhesive formulations provided more effective antihypertensive treatment as compared to oral conventional tablets.

Statistical significance ($p<0.001$) data after treatment with oral conventional tablets for 2 weeks showed significance only for triglycerides levels while all other parameters were insignificant. Treatment with core in cup tablets and bilayer buccal patches showed significance for all the parameters except heart rate while bilayer tablets formulated with chitosan microspheres showed all the significantly altered parameters. This data statistically highlighted that bilayer tablets with microspheres provided better antihypertensive treatment as compared to oral conventional tablets and other buccoadhesive formulations.

Again, treatment with buccoadhesive formulations exhibited better results when compared with oral conventional tablets justifying following therapeutic objectives of choosing buccal delivery systems,

i) Buccoadhesive formulations provide direct entry of Carvedilol to systemic circulation, bypass first pass metabolism and in turn increase bioavailability.

ii) Pharmacokinetic studies show that all formulated buccoadhesive dosage forms provided sustained release and are expected to minimize fluctuations in the plasma levels and thus effectively control the therapy.

iii) The impermeable backing layer of the bilayer formulations would have prevented the loss of drug to GIT through saliva and helped in improving bioavailability and in turn increased therapeutic activity of the drug.

13.2 Pravastatin sodium:

Optimized batches for core in cup tablets and bilayer patches were PCT5 and PBP5 respectively. Detailed composition of which is given in experimental part.

Surface pH exhibited by Pravastatin sodium core in cup tablets and bilayer patches were 6.10 ± 0.11 and 6.00 ± 0.19. Surface pH of these delivery systems was near to salivary pH of the buccal mucosa and will not cause any irritation to the mucosa because of alteration of pH due to delivery system.

The **swelling** of the core in cup tablets and bilayer patches in phosphate buffered saline pH 6.8 were 5.45 ± 0.18 % and 5.75 ± 0.18 % respectively. Maximum swelling was shown by bilayer patches. In patches, placebo patches showed less swelling as compared to medicated patches. It shows that presence of Pravastatin sodium in the patches affected the swelling.

Mechanical properties of bilayer patches were found suitable as it demonstrated relatively high TS (6.62 ± 0.59 $kgmm^{-2}$), high E/B (130.23 ± 4.98 % mm^{-2}) and high Strain (1.99 ± 0.35 kg) but a low EM (3.66 ± 0.10 $kgmm^{-2}$) indicating that the patch had both strength as well as elasticity.

In vitro mucoadhesive force shown by core in cup tablets and bilayer patches were 45 ± 2.58 and 44 ± 1.56 N respectively. Obtained value of in vitro mucoadhesive force was sufficient to prevent dislodgement of the system from buccal mucosa for at least 6 hr. Placebo patches showed high mucoadhesive force (53 ± 2.15 N) as compared to medicated patches. It implies that presence of Pravastatin sodium in the patches had negative effect on in vitro mucoadhesive force.

***In vitro* diffusion** of pure drug was 49.36 ± 4.87 %. Pure drug with sodium glycocholate showed 80.23 ± 3.15% diffusion, implying that sodium glycocholate effectively enhanced diffusion of Pravastatin sodium. Diffusion of Pravastatin sodium through core in cup tablets and bilayer patches was 62.98 ± 2.14 and 64.88 ± 2.34 % respectively.

In vitro drug dissolution

Bilayer patches showed slightly higher dissolution (94.12 ± 2.11 %) as compared to buccal core in cup tablets (92.11 ± 3.84 %), may be due higher surface area of patches available for dissolution. Core in cup tablets showed best fit for zero order model. The release data also fitted to Peppas model for core in cup tablets implying non-fickian diffusion pattern i.e. drug release is by diffusion mechanism. Bilayer patches showed best fit for zero order model,

implied diffusion is dominant Pravastatin sodium dissolution mechanism and followed non-fickian diffusion pattern.

Comparative in vitro dissolution profile showed that bilayer patches showed better sustained release of Pravastatin sodium as compared to core in cup tablets.

Pharmacokinetic studies:

The plasma concentration profile for buccal formulations showed sustained release of Pravastatin sodium as compared to oral administration. The Cmax for oral conventional tablets was 67.40 ± 9.23 ng/ml while that of core in cup tablets and bilayer patches were observed to be 72.36 ± 9.68 and 75.63 ± 6.98 ng/ml showing increase in absorption of Pravastatin sodium through buccal route.

Oral conventional tablets showed AUC of 130.33 ± 10.25 ng/ml/hr while core in cup tablets and bilayer patches showed AUC of 270.28 ± 10.98 and 311.10 ± 5.89 ng/ml/hr respectively. This shows increase in bioavailability due to bypassing first pass metabolism and increased permeation of drug by addition of permeation enhancer (sodium glycocholate). Core in cup tablets and bilayer patches showed 2.07 and 2.38 fold increase in bioavailability respectively as compared to oral conventional tablets.

When Pravastatin sodium buccal patches and core in cup tablets were compared for their pharmacokinetic efficacy, it was found that Pravastatin sodium buccal patches showed slightly high AUC (311.10 ± 5.89 ng/ml/hr) than buccal core in cup tablets (270.28 ± 10.98 ng/ml/hr). This may be because greater surface area available for absorption of Pravastatin sodium from the patch (14.00 mm) when compared with core in cup tablets (6.00 mm). Thickness of the core tablet, in core in cup tablet was 3.00 mm while that of medicated layer in bilayer patches was 1.00 mm, therefore diffusional path lengh for Pravastatin sodium in core in cup tablet was higher than that of bilayer patches. This might have resulted in increased absorption and AUC of Pravastatin sodium from bilayer patches.

The results obtained in these studies prove the justification of administering Pravastatin sodium through the buccal route for avoiding pre-systemic metabolism and improving bioavailability.

Histological study of buccal mucosa:

Light microscopy:

Sections treated with core in cup tablets and bilayer patches showed little modification in the epithelial layer may be because of use of sodium glycocholate which was used a permeation enhancer. Permeation enhancing effect of propylene glycol might have caused certain disruption of cells of epithelium in case of bilayer patches. It is clear from the results of the permeation experiments that no major alteration in the barrier function of the tissue was seen.

Scanning Electron Microscopy of buccal mucosa:

Slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue when treated with core in cup tablets. These changes may be due to use of sodium glycocholate which improved permeation of Pravastatin sodium by effectively decreasing resistance to paracellular pathway.

SEM of sample buccal mucosa treated with bilayer patches show slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue. These changes may be due to use of permeation enhancing effect of sodium glycocholate. Mechanism by which sodium glycocholate act is by solubilization of intercellular lipids which may have altered the structure of buccal mucosa.

It can be expected that these slight changes may be reversible and not affected overall structure, surface and function of the buccal mucosa.

***In vivo* acceptability testing:**

It can be concluded that the core in cup tablets and bilayer patches would be comfortable and acceptable by the patients and retained in the buccal cavity long enough for the complete drug release to occur.

Pharmacodynamic studies:

These studies were divided into 2 parts,

i) Development of hyperlipidemia

ii) Treatment with oral and buccoadhesive formulations

Development of hyperlipidemia:

Cholesterol-fed rabbit model was used to induce hyperlipidemia. The model was found suitable and using this model hyperlipidemia was induced in 8 weeks. Parameters like triglycerides (TG), very low density lipoproteins (VLDL) and low density lipoprotein levels (LDL) were elevated. These hyperlipidemic rabbits were then used for studying effect of

Pravastatin sodium when administered in the form of conventional oral as well as buccal core in cup tablets and bilayer patches.

Treatment with buccoadhesive formulations:

At the end of 4 weeks, reduction in TG (mg/dL) was found 15.70 % by oral conventional tablets while core in cup tablets and bilayer patches showed 25.13 and 31.41 % respectively. Reduction in VLDL (mg/dL) was found to be 15.78, 23.68 and 31.57 % by oral conventional tablets, core in cup tablets and bilayer patches respectively. LDL (mg/dL) reduction was found to be 15.00, 50.24 and 55.22 % by oral conventional tablets, core in cup tablets and bilayer patches respectively.

This clearly indicated that both the buccoadhesive formulations provided better antihyperlipidemic treatment as compared to oral conventional tablets.

Statistical significance ($p<0.005$) test after treatment with oral conventional tablets for 4 weeks shows significance only for triglycerides levels while all other parameters were not significantly altered. However, treatment with core in cup tablets and bilayer buccal patches showed all the significantly altered parameters.

This study highlighted that core in cup tablets and bilayer patches provided significantly better antihyperlipidemic treatment as compared to oral conventional tablets.

To summarize, it can be concluded that the buccoadhesive formulations of Pravastatin sodium have been able to justify the following therapeutic,

i) Buccoadhesive formulations provide direct entry of Pravastatin sodium to systemic circulation, bypass first pass metabolism and in turn increase bioavailability.

ii) Both formulated buccoadhesive dosage forms showed sustained release of Pravastatin sodium.

iii) The buccal formulation will overcome the limitation of oral route wherein food intake reduces the absorption of Pravastatin sodium. The absorption of Pravastatin sodium from buccal formulations through buccal route will be independent of food.

iv)Pravastatin sodium is acid labile, hence it undergoes degradation in stomach but when administered as buccoadhesive formulation it will not get exposed to the gastric conditions. Hence, effective concentration of drug available for absorption also increases.

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Cardiovascular diseases (CVDs) are one of the leading life threatening diseases of the world and are also responsible for placing a great burden on our national healthcare services. **Atherosclerotic vascular disease** (ASVD) is responsible for nearly 75% of all deaths from CVDs, and it is the leading cause of death for both men and women in India. Elevated cholesterol, specifically cholesterol contained in low-density-lipoprotein (LDL) particles, is an important risk factor for the development of ASVD. **Cardiac heart failure** (CHF) is also one of the most common causes of death and disability in industrialized nations and is among the syndromes most commonly encountered in clinical practice.

Presently, the cardiovascular diseases such as ASVD and CHF are treated by statins and β-blockers respectively. Loop diuretics e.g. furosemide, angiotensin-converting enzyme inhibitors e.g. captopril, β-blockers e.g. Carvedilol, Organic nitrates e.g. isosorbide mononitrate have been used to treat CHF. Statins have become the first-line agents for primary and secondary prevention of CHD in patients with elevated LDL levels because of their effectiveness, tolerability and safety.

Brief Profile of Carvedilol:

Carvedilol (Carvedilol) is a lipophilic, nonselective β-adrenergic blocking agent. On oral administration, it is extensively absorbed but its **oral bioavailability is 25%** because of substantial hepatic first pass metabolism. Carvedilol is used for the treatment of mild to moderate heart failure of ischemic or cardiomyopathic origin, alone or in conjunction with other agents. It is also indicated for management of essential hypertension. But its conventional dosage form (tablets, 6.250 mgs/three times a day) suffers from certain **disadvantages** such as,

1. Low oral bioavailability.
2. Extensive hepatic first pass metabolism.
3. Less patient compliance because of higher frequency of administration.

Brief profile of Pravastatin sodium:

Pravastatin sodium is most effective and best tolerated agent for treating ASVD. There is extensive first pass metabolism (up to 70%), as a consequence of which its **oral bioavailability is only 17%.** In ASVD, the therapy once initiated, usually lasts life long or for years; therefore precise treatment coupled with exact delivery system is required. But when Pravastatin sodium is administered as conventional dosage form (tablets, 10 mg/once a day) it suffers from certain **disadvantages** such as,

1. Extensive first pass metabolism.
2. Low oral bioavailability.
3. Unstable at acidic pH.
4. Reduction of absorption in presence of food.
5. Incomplete absorption through GIT.
6. Increase in liver load to metabolize the drug because therapy lasts for years.

The **logical way** of overcoming above listed drawbacks of Carvedilol and Pravastatin sodium is by administering Carvedilol and Pravastatin sodium in drug delivery systems, which is capable of bypassing first pass effect, improving bioavailability and providing sustained release to achieve maximum therapeutic benefits.

Hypothesis

Drugs having inherent drawbacks related to poor bioavailability due to first pass metabolism can be effectively delivered by buccal route as a buccoadhesive formulation to improve its bioavailability to considerable extent.

The investigations reported herein were based on the **objective** of designing and characterizing buccoadhesive formulations of Carvedilol and Pravastatin sodium aimed at reducing drawbacks of conventional formulations. It was envisaged that the proposed delivery systems will overcome first pass metabolism and result in increased bioavailability. It was expected to provide sustained release of Carvedilol and Pravastatin sodium which will reduce the dose and frequency of administration of conventional dosage form and will improve patient compliance.

- **Carvedilol:**

Three formulations were prepared for Carvedilol,

1. Core in cup tablets (CCT)
2. Bilayer patches (CBP)
3. Bilayer tablets formulated with chitosan microspheres (CTM)

- **Pravastatin sodium:**

Two formulations were prepared for Pravastatin sodium,

1. Core in cup tablets (PCT)
2. Bilayer patches (PBP)

The above formulations were prepared with *easy to manufacture* methods and the methods were found suitable. All the formulations were evaluated for critical aspects or parameters

required for optimized sustained buccoadhesive drug delivery system. Optimized formulations were selected on the basis of satisfactory in vitro properties.

CARVEDILOL:

Optimized batches for core in cup tablets, bilayer patches and bilayer tablets formulated with microspheres were CCT7, CBP7 and CTM1 respectively. Detailed composition of which is given in experimental part.

Surface pH exhibited by Carvedilol core in cup tablets, bilayer patches and bilayer tablets with microspheres were 6.15 ± 0.20, 6.01 ± 0.25 and 5.71 ± 0.17. As surface pH of these delivery systems was near to salivary pH of the buccal mucosa, it can be reasonably assumed that it will not cause any irritation to the mucosa because of alteration of pH due to delivery system.

The **swelling** of the core in cup tablets, bilayer patches and bilayer tablets with microspheres in phosphate buffered saline were 4.09 ± 0.39 %, 4.19 ± 0.35 % and 2.95 ± 0.29 % respectively. Maximum swelling was shown by bilayer patches and least by bilayer tablets with microspheres probably due to lower affinity of chitosan to phosphate buffer saline at basic pH. In patches, placebo patches showed less swelling as compared to medicated patches, indicating that the presence of Carvedilol in the patches affected the swelling.

Mechanical properties of bilayer patches were found to be suitable as it demonstrated relatively high TS (7.92 ± 0.34 $kgmm^{-2}$), high E/B (137.36 ± 7.49 % mm^{-2}) and high Strain (2.01 ± 0.34 kg) but a low EM (3.94 ± 0.11 $kgmm^{-2}$) indicating that the patch had both strength as well as elasticity.

In vitro mucoadhesive force shown by core in cup tablets, bilayer patches and bilayer tablets with microspheres were 50 ± 2.45, 53 ± 2.13 and 50 ± 1.84 x 10^3 dyne cm^{-2} respectively. Obtained value of in vitro mucoadhesive force was sufficient to prevent dislodgement of the system from buccal mucosa for at least 6 hr (as proved subsequently by in vivo acceptability testing). Placebo patches showed high mucoadhesive force (58 ± 3.01 x 10^3 dyne cm^{-2}) as compared to medicated patches. It implies that presence of Carvedilol in the patches had negative effect on in vitro mucoadhesive force.

***In vitro* diffusion** of pure drug was 93.02 ± 2.93 % in 2 hr as expected from its lipophilic characteristics and high partition coefficient. Diffusion of Carvedilol through core in cup tablets, bilayer patches and bilayer tablets with microspheres was 78.23 ± 3.56, 79.86 ± 2.99

and 71.12 ± 2.59 % respectively. Less Carvedilol diffusion was shown by bilayer tablets may be due high concentration of polymer and less swelling of tablets at salivary pH.

In vitro drug dissolution

It was observed that bilayer tablets with microspheres showed less dissolution (72.08 ± 3.05 %) as compared to other dosage forms. Bilayer patches showed highest dissolution may be due higher surface area of patches available for dissolution.

Core in cup tablets showed best fit for zero order. Peppas model also fitted for core in cup tablets, implying non-fickian pattern i.e. drug release is by diffusion mechanism. Bilayer patches showed best fit for zero order model, followed non-fickian diffusion pattern implying diffusion is the dominant release mechanism. Bilayer tablets with chitosan microspheres followed non-fickian release mechanism with super case II transport i.e. dissolution is the combination of diffusion and chain relaxation and showed best fit for zero order kinetics. Comparative in vitro dissolution profile of all formulations showed that bilayer tablets formulated with chitosan microspheres showed better sustained release of Carvedilol.

Pharmacokinetic studies:

The plasma concentration profile for buccal formulations showed sustained release of Carvedilol as compared to oral administration. The C_{max} for oral conventional tablets was 58.25 ± 4.26 ng/ml while that of core in cup tablets, bilayer patches, bilayer tablets with microspheres were observed to be 64.14 ± 7.36, 69.18 ± 6.69 and 71.26 ± 3.88 ng/ml respectively, showing increase in C_{max} of Carvedilol through buccal route. Oral conventional tablets showed AUC of 152.22 ± 8.43 ng/ml/hr while core in cup tablets, bilayer patches and bilayer tablets with microspheres showed AUC of 297.53 ± 8.20, 319.44 ± 6.65 and 390.75 ± 5.23 ng/ml/hr respectively. Thus, all buccal formulations were able to provide increase in bioavailability, probably due to bypassing first pass metabolism. Core in cup tablets, bilayer patches and bilayer tablets with microspheres showed 1.95, 2.09 and 2.52 fold increase in bioavailability respectively as compared to oral conventional tablets.

When Carvedilol buccal patches and core in cup tablets were compared for their pharmacokinetic efficacy, it was found that Carvedilol buccal patches (319.44 ± 6.65 ng/ml/hr) showed slightly higher AUC than buccal core in cup tablets (297.53 ± 8.20 ng/ml/hr). This may be because greater surface area and less diffusional path length

available for absorption of Carvedilol from the patch (14.00 mm) when compared with core in cup tablets (6.00 mm). Another reason behind higher bioavailability of bilayer patches could be permeation enhancing activity of propylene glycol used as a plasticizer in the formulation (as observed from in vitro diffusion studies).

Absorption of Carvedilol from the bilayer buccal tablets formulated with microspheres appeared slightly slow as compared to other buccal formulations. The lag time for the release of Carvedilol may be due to its slow rate of swelling of the microspheres present in the tablet matrix. Prolonged plasma levels (10.02 ± 0.95 ng/ml at 10^{th} hr) were exhibited by bilayer buccal tablets formulated with chitosan microspheres while core in cup tablets and bilayer patches showed sustained plasma levels up to 8 hr.

Amongst all formulations, bilayer buccal tablets formulated with chitosan microspheres were effective in sustaining plasma Carvedilol levels for maximum time and also showed highest increase in bioavailability.

The results obtained in these studies prove the justification of administering Carvedilol through the buccal route for avoiding pre-systemic metabolism and improving bioavailability.

Histological study of buccal mucosa:

Light microscopy:

Control buccal mucosa showed all the three distinctive layers of the oral mucosa, the epithelium, basement membrane, and connective tissues.

Buccal mucosa treated with core in cup tablets showed negligible changes in epithelium and basal membrane. The principal components of the permeation barrier appeared same as control at the end of the study. Sections of sample mucosa treated with bilayer patches showed little modification in the epithelial layer. Permeation enhancing effect of propylene glycol might have caused certain disruption of cells of epithelium. It is clear from the results of the permeation experiments that no major alteration in the barrier function of the tissue was seen.

Sections of sample mucosa treated with bilayer tablets formulated with chitosan microspheres showed slight modification to the epithelial layer because of chitosan which is characterized by absorption enhancing effects, as it improves the paracellular transport by

opening the tight junctions in the epithelial layer. It can also be assumed that the slight change in epithelial layer was may be due to retention of drug on mucosa.

It was clear from the permeation experiments that no major alterations were found in the mucosa and it can be concluded that the changes may be reversible.

Scanning Electron Microscopy of buccal mucosa:

SEM of the control buccal mucosa showed the presence of the superficial cells of the epithelium and showed that stratified squamous cells have intact cell junctions with microridges.

Sample buccal mucosa treated with core in cup tablets showed that the squamous cells are normal and to some extent similar to those of the control. But, slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue.

SEM of sample buccal mucosa treated with bilayer patches showed shrinkage of superficial cells. Use of dichloromethane in the buccal patches might have contributed to the shrinkage of cells.

Treatment of the buccal mucosa with the bilayer buccal tablets formulated with chitosan microspheres showed that the squamous cells are normal and similar to those of the control. Chitosan is characterized by permeation enhancing effect which opens paracellular junctions and results in shrinkage of superficial layers. From available literature it can be expected that these slight changes may be reversible and not affected overall structure, surface and function of the buccal mucosa.

***In vivo* acceptability testing:**

It can be concluded that the core in cup tablets, bilayer patches and bilayer tablet formulated with microspheres would be comfortable and acceptable by the patients and retained in the buccal cavity long enough for the complete drug release to occur. Comparative results showed that bilayer patches was most acceptable by the volunteers as compared to other formulations.

Pharmacodynamic studies:

These studies were divided into 2 parts,

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b. Treatment with oral and buccoadhesive formulations

Development of hypertension:

Fructose model was used to induce hypertension in rats. The model was found suitable and using this model hypertension was induced in 6 weeks. Parameters like mean arterial pressure (MAP), heart rate (HR/min), body weight (g) and triglyceride levels (mg/dl) were elevated. These hypertensive rats were then used for studying effect of Carvedilol when administered in the form of conventional oral as well as buccal core in cup tablets, bilayer patches and bilayer tablets formulated with chitosan microspheres.

Treatment with buccoadhesive formulations:

At the end of 2 weeks, reduction in MAP (mm Hg) was found to be 8.72 % by oral conventional tablets while core in cup tablets, bilayer patches and bilayer tablets formulated with microspheres showed 21.47, 23.48 and 25.50 % respectively. Reduction in HR/min was found to be 5.12, 11.46, 14.39 and 17.07 % by oral conventional tablets, core in cup tablets, bilayer patches and bilayer tablets respectively. Body Weight (gm) reduction was found to be 9.61, 18.84, 19.23 and 23.07 % by oral conventional tablets, core in cup tablets, bilayer patches and bilayer tablets respectively. Similarly triglycerides levels were reduced up to 33.80, 53.33, 56.66 and 59.05 % respectively.

This clearly indicated that all the buccoadhesive formulations provided more effective antihypertensive treatment as compared to oral conventional tablets.

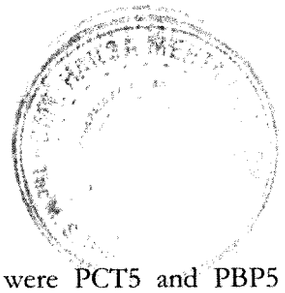
Statistical significance ($p<0.001$) data after treatment with oral conventional tablets for 2 weeks showed significance only for triglycerides levels while all other parameters were insignificant. Treatment with core in cup tablets and bilayer buccal patches showed significance for all the parameters except heart rate while bilayer tablets formulated with chitosan microspheres showed all the parameters were significantly altered. This data statistically highlighted that bilayer tablets with microspheres provided better antihypertensive treatment as compared to oral conventional tablets and other buccoadhesive formulations.

Again, treatment with buccoadhesive formulations exhibited better results when compared with oral conventional tablets justifying following therapeutic objectives of choosing buccal delivery systems,

i) Buccoadhesive formulations provide direct entry of Carvedilol to systemic circulation, bypass first pass metabolism and in turn increase bioavailability.

ii) Pharmacokinetic studies show that all formulated buccoadhesive dosage forms provided sustained release and are expected to minimize fluctuations in the plasma levels and thus effectively control the therapy.

iii) The impermeable backing layer of the bilayer formulations would have prevented the loss of drug to GIT through saliva and helped in improving bioavailability and in turn increased therapeutic activity of the drug.



PRAVASTATIN SODIUM:

Optimized batches for core in cup tablets and bilayer patches were PCT5 and PBP5 respectively. Detailed composition of which is given in experimental part.

Surface pH exhibited by Pravastatin sodium core in cup tablets and bilayer patches were 6.10 ± 0.11 and 6.00 ± 0.19. Surface pH of these delivery systems was near to salivary pH of the buccal mucosa and will not cause any irritation to the mucosa because of alteration of pH due to delivery system.

The **swelling** of the core in cup tablets and bilayer patches in phosphate buffered saline pH 6.8 were 5.45 ± 0.18 % and 5.75 ± 0.18 % respectively. Maximum swelling was shown by bilayer patches. In patches, placebo patches showed less swelling as compared to medicated patches. It shows that presence of Pravastatin sodium in the patches affected the swelling.

Mechanical properties of bilayer patches were found suitable as it demonstrated relatively high TS (6.62 ± 0.59 $kgmm^{-2}$), high E/B (130.23 ± 4.98 % mm^{-2}) and high Strain (1.99 ± 0.35 kg) but a low EM (3.66 ± 0.10 $kgmm^{-2}$) indicating that the patch had both strength as well as elasticity.

In vitro mucoadhesive force shown by core in cup tablets and bilayer patches were 45 ± 2.58 and 44 ± 1.56 x 10^3 dyne cm^{-2} respectively. Obtained value of in vitro mucoadhesive force was sufficient to prevent dislodgement of the system from buccal mucosa for at least 6 hr. Placebo patches showed high mucoadhesive force (53 ± 2.15 x 10^3 dyne cm^{-2}) as compared to medicated patches. It implies that presence of Pravastatin sodium in the patches had negative effect on in vitro mucoadhesive force.

***In vitro* diffusion** of pure drug was 49.36 ± 4.87 %. Pure drug with sodium glycocholate showed 80.23 ± 3.15% diffusion, implying that sodium glycocholate effectively enhanced diffusion of Pravastatin sodium. Diffusion of Pravastatin sodium through core in cup tablets and bilayer patches was 62.98 ± 2.14 and 64.88 ± 2.34 % respectively.

In vitro drug dissolution

Bilayer patches showed slightly higher dissolution (94.12 ± 2.11 %) as compared to buccal core in cup tablets (92.11 ± 3.84 %), may be due higher surface area of patches available for dissolution. Core in cup tablets showed best fit for zero order model. The release data also fitted to Peppas model for core in cup tablets implying non-fickian diffusion pattern i.e. drug release is by diffusion mechanism. Bilayer patches showed best fit for zero order model,

implied diffusion is dominant Pravastatin sodium dissolution mechanism and followed non-fickian diffusion pattern.

Comparative in vitro dissolution profile showed that bilayer patches showed better sustained release of Pravastatin sodium as compared to core in cup tablets.

Pharmacokinetic studies:

The plasma concentration profile for buccal formulations showed sustained release of Pravastatin sodium as compared to oral administration. The Cmax for oral conventional tablets was 67.40 ± 9.23 ng/ml while that of core in cup tablets and bilayer patches were observed to be 72.36 ± 9.68 and 75.63 ± 6.98 ng/ml showing increase in absorption of Pravastatin sodium through buccal route.

Oral conventional tablets showed AUC of 130.33 ± 10.25 ng/ml/hr while core in cup tablets and bilayer patches showed AUC of 270.28 ± 10.98 and 311.10 ± 5.89 ng/ml/hr respectively. This shows increase in bioavailability due to bypassing first pass metabolism and increased permeation of drug by addition of permeation enhancer (sodium glycocholate). Core in cup tablets and bilayer patches showed 2.07 and 2.38 fold increase in bioavailability respectively as compared to oral conventional tablets.

When Pravastatin sodium buccal patches and core in cup tablets were compared for their pharmacokinetic efficacy, it was found that Pravastatin sodium buccal patches showed slightly high AUC (311.10 ± 5.89 ng/ml/hr) than buccal core in cup tablets (270.28 ± 10.98 ng/ml/hr). This may be because greater surface area available for absorption of Pravastatin sodium from the patch (14.00 mm) when compared with core in cup tablets (6.00 mm). Thickness of the core tablet, in core in cup tablet was 3.00 mm while that of medicated layer in bilayer patches was 1.00 mm, therefore diffusional path lengh for Pravastatin sodium in core in cup tablet was higher than that of bilayer patches. This might have resulted in increased absorption and AUC of Pravastatin sodium from bilayer patches.

The results obtained in these studies prove the justification of administering Pravastatin sodium through the buccal route for avoiding pre-systemic metabolism and improving bioavailability.

Histological study of buccal mucosa:

Light microscopy:

Sections treated with core in cup tablets and bilayer patches showed little modification in the epithelial layer may be because of use of sodium glycocholate which was used a permeation enhancer. Permeation enhancing effect of propylene glycol might have caused certain disruption of cells of epithelium in case of bilayer patches. It is clear from the results of the permeation experiments that no major alteration in the barrier function of the tissue was seen.

Scanning Electron Microscopy of buccal mucosa:

Slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue when treated with core in cup tablets. These changes may be due to use of sodium glycocholate which improved permeation of Pravastatin sodium by effectively decreasing resistance to paracellular pathway.

SEM of sample buccal mucosa treated with bilayer patches show slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue. These changes may be due to use of permeation enhancing effect of sodium glycocholate. Mechanism by which sodium glycocholate act is by solubilization of intercellular lipids which may have altered the structure of buccal mucosa.

It can be expected that these slight changes may be reversible and not affected overall structure, surface and function of the buccal mucosa.

***In vivo* acceptability testing:**

It can be concluded that the core in cup tablets and bilayer patches would be comfortable and acceptable by the patients and retained in the buccal cavity long enough for the complete drug release to occur.

Pharmacodynamic studies:

These studies were divided into 2 parts,

i) Development of hyperlipidemia

ii) Treatment with oral and buccoadhesive formulations

Development of hyperlipidemia:

Cholesterol-fed rabbit model was used to induce hyperlipidemia. The model was found suitable and using this model hyperlipidemia was induced in 8 weeks. Parameters like triglycerides (TG), very low density lipoproteins (VLDL) and low density lipoprotein levels (LDL) were elevated. These hyperlipidemic rabbits were then used for studying effect of

Pravastatin sodium when administered in the form of conventional oral as well as buccal core in cup tablets and bilayer patches.

Treatment with buccoadhesive formulations:

At the end of 4 weeks, reduction in TG (mg/dL) was found 15.70 % by oral conventional tablets while core in cup tablets and bilayer patches showed 25.13 and 31.41 % respectively. Reduction in VLDL (mg/dL) was found to be 15.78, 23.68 and 31.57 % by oral conventional tablets, core in cup tablets and bilayer patches respectively. LDL (mg/dL) reduction was found to be 15.00, 50.24 and 55.22 % by oral conventional tablets, core in cup tablets and bilayer patches respectively.

This clearly indicated that both the buccoadhesive formulations provided better antihyperlipidemic treatment as compared to oral conventional tablets.

Statistical significance ($p<0.005$) test after treatment with oral conventional tablets for 4 weeks shows significance only for triglycerides levels while all other parameters were not significantly altered. However, treatment with core in cup tablets and bilayer buccal patches showed all significantly altered parameters.

This study highlighted that core in cup tablets and bilayer patches provided significantly better antihyperlipidemic treatment as compared to oral conventional tablets.

To summarize, it can be concluded that the buccoadhesive formulations of Pravastatin sodium have been able to justify the following therapeutic objective,

i) Buccoadhesive formulations provide direct entry of Pravastatin sodium to systemic circulation, bypass first pass metabolism and in turn increase bioavailability.

ii) Both formulated buccoadhesive dosage forms showed sustained release of Pravastatin sodium.

iii) The buccal formulation will overcome the limitation of oral route wherein food intake reduces the absorption of Pravastatin sodium. The absorption of Pravastatin sodium from buccal formulations through buccal route will be independent of food.

iv) Pravastatin sodium is acid labile, hence it undergoes degradation in stomach but when administered as buccoadhesive formulation it will not get exposed to the gastric conditions. Hence, effective concentration of drug available for absorption also increases.