

Development and Characterization of Mucoadhesive Buccal Gel Containing Lipid Nanoparticles of Triamcinolone Acetonide

Dandagi Panchaxari Mallappa¹, Fernandes Reesha Chelsea¹, Raikar Prasiddhi Ratnakar^{1,*}, Gadad Anand Panchakshari¹, Mastiholimath Vinayak Shivamurthi², Bhat Samritha Uppinangady²

¹Department of Pharmaceutics, KLE College of Pharmacy, A constituent unit of KAHER, JNMC Campus, Belagavi, Karnataka, INDIA.

²Department of Quality Assurance, KLE College of Pharmacy, A constituent unit of KAHER, JNMC Campus, Belagavi, Karnataka, INDIA.

ABSTRACT

Background: Triamcinolone Acetonide is a synthetic glucocorticosteroid which has been used for its immunosuppressive and anti-inflammatory activity belonging to BCS class IV, yet it is not recommended to be used at higher doses due to its possible adverse effects. Lipid nanoparticle systems are good carriers for poor water soluble drugs, improve permeability and show sustained drug release. **Materials and Methods:** Triamcinolone Acetonide was formulated as Nanostructured Lipid Carriers. High shear homogenization followed by ultra-sonication method was used with Precirol ATO 5 and Isopropyl myristate as lipids. Optimisation was based on their particle size and entrapment efficiency using the design expert software. **Results and Discussion:** Formulations F-5 and F-7 were selected as optimised formulations and had drug release patterns that fit Korsmeyer-peppas model kinetics. SEM and TEM studies showed that the Nano structured lipid carriers had size within the nanometre range and morphology resembling type II (amorphous type). The mucoadhesive gel of optimised formulations F-5 and F-7 were prepared as a 1% w/w hydrogel with carbopol 934. These gels showed pseudoplastic fluid nature and good physical properties and also showed a more sustained drug release in comparison to gel incorporated drug suspension. **Conclusion:** Based on the results obtained it was concluded that Triamcinolone Acetonide can be successfully formulated as a gel incorporated with Nanostructured lipid carriers for treatment of buccal diseases.

Key words: Triamcinolone Acetonide, Nanostructured lipid carriers, Mucoadhesive buccal gel, Factorial Design, Response Surface plot.

INTRODUCTION

Oral mucosal diseases are one of the most common disorders affecting humans, however their treatment is limited to only a few topical formulations and most of these are borrowed from dermatology. Since the oral cavity's structure, environment and functional features differ from the skin; these formulations may show limitations to mucosal drug delivery. Further, the presence of saliva in the mouth along with the mechanical motions during chewing, swallowing and phonation lead to majority of the drug being washed away from the site.¹ Nanostructured lipid carriers (NLCs) have

been considered as an excellent alternative drug carrier to conventional dosage forms.² In case of oral mucosal diseases, when a liquid dispersion of nanoparticles is directly applied onto the buccal cavity, it will be rapidly removed by salivary action. Incorporation of NLCs in hydrogels made with mucoadhesive polymers is a promising means to prevent washing away from the target site. Due to its adhesive property it can be in constant contact with the buccal mucosa and will be retained for a longer time.³ Triamcinolone Acetonide has been clinically proven for its immunosuppressive

Submission Date: 02-04-2020;

Revision Date: 17-06-2020;

Accepted Date: 23-08-2020

DOI: 10.5530/ijper.54.3s.149

Correspondence:

Ms. Prasiddhi R Raikar

Department of Pharmaceutics,
KLE College of Pharmacy, A
constituent unit of KAHER,
JNMC Campus, Belagavi-
590010, Karnataka, INDIA.
Phone: +91-9923943569
E-mail: prasiddhiraikar07@
gmail.com



www.ijper.org

and anti-inflammatory activity.⁴ The drug is currently employed as a 0.1 percent formulation for treatment of inflammatory disorders of the buccal cavity including recurrent aphthous stomatitis and oral lichen planes. It belongs to BCS class IV.⁵ Lipid nanoparticles have been found to be good carriers for poorly aqueous soluble drugs including antifungal agents like miconazole nitrate, amphotericin B and clotrimazole hence are assumed to be good carriers for triamcinolone acetonide.⁶

MATERIALS AND METHODS

Materials

Triamcinolone Acetonide IP and Precirol ATO 5 were received as gift samples from Cadila Pharmaceuticals Limited, Ahmedabad and Gattefossé, France respectively. Isopropyl Myristate, Tween 80 and Carbopol 934 were procured from Himedia Pvt Ltd., Mumbai. Triethanolamine was purchased from Merck Pvt Ltd Mumbai. All the chemicals used were of analytical grade.

Method

Preparation of Nanostructured Lipid Carriers by High Shear Homogenization Followed by Ultra-Sonication Method

In this method, the solid and liquid lipids were accurately weighed and heated together at 10°C above their melting point to form a molten mass. TA was added to the melted lipids and mixing was carried out under magnetic stirring. Tween 80 was added to distilled water in a separate beaker and heated to the same temperature as lipid phase. Molten lipid phase was then added drop wise into the aqueous phase under magnetic stirring. The dispersion formed was further mixed using high shear homogenizer at 13,000 rpm for 10 min followed by ultra-sonication at 45% amplitude for 15 min. The prepared formulations containing NLCs were then set aside to stabilise for 24 hr.

Optimisation by factorial design

The above given method was successfully used to formulate NLCs at different ratios of solid lipid: liquid lipid including 70:30, 50:50 and 30:70. However, in order to establish an optimised formulation, multiple ratios would need to be attempted and evaluated and hence factorial design was used. A 3² factorial design was adopted in this study. Design Expert v11 software (Stat-ease Inc., USA) was used for this purpose. The factors and the levels selected for optimization are showed in Table 1. Formulation design for F-1 to F-9 shown

in Table 1. Entrapment efficiency and particle size of NLCs were considered as responses for optimisation.

Preparation of gel

Gels incorporated with pure drug TA and with optimised NLC formulations were prepared using carbopol 934. NLC aqueous dispersion corresponding to 10 mg drug as per the drug content was taken and accurately weighed amount of carbopol 934 was added to it to obtain a 1% w/w carbopol hydrogel. The gel base was homogenised under magnetic stirring. Thus a 0.05% w/w gel of triamcinolone acetonide was achieved. Similarly, a suspension of pure drug was homogenised with weighed amount of carbopol 934 to obtain a gel with drug concentration of 0.05% w/w. The pH of the gel was adjusted adding Triethanolamine (TEA) drop wise and mixing was continued to obtain clear homogenized gel.

Evaluation of Nanoparticles

Analysis of Particle Size and PDI

Nanotracs Particle Size Analyser was used for evaluation of the NLCs. The formulations were diluted with millipore water and analysed. The size range of the analyser was set at 0.08 nm to 6.8 µm. Results generated were mean values of 6 runs. Polydispersity index (PDI) of the formulations was measured by the same instrument.

Entrapment Efficiency⁷

Entrapment efficiency was determined by using a modified method. 2 ml of each formulation was centrifuged for 60 min at 12,500 RPM under temperature at 4°C. The pellet formed after centrifugation was dissolved with ethanol. The resultant solution was then diluted using buffer pH 7.4. UV absorbance (UV-1900, Shimadzu, Japan) of each sample was determined at 242 nm and entrapment efficiency was determined by suitable calculation.

Drug content

The method for drug content determination involved using ethanol for release the drug into solution. This solution was then diluted with buffer pH 7.4 and centrifuged at 4°C for 45 min with 13,000 rpm speed to separate the free drug from lipid. The centrifuged solution was diluted with buffer pH 7.4. UV absorbance was taken at 242 nm.

Morphology studies

SEM of the formulated NLCs was carried out using JSM-6360LV Scanning Electron Microscope (JEOL, Japan) at 20 Kv and magnification 10X-10,000X. TEM

analysis of the NLCs was carried out on Transmission electron microscope (TEM, JEM-2100, JEOL, Japan) at 200 Kv magnifications 2000X-150000X. As shown in Figure 1.

In vitro Diffusion Study⁸

The dialysis bag method was adopted to study *in vitro* drug release. Dialysis membrane (Himedia Laboratories Pvt Ltd,) with pore size 2.4 nm and 12,000-14,000 molecular weight cut-off was used for this purpose. The membrane was soaked in PBS pH 7.4 for 12 h before use. 2 ml of formulation was transferred into the dialysis membrane ribbon and it was bound tightly at both ends. The dialysis bag was allowed to rotate freely in a beaker containing 200 ml phosphate buffer pH 7.4 which acts as the receptor compartment. Samples were taken at appropriate intervals while maintaining sink conditions. UV absorbance was measured at 242 nm. Drug release data obtained experimentally was fit into different mathematical models. These models include Zero order, First order, Higuchi matrix, Hixon and Korsmeyer-Peppas model.

Stability Studies

Stability studies of optimised formulations were carried out as per ICH guidelines for 30 days.

Evaluation of Gel

Rheological Properties

Brookfield viscometer CAP 2000+ was used to determine the viscosity of prepared NLC gel formulation. Samples of gel were applied on the plates of the viscometer and allowed to settle for 5 min. The spindle rotation speed was increased from 5 to 75 rpm at 37°C. The values of viscosity, shear stress and shear rate obtained were examined. The results were taken as average of three.

Mucoadhesive strength of gel⁹

Two pieces of rat skin of dimensions 2.5 x 3.5 cm were cut and soaked in phosphate buffer pH 7.4 for 2 hr. The rat skin pieces were then attached to glass slides using double sided tape, the other adhesive side of which was used to attach the slides to the pan and wooden board of the weighing balance respectively. 200 mg of NLC incorporated gel was applied between the two rat skin pieces and allowed to stabilise for 5 min. Weights were then added to the other pan till the slides separated indicating that mucoadhesive strength of the gel was overcome. Formula for calculation of mucoadhesive strength is as given below

$$\text{Mucoadhesive strength (mg/cm}^2\text{)} = \frac{\text{Weight required for detachment (mg)}}{\text{Area of applied gel (cm}^2\text{)}}$$

In vitro drug release from gel

Gels incorporated with optimised NLC formulations were compared with a gel incorporated with pure drug for *in vitro* drug release. The release studies were performed by Franz diffusion cell method. Dialysis membrane with pore size 2.4 nm and 12,000-14,000 mol. wt. cut-off was used for this purpose. 12 ml of PBS was taken in the receptor compartment and 1mg drug equivalent gel was placed in the donor compartment. Temperature was set at 37°C with continuous stirring of the receptor compartment. Samples were collected at appropriate time intervals while maintaining sink conditions. Aliquots were diluted with PBS pH 7.4 and analysed at 242 nm by Spectrophotometry.

RESULTS AND DISCUSSION

Lipid Screening

Among the solid lipids TA showed most solubility in Cetyl alcohol and the order of solubility was as follows: Cetyl alcohol > Precirol ATO 5 > Stearic acid.

In a similar manner for liquid lipids, solubility of TA was highest in Isopropyl myristate and the order of solubility was: Isopropyl myristate > Castor oil > Labrafil M2125.

The congealed mass of Cetyl alcohol with Isopropyl myristate and precirol ATO5 with isopropyl myristate when smeared on filter paper showed the presence of liquid droplets (rejected) and the latter showed no droplets, hence the lipids in the binary mixture was considered compatible.

Compatibility studies

Compatibility study was carried out using DSC and FTIR and it was concluded that all the ingredients used were compatible.

Table 1: Formulation design.				
Formulation no.	Drug (mg)	Solid lipid : liquid lipid ratio	Precirol ATO 5 (mg)	Isopropyl Myristate (mg)
F1	30	7:3	210	90
F2	30	3:2	180	120
F3	30	1:1	150	150
F4	30	7:2	233	67
F5	30	7:1	263	37
F6	30	3:3	150	150
F7	30	3:1	225	75
F8	30	1:3	75	225
F9	30	1:2	100	200

Particle size and entrapment efficiency

The particle size, PDI and entrapment efficiency was determined as mentioned in the procedure. The results of the optimised formulations given in Table 2.

Statistical optimization

The level of significance of the tested independent factors on the responses was tested by ANOVA. The independent variables (A, B) and responses (R1, R2) were correlated using polynomial equations. As shown in Table 3.

Response R1 – Particle size

From the polynomial equation derived by the software, it can be concluded that Factor B has a synergistic effect on the particle size whereas Factor A and Combination of factors A and B (AB) have an antagonistic effect on the particle size. The Response Surface Plot for Response R1 shows that particle size increases as the level of factor B increases. On the other hand Factor A has little effect on response R1, with the Particle size remaining near constant at all levels of factor A.

Response R2 – Entrapment Efficiency

The polynomial equation for Response R2 (Table) indicated that factor B had a more antagonistic effect on the response whereas factor A had a synergistic effect. In the response surface plot, the response R2 increased with increase in level of Factor B. Increase in level of factor A also showed an increase in response R2.

Establishing the Design space

From the two responses selected, particle size was required to be minimum and entrapment efficiency had to be maximum. These parameters were incorporated the design space by setting particle size to be in the range of 200-350 nm and setting entrapment efficiency range between 65-100%. The generated design space (overlay plot) is given in Figure. Formulations F-5 and

F-7 lie within the acceptable operating ranges of the design space; hence they were selected as optimized formulations.

Validation of the optimized formulations

A randomized 3^2 response surface design was used for optimization of the formulation. In order to validate the optimization, the predicted responses and experimental responses of the optimized formulation were compared as shown in Table 2.

Drug content

Drug content of formulations F1 to F9 were found to be in the range 78.29 ± 2.09 to 97.16 ± 0.46 . Formulation F3 with 1:1 ratio of solid lipid:liquid lipid showed the highest drug content.

In-vitro Drug

Diffusion Study

The drug release followed a biphasic pattern, wherein an initial burst release was followed by sustained release. The release data of all 9 NLC formulations were fitted into various kinetic models. The values of

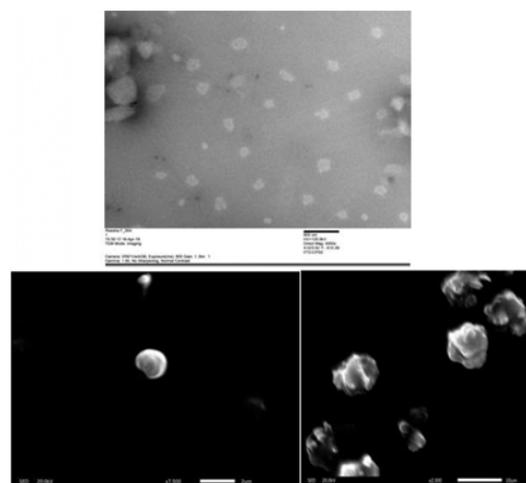


Figure 1: SEM and TEM.

Table 2: Experimental and predicted responses for optimized nlc formulations f-5 and f-7.

Responses	F-5		F-7	
	Predicted	Experimental	Predicted	Experimental
Particle Size (nm)	323.80	309.00	329.05	346
Entrapment Efficiency (%)	68.51	69.18	65.24	66.94

Table 3: Summary of statistical parameters – analysis of variance (anova).

Response	Sum of Squares	df	Mean Square	F-value	p-value	R ²	Adjusted R ²	Model significance
R1	1.729E+05	3	57621.68	14.26	0.0133	0.9145	0.8504	significant
R2	1055.25	2	527.63	6.75	0.0379	0.7298	0.6217	Significant

release constant and regression coefficient were used to determine the best fit model for each formulation.

Morphology studies

SEM of the optimised NLC formulation F-5 showed that the NLCs were near spherical with slight imperfections in their surface. This may be attributed to the use of special type liquid lipid- Isopropyl Myristate in the formulation which has been reported to give rise to type II (amorphous) NLCs.¹⁰ TEM analysis of the NLCs provided the shape and size of the NLCs. It may be observed that the NLCs were within the nanometre range.

Stability Studies

The samples stored for stability studies were tested for changes in particle size and entrapment efficiency after 15 days and 30 days. It was observed that the sample stored at cold temperature did not show much deviation from its original particle size and entrapment efficiency. On the hand the particle size of the sample stored at room temperature varied significantly on storage. Therefore, the formulation stored at $4\pm 20\text{C}/65\pm 5\%\text{RH}$ maintained its stability better than that stored at $25\pm 20\text{C}/65\pm 5\%\text{RH}$.

Evaluation of Gel

Rheological Properties

From the rheograms prepared it may be observed that there was an increase in shear stress as the shear rate was increased. However, with the increase in shear rate, viscosity decreased.

Mucoadhesive strength of gel

The mucoadhesive strength of the gel was found to be $6.57\pm 0.38\text{ g/cm}^2$. The mucoadhesive strength of a formulation depends on the concentration of mucoadhesive polymer added. Hence, the mucoadhesive strength of the gel can also be adjusted by changing the concentration of polymer used.

In vitro drug release from gel

Gels incorporated with optimised formulation were compared for *in vitro* drug release with a gel incorporated with pure drug. Formulation F-5 and F-7 loaded gels showed drug release of 8% and 7.6% respectively after 8 hr. The free drug incorporated gel showed a much higher drug release of 14.83% after 8 hr. The results match the previous research findings and prove the claims that NLCs provide a sustained drug release effect. The best fit models for NLC-loaded gels as well as free drug loaded gel was Higuchi kinetic model.

CONCLUSION

Conclusion: The mean particle size of NLCs appeared to increase as liquid lipid concentration was increased. It was also observed that increasing liquid lipid concentration caused a decrease in entrapment efficiency which may be attributed to the higher solubility of drug in solid lipid as compared to liquid lipid. Optimized formulations F-5 and F-7 had drug release patterns that followed Korsmeyer-peppas model kinetics. SEM and TEM studies of optimized formulation F-5 showed that the NLCs had size within the nanometer range and morphology resembling type II (amorphous type) NLCs. The gel of optimized formulations F-5 and F-7 showed pseudo plastic fluid nature, Spreadability, extrudability, pH and mucoadhesive strength characteristics which met the requirements for use in the buccal cavity. In vitro drug release from the NLC formulation incorporated gels indicated that the release profile showed a more sustained drug release as compared with gel incorporated with drug suspension.

ACKNOWLEDGEMENT

The authors are grateful to Cadila Pharmaceuticals Limited, Ahmedabad for providing the gift sample of Triamcinolone Acetonide IP and also to Gattefosse, France for the gift sample of Precirol ATO 5. The authors are also thankful to the Principal, KLEU College of Pharmacy, Belagavi and Dr. PK Basic Science Research centre, Belagavi for providing laboratory and necessary facilities to carry out research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

BSC: Biopharmaceutics Classification System; **SEM:** Scanning Electron Microscopy; **TEM:** Transmission Electron Microscopy; **NLCs:** Nanostructured Lipid Carriers; **TA:** Triamcinolone Acetonide; **TEA:** Triethanolamine; **PDI:** Polydispersity Index; **ICH:** International Conference on Harmonization.

REFERENCES

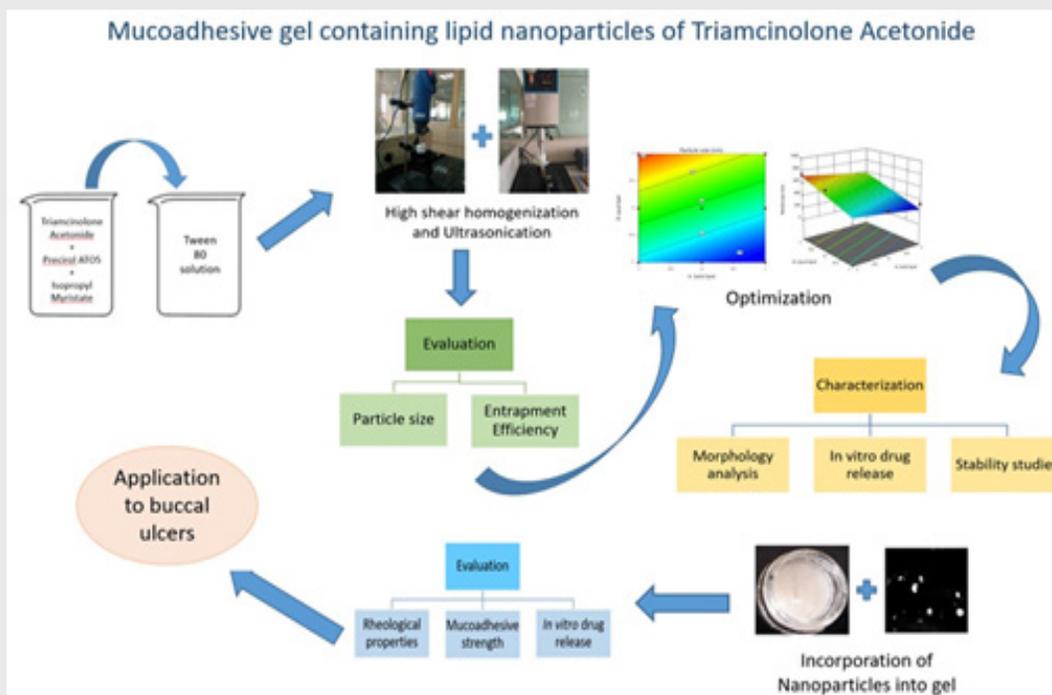
1. Wanda MH, Colin GR, Matthew AW. Chapter 7 - Skin and Oral Mucosa, Fundamentals of Toxicologic Pathology (Second Edition). Academic Press. 2010;135-161.
2. Triamcinolone Acetonide dental paste USP. 0.1%, <https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=ea6fac20-4c6b-42bb-8efd-c43458564fe7&type=display>
3. Triamcinolone - DrugBank: <https://www.drugbank.ca/drugs/DB00620>

- Padula C, Telò I, Dilanni A, Pescina S, Nicoli S, Santi P. Microemulsion containing triamcinolone acetonide for buccal administration. *Eur J Pharm Sci.* 2018;115:233-9.
- Florey K. Triamcinolone Acetonide. In: *Analytical Profiles of Drug Substances.* Volume 1. New York: Academic Press. 1972;397-421.
- Aditya N, Macedo A, Doktorovova S, Souto E, Kim S, Chang P, *et al.* Development and evaluation of lipid nanocarriers for quercetin delivery: A comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid nanoemulsions (LNE). *LWT: Food Science and Technology.* 2014;59(1):115-21.
- Kraisit P, Sarisuta N. Development of Triamcinolone Acetonide-Loaded Nanostructured Lipid Carriers (NLCs) for Buccal Drug Delivery Using the Box- Behnken Design. *Molecules.* 2018;23(4):982-9.
- Mendes AI, Silva AC, Catita JAM, Cerqueira F, Gabriel C, Lopes CM. Miconazole loaded nanostructured lipid carriers (NLC) for local delivery to the oral mucosa: Improving antifungal activity. *Colloids Surf B Biointerfaces.* 2013;11(1):755-63.
- Shelke S, Shahi S, Jalalpure S, Dhamecha D, Shengule S. Formulation and evaluation of thermo reversible mucoadhesive in situ gel for intranasal delivery of Naratriptan hydrochloride. *J Drug Deliv Sci Tec.* 2015;29:238-44.
- Tetyczka C, Griesbacher M, Absenger-Novak M, Fröhlich E, Roblegg E. Development of nanostructured lipid carriers for intraoral delivery of Domperidone. *Int J Pharm.* 2017;526(1-2):188-98.

SUMMARY

In the present study a mucoadhesive buccal gel containing NLCs was formulated and factorial design was applied to optimise the NLC preparation. The observations made concluded that mean particle size of NLCs appeared to increase as liquid lipid concentration was increased. It was also observed that increasing liquid lipid concentration caused a decrease in entrapment efficiency which may be attributed to the higher solubility of drug in solid lipid as compared to liquid lipid. The *in vitro* drug release from the NLCs varied between 44.88% (F-5) to 65.17% (F-1) at the end of 12 hr. Using the design space tool, two optimised formulations were selected. Optimised formulations F-5 and F-7 had drug release patterns that followed Korsmeyer-peppa model kinetics. SEM and TEM studies of optimised formulation F-5 showed that the NLCs had size within the nanometer range and morphology resembling type II (amorphous type) NLCs. Type II NLCs are reported to reduce drug expulsion during storage. The gel of optimised formulations F-5 and F-7 were prepared as a 1% w/w hydrogel with carbopol 934. These gels showed pseudoplastic fluid nature, Spreadability, Extrudability, pH and mucoadhesive strength characteristics which met the requirements for use in the buccal cavity. *In vitro* drug release from the NLC formulation incorporated gels indicated that the release profile followed Higuchi matrix model. They also showed a more sustained drug release as compared with gel incorporated with drug suspension.

PICTORIAL ABSTRACT



About Authors



Dr. Panchaxari M. Dandagi, is Professor in the department of Pharmaceutics, KLE College of Pharmacy, a Constituent unit of KAHER, Belagavi.



Dr. Vinayak S. Mastiholmath, is Professor in the department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, a Constituent unit of KAHER, Belagavi.



Ms. Reesha Fernandes was a Post graduate research scholar, completed her PG in the department of Pharmaceutics, KLE College of Pharmacy, a Constituent unit of KAHER, Belagavi.



Ms. Samritha Bhat was a Post graduate research scholar, completed her PG in the department of Pharmaceutics, KLE College of Pharmacy, a Constituent unit of KAHER, Belagavi.



Dr. Anand P. Gadad, is Professor in the department of Pharmaceutics, KLE College of Pharmacy, a Constituent unit of KAHER, Belagavi.

Cite this article: Mallappaa DP, Chelseaa FR, Ratnakar RP, Panchaksharia GA, Shivamurthib MV, Uppinangadyb BS. Development and Characterization of Mucoadhesive Buccal Gel Containing Lipid Nanoparticles of Triamcinolone Acetonide. Indian J of Pharmaceutical Education and Research. 2020;54(3s):s505-s511.