Pharmacokinetic Investigation to Study the In Vivo Bioavailability of Thiolated Chitosan Based Repaglinide Buccal Tablets

Raja Navamanisubramanian 1, Raghunandan Nerella 1, Shanmuganathan Seetharaman 2*

1 Balaji Institute of Pharmaceutical Sciences, Luknepally (V), Narsampet (M), Warangal Rural, Telangana, India.
2 Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India.

Abstract
Background: Repaglinide (REP) is an antihyperglycemic drug having low bioavailability due to its extensive first-pass metabolism. The present study aimed to develop and pharmacokinetic investigate the thiolated chitosan (TC) based buccal tablets of REP for improved bioavailability.

Methods: TC was prepared by conjugation of L-cysteine with chitosan. The amount of free thiol groups present in TC was determined by UV-spectrophotometry using Ellman’s reagent. TC based REP buccal tablets were prepared by two layers co-compression method and characterized for in vitro and ex vivo parameters. The in vivo performance of prepared REP buccal tablets was assessed by the pharmacokinetic study in New Zealand white rabbits.

Results: The prepared TC resulted in 87% w/w yield with 52.3±3.2 µM free thiol functional groups per 10 mg of TC. The prepared formulations have good flow nature and compressibility, acceptable thickness (2.02 to 2.1 mm), weight (60.11 to 61.06 mg), surface pH (6.59 to 6.81) and drug content (98.92 to 101.08 % w/w). The presence of TC significantly improved the mucoadhesion strength, sustained the drug content (98.92 to 101.08 % w/w). The prepared REP buccal tablets (V5) have area under the curve (712.22±15.91 ng/mL/h) and mean residence time (4.66±0.25 h) was 1.89 and 1.83 folds higher than oral bolus respectively.

Conclusion: TC based REP buccal tablets are capable of controlled transbuccal release of REP for a prolonged time and have better bioavailability than oral bolus.

Introduction
Repaglinide (REP) is a novel short-acting meglitinide class oral antihyperglycemic drug, used to regulate the prandial hyperglycemia in the treatment of type-II diabetes. REP is a highly preferred therapeutic alternative to sulfonylureas in treating patients with a new chemical class of insulin secretagogues. REP is heptically metabolized and is excreted principally (>90%) in the bile, thus it is a safe drug of choice to treat patients with chronic diabetes kidney disease and contraindicated to metformin. The pharmacokinetic report declares that REP has a short biological half-life (1 h) with low bioavailability (56%) due to profound and inconsistent hepatic first-pass effect.

Accordingly the literature reviewed, many attempts were made for sustained release of REP through matrix tablets, osmotic pump and dual crosslinked pectin–alginate microspheres. These formulations were reported to improve the relative bioavailability of REP around 111% to 113% compared to oral bolus. Further, bilayer matrix tablets for prolonged release of metformin hydrochloride and REP gastro retentive drug delivery of REP through mucoadhesive tablets, floating microspheres, mucoadhesive microsphere and mucoadhesive nanoparticles were reported. Yet, those formulations could not overcome the first-pass effect of REP due to GIT being their common site of administration.

Thiolated chitosan (TC), a novel multi-functional polymer has increased mucoadhesion and permeation enhancing properties that create interest to use TC in the transbuccal drug delivery system. So far reported methods have demonstrated the synthesis of TC by immobilizing a sulfydryl bearing agent like cysteine, N-acetylcysteine, homocysteine, thioglycolic acid, thioethylamidine, 4-thiobutylamidine, glutathione, thiolactic acid, and 6-mercaptocinotinic acid, covalently with the primary amino group at the second position of glucosamine subunits of chitosan.

In this study, TC was prepared by conjugating the L-cysteine with chitosan. The prepared TC was characterized, applied in the formulation of REP buccal tablets along with sodium carboxymethyl cellulose (NaCMC) and pectin.
as mucoadhesive polymers. The optimum formulas of REP buccal tablets were developed by pre-validated Box-Benken design (BBD) and characterized for expected quality parameters. The in vivo performance of developed REP buccal tablets was assessed through pharmacokinetic study in New Zealand white rabbits comparing with oral bolus.

Materials and Methods
Repaglinide (REP) and Carvedilol (CDL) were provided as a gift sample by KP Labs Ltd., Hyderabad, India. The chitosan gift sample was obtained from India Sea Foods, Cochin, Kerala, India. The pectin gift sample was received from Krishna pectin, Jalgaon, India. L-cysteine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) and Ellman’s reagent were purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra, India. Sodium carboxymethyl cellulose (NaCMC) was gifted by Dipti Cellulose Pvt. Ltd., Jalgaon, India. Ethyl cellulose (EC), micro crystalline cellulose (MCC), mannitol, talc, magnesium stearate and other chemicals were purchased from SD Fine-Chem Ltd, Mumbai, India.

Preparation and characterization of thiolated chitosan
TC was prepared by conjugating L-cysteine covalently to the primary amino group at 2-position of glucosamine subunit of chitosan via an amide or amidine linkage. The chitosan (1 g) was dissolved in 100 mL acetic acid (10% v/v). To this solution, L-cysteine (0.5 g) was added and mixed until dissolved. This mixture was added with EDAC (50 mM) as a coupling agent and the pH was adjusted to 5 by the addition of NaOH (1 N). After the incubation period of 3 h in dark with continuous stirring (100 rpm), the mixture was dialyzed twice with a dialysis tubing (MW cut-off 12 kDa) in dark, at 10°C with HCl (1 mM) containing NaCl (1%) for 1 day, thereafter, with HCl (1 mM) for 2 days. The resulted conjugate was lyophilized and stored at 4°C until used in experiments.14

The prepared TC was characterized for physical appearance and organoleptic properties. The amount of free thiol functional group present in TC was determined using Ellman’s reagent [5, 5’-dithiobis (2-nitrobenzoic acid)] (DTNB) as chromophore and absorbance was measured in UV/Visible spectrophotometer at λ-412 nm.24 The number of thiol functional group present in prepared TC was estimated through a calibration curve established using working stock solutions (50 to 250 µM) of L-cysteine. The adhesion property of TC was tested as shear strength by measuring the weight required to shear glass plates (5X5 cm) sandwiched with a TC solution (1%w/v). The bioadhesion property of TC was studied by measuring the weight influencing the complete detachment of TC pellets from an isolated porcine buccal membrane.14

Preparation of REP buccal tablets
The Box-Benken design (BBD) developed polynomial equations were employed to investigate the effect of polymers used on critical formulation characters such as mucoadhesion strength (MS), time taken for 50% drug release (T50%) and the average flux (f) across buccal membrane. The quantity levels of polymers used and optimization experiment was described by Navamanisubramanian et al., 2018.14 The optimized formulas for appropriate formulation characters were selected by the intended search for variables (polymers composition) via a model developed equations using the software Design-Expert 10 (Stat-Ease Inc., USA). The bilayer buccal tablets of REP were prepared by two layers co-compression method. The REP was mixed with polymers (NaCMC, Pectin, and TC) and other excipients, as per software guided combinations were shown in Table 1. This mucoadhesive core mixture (30 mg) was compressed using 6 mm diameter die in a single stroke 10-station rotary tablet press (Karnavathi Engineering, India) to result the primary layer. Next, the water impermeable backing layer was added by co-compressing the primary layer and EC (30 mg) together. The prepared bilayer REP buccal tablets were tested for in vitro and in vivo performance.

Table 1. Formulation composition of REP buccal tablets.

| Components | V1 | V2 | V3 | V4 | V5 | V6 |
|------------|----|----|----|----|----|----|
| REP        | 4  | 4  | 4  | 4  | 4  | 4  |
| NaCMC      | 3  | 5  | 4  | 5.5| 6  | 5  |
| Pectin     | 2.6| 4  | 3  | 4.8| 4.5| 3.8|
| TC         | 4  | 2  | 2  | 2  | 1  | 1  |
| MCC        | 7.65| 6.25| 8.25| 4.95| 5.75| 7.45|
| Mannitol   | 8  | 8  | 8  | 8  | 8  | 8  |
| Talc       | 0.75| 0.75| 0.75| 0.75| 0.75| 0.75|
| Core layer | 30 | 30 | 30 | 30 | 30 | 30 |
| Ethyl cellulose | 30 | 30 | 30 | 30 | 30 | 30 |
| Total weight | 60 | 60 | 60 | 60 | 60 | 60 |

All weights of ingredients and total formulation were taken in mg.

Physico-chemical characterization of REP buccal tablets
The optimized REP buccal tablets were evaluated for weight variation (n=10), thickness uniformity (n=6), hardness (n=6) and friability (n=20) to assess the physical and mechanical properties.25,26 The drug content was assessed as percentage of REP present in prepared buccal tablets.14 Randomly selected tablet samples (n=5) were crushed, powder equivalent to 4 mg of REP (n=3) was added to 50 mL methanol and stirred for 30 min in sealed condition. The mixture was filtered through Whatman filter paper (0.45 µm), 1 mL of filtrate was made up to 5 mL with methanol and analyzed at 290 nm on a UV spectrophotometer (PG Instrument, United Kingdom).

Ex vivo mucoadhesion strength
Physical balance with needed modifications was used to measure the mucoadhesion strength of REP buccal tablets.27 The isolated mucosal membrane from freshly obtained porcine buccal pouch was equilibrated in Krebb’s-Ringer bicarbonate buffer solution (pH 7.4) at 37±0.5°C up
to 30 min. Afterward, this buccal membrane was mounted on a metal cylinder like so the mucus layer facing upward and moistened with 0.1 mL of artificial mucin solution (5% w/w). The EC layer of the testing buccal tablet was glued at the bottom of a glass vial connected with the balance. The tablet (mucoadhesive core layer) was allowed to contact with porcine buccal membrane for 5 min with a load weight of 5 g. Next, the load weight was removed and the weight (g) required to detach the adhered tablet from the mucous membrane was measured as described by Navamanisubramanian et al.\textsuperscript{28}

**In vitro drug dissolution**

The *in vitro* dissolution profile of REP bilayer buccal tablets was studied (n=3) in rotating paddle (USP-II) dissolution apparatus (DS 8000, Labindia Analytical) using simulated saliva (pH 6.8, 250 mL) as the medium with controlled conditions 37±0.5°C and 50 rpm.\textsuperscript{29} At scheduled intervals 5 mL samples were withdrawn via syringe filters (0.45µm) with immediate replacement of fresh medium and amount of REP exist in the samples were determined by UV-spectrophotometer at 290 nm.\textsuperscript{14} Furthermore, the release kinetics of REP from prepared buccal tablets were investigated by fitting the dissolution profile to various release models (zero order, first order) and release mechanism (Higuchi's and Korsmeyer's model).\textsuperscript{30,31}

**Ex vivo drug permeation**

As per the method described by Navamanisubramanian et al., "the *ex vivo* buccal permeation of REP from prepared buccal tablets across porcine buccal mucosa was studied on Franz diffusion cell. An equilibrated porcine buccal mucosa was mounted on the diffusion cell like so the mucous surface facing the donor compartment. The mucoadhesive layer of prepared REP buccal tablet was placed on the mucosa and slightly wetted with 1 mL of simulated saliva. The receptor compartment was loaded with isotonic phosphate buffer pH 7.4, kept in controlled conditions at 37±0.2°C and 100 rpm.\textsuperscript{34} At scheduled time intervals 1 mL of sample were withdrawn, diluted appropriately and analyzed for the drug content at 290 nm in UV-spectrophotometer\textsuperscript{14}.

**Stability study**

The short term (stress induced) stability study was conducted according to the standard guidelines of the International Council for Harmonization (ICH).\textsuperscript{32} The optimized REP buccal tablets were packed in air tight self-seal pouches and stored in stability chamber at 40±0.5°C and 75±5% RH. At the end of 3 months, the study samples were withdrawn and subjected to study the changes in physical appearance, mucoadhesion strength, drug content uniformity, *in vitro* dissolution and *ex vivo* permeation profile of REP, if any. All those estimates were compared with initial time results and the significance of changes in stability profile of REP buccal tablets was assessed.

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### In vivo study

**Study design**

The *in vivo* study of REP buccal tablets was performed in New Zealand white rabbits as per the protocol (IAEC/52/SRU/578/2017) approved by the institutional animal ethical committee of Center for Toxicology and Developmental Research, Sri Ramachandra University, Chennai. Healthy male rabbits (n=6) having body weight 2.5±0.25 kg were selected, divided into two groups (test, standard) and fasted overnight before the study. During each experiment the rabbits were placed in a restrainer and the oral cavity was kept as slightly opened with the help of a mouth gag. A single dose of REP tablet (4 mg) was administered by the oral route as standard (n=3) and the optimized buccal tablet (V5, n=3) was placed on the buccal region (cheek mucosa) as the core layer facing the buccal membrane, with gentle pressure using a forceps. The blood samples (2 mL) were collected at 0, 0.5\textsuperscript{th}, 1, 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th} and 6\textsuperscript{th} h after the dosing, via the marginal ear vein, in heparin coated blood sampling tube. The collected blood samples were immediately centrifuged at 5000 g for 15 min in a refrigerated centrifuge (4°C) and the supernatant plasma was separated. To 200 µL rabbit plasma 800 µL of acetonitrile (ACN) was added as an extraction reagent, 20 µL of CDL (5 µg/mL) was added as an internal standard. Next, the resulted mixture was subjected to refrigerated centrifuge at 5000 g for 15 min at 4°C. The protein free clear supernatant (500 µL) was shifted into a fresh Eppendorf tube (1 mL) and evaporated to dryness in a vacuum evaporator. Thereafter, the residue was reconstituted in 100 µL mobile phase, capped, vortexed for a min and centrifuged again at 5000 g for 15 min at refrigerated condition (4°C). The particle free clear supernatant (20 µL) was allowed to inject into the HPLC column [C18 (2), 250×4.6 mm; 5µ particle size (Luna®, Phenomenex)] to develop the chromatogram by isocratic elution using a fixed composition of ACN:0.05% trifluoroacetic acid (TFA) in water (55:45, %/v) mixture as mobile phase, pumped with constant flow rate (1 mL/min) and 285 nm was set as detection wavelength for entire study.\textsuperscript{33}

**Pharmacokinetic analysis**

The pharmacokinetics information of REP was studied from the time dependent plasma concentration profile of REP. The pharmacokinetic parameters according to compartment and non-compartment modeling were estimated by the derived mathematic formulas. The elimination rate constant (K\textsubscript{e}) describes the velocity by which the drug is removed from the physiological system per unit time. The K\textsubscript{e} was calculated from the slope at the terminal log-linear elimination phase of plasma concentration-time data plotted by Microsoft-Excel\textsuperscript{a} and the absorption rate constant (K\textsubscript{a}) was estimated from the slope of the residual line resulted from a plot of log-residual concentration of drug Vs time. The mean biological half-life (t\textsubscript{1/2,b}) and mean absorption half-life (t\textsubscript{1/2,a}) were calculated as 0.693/K\textsubscript{e} and 0.693/K\textsubscript{a} respectively. The maximum plasma concentration...
was estimated by applying linear trapezoidal approximation. Furthermore, the AUC between time ‘t’ to ‘infinity’ (AUC∞) was estimated by C∞/Kd (where C∞ represents the estimated REP concentration in rabbit plasma at end time of the study). The sum of AUC0,t and AUC∞ yields a total integrated area under the curve between time ‘0’ to ‘infinity’ (AUC∞). Likewise, the area under first moment graph (AUMC0,t) was measured from the plot made by product of REP plasma concentration*time versus time. The estimation volume of distribution (Vd) was done by FXd/(Kd*AUC0,t) and total body clearance (CL) was done by Vd*Kd. The mean residence time (MRT) of REP was calculated by the ratio of AUMC0,∞ and AUC0,∞.

**Data analysis**

"The estimation of mean and standard deviation of results was done by MS excel 2007. The effect of factors on the responses for optimization was studied by the software Design-Expert 10 (Stat-Ease, Inc., USA). Calculation of mean, standard deviation, tests of significance (paired t-test, one-way ANOVA) for other data was performed with Microsoft Excel, 2007".

**Results and Discussion**

**Characterization of thiolated chitosan**

TC was prepared by immobilization of a thiol (-SH) functional group containing molecule with the primary amino group of chitosan. For this, L-cysteine was used as a source for –SH functional group, immobilized by establishing amide linkage between carboxylic acid of L-cysteine with the primary amino group of chitosan, that was mediated by EDAC. The lyophilized TC appears as slight yellowish-white, fibrous solid with a distinct odor was harmless to the buccal mucosa. The friability was found to be 6.59±0.09 and 6.81±0.11 kg/cm2 respectively. The adhesion and bioadhesion properties of prepared TC were assessed by shear strength and bioadhesion strength respectively. The shear strength of TC (12.85 g) was measured as 1.64 fold higher than chitosan (7.84 g) and the bioadhesion strength of TC (83.5 g) on the porcine buccal membrane was found to be 4.93 folds higher than chitosan (16.94 g). Improved mucoadhesion of TC with mucus membrane may caused by establishment of disulfide bonding between TC and mucus layer that is the target feature of the conjugate.

**Pre-compression and post-compression characterization**

The formulations of REP bilayer buccal tablets were studied for micromeritics and formulation properties. The preformulation characters; bulk density and tapped density varied from 0.324±0.009 g/mL to 0.372±0.012 g/mL and 0.385±0.013 g/mL to 0.452±0.021 g/mL respectively. The result of compressibility index was found as 11.31 to 15.84 and Hausner’s ratio resulted as 1.13 to 1.22 indicates the powder blends have fair to good flow property with adequate packing ability (Table 2). The total weight of REP bilayer buccal tablets was designed to 60 mg and the average weight was found to be varied from 60.11±0.39 mg to 61.06±0.42 mg. The prepared buccal tablets have thickness ranged between 2.02±0.009 mm to 2.1±0.012 mm and hardness from 3.9 kg/cm2 to 4.3 kg/cm2. The surface pH of the mucoadhesive layer of REP buccal tablets resulted between 6.59±0.09 and 6.81±0.11 is harmless to the buccal mucosa. The friability was found to be <1%w/w and estimated REP content among the prepared buccal tablets was ranged within 98.92±2.26 %w/w to 101.08±2.42 %w/w (Table 2). All these results estimated were found satisfactory as per the requirements of compendia.

**Mucoadhesion strength**

Mucoadhesion strength is the function of hydration extent and amount of polymers incorporated in the formulation.
The mucoadhesion strength of prepared REP buccal tablets was found to be in the range of 38.27 g to 50.42 g (Table 3) is good enough to retain the tablet at buccal mucosa. Among the prepared REP buccal tablets, mucoadhesion strength was varied proportional to total polymer content in the formulation. The presence of NaCMC and pectin in buccal tablets resulted in varied hydration of the matrix accordingly the composition among them and mucoadhesion was established by non-covalent or hydrogen bonding with the mucosal layer. Further, the addition of TC resulted in higher mucoadhesion strength as the thiomer (-SH) interacts with cysteine-rich subdomains of mucus glycoprotein via disulfide linkage (-S-S-) or by a simple oxidation process. The formula V5 shown superior mucoadhesion strength as it consists an optimum composition of polymers that provides faster hydration of polymer matrix to cover a larger surface area, cross entanglement to form covalent bonding with the mucus polysaccharide.

In vitro dissolution study

The time dependent in vitro release profile of REP buccal tablets was shown in Figure 1 and the results of \( T_{50\%} \) ranged from 56.33 min to 73.33 min (Table 3). The in vitro dissolution rate of REP from the buccal tablets containing increased polymer level was retarded due to the formation of a swollen gel layer around the tablet that restricts the drug release. Further, the increased proportion of NaCMC in the total polymer that promoted drug release due to faster swelling and erosion of the hydrophilic polymer matrix than pectin. The addition of multifunctional TC rendered higher release of REP with pectin and was retarded the REP release while adding with NaCMC. This could be resulted due to the difference in hydration rate of TC which is relatively higher than Pectin and relatively lower than NaCMC.

**Ex vivo permeation study**

To describe ex vivo permeation of REP the term average flux (f-value) of the drug across the porcine buccal membrane was used. The ex vivo permeation profile of prepared REP buccal tablets was depicted in Figure 2 and the average flux of REP across mucus membrane was found between 0.09 mg/cm\(^2\)/hr to 0.119 mg/cm\(^2\)/hr (Table 3). The ex vivo permeation of REP (f) was increased proportionally to level of TC in buccal tablets. This would be resulted by the interaction of cationic TC with the buccal mucosa through disulfide linkage (-S-S-), resulting in a structural reorganization of tight junction-associated proteins for enhanced REP permeability. Other polymers NaCMC and pectin were retarded the permeation by controlling the release of REP from the matrix, without altering the membrane permeability. The results of correlation study between the predicted responses via statistical optimization model and observed

### Table 3. Correlation study results of predicted and experimental values of desired responses with regression coefficient.

| Responses | Formulation | Exp. Value | Pred. Value | %Error | \( R^2 \) | Adjusted \( R^2 \) | Adeq. Precision | %CV |
|-----------|-------------|------------|-------------|--------|---------|----------------|----------------|-----|
| MS        | V1          | 38.27      | 36.43       | 4.81   | 0.8102  | 0.7584         | 11.07          | 7.61|
|           | V2          | 40.7       | 41.74       | -2.56  |         |                |                |     |
|           | V3          | 39.82      | 37.28       | 6.38   |         |                |                |     |
|           | V4          | 45.29      | 45.02       | 0.59   |         |                |                |     |
|           | V5          | 50.42      | 51.57       | -2.28  |         |                |                |     |
|           | V6          | 46.37      | 47.18       | -1.75  |         |                |                |     |
| \( T_{50\%} \) | V1          | 56.33      | 54.92       | 2.5    | 0.8296  | 0.7831         | 12.44          | 10.8|
|           | V2          | 67.67      | 68.18       | -0.75  |         |                |                |     |
|           | V3          | 64.67      | 67.3        | -4.07  |         |                |                |     |
|           | V4          | 67.67      | 66.06       | 2.38   |         |                |                |     |
|           | V5          | 73.33      | 76.04       | -3.69  |         |                |                |     |
|           | V6          | 72         | 73.84       | -2.56  |         |                |                |     |
| Flux (f)  | V1          | 0.119      | 0.105       | 11.76  | 0.7206  | 0.6443         | 9.4            | 15.35|
|           | V2          | 0.102      | 0.083       | 18.63  |         |                |                |     |
|           | V3          | 0.105      | 0.0905      | 13.81  |         |                |                |     |
|           | V4          | 0.1        | 0.084       | 16     |         |                |                |     |
|           | V5          | 0.09       | 0.074       | 17.78  |         |                |                |     |
|           | V6          | 0.097      | 0.078       | 19.59  |         |                |                |     |

Note: MS-Mucoadhesion strength, \( T_{50\%} \)-Time taken for 50% drug to release, f-Average flux of REP across porcine buccal membrane.
responses estimated by software (State-Ease 10) were described in table 3. The observed responses (MS, \(T_{50}\) and \(f\)) were agreed with the predicted responses. The results of correlation coefficient values (\(R^2\)) and percentage coefficient of variance (%CV) indicate the REP buccal tablets developed were significantly obeying the optimization model used.

**Stability studies**
The physico-chemical stability of REP buccal tablets during storage was studied as per ICH guidelines for accelerated stability testing of finished products. The physical appearances of all prepared buccal tablets were found unchanged with adequate hardness (≥3.5 kg) and acceptable friability (≤0.43 %w/w). The chemical degradation rate of REP at storage was studied by a change in assay value of buccal tablets at the end of 3rd month. The maximum drug degradation rate was found to be 0.0276 mg/month and expected \(T_{90}\) resulted as 15.07 months. The statistical significance of the difference in assay values of REP between initial and end of 3 months was assessed by the test of significance (paired t-test) and the results found as \(p\geq0.24\), which indicates the differences estimated for the formulations were insignificant (Table 4). The t-test results of mucoadhesion strength (\(p\geq0.074\), \(T_{50}\) (\(p\geq0.057\)) and average flux of REP across the buccal membrane (\(p\geq0.053\)) indicate no significant changes in these parameters during the testing period. *In vitro* release profile of aged formulations was compared with zero month release profile and the similarity factor (f2) among the two release profiles was found between 74.6±3.16 to 95.12±3.23 indicates no significant changes in *REP* release profiles of developed buccal tablets. All these results confirm the physical and chemical stability of REP buccal tablets in the entire study.

**In vivo study and pharmacokinetic analysis**
The chromatographic separation of REP and CDL was done in RP-HPLC using the mobile phase comprising ACN:0.05% TFA in water (55:45, v/v) using reverse phase analytical column (250×4.6 mm; 5 µ particle size) at ambient condition. The retention times (Rs) of REP and CDL were found as ~4.3 and 5.1 min respectively (Figure 3). The calibration curve established for plasma samples with a known concentration of REP was found linear (Y=0.0014X+0.0002) between 10 to 1000 ng/mL with \(r^2\) value 0.9988.

![Figure 3. Chromatogram of rabbit plasma sample collected 1 h after oral dosing of 1) REP (4 mg) and spiked with 2) CDL.](image)

**Table 4. Results of short term stability test of REP buccal tablets.**

| Batch code | Assay       | Mucoadhesion strength | Flux          |
|------------|-------------|-----------------------|---------------|
|            | 0 Month     | 3 Month               | p-value*      | 0 Month     | 3 Month               | p-value*      |
| V1         | 98.59±1.78  | 97.96±1.97            | 0.53          | 38.27±2.79  | 37.05±3.15            | 0.074          |
| V2         | 100.45±1.61 | 98.37±1.32            | 0.31          | 40.70±2.01  | 41.33±2.53            | 0.47           |
| V3         | 100.23±1.76 | 99.44±2.51            | 0.34          | 39.82±2.36  | 39.12±0.94            | 0.58           |
| V4         | 99.58±2.13  | 99.26±4.23            | 0.82          | 45.29±1.42  | 43.82±2.93            | 0.33           |
| V5         | 101.08±2.42 | 98.97±2.29            | 0.51          | 50.42±2.71  | 49.67±0.64            | 0.59           |
| V6         | 100.56±1.95 | 101±3.29              | 0.24          | 46.37±0.91  | 45.95±1.31            | 0.66           |

| Batch code | \(T_{50}\)   | Flux          |
|------------|---------------|---------------|
|            | 0 Month       | 3 Month       | p-value*      | 0 Month     | 3 Month               | p-value*      |
| V1         | 57.33±2.08    | 55.33±3.51    | 0.32          | 0.119±0.007 | 0.115±0.0005          | 0.053          |
| V2         | 67.67±3.06    | 69.33±3.79    | 0.42          | 0.102±0.005 | 0.099±0.006           | 0.094          |
| V3         | 64.67±3.21    | 66.67±3.51    | 0.23          | 0.105±0.008 | 0.104±0.006           | 0.58           |
| V4         | 67.67±3.79    | 69.33±4.93    | 0.13          | 0.1±0.005   | 0.097±0.004           | 0.12           |
| V5         | 73.33±3.79    | 71.67±2.52    | 0.19          | 0.09±0.008  | 0.087±0.006           | 0.15           |
| V6         | 72±2.65       | 73.33±3.06    | 0.057         | 0.097±0.007 | 0.098±0.008           | 0.58           |

Values represent mean±SD, n=3; *p-value of t-test.
Note: \(T_{50}\)-Time taken for 50% drug to release.
The in vivo study of REP buccal tablets was performed in New Zealand white rabbit as per the approved protocol described in the study design and the time dependent plasma concentrations of REP were estimated by the RP-HPLC method using CDL as internal standard (Figure 4).

The compartmental and non-compartmental pharmacokinetic parameters of REP from buccal tablets were compared with oral bolus (Table 5). The absorption rate was slower in the buccal route (1.15±0.02 h⁻¹) than oral bolus (2.27±0.6 h⁻¹) and the absorption half-life was increased from 0.32±0.09 h (oral) to 0.60±0.01 h (buccal). This would be happened by sustained release of REP from the buccal tablet that extended the REP absorption in buccal route than oral bolus. As a result of the continuous addition of REP to plasma by sustained release from buccal tablet, the reduction rate of REP from plasma was decreased. This was perceived from a reduction of REP elimination rate up to 50% (oral/buccal: 0.54/0.27 h⁻¹) and increased biological half-life of REP around 2 folds (oral/buccal: 1.3/2.61 h) by transbuccal administration. The C_{max} value of buccal tablet (122.71±2.94 ng/mL) was resulted as relatively lesser than oral bolus (136.24±3.94 ng/mL) and the apparent volume of distribution (V_{app}) of REP was estimated as 21.13±0.78 L for buccal tablets was relatively higher than 19.85±1.06 L of the oral bolus. The total clearance (CL) value of REP from buccal tablets and oral bolus was found to be 5.62±0.12 L/h and 10.63±0.72 L/h respectively, indicates the removal of REP form plasma was relatively reduced for buccal tablets than oral bolus.

In general, the overall biological performance of drugs will be assessed through AUC,^α was found to be 712.22±15.91 ng/mL/h for buccal tablets and 377.49±26.22 ng/mL/h for oral bolus. The relative AUC values indicate the buccal tablets produced 1.89 fold increased bioavailability than oral bolus. These results indicate the transbuccal administration of REP into systemic circulation eliminates the first-pass metabolism and results in increased bioavailability. These results were further supported by the non-compartmental pharmacokinetic parameter, MRT of REP was found to be 4.66±0.25 h for buccal tablets and 2.54±0.46 h for oral bolus that indicates the REP administered through buccal tablet resides in the body for a longer period than oral bolus.

### Conclusion

The TC based REP buccal tablets were successfully developed using optimized combinations of NaCMC, Pectin and TC. The developed buccal tablets of REP were found to be stable and firmly adhere to the buccal mucosa. The administration of REP via buccal tablets may promote compliance due to its easy administration and sustained release for prolonged time that reduces the frequency of administration. The pharmacokinetics study results certify that the REP buccal tablets have superior bioavailability than an oral bolus. This may help to attempt dose reduction.

### Table 5. Pharmacokinetic results for in vivo study of REP buccal tablet and oral bolus in rabbit.

| PK parameters | Unit | Oral | SE | Mean* | SD | SE |
|---------------|------|------|----|-------|----|----|
| C_{max}       | ng/mL| 136.24| 3.90| 2.11  | 122.71| 2.94| 1.60 |
| T_{max}       | h    | 0.85 | 0.13 | 0.07  | 1.65 | 0.03| 0.02 |
| K_{a}         | h⁻¹  | 2.27| 0.60 | 0.30  | 1.15 | 0.02| 0.01 |
| T_{1/2(ab)}   | h    | 0.32| 0.09 | 0.05  | 0.60 | 0.01| 0.004 |
| K_{e}         | h⁻¹  | 0.54| 0.02 | 0.01  | 0.27 | 0.01| 0.003 |
| T_{1/2(el)}   | h    | 1.30| 0.05 | 0.03  | 2.61 | 0.06| 0.03 |
| AUC_{0-6}     | ng/mL/h| 353.42| 34.28| 18.66 | 528.08| 34.40| 18.35 |
| AUC_{6-∞}     | ng/mL/h| 24.40| 12.84| 6.63  | 184.14| 18.97| 10.32 |
| AUC_{0-6}     | ng/mL/h| 377.49| 26.22| 13.57 | 712.22| 15.91| 8.02 |
| AUMC_{0-6}    | ng·h/mL| 768.88| 92.00| 46.96 | 1520.17| 76.42| 40.34 |
| AUMC_{6-∞}    | ng·h/mL| 186.01| 91.97| 47.10 | 1797.22| 184.85| 99.62 |
| MRT           | h    | 2.54| 0.46 | 0.25  | 4.66 | 0.25| 0.13 |
| V_{app}       | L    | 19.85| 1.06| 0.55  | 21.13| 0.78| 0.43 |
| CL            | L/h  | 10.63| 0.72 | 0.37  | 5.62 | 0.12| 0.06 |

^αvalues represent mean determined from n=3.

Note: C_{max}-Peak plasma concentration of REP, T_{max}-Time taken to reach C_{max}, K_{a}-Absorption rate constant, T_{1/2(ab)}-Absorption half-life, K_{e}-Elimination rate constant, T_{1/2(el)}-Biological half-life, AUC-area under the curve, AUMC-Area under first moment curve, MRT-Mean residential time, V_{app}-Apparent volume of distribution, CL-Total body clearance.
of REP in transbuccal administration that may eliminate dose induced adverse effects. It was concluded from the results that the developed TC based REP buccal tablets are capable of controlled delivery of REP into systemic circulation bypassing first-pass metabolism. Further, clinical studies are required to determine the clinical benefits of the developed REP buccal tablets and toxicity if any.

**Ethical Issues**
The authors declare that the experiments involved animals described in this manuscript were conducted in compliance with the guidelines for experiments on animals issued by CPCSEA, India.

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**Conflict of Interest**
The authors declare they have no conflict of interest.

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