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Research Article

Transbuccal Delivery of 5-Fluorouracil: Permeation Enhancement and Pharmacokinetic Study

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Abstract. The purpose of this study was to determine the effect of permeation enhancers on the transbuccal delivery of 5-fluorouracil (FU). The effect of permeation enhancers on *in vitro* buccal permeability was assessed using sodium deoxycholate (SDC), sodium dodecyl sulphate (SDS), sodium tauroglycocholate (STGC), and oleic acid and their concentrations for absorption enhancement were optimized. STGC appeared to be most effective for enhancing the buccal permeation of FU than the other enhancers. These enhancements by STGC were statistically significant ($p<0.05$) compared to control. The order of permeation enhancement was STGC > SDS > SDC > oleic acid. Histological investigations were performed on buccal mucosa and indicated no major morphological changes. The enhancing effect of STGC on the buccal absorption of FU was evaluated from the mucoadhesive gels in rabbits. The absolute bioavailability of FU from mucoadhesive gels containing STGC increased 1.6-fold as compared to the gels containing no permeation enhancer. The mean residence time and mean absorption time considerably increased following administration of gel containing penetration enhancer compared with the gel without penetration enhancer.

KEY WORDS: 5-fluorouracil; buccal; enhancer; gel; *in vivo*; mucoadhesive; permeation.

INTRODUCTION

The buccal mucosa offers excellent opportunities for the delivery of both locally and systemically active drugs. It has potential advantages over other mucosal routes available: it avoids the degradation by the gastrointestinal enzymes and acids and first-pass metabolism. Because of its excellent accessibility, self-placement of a dosage form is possible. Moreover, the drug can be removed at any time. Clinical success following oral mucosal delivery depends on the ability of a formulation to achieve and maintain plasma drug level for a defined period of time for the desired therapeutic response (1,2). However, most of the drugs administered in the form of buccal or sublingual tablets have exhibited low bioavailability due to the low mucosal membrane permeability. In order to overcome this problem, one approach consists of increasing the permeability of the buccal mucosa using penetration enhancers, which are able to decrease the barrier capacity of the tissue (3–6). The permeation enhancement approach was considered in order to further improve buccal mucosal membrane permeability of various drug delivery systems (7–9). However, it is unclear whether they act by transiently altering the mucosa or by damaging it (10).

Oropharyngeal cancer develops in the part of the throat just behind the mouth, called the oropharynx. Oropharynx includes the base of the tongue, soft palate, tonsils, and tonsillar pillars and back wall of the throat and it helps in breathing, eating, and talking. Therapies being used to treat oropharyngeal cancer are surgery, radiation therapy, and chemotherapy. Chemotherapy given to shrink cancer before surgery or radiation treatment is called neoadjuvant chemotherapy (www.cancer.org/docroot/cri/content).

Most of the anticancer drugs have to be administered parenterally as they have poor bioavailability when administered orally either due to poor absorption or due to significant first pass metabolism. However, this route is associated with pain on administration, formulations need to be sterile, and it is time consuming for doctors and patients (11). In addition, certain health risks are associated with this route (including psychological distress, occasional allergies, hypertrophy, or atropy of the subcutaneous fat at the injection site) (12). A recent study concerning patient preference showed that the large majority, approximately 90%, of cancer patients preferred oral administration to intravenous administration (13). However, patients suffering from oropharyngeal cancer have difficulty in swallowing (14). Systemic delivery of drugs through the mucosal routes presents a solution to the problems of hepatic and gastrointestinal metabolism associated with the oral drug-delivery route and the health risks associated with the parenteral route.

5-Fluorouracil (FU) is an antimetabolite with promising antineoplastic activity in head and neck cancer. Chemically, it is a diprotic acid with p*K*a values of 8.0 and 13.0 and is highly polar in nature (log *P* octanol/water=−0.89) (15,16). FU has been widely used in the treatment of oropharyngeal cancer.

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After oral administration, FU is poorly absorbed with significant variation in bioavailability ranging between 0 and 80%. Following parenteral administration of FU, it is rapidly eliminated with an apparent terminal half-life of approximately 8–20 min (17–19). These two problems make FU a suitable candidate for transbuccal delivery.

Bile salts such as sodium deoxycholate (the sodium salt of deoxycholic acid), sodium tauroglycocholate have been employed to enhance the absorption of drugs through buccal membrane. In earlier reports permeation across buccal mucosa was studied for morphine sulphate using sodium glycodeoxycholate (6). Sodium glycocholate was able to enhance permeation of morphine hydrochloride through the buccal mucosa at 100 mM concentration with an enhancement factor of 2 (20). Attia *et al* have studied permeation of piroxicam across buccal mucosa using sodium lauryl sulphate, sodium deoxycholate, and sodium tauroglycocholate (21). Many other drugs like triamcinolone acetonide (8), flecainide, and sotalol (22) have been studied for permeation enhancement via buccal route.

Gantrez® S 97 is polymethyl ether/maleic acid with molecular weight of 1,200,000. It is widely used in toothpastes and mouthwashes and is reported to be bioadhesive. Gantrez® S copolymers are water-soluble, giving clear, tacky solutions (23). HPMC is partly *O*-methylated and *O*-(2-hydroxypropylated cellulose). HPMC has been reportedly used as bioadhesive in gel formulations (22,24).

The aim of the present work was to investigate permeation properties of FU across buccal mucosa and to study the effect of various permeation enhancers on buccal permeation of FU. Bioavailability of FU in rabbits by buccal administration of the bioadhesive controlled release gel formulations containing penetration enhancer was evaluated.

EXPERIMENTAL WORK

Materials and Methods

FU was obtained as a gift sample from BDH Pharma, Mumbai (India), while sodium dodecyl sulphate (Himedia Labs, Mumbai), sodium deoxycholate (Himedia Labs, Mumbai), sodium tauroglycocholate (SD Fine chemicals, Mumbai), and oleic acid (Sigma, Mumbai) were used as received. HPMC K15 M was kindly provided by Alembic Ltd., Baroda. Gantrez® S-97 was received from ISP International Ltd., Mumbai as gift sample. Poloxamer-407 (P-407) was procured from BASF, Mumbai. All other chemicals and solvents used were of AR grade.

Tissue Preparation

Guinea pig buccal tissue was chosen, because its non-keratinized morphology is quite similar to human buccal epithelium (22). Buccal tissue was removed after sacrificing the animal and was stored in phosphate buffer (pH 6.8) and immediately transported to the experimental setup. The buccal mucosal membranes were separated by removing the underlying connective tissues using surgical scissors making sure that the basal membrane was still present. The tissue was rinsed and then stored in ice-cold phosphate buffer until mounted in the Franz diffusion cell (within 1 h upon removal) (21). Slice thickness ranged from 2.2 to 2.5 mm and was mounted between donor and receiver chambers of the diffusion cells for permeation studies.

Analytical Method

The concentration of FU was quantitated by reversed phase high-performance liquid chromatography (HPLC) by a modified method of that described by Nassim *et al*. was used (25). The method was validated for linearity, specificity, accuracy, and precision. Dionex isocratic HPLC with a UV-visible detector was used for HPLC analysis. The mobile phase, consisting of potassium dihydrogen phosphate (KH_2PO_4, pH 6.8), was prepared at 50 mM concentration with distilled deionised water followed by degassing of the solution and filtering through a 0.45 μ inorganic filter. BDS Hypersil C_{18} column (Thermo Hypersil Keystone), 150× 4.6 mm with 5 μ particles was used. The required parameters were programmed using Chromeleon software. Program parameters were as follows: flow rate—0.5 ml/min, detection wavelength—265 nm, run time—15 min. Twenty microliters of sample was loaded using syringe through rheodyne injector. Retention time was 5.8 min.

For analysis of FU from plasma samples, the proteins were precipitated by addition of acetonitrile and then the drug was extracted twice by addition of ethyl acetate. The extract was evaporated to dryness and diluted with mobile phase and injected into the injector. Program parameters were as follows: flow rate—0.5 ml/min, detection wavelength—265 nm, run time—15 min. Retention time was 6.3 min.

In Vitro Diffusion Study

The isolated buccal mucosa was mounted in Franz diffusion cells with a diffusion area of 3.7 cm^2 and a compartment volume of 20 ml. After an equilibrium period with phosphate buffer on both sides, the acceptor side was filled with phosphate buffer and donor side with FU solution (0.5% *w*/*v*) in phosphate buffer alone or with the permeation enhancers. The solution in the receptor compartments was continuously stirred at 600 rpm using a Teflon-coated magnetic stirring bead on electric stirrer (Remi, Mumbai). The diffusion studies were carried out at 37±1°C. Samples of 1 ml were taken from the acceptor side at 1 h intervals for 8 h, replaced with the same amount of fresh buffer solution, and subsequently assayed using HPLC.

Treatment of Permeation Data

The cumulative amount of permeated drug (from mucosal side to serosal side) was plotted *versus* time and the flux was calculated from the steady state part of the curve. The permeability coefficient (K_p) was calculated using the Eq. 1 described by Senel *et al*. (26).

$$K_p = {}^{(dQ/dt)}/_{(\Delta C \times A)} \quad (1)$$

dQ/dt	average slope from steady state portion of the curve
ΔC	concentration difference across the mucosa
A	area of diffusion

The efficacy of the different enhancers was determined by comparing the permeation rate of FU in the presence and absence of enhancers. It was defined as the enhancement factor (EF) which was calculated using the following Eq. 2 (24):

Enhancement factor,

$$EF = \frac{\text{FU permeation rate at steady state in the presence of enhancer}}{\text{FU permeation rate at steady state in the absence of enhancer}} \quad (2)$$

One-way analysis of variance (ANOVA) was used for statistical evaluation.

Histology Study

Histological studies on buccal mucosa were performed by mounting on diffusion cells and exposing mucosal side to penetration enhancer and serosal side to pH 6.8 phosphate buffer. Control (buccal mucosa untreated with enhancer) was exposed to buffer on both sides. Study was performed for 1, 2, 4, 6, and 8 h for the tissues.

At the end of the exposure period, small portions of tissue were fixed in 10% buffered formalin solution and dehydrated. All specimens were then embedded in paraffin wax and sections were cut with a microtome in a direction perpendicular to the epithelial surface. They were then stained with hematoxylin eosin (HE) and examined under light microscope (Olympus, Japan) (27).

Preparation of FU Mucoadhesive Gels

Poloxamer 407 gels were prepared using the method described by Schmolka, I.R. (28). Poloxamer-407 (17 g) was dissolved in water (30 ml) with moderate stirring. The solution was kept in a refrigerator overnight to ensure complete polymer dissolution. FU (1 g) and sodium tauroglycocholate (1.15 g) were dissolved in water (60 ml) and then HPMC K15M (2 g) and Gantrez S-97 (3 g) were added afterwards. Then both the solutions (Poloxamer and drug solution with HPMC and Gantrez) were mixed and stirred at 40–45°C for 2 h till a gel was formed. Total amount of water used in the formulation was 90 ml. The prepared gel formulations were filled in well-stoppered glass containers and stored at room temperature. These gels were evaluated for compatibility between drug and polymers, assay, pH, bioadhesion, viscosity, *in vitro* release across cellulosic membrane, and *in vitro* diffusion across buccal mucosa. The details of formulation and characterization of buccal bioadhesive gels containing FU are reported in our earlier work (29).

Stability of Bioadhesive Gels

Gels were stored in glass containers (well-stoppered) for 3 months in the dark at room temperature (25±1°C). They were checked after preparation and throughout storage period. Evaluation of stability of the samples was carried out by visual inspection, assay, and rheology test (30).

In Vivo Bioadhesion of Gels

Radiolabelling of the Gels and their Application

One milliliter of technetium-99m (^{99m}Tc) (2 mCi/ml) was mixed with 0.1 ml of stannous chloride solution (1 mg/ml) and the pH was adjusted to 7 using sodium bicarbonate solution. Ten microliters of above aqueous solution of ^{99m}Tc was added to approximately 2 g of gel formulation (placebo) to be tested and thoroughly mixed. The average final activity per dose and per subject, at the time of administrations ranged from 3,995 to 4,210 kcpm (kilocounts per minute). Rabbits were anesthetized by an i.m. injection of a 1:5 mixture of diazepam (4.6 mg/kg) and ketamine (9.3 mg/kg) and 200 mg of the gel formulation was applied with a small Teflon spatula on an area of approximately 1 cm^2 of the oral mucosa. Each animal was used only once throughout these trials (31,32).

Gamma Scintigraphy

For the determination of residence time of gel formulations in the buccal cavity, the rabbits were fixed on a board and imaging was performed using a single photon emission computerized tomography gamma camera (SPECT, LC 75–005, Diacam, Siemens, USA).

Animal Study and Pharmacokinetics

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of MS University of Baroda (protocol no: MSUP 031/06) and are in accordance with the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. White male rabbits weighing 2.0–2.6 kg were put up separately for 2 weeks in an environment with controlled temperature (20–25°C) and the relative humidity between 50% and 60%. They were fasted for 24 h before drug administration.

Animals were lightly anesthetized by an i.m. injection of a 1:5 mixture of diazepam (4.6 mg/kg) and ketamine (9.3 mg/kg). Following induction of anesthesia, a catheter was placed in the marginal ear vein for blood sample collection. Blood sample, 0.5 ml, (control) were taken before and 1, 2, 3, 4, 6, 8, and 24 h after buccal administration of the mucoadhesive buccal gels (containing 20 mg 5-FU). The sampling periods were 30 min and 1, 2, 4, and 6 h after intravenous (containing 20 mg 5-FU), oral tablet, and oral solution (10% *w*/*v*) (both containing 20 mg 5-FU) administration. The blood samples were collected into heparinized microcentrifuge tubes.

The light level of anesthesia was maintained by an i.m. injection of one-third of the initial dose of diazepam and ketamine mixture as needed. Then the samples were subjected to centrifugation on a cooling laboratory centrifuge (Sigma, 3K30; Germany) at 10,000 rpm for 10 min at 0°C and supernatant plasma was collected into another microcentrifuge tube and kept at −20°C until analysis (33).

The noncompartmental pharmacokinetic analysis was performed (9). The area under the plasma concentration time curve (AUC) and the area under the first moment curve (AUMC) were estimated by the linear trapezoidal rule and extrapolated to infinity using standard techniques. The mean

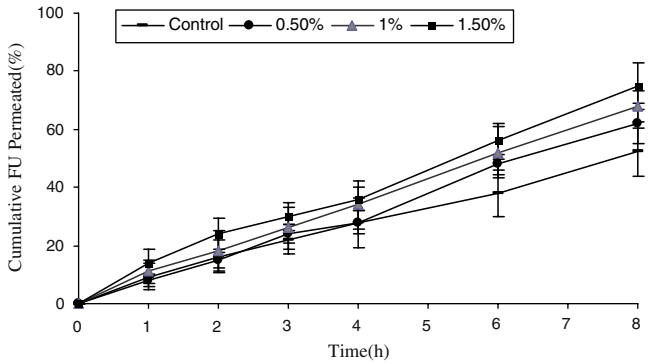

Fig. 1. Permeation of FU in presence of SDS across buccal mucosa

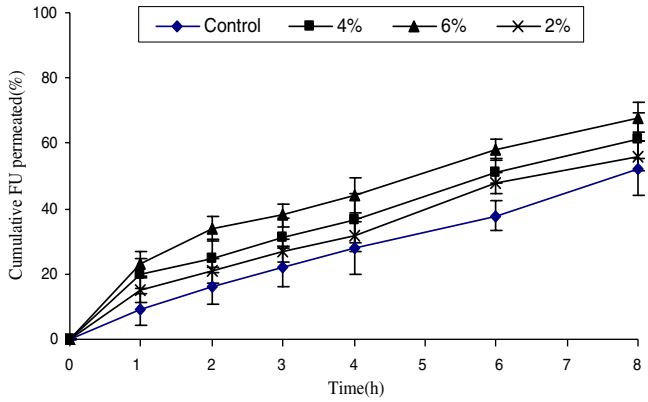

Fig. 3. Permeation of FU in presence of SDC across buccal mucosa

residence time (MRT) of the drug in the body was obtained from the ratio of AUMC to AUC. The rate of absorption of FU after oral administration was estimated by the mean absorption time (MAT) based on differences in MRT after buccal and intravenous administration (34).

All the pharmacokinetic parameters were calculated using MS-Excel software. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were determined by visual inspection of the experimental data as well as the plasma concentration curve using MS-Excel software. The absolute bioavailability (A.B.) of FU after buccal and i.v. administration was calculated by using Eq. 3 as given below (9):

$$\text{A.B.} = \frac{(\text{AUC})_{\text{sample}}}{(\text{AUC})_{\text{i.v.}}} \times \frac{(\text{Dose})_{\text{i.v.}}}{(\text{Dose})_{\text{sample}}} \times 100 \qquad (3)$$

The pharmacokinetic parameters were tested for statistical significance using the ANOVA test. Differences were considered to be significant when $p<0.05$. All the values were reported as mean±standard deviation (SD) of six determinations.

RESULTS AND DISCUSSION

Effect of Permeation Enhancers on buccal permeation of FU

The permeation profiles of FU in presence of various enhancers are reported in Figs. 1, 2, 3, and 4, while their permeability coefficients (K_p) and the enhancement factors (EF) are reported in Table I. In case of control, 52.3±8.1% FU was found to permeate through the buccal mucosa in 8 h with permeability coefficient of 0.799 (±0.31)×10^{-4} cm/s. In order to improve buccal permeation and hence absorption of FU, various permeation enhancers such as SDS, oleic acid, SDC, and STGC were used. EF as a function of the K_p is plotted into a graph (Fig. 5).

In case of SDS (0.5%, 1.0%, and 1.5%), significant improvement ($p<0.05$, ANOVA followed by Dunnett's Multiple Comparison Test) in permeation was discerned. SDS at 1.5% showed 75±8.2% of FU permeation with K_p value 4.13 (±0.74)×10^{-4} cm/s and EF as 1.43 (Fig. 1 and Fig. 5). As the concentration of SDS was increased, increase in permeation was observed. On using oleic acid, no significant improvement was noticed ($p>0.05$) in permeability coefficient and EF as the values were 0.879 (±0.58)×10^{-4} cm/s and 1.08, respectively (Table I, Figs. 2 and 5). Bile salts i.e. SDC and STGC, displayed improvement in permeation as evident from K_p and EF values. SDC and STGC at the concentrations of 4% and 3%, respectively, were able to enhance the buccal permeation of FU significantly. SDC showed 68±5.6% permeation across the buccal mucosa, while the K_p and EF values were 3.17(±0.69)×10^{-4} cm/s and 1.30, respectively, which was significantly different from control ($p<0.05$).When STGC was used, highly significant increase ($p<0.001$) in permeation was observed, with 90.13±7.2% FU permeated through the mucosal membrane. K_p (5.80 (±0.62)×10^{-4} cm/s) and EF (1.72) values were also increased significantly. This

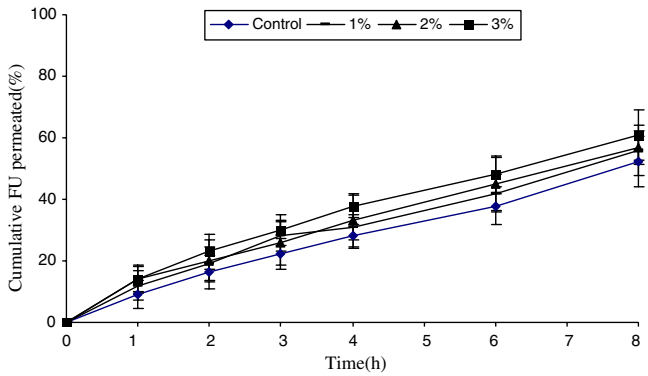

Fig. 2. Permeation of FU in presence of oleic acid across buccal mucosa

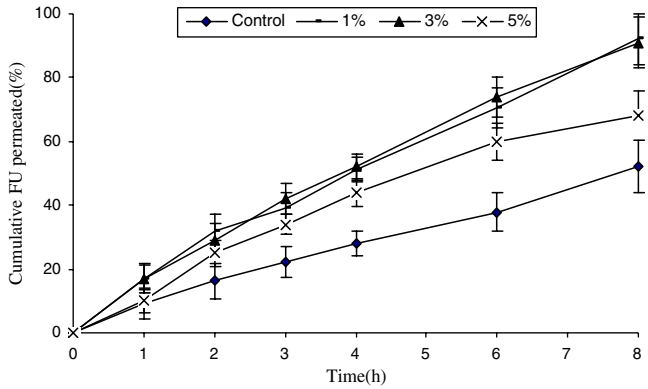

Fig. 4. Permeation of FU in presence of STGC across buccal mucosa

Table I. Permeability of FU Across Buccal Mucosa Using Different Penetration Enhancers

Batch	Permeability coefficient (K_p) ($\times 10^{-4}$ cm/s)	Permeation rate (%/h)	Enhancement factor
Control	0.799 (±0.31)	6.54	1
STGC (1%)	0.99 (±0.49)	8.5	1.3
STGC (3%)	5.80 (±0.62)	11.27	1.72
STGC (5%)	2.93 (±0.53)	10.59	1.62
SDS (0.5%)	3.21 (±0.48)	8.15	1.25
SDS (1%)	3.44 (±0.82)	8.50	1.30
SDS (1.5%)	4.13 (±0.74)	9.38	1.43
SDC (2%)	3.01 (±0.22)	7.00	1.07
SDC (4%)	3.17 (±0.69)	8.5	1.3
SDC (6%)	3.07 (±0.81)	7.5	1.14
Oleic acid (1%)	0.815 (±0.95)	6.75	1.03
Oleic acid (2%)	0.869 (±0.54)	7.00	1.07
Oleic acid (3%)	0.879 (±0.58)	7.12	1.08

Oleic acid dissolved in ethanol and then in water. Proportion of ethanol–water was 5:95. n=6; ±SEM

increase was observed at 3% concentration of STGC, and at higher concentration 5%, no further enhancement in permeation was observed.

The order of permeation enhancement was STGC > SDS > SDC > oleic acid. Permeability of mucosa to FU was rather low (K_p 0.799(±0.31)$\times 10^{-4}$ cm/s), whereas permeability increased most significantly ($p<0.001$) in the presence of STGC. The value of K_p augmented 7.25-fold in presence of STGC as compared to FU alone. Hence, STGC was selected for further studies.

These results further confirmed the efficacy of the bile salts in enhancing the permeation of hydrophilic drugs by affecting transport through the paracellular route. (e.g. In earlier reports, sodium glycocholate was shown to enhance the buccal transport of flecainide acetate and not the more lipophilic flecainide base, which was attributed to the different pathways for each permeant and the ability of the bile salt to affect only the paracellular route) (22,35).

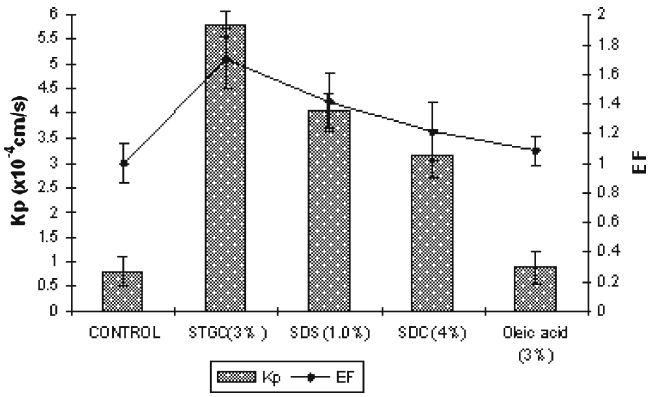

Fig. 5. Permeability coefficients (K_p) and enhancement factor (EF) for FU across buccal mucosa in presence and absence of penetration enhancers

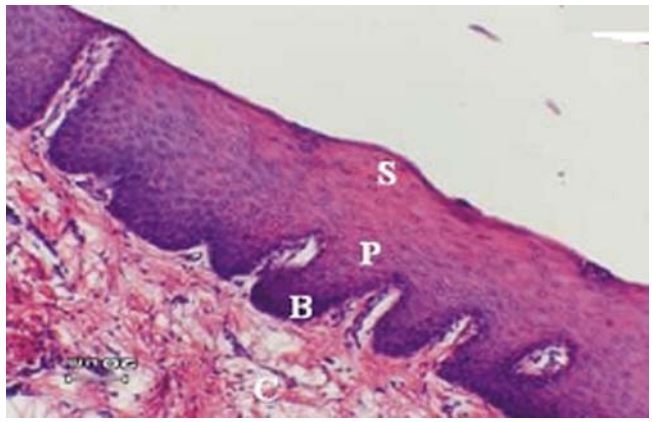

Fig. 6. Photomicrograph (×40) of buccal mucosa (control); *S* superficial cells, *P* prickle cells, *B* basal cells, *C* connective tissue

Histology Studies

Figure 6 shows a photo micrograph of buccal mucosa depicting the outermost layer of stratified squamous epithelium, below which can be seen the basement membrane, lamina propria, and submucosa. The boundary between the buccal epithelium and the connective tissue is delineated by the basement membrane.

Typical photo micrographs of buccal epithelium after 2 h treatment with 3% STGC is shown in Fig. 7 where epithelial layer is intact. No noticeable morphological changes are visible in the underlying layers (like in prickle cells). While after 6 h treatment, no significant loss of superficial cell layers was observed, but there is formation of vacuoles especially in the prickle cell layers (Fig. 8). In addition, an increase in intercellular space and swelling, especially inside the cells was observed. Thus, it can be concluded that STGC altered the histology of the buccal epithelium and with increasing the time of STGC exposure, the morphological changes became more progressive in the tissue. These histological findings explain well the permeation results where the permeation of FU through the buccal mucosa increased significantly in presence of STGC.

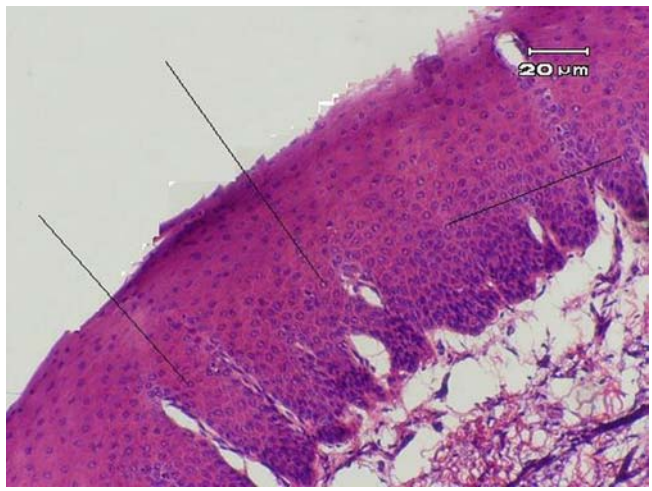

Fig. 7. Photomicrograph (×40) of buccal mucosa (after 2 h exposure). *Arrows* indicate formation of vacuoles

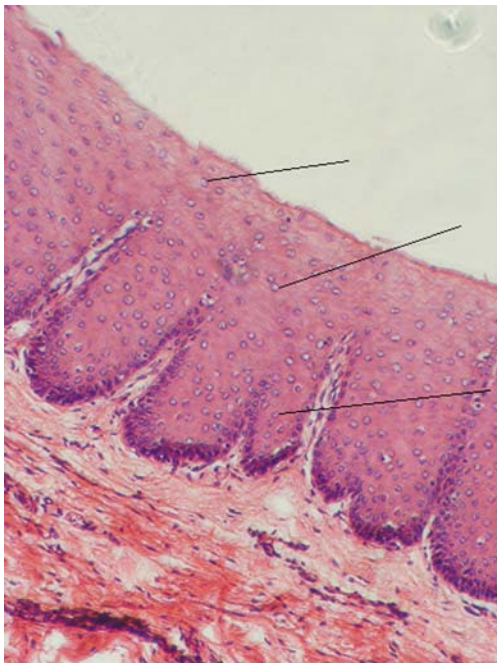

Fig. 8. Photomicrograph (×40) of buccal mucosa (after 6 h exposure). *Arrows* indicate formation of vacuoles

This can be collaborated by the reports that bile salts (like STGC and SDC) accumulate in the tissue after penetration into the tissue without causing a loss of superficial cell layers and then interact with the intercellular or membrane lipids thus increasing the permeability of the permeant through the epithelium (6). Swelling of the cells may be due to the diffusion of STGC into cells as the tissue only with phosphate buffer (control) when subjected to incubation for same time does not show any swelling. The effect of bile salts on the permeability barrier has been reported to be reversible and dependent on the concentration of the bile salts (5,10).

Preparation of FU Mucoadhesive Gels

The prepared gels were clear and yellowish brown in color. The bioadhesive strength and viscosity of the optimized formulation were 12.8 (±1.4)×10^3 dyne/cm^2 and 544.49 (± 5.9) cps, respectively (29). No interactions were found between formulation ingredients and FU (29).

Stability of FU in the Gels

Table II depicts results of stability study of FU bioadhesive gels. No macroscopical physical changes were observed during storage. Assay, pH, and viscosity values of the formulations carried out at 3 months showed no significant differences. Hence, it was concluded that the FU bioadhesive gels exhibited good stability.

Table II. Stability of FU in the Gels

		2 months	3 months
Parameter	Initial	25±2°C	25±2°C
pH	6.12 (±0.028)	6.08 (±0.112)	6.23 (±0.217)
Assay (% *w*/*w*)	99.74 (±0.225)	99.05 (±0.201)	97.05 (±0.095)
Viscosity (cps)	544.49 (±5.9)	534.9 (±3.7)	532.12 (±6.4)

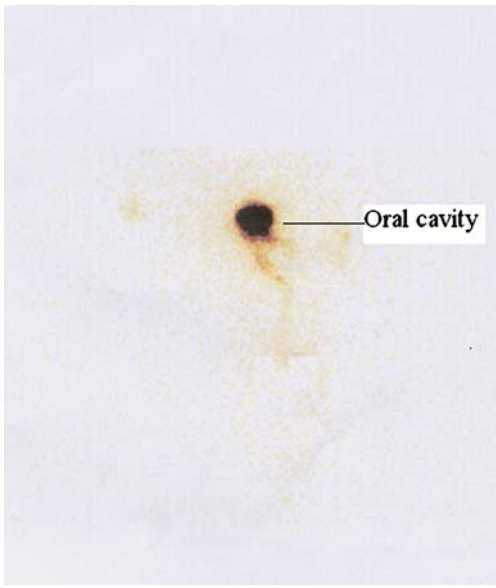

Fig. 9. Scintigraphic image of rabbit 6 h after gel administration

In Vivo Bioadhesion

The γ-scintigraphic images (Fig. 9) of the rabbits taken after 6-h post-administration of buccoadhesive gels showed the presence of major portion of gels in the buccal cavity indicating *in vivo* bioadhesion of the gels.

Pharmacokinetics

The plasma concentration–time curve for FU after the administration of buccal gels and i.v. injection to rabbits of a single 20 mg dose (Marketed FU injection, I.P., Fluracil®, Biochem Pharmaceutical Industries, Mumbai) is shown in Fig. 10 and the plasma concentration–time curve for FU

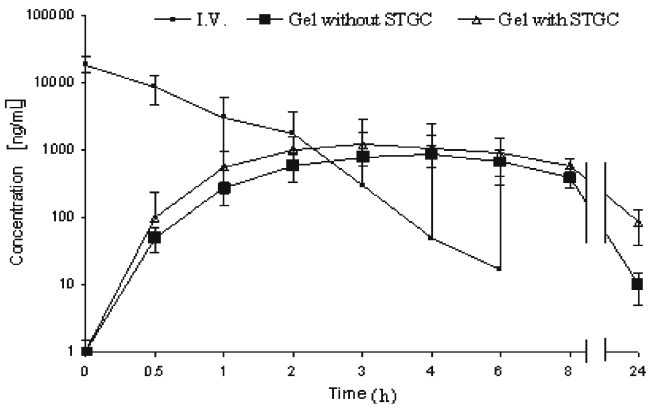

Fig. 10. Plasma concentration–time profile of FU following i.v. administration and buccal administration of the mucoadhesive gels to rabbits ($n=6$)

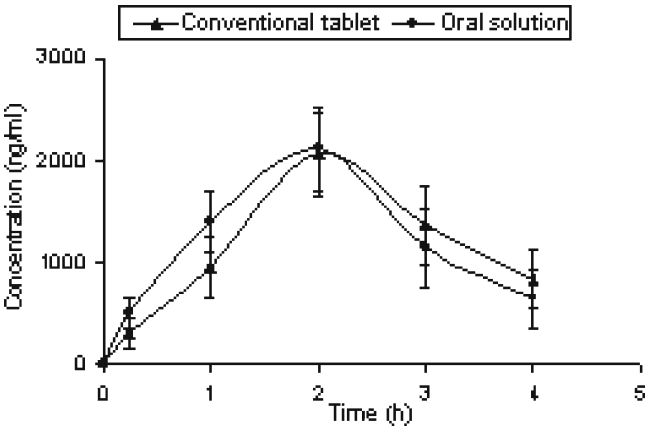

Fig. 11. Plasma concentration–time profile of FU following oral administration as conventional tablets and solution to rabbits ($n=6$)

following oral administration as conventional tablets and solution to rabbits is shown in Fig. 11. The pharmacokinetic parameters of FU following buccal administration of bioadhesive gels, oral tablet, oral solution, and the intravenous injection are summarized in Table III.

The average area under the plasma concentration–time curve, the value of AUC for the intravenuous administration was 21,467.79±1,701.12 hng/ml. Following buccal administration of a single 20 mg dose of FU to rabbits, the value of AUC of buccal administration of gels with enhancer was 12,653.65±1,405 hng/ml and that without enhancer (control) was 7,787.99±1,054 hng/ml. Within each study, no significant differences were observed among the formulations ($p>0.05$). Absolute bioavailability for oral solution and conventional tablets was 32.92% and 32.16%, respectively, and the difference is statistically insignificant. The absolute bioavailability of gel following buccal administration without an enhancer (control) was 36.28% compared with intravenous administration while the absolute bioavailability of FU gel containing STGC was 58.52% compared with intravenous administration. The lower absolute bioavailability for oral solution and conventional tablets could be due to the extensive first pass metabolism of FU (17). Thus, the buccal absorption of FU from the gel containing STGC as an enhancer was significantly higher ($p<0.05$) than that from the gel without enhancer. Among all the formulations, gels with enhancer displayed higher bioavailability than oral formulations. The difference in bioavailability from oral formulations (i.e. conventional tablets and oral solution) and gels with enhancer is statistically significant ($p<0.05$).

The sustained-release characteristics of the bioadhesive gels were also reflected in the MRT and the MAT of FU in the body. Both these parameters were considerably increased following buccal administration of the gels as compared to i.v. administration. The average MRT after buccal administration for control group and enhancer group were 6.06±1.54 h and 8.08±1.85 h, respectively, while the MAT values were 4.99± 0.79 h and 7.01±1.22 h for control group and enhancer group, respectively.

Statistical analysis of the C_{max} and t_{max} values observed following the buccal administration of the FU formulations showed that enhancer group exhibited higher average C_{max} values of 1,189.75±362 ng/ml than those of 860±214 ng/ml which was achieved by the control group, but those differences were statistically insignificant ($p>0.05$). The t_{max} of the gel with enhancer (STGC) was 3.0±1.25 h while it was 4.0± 1.08 h from the control group (Table III). The buccal administration of gels containing enhancer showed sustained and enhanced absorption. Between the subjects the difference was non-significant ($p>0.05$) indicating that there was less subject-to-subject variation.

CONCLUSION

STGC emerged as the most efficient enhancer among the enhancers investigated in the current study for buccal absorption of FU. At 3% concentration, it showed highest K_p and EF across the buccal mucosa. Histological investigations revealed an increase in intercellular space and swelling, especially inside the cells which may be responsible for the permeation enhancement. *In vivo* study portrays an increase in absolute bioavailability of FU from buccoadhesive gels with STGC as compared to the control.

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Table III. Pharmacokinetics of FU from the Buccal Oral Tablets, Mucoadhesive Gels and Intravenous Injection

Parameters#	Oral conventional tablet	Oral solution	Gel without STGC (control)	Gel with STGC	i.v.
AUC (ng h/ml)	6,562.73±854	7,069±902	7,787.99±1,054	12,653.65±1,405	21,467.79±1,701.12*
C_{max} (ng/ml)	2,058.25±214	2,112.25±314	860±214	1,189.75±362	–
T_{max} (h)	2.0±0.75	2.0±0.82	4.0±1.08	3.0±1.25	–
MRT (h)	3.23±1.05	2.72±1.34	6.06±1.54	8.08± 1.85**	1.15±0.86
MAT (h)	1.96±0.47	1.57±0.64	4.99±0.79	7.01±1.22	–
A. B. (%)	32.16	32.92	36.28	58.52	100

Each value represents the mean±SD of six determinations
A.B. absolute bioavailability to IV AUC (%)
*$P<0.001$; **$P<0.05$

REFERENCES

1. M. E. DeVries, E. Bodde, J. Coos, and H. E. Junginger. Development in buccal drug delivery. *Crit. Rev. Ther. Drug Carrier Syst*. 271–303 (1991).
2. D. Harris, and J. R. Robinson. Drug delivery via the mucous membranes of the oral cavity. *J. Pharm. Sci.* **81**(1):1–10 (1992).
3. B. J. Aungst, and N. J. Rogers. Comparison of the effects of various transmucosal absorption promoters on buccal insulin delivery. *Int. J. Pharm.* **53**(3):227–235 (1989).
4. V. H. Lee, A. Yamamoto, and U. B. Kompella. Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Crit. Rev. Ther. Drug Carrier Syst.* **8**(2):91–192 (1991).
5. A. J. Hoogstraate, S. Senel, C. Cullander, J. Verhoef, H. E. Junginger, and H. E. Boddé. Effects of bile salts on transport rates and routes of FITC-labelled compounds across porcine buccal epithelium *in vitro*. *J. Control. Rel.* **40**:211–221 (1990).
6. S. Senel, Y. Çapan, M. F. Sargon, G. Ikinci, D. Solpan, O. Güven, HE. Boddé, and A. A. Hincal. Enhancement of transbuccal permeation of morphine sulfate by sodium glycodeoxycholate *in vitro*. *J. Control. Rel.* **45**(2):153–162 (1997).
7. J. Lee, and I. W. Kellaway. Combined effect of oleic acid and polyethylene glycol 200 on buccal permeation of [D-Ala^2, D-Leu^5]enkephalin from a cubic phase of glyceryl monooleate. *Int. J. Pharm.* **204**(1–2):137–144 (2000).
8. S. C. Shin, J. Y. Kim, and I. J. Oh. Mucoadhesive and physicochemical characterization of carbopol-poloxamer gels containing triamcinolone acetonide. *Drug. Dev. Ind. Pharm.* **26**:307–312 (2000a).
9. S. C. Shin, J. P. Bum, and J. S. Choi. Enhanced bioavailability by buccal administration of triamcinolone acetonide from the bioadhesive gels in rabbits. *Int. J. Pharm.* **209**(1–2):37–43 (2000b).
10. J. Zhang, S. Niu, C. Ebert, and T. H. Stanley. An *in vivo* dog model for studying recovery kinetics of the buccal mucosa permeation barrier after exposure to permeation enhancers: apparent evidence of effective enhancement without tissue damage. *Int. J. Pharm*. **101**(1–2):15–22 (1994).
11. M. J. Rathbone, B. K. Drummond, and I. G. Tucker. The oral cavity as a site for systemic drug delivery. *Adv. Drug. Del. Rev.* **13**:1–22 (1994).
12. M. Nair, and Y. W. Chien. Buccal delivery of progestational steroids. I. Characterisation of barrier properties and effect of penetrant hydrophilicity. *Int. J. Pharm*. **89**:41–49 (1993).
13. G. Liu, E. Franssen, M. I. Fitch, and E. Warner. Patient preferences for oral *versus* intravenous palliative chemotherapy. *J Clin. Oncol.* **15**:110–115 (1997).
14. www.cancer.gov
15. B. C. Rudy, and B. Z. Senkowski. Fluorouracil. In K. Florey (ed.), *Analytical Profiles of Drug Substances*, 2:Academic Press, New York, 1973, pp. 221–244.
16. A. C. Williams, and B. W. Barry. Terpenes and the lipid-protein partitioning theory of the skin penetration enhancement. *Pharm. Res*. **8**:17–24 (1991).
17. Dollery Collin. (Ed.), Therapeutic Drugs. Vol 1, 2nd edition, Churchill Livingstone, Edinburgh, 1999; F 103–108.
18. R. B. Diasio, and B. E. Harris. Clinical pharmacology of 5-fluorouracil. *Clin. Pharmacokinet.* **16**:215–237 (1989).
19. B.N. Singh, R. B. Singh, and J. Singh. Effects of ionization and penetration enhancers on the transdermal delivery of 5-fluorouracil through excised human stratum corneum. *Int. J. Pharm.* **298**(1):98–107 (2005).
20. S. Senel, and A. A. Hıncal. Drug permeation enhancement via buccal route: possibilities and limitations. *J. Control. Rel.* **72**:133–144 (2001).
21. M. A. Attia, I. E. Gibaly, S. E. Shaltout, and G. N. Fetih. Transbuccal permeation, anti-inflammatory activity and clinical efficacy of piroxicam formulated in different gels. *Int. J. Pharm.* **276**(1–2):11–28 (2004).
22. V. H. M. Deneer, G. B. Drese, P. E. H. Roemele, J. C. Verhoef, L. A. Huen, J. H. Kingma, J. R. B. J. Brouwers, and H. E. Junginger. Buccal transport of flecainide and sotalol: effect of a bile salt and ionization state. *Int. J. Pharm.* **241**:27–134 (2002).
23. Gantrez ® Copolymers, International Speciality Products. New Jersey, USA, 2005.
24. S. C. Shin, C. W. Cho, and K. H. Yang. Development of lidocaine gels for enhanced local anesthetic action. *Int. J. Pharm.* **287**:73–78 (2004).
25. M. A. Nassim, F. Shirazi, C. M. Cripps, S. Veerasinghan, M. J. Molepo, M. Obrocea, D. Redmond, S. Bates, D. Fry, D. J. Stewart, and R. Goel. An HPLC method for the measurement of 5-fluorouracil in human plasma with a low detection limit and a high extraction yield. *Int. J. Mol. Medicine.* **10**:513–516 (2002).
26. S. Senel, A. J. Hoogstraate, F. Spies, J. C. Verhoef, G. A. Bosvan, H. E. Junginger, and H. E. Boddé. Enhancement of *in vitro* permeability of porcine buccal mucosa by bile salts: kinetic and histological studies. *J. Control Rel.* **32**:45–56 (1994).
27. A. Aioi, K. Kuriyama, T. Shimizu, M. Yoshioka, and S. Uenoyama. Effects of vitamin E and squalene on skin irritation of a transdermal absorption enhancer, laurylsarcosine. *Int. J. Pharm.* **93**:1–6 (1993).
28. I. R. Schmolka. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. *J. Biomed. Mater. Res*. **6**:571–582 (1972).
29. M. Dhiman, P. D. Yedurkar, and K. K. Sawant. Formulation, Characterization and *in vitro* evaluation of bioadhesive gels containing 5-Fluorouracil. *Pharm. Dev. Tech.* **13**(1):15–25 (2008).
30. C. Tas, Y. Ozkan, A. Savaser, and T. Baykara. *In Vitro* and *ex vivo* permeation studies of chlorpheniramine maleate gels prepared by carbomer derivatives. *Drug Dev. Ind. Pharm.* **30** (6):637–647 (2004).
31. N. Subramanian, *et al*. Radiolabeling, Biodistribution and Tumor Imaging of Stealth Liposomes Containing Methotrexate. Alasbimn Journal 6(22): October 2003. Article No.AJ22–6.
32. Y. Jacque, T. Nguyen-Xuan, E. Ionescu, G. P. Ravelli, P. Buri, P. Baehnid, and R. Gurny. *In vivo* evaluation of hydrophilic and hydrophobic mucoadhesive semi-solid formulations containing sucralfate and lidocaine for intraoral use. *Eur. J. Pharm. Biopharm.* **43**:59–63 (1997).
33. H. H. Alur, I. S. Pather, A. K. Mitra, and T. P. Johnston. Transmucosal sustained-delivery of chlorpheniramine maleate in rabbits using a novel, natural mucoadhesive gum as an excipient in buccal tablets. *Int. J. Pharm.* **188**(1):1–10 (1999).
34. M. Gibaldi. Biopharmaceutics and clinical pharmacokinetics, Lea and Febiger, Philadelphia, 1984.
35. J. A. Nicolazzo, B. L. Reed, and B. C. Finnin. Buccal penetration enhancers—How do they really work. *J. Control. Rel.* **105**(1–2):1–15 (2005).