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RESEARCH PAPER

Peptide Washout and Permeability from Glyceryl Monooleate Buccal Delivery Systems

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ABSTRACT

Simultaneous evaluation of the permeation and washout of a peptide from the mucoadhesive liquid crystalline phases of glyceryl monooleate (GMO) has been investigated using a donor compartment flow-through diffusion cell. [D-Ala^2, D-Leu^5]enkephalin (DADLE) was incorporated into the cubic and lamellar liquid crystalline phases of GMO and applied to excised porcine buccal mucosa mounted in the donor compartment flow-through cell. Phosphate-buffered saline pH 7.4 (PBS) was pumped across the upper surface of the liquid crystalline phases to mimic salivary flow. The steady-state fluxes of DADLE and GMO from the cubic phase were significantly greater than that from the lamellar phase ($P < 0.01$). There was no statistical difference between the amounts of DADLE and GMO washed out from the lamellar and cubic phases ($P > 0.05$). The donor compartment flow-through diffusion cell was found to be a useful tool to evaluate the impact of salivary washout on mucoadhesive oral mucosal delivery systems.

Key Words: *Diffusion cell; Glyceryl monooleate; Mucoadhesive liquid crystalline phases; Peptide buccal delivery; Washout*

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INTRODUCTION

Since the 1980s, peptides have been increasingly investigated as therapeutic agents. Parenteral administration is usually preferred to deliver such drugs effectively. However, due to their short biological half-lives, side effects associated with repeated injection are often problematic.[1] The buccal mucosa has gained prominence as an alternative route for the delivery of peptides. However, this route also suffers from low bioavailability due largely to the low mucosal membrane permeability, a relatively small surface available for absorption, and poor retention of drug and delivery systems.[2] Buccal delivery systems formulated with mucoadhesive materials can offer a number of opportunities to overcome some of the limitations associated with poor absorption. For example, a mucoadhesive buccal delivery system can establish and maintain intimate contact with the mucosal membrane for a longer period of time, and consequently prolong the delivery of drugs and achieve high drug concentrations at the site of administration. Furthermore, it also offers possibilities to improve local permeability and to inhibit the activities of metabolic enzymes, which are extremely advantageous for peptide drugs.[3]

Of the many mucoadhesives, lamellar and cubic liquid crystalline phases of GMO have demonstrated a water concentration-dependent mucoadhesive property[4] and feasibility to be used as buccal delivery carriers for peptides.[5–7] Since the mucoadhesive liquid crystalline phases of GMO are semi-solid at ambient temperature they can easily be applied manually to the mucosal surfaces in the oral cavity such as buccal, sublingual, and gingival mucosae. Although the liquid crystalline phases require aqueous media such as saliva to exhibit mucoadhesion, the drug incorporated into the liquid crystalline phases will be lost primarily into continuous oral secretions such as salivary flow. This washed out fraction of a drug will certainly result in decreased bioavailability. To evaluate the extent of drug washed out, we designed a donor compartment flow-through diffusion cell in which an aqueous medium continuously flows over the liquid crystalline phases adhering to the buccal mucosa. The aim of this study was to provide a donor compartment flow-through diffusion cell that can measure the washout of drug from mucoadhesive formulations applied to the oral mucosa and simultaneously the transport of drug across buccal tissue and into the receptor compartment. We chose a model peptide for this work, [D-Ala^2, D-Leu^5]enkephalin (DADLE, Mw = 569.7), due to its relative stability and bioavailability.[8–11]

MATERIALS AND METHODS

Materials

A commercially available grade of distilled glyceryl monooleate was obtained from Danisco Ingredients (Copenhagen, Denmark) and used as received. The peptide DADLE was obtained from Sigma-Aldrich Company (Poole, U.K.). [*Tyrosyl*-3,5-^{3}H(N)]DADLE ([^{3}H]DADLE, specific activity: 33.50 Ci/mmol) and monooleoyl-*rac*-glycerol [oleic 1-^{14}C] ([^{14}C]GMO, specific activity: 5 mCi/mmol) were purchased from Du Pont (Hertfordshire, U.K.) and American Radiolabeled Chemicals (St. Louis, MO), respectively. Liquid scintillation cocktail (HiSafe OptiPhase III) was from Fisher Scientific (Loughborough, U.K.). The NCS-II Tissue Solubilizer was supplied by Amersham Corporation (Arlington Heights, IL). All other chemicals were analytical grade, except acetonitrile and trifluoroacetic acid which were HPLC grade. Freshly prepared distilled water was used throughout. Phosphate-buffered saline pH 7.4 (PBS) was prepared using PBS tablets obtained from Sigma-Aldrich Company (Poole, U.K.).

Partition Coefficient of DADLE

The *n*-octanol/PBS partition coefficient was measured prior to investigating the DADLE transport across the porcine buccal mucosa. The PBS and *n*-octanol were co-saturated for 24 hr at 37°C. The two phases were then separated by centrifugation. The aqueous phase was used to prepare a DADLE solution (50 μg/mL). The aqueous DADLE solution (3 mL) was added to the organic phase (3 mL) and shaken in a shaker-bath at 37°C. After equilibration (3 hr), the two phases were separated following centrifugation at 2000 rpm for 10 min. Samples were taken from the aqueous phase and analyzed for DADLE by high-performance liquid chromatography (HPLC). The partition coefficient (K_p) was calculated from the following equation:

$$K_p = \frac{[C_1 - C_2]}{C_2}$$

where, C_1 is the original concentration of DADLE in aqueous phase and C_2 is the final concentration of DADLE in aqueous phase.

Stability of DADLE

Effect of Temperature on the Stability of DADLE in PBS

An aqueous solution of DADLE was made up with PBS at a concentration of 10 μg/mL. This solution (5 mL) was stored at 4, 22 (room temperature), and 37°C. Samples were withdrawn at set time intervals over 24 hr and stored in a freezer (−20°C) until assayed by HPLC.

Metabolic Stability of DADLE in Porcine Buccal Mucosal Tissue Homogenates

The buccal mucosal tissue homogenates were prepared as follows. Freshly excised porcine buccal mucosa was rinsed with ice-cold PBS. Any underlying muscle tissue was removed using a pair of fine tweezers and scissors. The tissue was then weighed, finely divided, and transferred to the ice-cold PBS (5 mL). The tissue sample was homogenized in an ice bath using a Polytron homogenizer (Model PT 20-00, The Northern Media Supply Ltd., Hull, U.K.). Pooled aliquots were centrifuged at 2000 rpm for 20 min at 4°C. The total protein concentration of supernatant was determined by a Pierce BCA protein assay kit (Pierce & Warriner Ltd., Chester, U.K.). The tissue homogenates were diluted with cold BPS to give a protein concentration of 10 mg/mL.

The buccal homogenates (100 μL) were preincubated for 15 min, mixed with 300 μL of DADLE solution (1 mg/mL in PBS), and kept at 37°C. Samples (50 μL) were withdrawn at predetermined time intervals and added to acetonitrile (100 μL) to terminate the reaction by precipitating the tissue protein. This mixture was centrifuged at 100,000 rpm for 10 min and the supernatant was assayed for DADLE by HPLC.

HPLC Assay of DADLE

The concentration of DADLE was measured by isocratic reverse-phase HPLC (RP-HPLC) using a Hypersil C_{18} column (Hichrom Limited, Reading, U.K.) at ambient temperature, with detection at 214 nm The RP-HPLC system consisted of a ConstaMetric 3000 solvent delivery system (Laboratory Data Control, Stone, U.K.), SpectraSYSTEM UV1000 detector (Thermoquest, Withenshaw, U.K.), and Berthold integration software (Berthold Instruments, Herts, U.K.). The mobile phase was acetonitrile–0.1% trifluoroacetic acid 30:70 v/v) at a flow rate of 1.0 mL/min.

Buccal Permeation Studies

Preparation of Liquid Crystalline Phases

The cubic and lamellar liquid crystalline phases of GMO contained 35% w/w and 16% w/w water, respectively. The required amount of [^{3}H]DADLE (6 μCi/g) and [^{14}C]GMO (3 μCi/g) in ethanol was dispensed into a glass vial and the storage solvent was evaporated under a nitrogen stream at room temperature. Water and DADLE (200 μg/g) were also added to the vial and kept at 50°C. This aqueous mixture was added to molten GMO. Samples were incubated for 3 days and then allowed to equilibrate at room temperature for 5 days.

Buccal Permeation and Washout of DADLE

The donor compartment flow-through diffusion cell (Fig. 1) was used to simultaneously measure the amounts of DADLE and GMO permeated across the porcine buccal mucosa and the amounts of DADLE and GMO washed out from the liquid crystalline

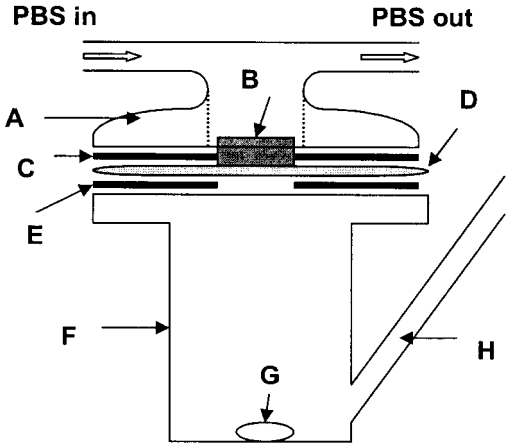

Figure 1. Schematic illustration of the donor compartment flow-through diffusion cell assembly. Key: A, donor compartment; B, liquid crystalline phase; C, top washer; D, porcine buccal mucosa; E, bottom washer; F, receiver compartment; G, magnetic stirrer; H, sampling arm.

phases applied onto the surface of the porcine buccal mucosa. Porcine buccal tissue was freshly excised and stored in PBS at 4°C. The mucosal membrane was separated by removing the underlying connective tissue with tweezers and surgical scissors. The liquid crystalline phases were applied onto the porcine buccal mucosa and the diffusion cell was assembled by a previously reported method.[7] The PBS was pipetted into the receiver compartment to initiate a diffusion experiment. The PBS was separately maintained at 37°C and pumped over the liquid crystalline phases applied onto the porcine buccal mucosa at a constant rate of 1.0 mL/min to mimic the salivary flow on the formulation.[12] The PBS that flowed through the donor compartment was collected in a beaker and stirred by a magnetic stirrer. Samples (0.2 mL) were withdrawn from the receiver compartment and from the beaker, and added to 3 mL of liquid scintillation cocktail. Fresh PBS replaced both samples. A DADLE permeation study using a standard Franz diffusion cell was also performed with the same experimental conditions. Liquid scintillation counting was used to determine levels of radioactivity that permeated into the receiver compartment and washed out.

At the end of the experiment (8 hr), the buccal tissue was thoroughly washed with 50% ethanol to remove the residual liquid crystalline phases and weighed. The tissue was then digested with 1 mL of NCS II Tissue Solubilizer at 37°C for 3 days. Acetic acid (30 μL) was added to neutralize the buccal tissue solution. Assay for the radioactivity found in the buccal tissue was carried out by liquid scintillation counting after the addition of 3 mL of liquid scintillation cocktail.

Data Analysis

The buccal permeation results were statistically analyzed using Analysis of variance (ANOVA) and P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Stability studies and partition coefficient measurement of DADLE were carried out since buccal absorption of peptides can be related to these two parameters.[13] The apparent *n*-octanol/PBS partition coefficient ($\log K_p$) of DADLE at 37°C was −0.13, indicating that DADLE is a rather hydrophilic peptide and therefore rapid permeation across the porcine buccal mucosa was not expected.[14]

The stability of DADLE was determined in PBS at three different temperatures. The DADLE exhibited no substantial degradation at the temperatures tested. No detectable degradation products were found in the chromatogram. Over 98% of DADLE was intact after 24 hr in PBS maintained at 4, 22, and 37°C. The degradation rate of DADLE after incubation with the porcine buccal mucosa homogenates was determined by measuring the disappearance of DADLE by RP-HPLC. One unknown degradation product of DADLE was detected. The DADLE disappearance in the buccal homogenates followed first-order kinetics, and therefore the degradation rate constant (k) was calculated from the degradation data using the following equation:

$$k = \frac{2.303}{t} \log \frac{A}{A - x}$$

where A is the initial amount of DADLE and x is the amount degraded in time t. The half-life of DADLE was 25.01±1.66 hr, calculated from the first-order rate constant. Uchiyama et al.[11] have compared the metabolic stability of DADLE with leucine enkephalin in the rat intestinal homogenates. They observed that DADLE is highly resistant to degradation by aminopeptidases, carboxypeptidases, and dipeptidylaminopeptidases compared with leucine enkephalin due to the substitution of D-Ala for L-Gly at the second amino acid and D-Leu for L-Leu at the C-terminal amino acid. The half-life of DADLE in the buccal homogenate was much longer than those measured in jejunal (126±3.2 min) and colonic (240±5.8 min) homogenates.[11] This is probably because the proteolytic activity in the buccal mucosa is lower than in the intestinal mucosa.[15]

The liquid crystalline phases of GMO have acted as permeation enhancers for peptides, and the permeation-enhancing effect of the cubic phase is greater than that of the lamellar phase.[6] For this reason, a greater permeation of DADLE was achieved from the cubic phase than the lamellar phase (Fig. 2). The GMO liberated from the liquid crystalline matrix permeated the porcine buccal mucosa in the donor compartment flow-through cell and the permeation of GMO was also greater from the cubic phase than from the lamellar phase (Fig. 2). The greater permeation of GMO from the cubic phase can be

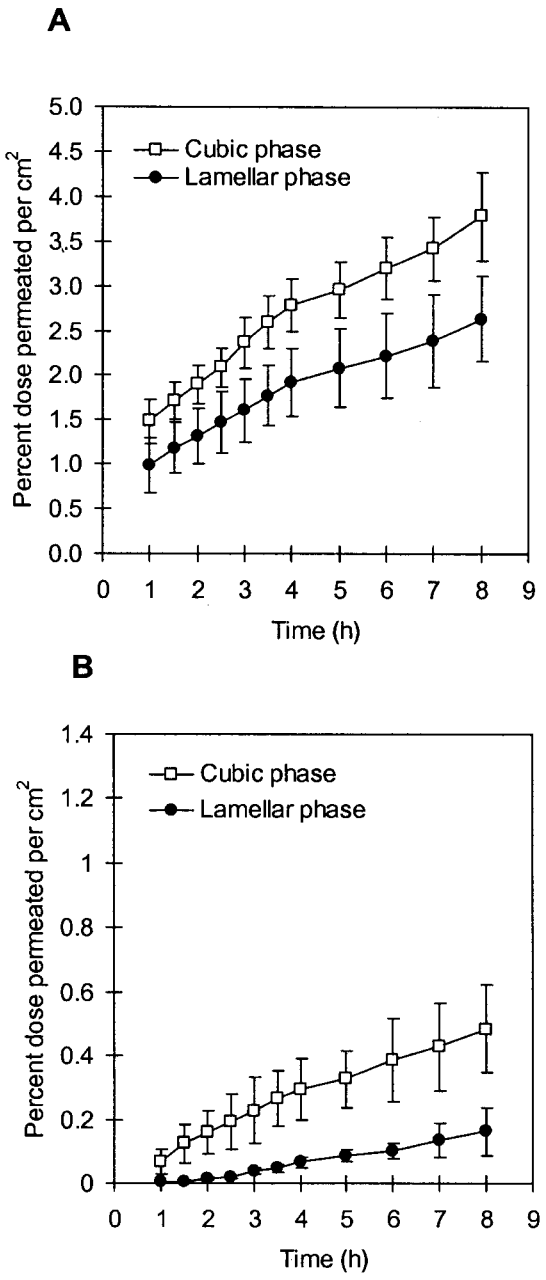

Figure 2. Buccal permeation profiles of DADLE (A) and GMO (B) from the cubic and lamellar liquid crystalline phases in the donor compartment flow-through diffusion cell. Mean±SD, n=5.

attributed to its greater in vitro release profile.[6] Since DADLE and GMO released from the cubic and lamellar phases permeate the buccal mucosa by co-transport mechanism,[6,7] the permeation rates of DADLE and GMO are mainly influenced by the release rates of DADLE and GMO from the liquid crystalline phases.

The permeation rates obtained using the donor compartment flow-through cell were smaller than those obtained using the static Franz cell, probably due to the continuous loss of initial applied dose. When a PBS stream of 1.0 mL/min was applied to the dose on the buccal mucosa, the steady-state (t < 4 hr) flux of DADLE from the cubic phase was significantly (P<0.01) decreased from 1.21±0.32 to 0.44±0.05% dose/cm^2/hr (Fig. 3). The DADLE permeation from the lamellar phase also significantly (P<0.01) decreased from 1.15±0.11 to 0.31±0.03% dose/cm^2/hr, when measured using the donor compartment flow-through cell (Fig. 3). Like DADLE, the flux values of GMO (0.07±0.02 and 0.02±0.01% dose/cm^2/hr for cubic and lamellar phases, respectively) obtained using the donor compartment flow-through cell decreased compared to those obtained using the static Franz cell (0.10±0.03 and 0.07±0.00% dose/cm^2/hr for cubic and lamellar phases, respectively) (Fig. 3). However, there was no statistical difference between the GMO fluxes from the cubic phases in the donor compartment flow-through cell and static cell. Therefore it can be considered that the cubic phase is more resistant to the flow of biological fluids, such as saliva, that may erode the dosage form.

The measurement of DADLE and GMO washed out revealed no statistical differences between the lamellar and cubic phases (Fig. 4). This is probably because the PBS that flowed through the donor compartment caused a rapid transformation of the lamellar phase to the cubic phase at the interface between the liquid crystalline phases and PBS stream. Indeed, the cubic phase of GMO is formed from the lamellar phase by increasing the temperature or increasing the water content of the lamellar phase.[16]

The quantity of DADLE and GMO found in the liquid crystalline phases (donor compartment), receiver compartment, buccal tissue, and washout fraction after 8 hr of the experiment is presented in Fig. 5 as the percentage of total amount applied to the tissue. The washout of DADLE is much greater than that of GMO. This may indicate that the loss of DADLE is mainly caused by transport of the peptide through the water channels in the liquid crystalline phases, rather than by the erosion of liquid crystalline matrices.

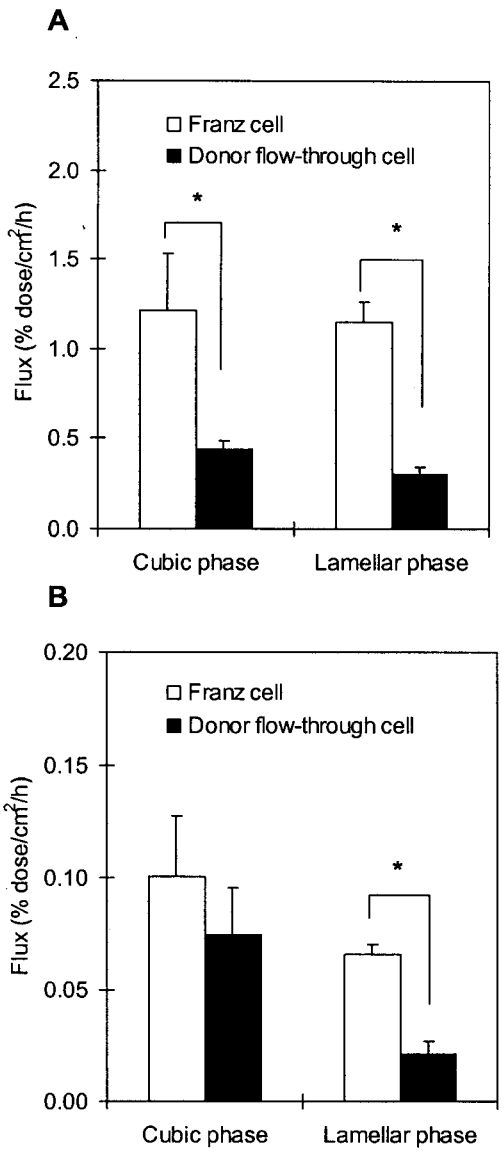

Figure 3. Comparison of flux values of DADLE (A) and GMO (B) from the cubic and lamellar liquid crystalline phases in Franz cell (white columns) and donor compartment flow-through cell (black columns). Mean±SD, n=5. $^{*}P<0.01$.

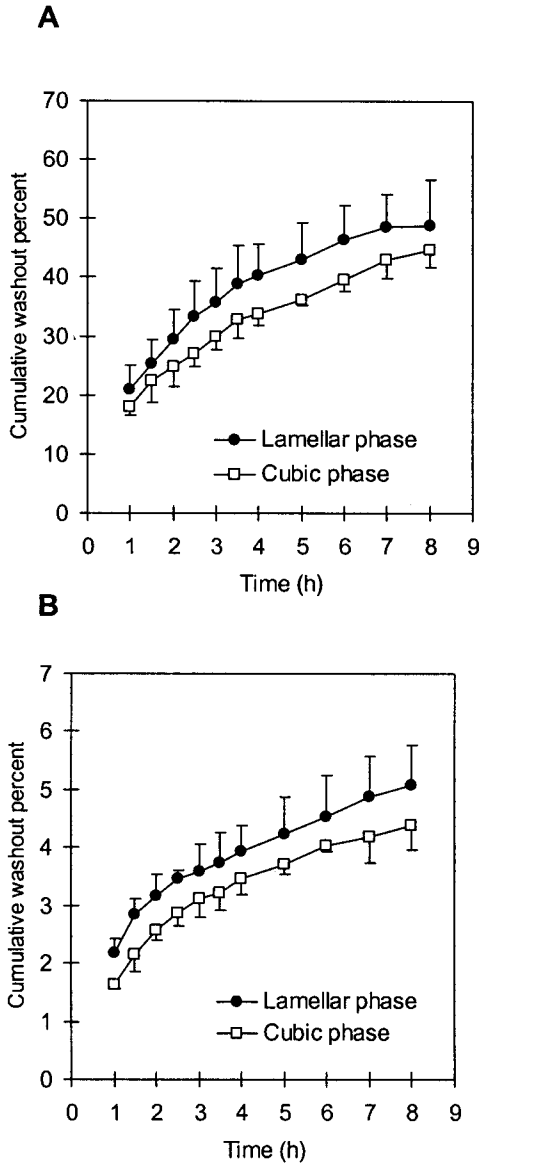

Figure 4. Percentage of DADLE (A) and GMO (B) washed out from the cubic and lamellar liquid crystalline phases in the donor compartment flow-through diffusion cell. Mean±SD, n=5.

CONCLUSIONS

In DADLE, we appear to have a suitable model peptide to investigate the ex vivo buccal transport, as it was relatively stable to enzymatic degradation. The half-life of DADLE in buccal mucosa homogenates was much longer compared to jejunal and colonic homogenates. This may imply that the buccal mucosa is an effective site for delivering peptide and protein drugs into the systemic circulation.

The donor compartment flow-through diffusion cell made it possible to measure simultaneously the permeation and washout of DADLE from the liquid crystalline phases of GMO. The transport of DADLE from the liquid crystalline phases into the PBS

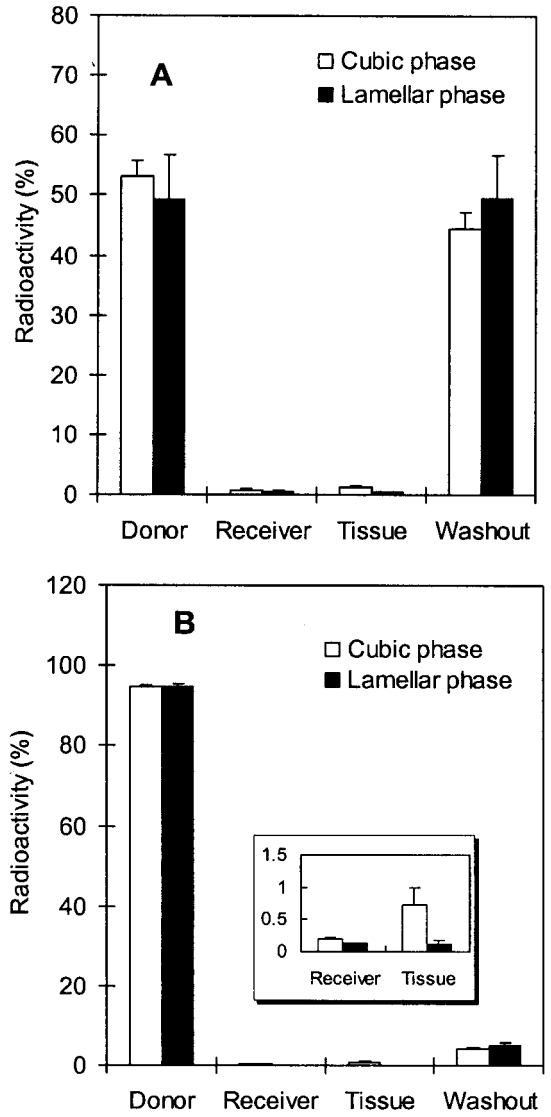

Figure 5. Amounts of DADLE (A) and GMO (B) found in donor and receiver compartments, digested tissue, and washout fraction after 8 hr. Mean±SD, n=5.

medium resulted in a decrease in the permeation rate of DADLE. The GMO was also washed out from the liquid crystalline phases, probably indicating the erosion of the liquid crystalline phases. However, the extent of the washout of GMO was very low, implying that there was no substantial breakdown of liquid crystalline matrices by the flow of PBS. The donor compartment flow-through diffusion cell is therefore a useful tool to study the impact of salivary washout on mucoadhesive oral mucosal delivery systems.

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