Microemulsion containing triamcinolone acetonide for buccal administration

Elsevier Editorial System(tm) for European Journal of Pharmaceutical Sciences
Manuscript Draft

Manuscript Number: EJPS-D-17-01504R2

Title: Microemulsion containing triamcinolone acetonide for buccal administration.

Article Type: Research Paper

Keywords: triamcinolone acetonide, buccal delivery, microemulsion,. chitosan

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Manuscript Region of Origin: ITALY

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The aim of the present work was to investigate the potential of microemulsions for the buccal administration of triamcinolone acetonide. ME were developed by the construction of pseudoternary phase diagrams, using the aqueous titration method. Among all microemulsions prepared and tested for stability, three were selected and submitted to characterization and in vitro permeation/retention experiments, using pig esophageal epithelium, an accepted model of the buccal mucosa. Furthermore, one microemulsion was added of excipients (stearylamine, CTAB and chitosan) able to alter the charge of droplets.
The results obtained show that the permeation of triamcinolone acetonide across pig esophageal epithelium was not influenced by the droplet size nor by the composition, but only by the presence chitosan, polysaccharide able to increase the transport across mono and stratified epithelia. The determination of the permeation parameters allowed us to show that chitosan acts on the diffusion parameter across the tissue and not on the partitioning parameter; for the same reason the tissue retention of triamcinolone acetonide was not modified. Triamcinolone flux (2.6 µg cm-2 h-1) was too low to make systemic administration feasible (dose required 2.5 to 60 mg/day).
The amount of triamcinolone acetonide recovered in the mucosa after only 10 min. of microemulsion application was much higher than after overnight application of the commercial paste Omicilon® A. This suggests that triamcinolone acetonide microemulsions can be an interesting alternative to the commercial formulation to treat diseases of the buccal mucosa. Owing to the fast uptake by the tissue, the formulation can be used as a mouthwash.

UNIVERSITÀ
DI PARMA

**DIPARTIMENTO DI SCIENZE
DEGLI ALIMENTI E DEL FARMACO**

Parma, 15 January, 2018

Dear Editor,

With this letter, I wish to submit the revised version of the manuscript entitled "**Microemulsion containing triamcinolone acetonide for buccal administration**" to be considered for the publication in the *European Journal of Pharmaceutics Sciences*.
All reviewer's comments were taken into account.
Waiting to receive the editorial decision, I send you my best personal regards and my greetings for the incoming holydays.

Patrizia Santi

## Abstract

The aim of the present work was to investigate the potential of microemulsions for the buccal administration of triamcinolone acetonide. *ME were developed by the* construction of pseudoternary phase diagrams, using the aqueous titration method. Among all microemulsions prepared and tested for stability, three were selected and submitted to characterization and *in vitro* permeation/retention experiments, using pig esophageal epithelium, an accepted model of the buccal mucosa. Furthermore, one microemulsion was added of excipients (stearylamine, CTAB and chitosan) able to alter the charge of droplets.

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***Response to Reviewers**

Reviewers' comments:

Reviewer #2: Report about EJPS-D-17-01504R1

Minor comments
Graphical Abstract
- It is still not straightforward, nor understandable without reading the entire manuscript, even though, is much better than the first one. I suggest the authors to modify it by using figures, which is better than graphics to tell the story.

**A: The graphical abstract has been modified, following to the reviewer's suggestion.**

Abstract
- The abstract is concise and direct. Even though, the sentence about the systemic use could be reviewed.

**A: The sentence about the systemic use has been changed to the following: "Triamcinolone flux (2.6 µg $cm^{-2}$ $h^{-1}$) was too low to make systemic administration feasible (dose required 2.5 to 60 mg/day).".**

Results
- Page 14 - Line 338 - Is the administration into the mouth classified as topical?

**A: Drug delivery to the mouth, to treat diseases of the oral mucosa, is considered topical (cfr. Topical corticosteroids and lesions of the oral mucosa, D.N. Thorburn and M.M. Ferguson, Adv. Drug Del. Rev., 13 (1-2), 135-149 (1994)).**

*Graphical Abstract (for review)

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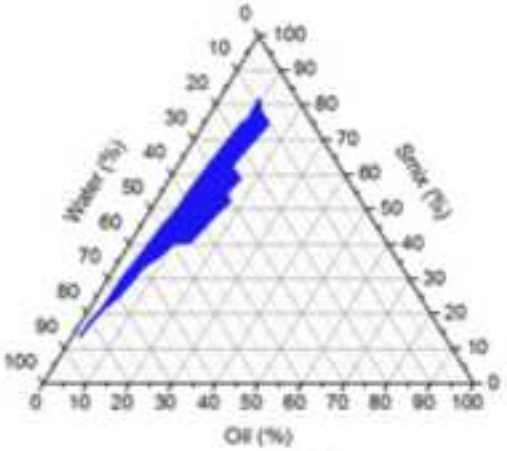
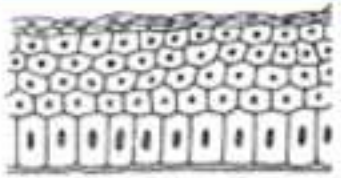
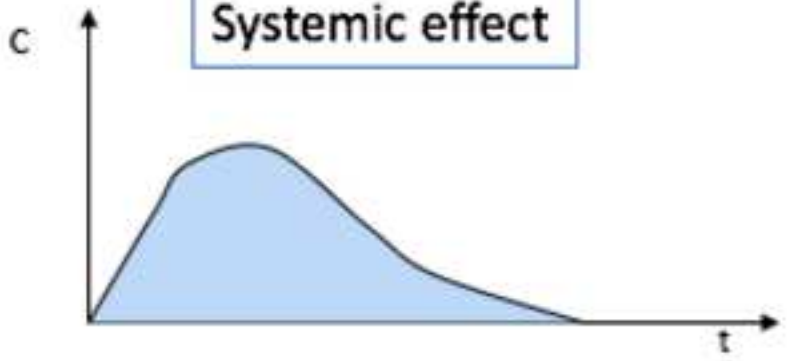



Topical effect



Buccal mucosa




Systemic effect

# Microemulsion containing triamcinolone acetonide for buccal administration.

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**Abstract**

The aim of the present work was to investigate the potential of microemulsions for the buccal administration of triamcinolone acetonide. ME were developed by the construction of pseudoternary phase diagrams, using the aqueous titration method. Among all microemulsions prepared and tested for stability, three were selected and submitted to characterization and *in vitro* permeation/retention experiments, using pig esophageal epithelium, an accepted model of the buccal mucosa. Furthermore, one microemulsion was added of excipients (stearylamine, CTAB and chitosan) able to alter the charge of droplets.

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**Key-words:** triamcinolone acetonide, buccal delivery, microemulsion, chitosan

## 1 Introduction

In the last decades microemulsions (MEs) have been studied as an interesting delivery vehicle for lipophilic drugs. These systems are easy to prepare and characterized by high thermodynamic stability; they can, in principle, solubilize both hydrophilic and lipophilic drugs and have been demonstrated to be able to enhance tissue uptake. MEs have been widely investigated for skin application, but only a few studies can be found in the literature for buccal drug delivery. Ceschel et al. tested microemulsions for the buccal delivery of S*alvia desoleana* essential oil [1], while Chukaewrungroj studied the use of microemulsions for the delivery of fluocinolone acetonide [2]. In 2004, the efficacy of a microemulsion loaded with mometasone furoate for the treatment of oral lichen planus was demonstrated *in vivo* [3]. Recently, promising results were obtained from the use of microemulsions, loaded into laminated sponges, for the transbuccal delivery of carvedilol [4]. Despite the few studies, the properties of MEs can be very interesting for buccal application because the presence of a specific combination of excipients can enhance and accelerate drug uptake, issue particularly relevant given the good barrier properties of the buccal epithelium and the short residence time that characterizes buccal administration.

MEs can, in principle, be used in different forms such as mouthwashes, oral sprays or gels. Liquid dosage forms, such as mouthwashes, allow the drug to reach all the areas of the oral mucosa, thus can be used to treat diffuse diseases that affect different parts of the oral cavity [5]. The application of a liquid vehicle can be useful in the case of painful diseases, because - if formulated with non-irritant excipients - liquid formulations are better accepted compared to the application of a solid dosage form. Oral sprays share the same advantages as liquid formulations; in addition, the drug is protected from the external environment and can achieve higher concentrations at the absorption site. Finally, MEs can be thickened to obtain mucoadhesive semisolid formulations, more suitable for treating easily accessible and localized mucosal diseases.

Triamcinolone acetonide (TA) is a long acting corticosteroid used topically and systemically (by the parenteral and oral routes); the typical dose by injection (½ to 1/3 of the oral dose), ranges from 2.5

to 60 mg/day according to the disease, whereas topically, 0.1% formulations are used. The drug is currently employed for many inflammatory conditions of the oral cavity, such as recurrent apthous stomatitis and lichen planus [6, 7]. Its permeation across and distribution into the buccal tissue has been studied *in vitro* [8] and *in vivo* [9, 10, 11] from several topical formulations (tablets, mouthwashes, bioadhesive gels, etc.). To increase TA transport and/or retention, penetration enhancers, such as bile salts and nonionic surfactants [12], liposomes [13], ethanol [14] and Azone® [15], were tested. In particular, Azone® doubled TA partitioning into the buccal mucosa and increased its permeation 4 times [16].

The aim of the present work was to investigate the potential of MEs for the buccal administration of TA. Microemulsion development was performed following a simple protocol based on a) drug solubility studies and excipients selection; b) construction of pseudoternary phase diagrams, using the aqueous titration method; c) accelerated stability tests. The prepared MEs were characterized for pH, structure, conductivity, droplet size distribution and zeta potential. Additives able to change the charge of the ME (stearyalmine, CTAB and chitosan) were also used.

Then, selected MEs were tested across pig esophageal mucosa, an accepted model of human buccal mucosa [17]. Permeation studies were performed in order to investigate TA transport across the tissue (in view of systemic administration) and mucosa retention (in view of local application) of the drug, in comparison with the commercial paste Omicilon® A.

## 2 Materials and methods

### 2.1 Materials

Triamcinolone acetonide was purchased from Metapharmaceutical (Barcelona, Spain) while, Transcutol® HP (diethylene glycol monoethyl ether), Labrasol® (PEG-8 caprylic/capric glycerides), Peceol® (glyceryl monoleate), Labrafac lipophile® (caprylic/capric triglyceride), Labrafac® PG (propylene glycol dicaprylocaprate), Lauroglycol® 90 (propylene glycol monolaurate), Plurol

oleique® (polyglyceryl-3 dioleate), Maisine® 35-1 (glyceryl monolinoleate), Capryol® 90 (Propylene glycol monocaprylate) were a gift from Gattefossé (Saint-Priest Cedex, France). Steraylamine, CTAB (cethyl-trimethyl ammonium bromide), Brij® 78 P (eicosaethylene glycol octadecyl ether) and Tween® 20 (polyoxyethylene (20) sorbitan monolaurate) were purchased from Sigma Aldrich (St. Louis, MO, USA). Chitosan (m.w. 10-1000 kDa), PEG 200, 400, 600, isopropyl myristate, myristyl myristate and propylene glycol were obtained from A.C.E.F. (Fiorenzuola, Italy). Oleic acid was obtained from Alfa Aesar (Karlsruhe, Germany) and TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate) from BASF (Ludwigshafen, Germany). Sorbitan monoleate 80 (Span® 80) was a gift from Croda Ibérica SA, Spain.

For HPLC analysis, MilliQ® water was used. Acetonitrile and methanol were of HPLC grade; all other reagents were of analytical grade. Omicilon® A Orabase (Bristol-Myers Squibb Farmacêutica S.A., San Paulo, Brazil) is a dental paste composed of gelatin, pectin, and carboxymethylcellulose sodium in Plastibase® (Plasticized Hydrocarbon Gel, a polyethylene and mineral oil gel base) and contains 0.1% of TA.

## 2.2 Methods

### 2.2.1 Solubility studies

An excess amount of TA was added to 2 ml of vehicle. Suspensions were left under magnetic stirring for 24 h at room temperature, then centrifuged for 10 minutes at 13000 rpm. The concentration of TA in the supernatant was determined by HPLC analysis after appropriate dilution.

### 2.2.2 Construction of pseudoternary phase diagrams

In order to identify the region of existence of microemulsions, pseudoternary phase diagrams were built (software Origin, OriginLab, Northampton, MA, USA). Beacuse microemulsions are made of 4 components, one axis reports “smix”, the total percentage of the mixture of surfactant/co-

surfactant; the following ratios of smix were prepared: 1/1, 1/2, 2/1, v/v. Then, mixtures of oil/smix in different ratios (1/9, 1.2/8.8, 1.25/8.75, 1.5/8.5, 1.7/8.3, 2/8, 2.3/7.7, 2.5/7.5, 3/7, 3.4/6.6, 4.5/5.5, 5/5, 6/4, 7/3, 8/2, 9/1, v/v) were prepared and added of known volumes of water, in order to obtain water concentrations between 5% and 95%; after each addition, the mixtures were visually inspected for transparency, opalescence, fluidity, and phase separation.

One ME was added of stearyalmine (0.5% w/v), CTAB (0.5% w/v) or chitosan (1.0 % w/v); these components were dissolved in the water phase and the ME was prepared as before.

### 2.2.3 Thermodynamic stability studies

For each pseudoternary diagram, five MEs were selected from the region of existence. The selected MEs were added of TA at 0.1 % w/v. Accelerated stability tests were then performed: formulations were first centrifuged for 30 min at 3500 rpm, then submitted to 6 cycles of heating (40°C) and cooling (4°C) of 48 hours each and then to 3 cycles of freezing (-20°C) and thawing (25°C) of 24 hours. MEs that did not pass these preliminary tests were not included in the following phase.

### 2.2.4 Microemulsions characterization

Droplet size and charge were measured using the light scattering technique, at 25° C with an incidence angle of 90°. The measures were performed on the native MEs, using a Brookhaven Instrument (PALS Zeta Potential Analyzer). Zeta potential was measured after 1:10 dilution in 1 mM KCl. The pH of o/w MEs was measured using an Orion 4 Star pH meter (Thermo Scientific, Waltham, Massachusetts, United States), at room temperature.

In order to assess isotropy, MEs were observed under a cross-polarized light microscope (Nikon, Shinjuku, Japan). Samples were deposited on a glass slide with a spatula, then covered with a covering slide in order to prevent the water evaporation.

MEs conductivity was measured at room temperature, using an AMEL 160 conductivity meter (Amel S.r.L., Milan, Italy) just after preparation and after 24 h.

MEs were analyzed for TA content by HPLC, after appropriate dilution.

### 2.2.5 *In vitro* permeation and retention studies

Permeation experiments were performed using Franz type diffusion cells, with a permeation area of 0.6 $cm^2$ (DISA, Milan, Italy). Pig esophageal epithelium was prepared according to Del Consuelo et al. [18]. The esophageal mucosa was separated from the outer muscle layer with a scalpel and the epithelium was peeled off from the connective tissue, after immersion in distilled water at 60° C for 60 s. The samples were frozen and used within 3 months. When needed, the tissue was thawed for 30 minutes in saline before mounting on the diffusion cells**.** The receptor compartment was filled with about 4 ml of a NaCl 0.9 % solution, previously degassed in order to avoid the formation of bubbles at the tissue interface. This solution was kept at 37°C under magnetic stirring. Experiments were performed at infinite dose (1.2 ml/$cm^2$ of formulation) at 37 °C, using MEs and commercial formulation Omicilon® A as donor. Samples of receptor solution were taken hourly for 7 h, replaced with fresh solution, and analyzed by HPLC. Control experiments, using blank ME, ensured no interference of the formulation on TA analysis.

At the end of the experiment, the donor solution was removed, the cell was dismantled and the tissue was carefully washed and cleaned with a cotton swab to remove any residue of donor solution. A disc of tissue was cut, fitting the area covered by donor compartment (0.6 $cm^2$), placed in pre-weighed plastic test tubes, weighed again to determine the amount of tissue and submitted to extraction. A control experiment, in which Omicilon® A and MEs were applied and immediately removed, was performed: tissue extraction and analysis showed no traces of TA, indicating that the cleaning procedure was able to remove all the residues of formulation.

### 2.2.6 Validation of extraction

For the validation of TA extraction, tissue samples (which had not previously been in contact with the drug) were used in specificity and recovery determination. Some of the blank tissues were submitted to the extraction procedure and the retention time of extracted endogenous compounds was compared with that of TA. Then, a known amount of TA (5 µl of TA solution 150 µg/ml in ethanol) was added to samples of the blank tissue. After 1 hour of contact, the tissues were submitted to the above-described procedure of extraction and analysis. The extraction recovery, obtained using different solvents (**Table 1),** was determined by computing the ratio of the amount of TA extracted from spiked tissue to the amount of TA added (determined by direct injection of spiking solutions in the absence of tissue).

### 2.2.7 HPLC analysis

For the quantitative determination of TA, HPLC analysis was performed using an Agilent 1260 Infinity instrument (Agilent Technologies, Santa Clara, CA, USA), equipped with a quaternary pump and autosampler. The column was a Bondclone 10 µm C18 300 x 3.9 mm (Phenomenex Columbus, USA). The mobile phase was water:acetonitrile (60:40, v:v), eluted at 1 ml/min. Using these conditions, the retention time was about 9 min. The injection volume was 100 µl and the UV detection was performed at 240 nm. The method was validated according to the FDA Guidance for industrial bioanalytical method validation.

### 2.2.8 Data analysis

In the permeation experiments, the cumulative amount of drug permeated was plotted as a function of time. The permeation profiles were fitted to a solution of Fick's law at non-steady-state (Eq. 1) ([19]) to calculate the relevant permeation parameters:

$$Q = (K H) C_{veh} \left[ \frac{D}{H^2} t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left( \frac{-D n^2 \pi^2 t}{H^2} \right) \right] \quad \text{Eq. (1)}$$

where Q is the cumulative amount of drug permeated per unit area at time t, $C_{veh}$ is the concentration of the drug in the donor vehicle, K is the tissue/vehicle partition coefficient, D the diffusion coefficient and H the diffusion path-length. The permeability coefficient P was calculated as the product between KH and $D/H^2$. The fitting was performed using KaleidaGraph® 4.5 (Synergy Software) running on a MacIntosh MacBook Pro. The average error associated with each fitted value was in the range 10-20%.

The numerical data are reported as mean values ± sd; for sake of clarity, figures report mean values ± sem.

## 3 Results and discussion

### 3.1 Validation of TA extraction from pig esophageal epithelium

The recovery of TA from the mucosa was tested in different experimental conditions; the results are illustrated in **Table 1**. By modifying the composition and the volume of the solvent mixture, a quantitative recovery was obtained. When blank tissue samples were analysed, no interferences with the same retention time of TA were found.

### 3.2 Microemulsions development

For the development of MEs, a simple procedure, largely described in literature, was followed [20]. The first step was the evaluation of TA solubility in different excipients. TA is a corticosteroid anti-inflammatory drug, with a molecular weight of 434.49 g/mol, sparingly soluble in water (21 μg/ml at 28 °C [21]) and in ethanol. TA has a logP value of 2.53 [22]; this is generally considered lipophilic, although it is reported to be partially soluble in vegetable oils and insoluble in mineral oils. The different vehicles tested were chosen among oil phases, surfactants, co-surfactants and co-

solvents. The solubility in the following solvents was lower than 1 mg/ml: oleic acid, isopropyl myristate, myristil myristate, Labrafac® lipophile (caprylic/capric triglyceride), Labrafac® PG (propylene glycol dicaprylocaprate), Lauroglycol® 90 (propylene glycol monolaurate), Maisine® 35-1 (glyceryl mono-linoleate), Plurol Oleique® (polyglyceryl-3 dioleate), Brij® 78 (5% w/v solution), Span® 20, Tween® 20 (5% w/v solution), TPGS (5% w/v solution). The values of solubility higher than 1 mg/ml are reported in **Table 2**.

The solubility of TA, despite its lipophilic nature, was lower than 1 mg/ml in almost all the lipophilic phases tested; among surfactants, the highest solubility was found for polyoxyethylene derivatives (such as Labrasol®). On the basis of the solubility results obtained, the following excipients were selected: Peceol® as oily phase, Labrasol® as surfactant and Transcutol® as co-surfactant. The choice was linked not only to solubility, but also to other aspects relevant for buccal administration, such as the irritation potential, the mucoadhesive properties and the organoleptic characteristics. Peceol® has shown mucoadhesive properties: Lee at al., studied the mucoadhesive properties of the liquid crystalline phase of Peceol®, demonstrating that are dependent of the water uptake into a water rich environment [23]. The surfactant chosen in ME formulation must be able to reduce the interfacial tension to suitable values, i.e. $10^{-2}/10^{-3}$ mN/m [24]. Usually it is not possible to reach these values with the presence of a single surfactant (Labrasol®), but it can be reeached with the addition of a co-surfactant, in this case Transcutol®. The combination helps the dispersion process, forming a flexible film around the droplets. Additionally, Transcutol® is a known solubilizer and enhancer for the skin and for the buccal mucosa [1].

The various proportions at which microemulsions were formed were plotted to generate a pseudoternary phase diagram, shown in **Fig. 1**. Three different smix ratios were chosen (1/1, 1/2, 2/1, v/v), using Labrasol® and Transcutol®. When the ratio of smix was 1/1 (**Fig. 1A**), 5 % of oil was solubilized by about 25 % of smix. Increasing the concentration of smix up to 50 %, it was possible to solubilize a maximum of 15 % of oil. Interestingly, increasing the percentage of Transcutol® (smix 1/2) (**Fig. 1B**), the area of existence of ME changed completely and, with the

same percentage (50%) of smix, up to 40 % of oil was solubilized. When the smix contained a higher percentage of Labrasol® (smix 2/1) (**Fig. 1C**) the region of existence increased, but a lower % of oil could be included in the ME: it was possible to reach 30 % of oil included using 60 % of smix. This confirms that the use of the co-surfactant is crucial.

From each diagram, five formulations were selected (**Table 3**). We decided to limit the concentration of smix to 50 %, because it is well reported that large amounts of surfactants (even if non-ionic, thus better tolerated) can cause irritation. The fifteen formulations were added of TA at 0.1 % w/v and then submitted to accelerated stability tests, such as heating/cooling cycles and centrifugation, in order to assess the stability. All formulations remained clear and transparent after TA addition. The accelerated stability tests were successfully passed for all MEs prepared from the smix ratio 1/1 (ME 1, ME 4, ME 5, ME 6, ME 7). When the smix was 1/2, meaning higher percentage of co-surfactant, only the formulation composed of 40 % oil, 50 % smix and 10 % water (ME 2) was stable in all conditions tested; this is the formulation with the lower water percentage, being only 10 %, indicating that Transcutol® favors the formation and stability of more hydrophobic systems. When the 2/1 smix was used, the system was stable only in the smix range 35 - 45 % and water range 40 – 60 % (ME 3, ME 13, ME 14). The results are not surprising, since using a surfactant with HLB of 12 the formation of an o/w system is favored.

Three formulations, ME 1, ME 2 and ME 3 were selected for further investigations.

### 3.3 Microemulsions characterization

The formulations were firstly observed under polarized light microscopy: no images were visualized, indicating the absence of liquid crystals or other birefringent structures in the material, thus confirming the microemulsion character of the formulations.

MEs were then characterized for pH, conductivity, droplet size and zeta potential and the results are reported in **Table 4**.

The pH was approximately 4, compatible with the pH of the oral environment [25].

MEs can be o/w, w/o or bicontinuous; in the case of dispersed ME, another important parameter is the size of the dispersed phase. It is generally accepted that to have what is called a microemulsion, a system visually clear and transparent, the size of the dispersed phase should be lower than 150 nm [24]. DLS analyses of ME 1 and ME 3, showed a dispersed droplets size of 86 and 120 nm, respectively, whereas ME2 was characterized by a smaller size, approx. 6 nm (**Table 4**), compatible with an inverse micellar solution (w/o); the high PI indicates the presence of different populations of micelles. ME1 and ME3 contained a population of small-sized droplets (approx. 5-10 nm) as well; due to the high concentration of surfactant, the formation of micelles is possible.

The conductivity data reported in **Table 4** confirm the structural analogy of ME1 and ME3 (high conductivity, typical of an o/w system), while ME2 is a non-conductive system, compatible with a w/o structure. The conductivity after 24 h did not change, suggesting that no short-term changes take place. The zeta potential of ME1 and ME3 was very close to zero, indicating that the MEs were not electrically charged.

### 3.4 *In vitro* permeation experiments

*In vitro* evaluation of TA permeation through porcine esophageal epithelium was performed. The porcine buccal mucosa is a well characterized model for human buccal mucosa and it is widely used for accumulation and permeation experiments. However, even if porcine tissues are more easily available than human tissue, they are frequently damaged by mastication. Moreover, the separation of the mucosa from the underlying muscular tissue is not an easy task. For these reasons, Diaz del Consuelo at al., proposed in 2005 the esophageal porcine mucosa as an alternative to buccal porcine mucosa because it is easier to prepare and less damaged by chewing, compared to buccal mucosa. The lipid characterization and the permeation studies showed that the esophageal mucosa is a suitable model for buccal human mucosa [26].

We used this model to investigate the performance of ME1, ME2 and ME3 previously prepared and characterized. The aim was to explore the possibility of achieving a systemic delivery, using the buccal route as (non-invasive) alternative to the oral route, which shows a bioavailability of 10-20 % [27, 28] compared to injection. At the same time, mucosa retention was measured, in view of local delivery of TA, which is already used in therapy for the treatment of mucosa diseases.

In **Fig. 2** the permeation profiles of the selected MEs, applied on the mucosa at infinite dose, are reported. Data are shown as amount of drug permeated per unit area ($\mu g/cm^2$) as a function of time. Surprisingly, the permeation profiles were all superimposed, despite the different nature of ME2 (o/w) with respect of the other two (both o/w).

The permeation profiles were fitted to a solution of Fick's law at non-steady-state [19], to calculate the relevant permeation parameters reported in **Table 5**: partitioning parameter (KH), diffusive parameter ($D/H^2$), permeability coefficient (P), flux (J) and time lag. As can be expected from the profiles, the parameters were not significantly different, although the variability of the data was particularly high.

It is interesting to observe that TA permeation across the mucosa was relatively slow, with a lag-time of 2-3 h; this has been already reported in the literature [15]. TA flux is in the order of 1.5 µg $cm^{-2}$ $h^{-1}$, with a permeability coefficient of approx. $1*10^{-3}$ cm $h^{-1}$ - value slightly lower compared to the one obtained by [16] ($1*10^{-2}$ cm $h^{-1}$) and by [12] ($1.47\ 10^{-2}$ cm $h^{-1}$) from a TA aqueous solutions across pig buccal mucosa; the use of a different solvent in the donor solution explains this small difference.

Then, with the aim of increasing TA penetration/retention, the charge of the ME – in particular of ME1 – was altered by adding small percentages of stearylamine (0.5%), CTAB (0.5%) or chitosan (1%). This approach derives from the observation that positively charged MEs increase tissue and mucosa penetration, due to charge interaction [29, 30]. Additionally, chitosan – positively charged polysaccharide - has been demonstrated to be able to act as penetration enhancer in mono or stratified epithelia, with or without tight junctions [31]. Sandri et. al demonstrated that chitosan acts

by partly disarranging desmosomes and repackaging of the epithelial cells in buccal mucosa (stratified without tight junctions); other authors [32] suggested that chitosan can act on the organized intercellular lipid lamellae.

The characteristics of the new MEs prepared are reported in **Table 4**, where it can be observed that only chitosan was able to alter the charge of ME1 to a significant extent. The droplet size changed as well, although the high PI obtained suggests the presence of aggregates.

The permeation profiles obtained in the presence of chitosan were significantly higher compared to ME1 (**Fig. 2**) as demonstrated also by the permeation data reported in **Table 5**. On the contrary, CTAB and stearylamine did not have any effect. The increased permeation obtained with chitosan resulted due to an increase in the diffusive parameter, in agreement with the hypothesis mentioned above of interference with the permeation barrier of the tissue.

Considering the average dose of TA administered systemically (2.5 to 60 mg/day by injection), buccal administration does not allow to reach a therapeutic systemic effect, because the max. flux obtained (2.6 μg $cm^{-2}$ $h^{-1}$) is too low.

### 3.5 TA tissue retention

The following set of experiments was performed to evaluate tissue retention, in view of topical application of MEs. At the end of the permeation experiments, the mucosa was submitted to TA extraction, after method validation (see above), and then TA was quantified by HPLC.

First of all, the performance of the commercial formulation Omicilon® A was studied: the product was applied overnight, as directed by the producer, and the resulting tissue concentration was very low, namely 0.08±0.03 μg/$cm^2$ (equivalent to 13±7 μg/g). The commercial formulation is a dispersion of triamcinolone acetonide (0.1% w/w) in a dental paste containing gelatin, pectin, and carboxymethylcellulose sodium in Plastibase® (Plasticized Hydrocarbon Gel), a polyethylene and mineral oil gel base.

The literature reports controversial data on the commercial paste performance *in vitro*; some authors reported a very high mucosa retention, 1 µg/cm$^2$ after 4 h [15], whereas others [14, 33] reported values (approx. 1 µg/g) even lower than the one we have obtained (13±7 µg/g of tissue). The same authors [33] underlined the same difficulties we experienced in removing the formulation, beacause it contains carboxymethyl cellulose which adheres very firmly to the mucosa. Consequently, the amount of drug measured in the mucosa could be due to the drug remaining on the surface. This is not the case of our data, in which the cleaning procedure was validated (see Materials and methods). The results obtained for ME1, containing stearylamine, CTAB or chitosan, applied for 7 h are reported in **Fig. 3**.

The addition of stearylamine decreased TA retention in the tissue, although it did not influence its permeation across the tissue: the presence of stearylamine in the formulation increased the droplet size, although due to the increased polydispersity no conclusions can be drawn. The only other property changed was pH: in the presence of stearyalmine the pH of the ME shifted from 3.76 to 6.25. However, since the formulation does not contain ionized components (neither the drug, nor the excipients) the change in pH per se cannot explain the low retention. The only possible reason is a reduction of the partition coefficient in the presence of the amine; in effect, the presence of stearylamine reduced the partitioning parameter (see **Table 4**), although the difference is not statistically significant.

On the other hand, chitosan increased TA permeation, but not its retention which resulted comparable to that of the native ME1.

Because there was not difference with and without chitosan, CTAB or stearylamine, the following experiments, aimed at verifying the effect of the application time, were performed using the original MEs. The results obtained are compared with the commercial formulation, applied overnight, in **Fig. 4**. TA was found in the epithelial tissue, already after 10 minutes: this is in agreement with the observation of Nicolazzo et al. [15] and other authors that, particularly with lipophilic molecules, the permeant is rapidly taken up by partitioning onto the buccal epithelium and is then slowly

transferred into the systemic circulation. Prolonging the application time to 1 h did not modify TA tissue retention, whereas after 7 h the amount recovered was significantly higher. The results obtained with overnight application of Omicilon® A (for which after 10 min. no TA was found in the epithelial tissue) are much lower, probably because the partitioning between a very lipophilic formulation and the tissue is not as favored as in the case of MEs.

## 4 Conclusions

The results obtained in the present work confirm the usefulness of ME for the buccal delivery of TA. Among all the microemulsion prepared and tested for stability, three were selected and submitted to characterization and *in vitro* permeation/retention experiments. The results obtained show that the performance of the formulation, in terms of permeation across pig esophageal epithelium, was not influenced by droplet size or composition, but only by the addition of chitosan, polysaccharide able to increase the transport across mono and stratified epithelia. The determination of the permeation parameters allowed us to identify that chitosan acts on the diffusion parameter across the tissue and not on the partitioning parameter; for the same reason the tissue retention of TA was not modified by chitosan. The results indicate that microemulsions, even those containing chitosan, cannot be used for buccal administration in view of a systemic effect; only the association with an efficient enhancing strategy (physical or chemical) might make it possible.

Concerning local application, the comparison of the results with the commercial paste Omicilon® A revealed a very marked difference: the amount of TA recovered in the mucosa after 10 min. was much higher than that recovered after overnight application of the commercial paste. This suggests that TA microemulsions can be an interesting and versatile alternative to commercial formulation for local delivery, to treat diseases of the buccal mucosa. Owing to the fast uptake by the tissue, the mucosa could be simply rinsed with the formulation, used as a mouthwash; in alternative, the formulation can be gelled or adsorbed on an inert support and applied locally.

## Acknowledgements

This work did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

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Figure caption

**Figure 1.** Pseudoternary phase diagrams. The smix was always composed of Labrasol® and Transcutol® but in different ratio: a= 1/1, b= 1/2, c=2/1.

**Figure 2**. TA permeation profiles across pig esophageal epithelium from MEs containing 0.1 % of TA. ME1 was added of: stearylamine (0.5% w/v), CTAB (0.5% w/v) or chitosan (1.0% w/v) (mean values ± sem).

**Figure 3.** TA esophageal epithelium retention from ME1 and from ME1 containing stearyamine (0.5% w/v), CTAB (0.5% w/v) or chitosan (1.0% w/v); all MES were loaded with 0.1 % (w/v) of TA (mean values ± sem).

**Figure 4.** TA pig esophageal epithelium retention following MEs application for different times (mean values ± sem). As comparison Omicilon® A was applied overnight. TA concentration was 0.1% (w/v) in all cases.

**Table 1.** Results of validation of TA extraction from pig esophageal epithelium (mean values ± sd)

490

| Solvent (% v) | | | Extraction volume (ml) | Recovery (%) |
|---|---|---|---|---|
| **$CH_3CN$** | **Water** | **Transcutol®** | | |
| **75** | 25 | - | 1.0 | 58.1±6.6 |
| **90** | 10 | - | 1.0 | 67.4±6.9 |
| **70** | 15 | 15 | 1.5 | 82.2±1.87 |
| **75** | 25 | - | 1.5 | 104.2±7.5 |

**Table 2**. Estimated and measured TA solubility in different vehicles (mean values ± sd).

| Vehicle | m.w. | HLB | TA solubility (mg/ml) |
|---|---|---|---|
| **Transcutol®** | 134.2 | - | 25.87±3.28 |
| **PEG 200** | 190-210 | - | 17.65±1.46 |
| **PEG 400** | 380-420 | - | 14.66±4.26 |
| **PEG 600** | 570-630 | - | 8.41±0.88 |
| **Peceol®** | 356.5 | 1.0 | 1.09±0.29 |
| **Capryol® 90** | 220.3 | 5.0 | 5.71±0.55 |
| **Labrasol®** | 300.0 | 12.0 | 19.00±3.05 |

495 **Table 3**. Composition (% v/v) and stability of selected formulations for accelerated stability tests

| CODE | PECEOL® | WATER | SMIX (LABRASOL®/ TRANSCUTOL® 1/1) | SMIX (LABRASOL®/ TRANSCUTOL® 1/2) | SMIX (LABRASOL®/ TRANSCUTOL® 2/1) | STABILITY (Y/N) |
|---|---|---|---|---|---|---|
| **ME 1** | 10 | 40 | 50 | - | - | Y |
| **ME 2** | 40 | 10 | - | 50 | - | Y |
| **ME 3** | 15 | 40 | - | - | 45 | Y |
| **ME 4** | 10 | 45 | 45 | - | - | Y |
| **ME 5** | 5 | 55 | 40 | - | - | Y |
| **ME 6** | 5 | 60 | 35 | - | - | Y |
| **ME 7** | 5 | 65 | 30 | - | - | Y |
| **ME 8** | 30 | 20 | - | 50 | - | N |
| **ME 9** | 5 | 60 | - | 35 | - | N |
| **ME 10** | 5 | 55 | - | 40 | - | N |
| **ME 11** | 7 | 55 | - | 38 | - | N |
| **ME 12** | 10 | 50 | - | - | 40 | N |
| **ME 13** | 10 | 60 | - | - | 30 | Y |
| **ME 14** | 5 | 60 | - | - | 35 | Y |
| **ME 15** | 5 | 70 | - | - | 25 | Y |

**Table 4**. Physico-chemical properties of the microemulsions prepared (mean values ± sd)

| ME | Additive | pH | Conductivity (µS/cm) | | Droplet size (nm) | Polydispersity Index | Zeta Potential (mV) |
|---|---|---|---|---|---|---|---|
| | | | $t_0$ | $t_{24}$ | | | |
| **ME1** | - | 3.76 | 16.86±0.11 | 16.76±0.15 | 86.1 | 0.194 | -1.20±0.75 |
| **ME1** | CTAB 0.5% | 3.68 | n.d. | n.d. | 73.7 | 0.300 | +0.37±1.25 |
| **ME1** | Stearylamine 0.5% | 6.25 | n.d. | n.d. | 121.8 | 0.236 | +2.21±0.28 |
| **ME1** | Chitosan 1% | 4.68 | n.d. | n.d. | 251.9 | 0.283 | +23.10±2.22 |
| **ME2** | - | n.d. | 1.21±0.01 | 1.23±0.00 | 5.8 | 0.262 | n.d. |
| **ME3** | - | 3.80 | 16.12±0.05 | 16.46±0.04 | 119.5 | 0.188 | -1.88±1.84 |

n.d. not determined

500 **Table 5.** Permeation parameters of TA across pig esophageal epithelium (mean values ± sd)

| ME | Additive | KH (cm) $*10^2$ | $D/H^2$ ($h^{-1}$) $*10^2$ | P (cm $h^{-1}$) $*10^3$ | J (µg $cm^{-2}$ $h^{-1}$) | Time lag (h) |
|---|---|---|---|---|---|---|
| **ME1** | - | 2.02±1.20 | 6.93±2.73[a] | 1.34±0.81[b] | 1.34±0.81 | 2.72±0.98 |
| **ME1** | CTAB 0.5% | 1.68±0.33 | 8.52±0.49 | 1.43±0.23 | 1.43±0.23 | 1.96±0.11 |
| **ME1** | stearylamine 0.5% | 1.39±0.44 | 8.69±3.11 | 1.16±0.31 | 1.16±0.31 | 2.06±0.61 |
| **ME1** | chitosan 1% | 2.07±0.47 | 13.4±3.56[a] | 2.58±0.33[b] | 2.58±0.33 | 1.35±0.37 |
| **ME2** | - | 2.42±1.50 | 4.87±2.20 | 1.01±0.35 | 1.01±0.35 | 3.85±1.29 |
| **ME3** | - | 1.78±0.82 | 8.35±4.78 | 1.35±0.70 | 1.35±0.70 | 2.52±1.20 |

[a]$p<0.05$; [b]$p<0.05$

**Figure1**

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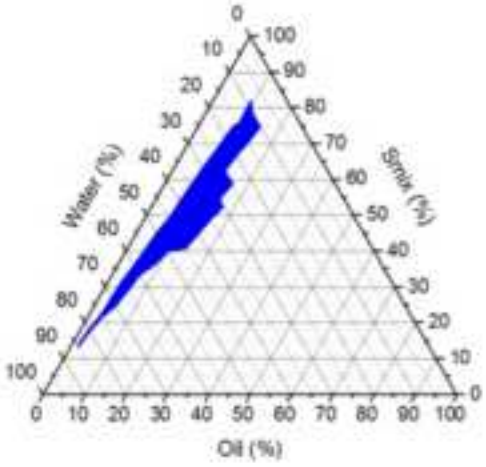


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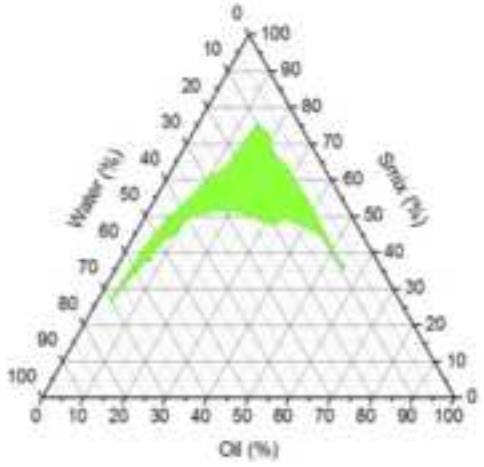


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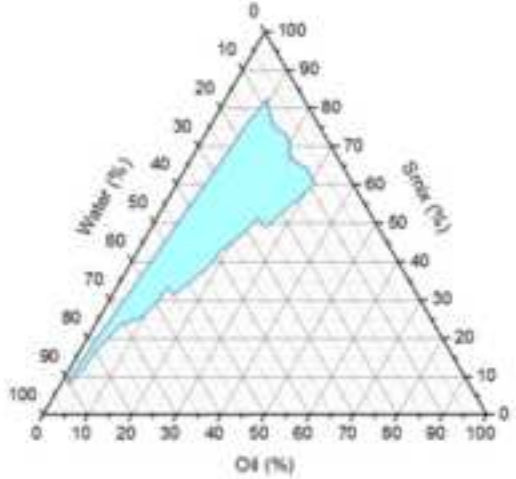


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Figure2

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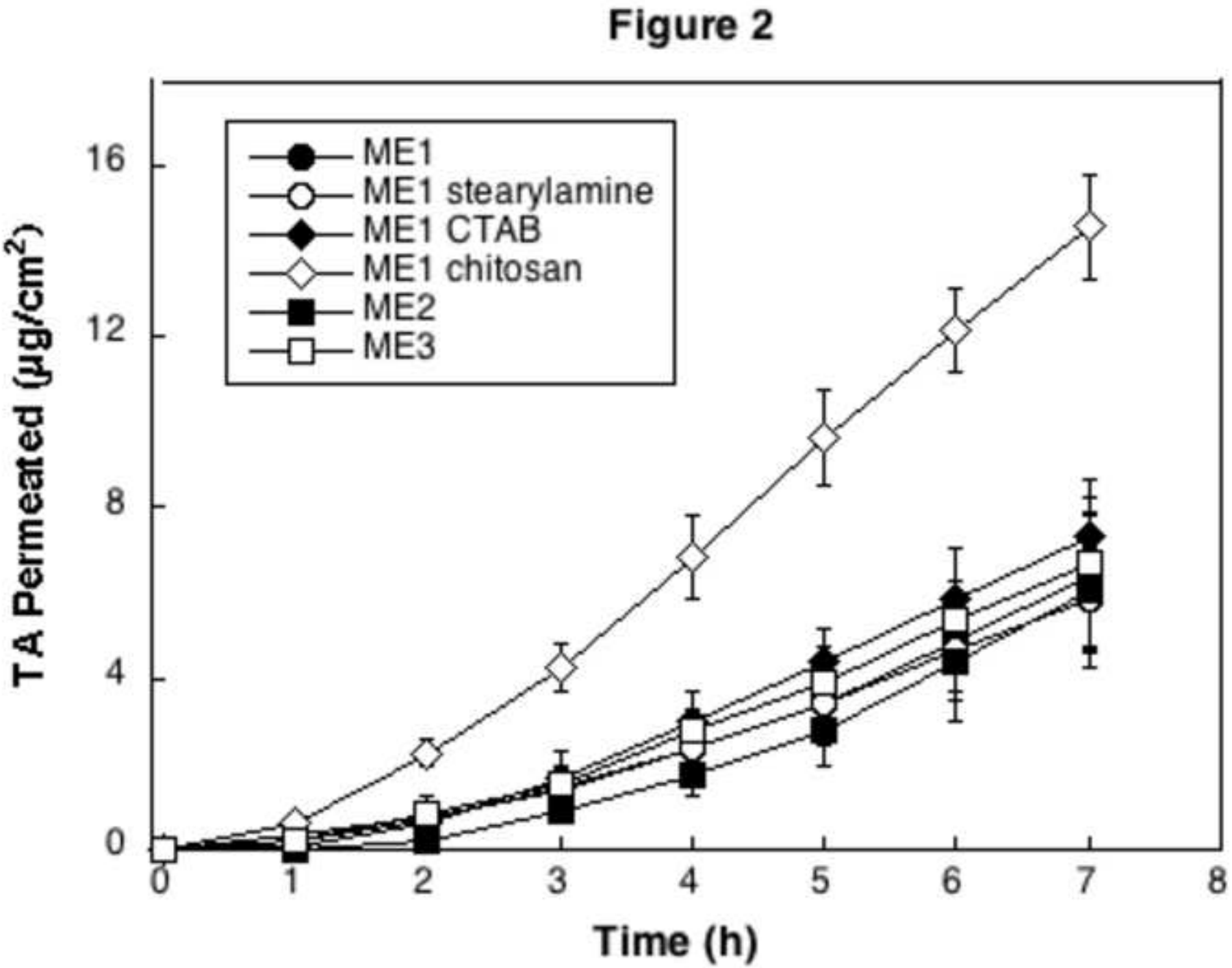

Figure3

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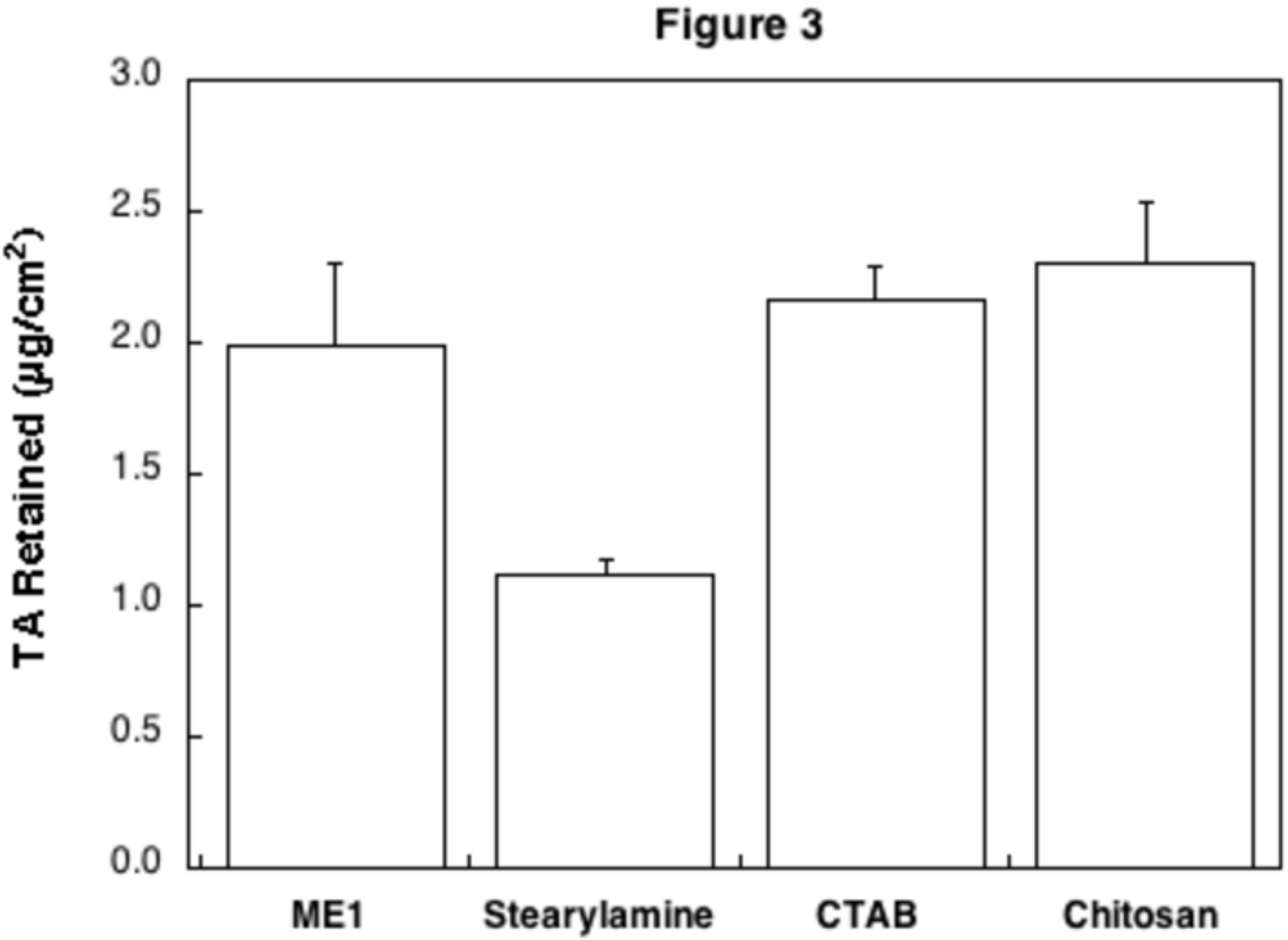

Figure4

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