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Galenic and Biopharmaceutical Study of the Triamcinolone Acetonide and Lidocaine Hydrochloride Semisolid Formulations for Buccal Administration †

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† Presented at the 1st International Electronic Conference on Pharmaceutics, 1–15 December 2020; Available online: https://iecp2020.sciforum.net/.

Abstract: The mouth can be affected by important inflammatory processes resulting from localized or systemic diseases, such as diabetes, AIDS, and leukemia, among others, which are manifested in various types of buccal sores, typically presenting pain [1]. The present work focuses on the design, formulation, and characterization of four semi-solid formulations for oral mucosa in order to symptomatically treat these painful processes. The formulations have two active pharmaceutical ingredients: triamcinolone acetonide (TA) and lidocaine hydrochloride (LIDO). The formulations also contain Orabase® as an excipient, which is a protective, hydrophobic, and anhydrous adhesive vehicle, used to retain or facilitate the application of active pharmaceutical ingredients (APIs) to the oral mucosa. After designing the formulations, the validation of the analytical method was performed to achieve reliable analytical results. Franz-type diffusion cells were used to perform drug release studies using synthetic membrane, and permeation studies using buccal mucosa, permitting the estimation of the amount and rate of TA permeated across this mucous membrane. Further, the amount of TA retained within the tissue was estimated, as this is where it performs its anti-inflammatory activity, and showed no significant differences between the 0.05% TA + LIDO and 0.1% TA + LIDO formulations ($p > 0.05$). Therefore, the results demonstrate the suitability of the administration of the lowest concentration of TA tested, which achieved a similar efficacy as higher concentrations and reduced the potential systemic effects of corticoid administration. Furthermore, sublingual permeation studies were carried out to evaluate a scenario of continuous contact of the tongue with the applied formulation. The four formulations studied show pseudoplastic and thixotropic behavior, ideal for topical application. These results provide evidence for the potential of these topical formulations for the treatment of inflammatory processes in the buccal mucosa.

Keywords: triamcinolone acetonide; buccal administration; semisolid formulations; thixotropic behavior; lidocaine hydrochloride; Franz-type diffusion cells

Citation: Márquez Valls, M.; Martínez Labrador, A.; Halbaut Bellowa, L.; Bravo Torres, D.; Granda, P.C.; Limón, D.; Calpena Campmany, A.C. Galenic and Biopharmaceutical Study of the Triamcinolone Acetonide and Lidocaine Hydrochloride Semisolid Formulations for Buccal Administration. **2021**, *78*, 57. https://doi.org/10.3390/IECP2020-08681

Published: 1 December 2020

1. Introduction

The mouth can be affected by important inflammatory processes resulting from localized or systemic diseases, such as diabetes, AIDS, and leukemia, among others, which can be manifested in various types of buccal sores, such as canker sores or lichen planus, conditions that typically present inflammation and pain [1]. Furthermore, although the

oral cavity has its own bacterial flora, a qualitative and quantitative imbalance of this ecosystem leads to infections, also causing inflammatory reactions.

The present work shows the design and development of four semisolid formulations for administration in the buccal mucosa, with the aim of symptomatically treating painful processes in this cavity. These formulations have one or two active pharmaceutical ingredients (APIs): triamcinolone acetonide (TA) and lidocaine hydrochloride (LIDO). TA is a synthetic glucocorticosteroid with immunosuppressive and anti-inflammatory activity [2] while lidocaine hydrochloride (LIDO) is a local anesthetic that blocks sodium ion channels. The formulations contain Orabase® as an excipient, which is a protective adhesive vehicle, hydrophobic and anhydrous, used to retain or facilitate the application of APIs in buccal mucosa. It has poor solubility and contains gelling agents that allow the adherence to the mucosa for periods between 15 min and 2 h [3].

The aim of this research was to evaluate the mechanical and biopharmaceutical properties of the semisolid formulations and determine the influence of the concentration of TA or the presence or absence of lidocaine hydrochloride on these properties. The suitability for a topical application was therefore evaluated by performing rheology studies, while the amount and rate of TA that could be released from the formulation was determined. Furthermore, the ability to permit the permeation of TA across either buccal or sublingual mucosa was studied using Franz cells. Moreover, the amount of TA retained within the buccal mucosa, where the drug performs its anti-inflammatory activity, was calculated. In order to obtain fully reliable results from release, permeation, and retention studies, we designed and validated an analytical method using high-performance liquid chromatography (HPLC), which was found to be linear and accurate in the rage of concentrations studied.

2. Experiments

2.1. Materials

Triamcinolone acetonide, lidocaine hydrochloride, Orabase®, and liquid paraffin were purchased from Fagron. Transcutol P was purchased from Gattefossé. Acetonitrile (ACN) was purchased from Fisher Chemical. Ammonium acetate (≥98%) was purchased from Panreac.

2.2. Composition of the Formulations

Four different formulations containing TA for topical administration were developed to evaluate the influence of TA concentration and the presence or absence of lidocaine hydrochloride on the mechanical and biopharmaceutical properties of the formulation (Table 1).

Table 1. Composition of the four different formulations.

Composition	0.05% TA	0.05% TA + LIDO	0.1% TA	0.1% TA + LIDO
TA	0.05%	0.05%	0.1%	0.1%
Lidocaine HCl	-	2%	-	2%
Liquid paraffin	5%	5%	5%	5%
Orabase®	q.s	q.s	q.s	q.s

2.3. Rheological Properties

The rheological characterization of the formulas was performed in duplicate at 25 °C, using a Thermo Scientific Haake Rheostress 1 rheometer (Thermo Fischer Scientific, Kalsruhe, Germany) equipped with a cone-plate geometry (C60/2° Ti) and connected to a temperature control device (Thermo Haake Phoenix II + Haake C25P) and operated using Haake Rheowin® Job Manager v. 3.3 software. The viscosity and flow curves were obtained in rotational mode, performing an ascendant shear rate ramp from 0 to 100 s^{-1} over 3 min, followed by 1 min at a constant rate of 100 s^{-1}, and then from 100 s^{-1} to 0 s^{-1} over 3 min.

The data obtained for each formulation were adjusted to different mathematical models: Newton, Bingham, Casson, Ostwald, Herschel–Bulkley and Cross.

2.4. Analytical Method Validation

The validation of the analytical method for TA using high performance liquid chromatography was carried out in a Waters HPLC system equipped with a Waters pump 1525, a UV-vis 2487 detector (Waters, Milford, EE. UU.), and a Supercosil LC-ABZ (15cm; 4.6 mm and 5 μm) column. The data were collected and processed using the Empower Pro software (Waters, Milford, MA, USA). The mobile phase consisted of 50:50 (*v*/*v*) water/methanol. Then, 10 μL samples were injected and TA was detected at 232 nm according to a method validated for a different route of administration [4]. TA was initially dissolved in Transcutol P and further diluted using a mixture of acetonitrile and ammonium acetate buffer at pH 4.7 (10:90) [5]. Six different calibration curves were produced by preparing stock solutions of 205 μg/mL TA, and further dilutions of 102.5 μg/mL, 68.3 μg/mL, 41 μg/mL, 20.5 μg/mL and 10.25 μg/mL. Linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were estimated as follows.

2.4.1. Linearity and Range

Linearity of the method in the defined range of concentrations was evaluated by performing a least squares regression for the experimental data and evaluating the correlation coefficient (r) based on Equation (1):

$$y = Sb \cdot x + a \quad (1)$$

where x is the concentration, y is the chromatographic area, Sb is the value of the slope, and a is the y-intercept [6].

2.4.2. Accuracy and Precision

Accuracy at each concentration was expressed as the mean percentage deviation or relative error (*RE*, %) and calculated using Equation (2):

$$\%RE = [(Cobs - Cnom)/Cnom]\ 100 \quad (2)$$

where *Cobs* is the observed concentration and *Cnom* is the nominal concentration of each standard solution.

Precision was calculated and expressed as the relative standard deviation (*RSD*, %) of each replicate series using Equation (3):

$$\%RSD = (SD/Cobs)\ 100 \quad (3)$$

where *SD* is the standard deviation and *Cobs* is the nominal concentration.

2.4.3. Determination of Limits

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve using the following equation:

$$LOD\ or\ LOQ = K\ SDsa/Sb \quad (4)$$

where *K* is a factor related to the level of confidence (3 for *LOD* and 10 for *LOQ*), *SDsa* is the standard deviation of the intercept (a) and *Sb* is the slope of the calibration line [7].

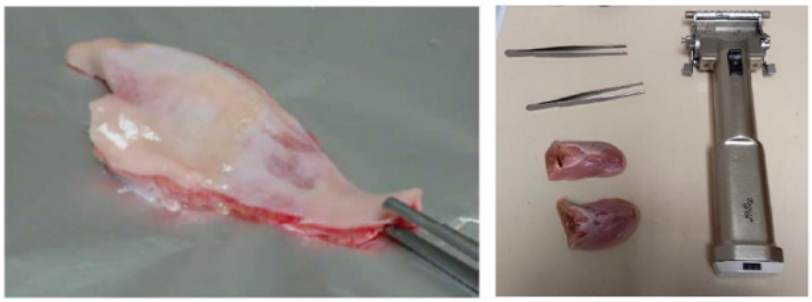
2.5. Release Studies

To assess the release of TA from the four different types of formulations, drug release experiments were performed in triplicate using Franz-type diffusion cells (FDC 400, Crown Glass, Somerville, NY), being the donor and receptor chambers separated by nylon synthetic membranes (Type NY41 41 μm). The receptor chambers were filled with an acetonitrile mixture consisting of ammonium acetate buffer pH 4.7 (10:90) and Transcutol,

complying with SINK conditions. The Franz-type diffusion cells were connected with a temperature-controlled circulating bath at 37 °C. Samples at known intervals were collected with the micropipette MODEL 5000 (Gilson) and directly stored in HPLC vials for their analysis.

2.6. Permeation and Retention Studies

Ex vivo permeation and retention studies were conducted in Franz-type diffusion cells with a setup that was similar to that of release studies, but with the membrane replaced with either porcine buccal (Figure 1a) or sublingual mucosa (Figure 1b).



(**a**) (**b**)

Figure 1. (**a**) Porcine buccal mucosa; (**b**) porcine tongues, dermatome, and tweezers.

The mucosa samples were frozen at −20 °C and longitudinally cut in 700 μm slabs with a dermatome GA 630 (Figure 1b). Mucous membrane samples were placed between the receptor and donor compartments with the proximal side in contact with the receptor medium and the mucous side in contact with the donor chamber [8]. The flux values of TA (μg/h) across mucous membranes were estimated through the slope of the cumulative amount of TA permeated vs. the time for each formulation. Moreover, the retention (%) of TA was estimated in the mucous membranes after the permeation experiment.

2.7. Statystical Analysis

Non-parametric Student's *t*-tests were performed using GraphPad Prism 3 to compare the different formulations.

3. Results and Discussion

3.1. Composition of the Formulations

Four different formulations containing TA for topical administration were developed to evaluate the influence of TA concentration and the presence or absence of lidocaine hydrochloride on the mechanical and biopharmaceutical properties of the formulation (Table 1). For instance, TA was prepared at 0.05% and 0.1% (*w*/*v*), and lidocaine hydrochloride was tested at 2% concentration. Liquid paraffin and Orabase® were included as excipients for promoting the formation of a homogeneous and consistent hydrophobic film upon application in order to maximize the retention of the API in the area of application.

3.2. Rheological Properties

The rheological characteristics of formulations play an important role in physical stability and are an important attribute in the development of topical drug products [9]. In order to identify the mechanical properties of the formulations, rheology studies were performed and revealed shear thinning and apparently thixotropic behavior in all the formulations (Figure 2), both being desirable characteristics for topical application, allowing the formation of a consistent film covering the application area which facilitates the diffusion of the drug through the matrix [10–13].

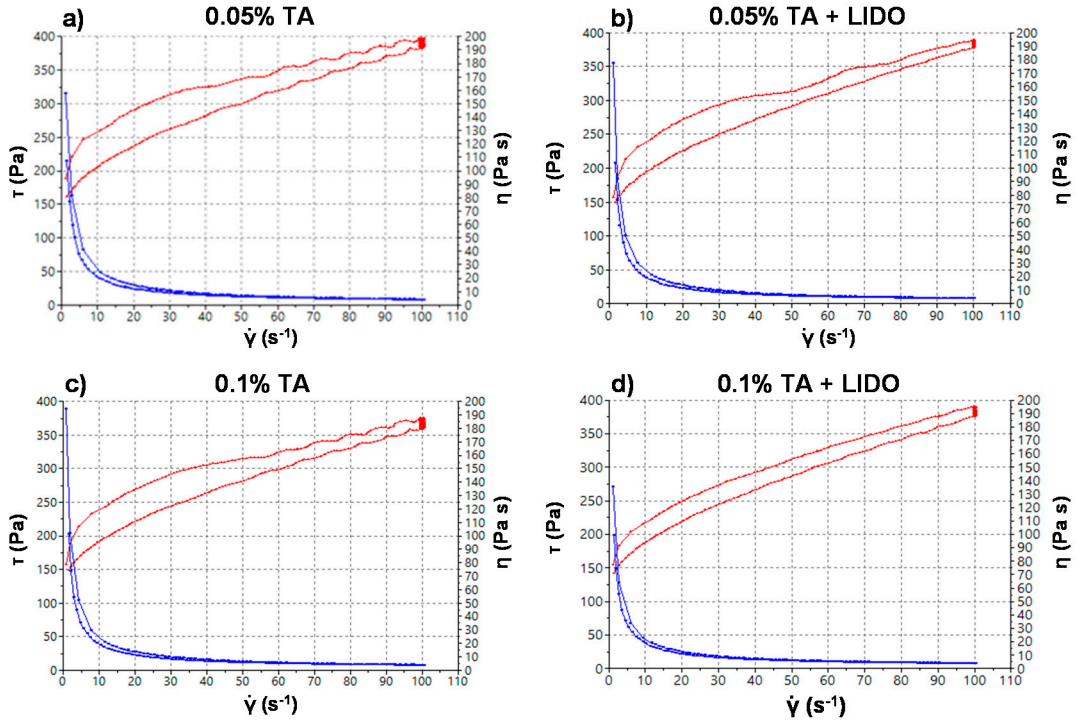


Figure 2. Viscosity curve (blue line) and flow curve (red line) of the four formulations. (**a**) 0.05% TA; (**b**) 0.05% TA + lidocaine; (**c**) 0.1% TA; (**d**) 0.1 TA + lidocaine.

On the other hand, Table 2 shows that the viscosity was similar in all formulations, except for 0.1% TA which was slightly lower. All the formulations followed a Cross model (Equation (5)) for both the ascendant and descendant sections.

$$\text{Cross equation: } \tau = \dot{\gamma} \cdot \frac{\eta_\infty + (\eta_0 - \eta_\infty)}{1 + \left(\frac{\dot{\gamma}}{\dot{\gamma}_0}\right)^n} \quad (5)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (1/s), $\dot{\gamma}_0$ is the zero shear rate (1/s), η_∞ is the infinite shear rate viscosity, η_0 is the zero shear rate viscosity (Pa·s), and n is a dimensionless rate constant.

Table 2. Rheological evaluation of the different formulations at 100 s^{-1}.Values represent means ± SD (n = 2).

Formulations	Viscosity (mPa·s) at 100 s^{-1}
0.05% TA	3890.0 ± 39.8
0.05% TA + LIDO	3833.0 ± 27.9
0.1% TA	3662.0 ± 42.3
0.1% TA + LIDO	3819.0 ± 39.8

3.3. Analytical Method Validation

The analytical method for TA using high performance liquid chromatography was validated in order to obtain consistent, reliable, and accurate data [14,15] for the formulations of topical administration, as analytical methods for TA have been validated only for other routes of administration [4,5]. Linearity is the ability to obtain, within a defined range, results directly proportional to the concentrations (amount) of the analyte in the sample. The range is the interval defined by the upper and lower concentrations of the tested drug, for which it has been proved that the method has a suitable level of accuracy, precision and linearity [16]. Figure 3 shows a typical chromatogram obtained in the analysis of samples containing TA.

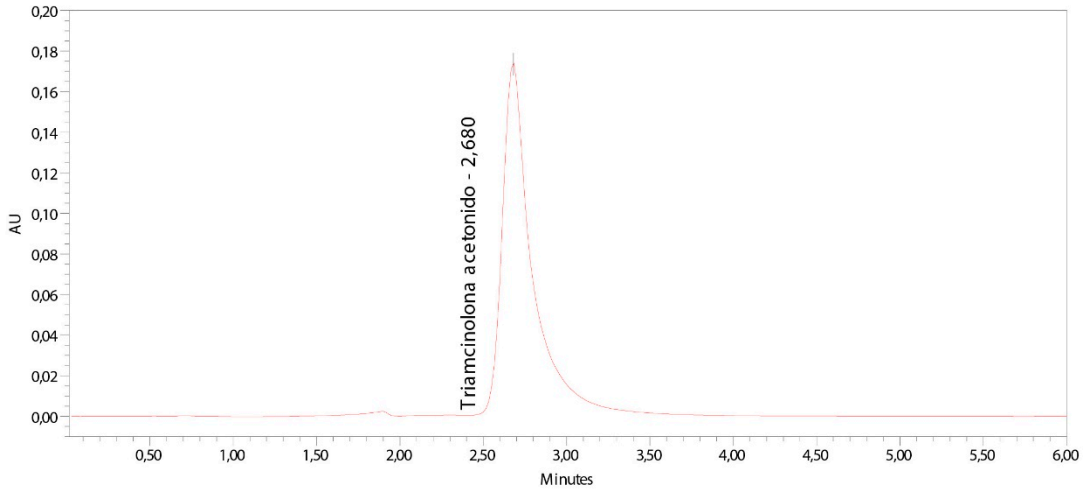


Figure 3. Chromatogram of the TA standard solution.

The results of the analytical method validation show that the six calibration lines were linear from 6.26 to 100.20 µg/mL, showing a correlation coefficient (r) in the range of 0.9993 to 0.9998 for each line. The method was accurate and precise in the range of 6.26 µg/mL to 100.20 µg/mL, with an accuracy of 92.49% and precision of 98.23% (at 6.26 µg/mL). Finally, the LOD of the method was 2.63 ± 1.19 µg/mL and the LOQ calculated was 7.97 ± 3.60 µg/mL.

3.4. Release Studies

In order to confirm that the APIs can be released from the matrix of the pharmaceutical form and can reach the biophase, drug release studies were performed using Franz-type diffusion cells. For each formulation, the cumulative released amount of TA (µg) versus the time (h) was obtained in triplicate (Figure 4), all of them following a Boltzmann sigmoidal model according to the coefficients of determination (r^2) ≥ 0.98.

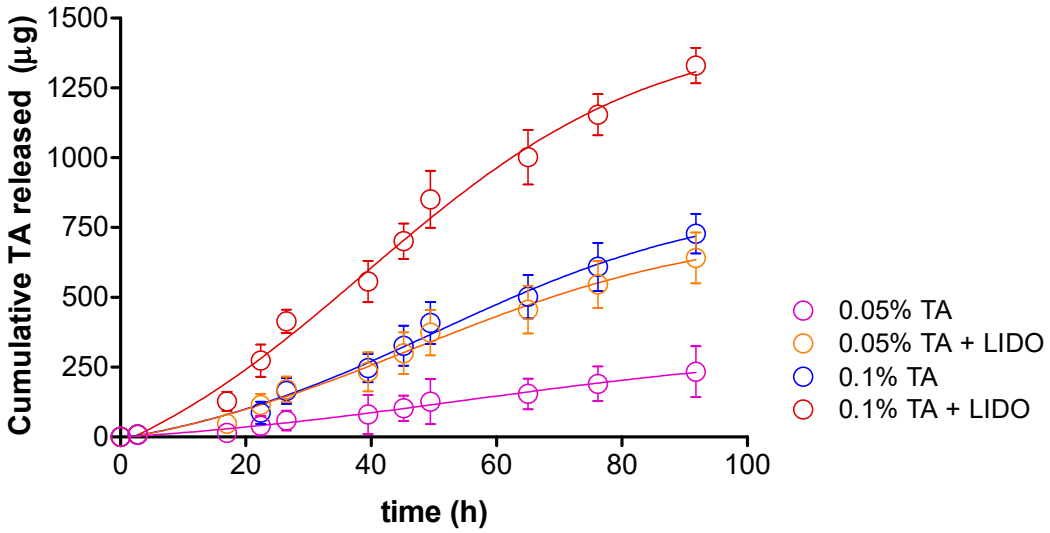


Figure 4. Cumulative amount (µg) of TA released versus time (h) from the four different formulations. Values represent means ± SD (n = 3).

TA was released to different extents depending on the formulation: after 76.2 h 1154.33 µg was released from the TA 0.1% + LIDO, 609.11 µg from the TA 0.1%, 546.33 µg from the TA 0.05% + LIDO, and 190.78 µg from the TA 0.05% formulations. Therefore, the presence of lidocaine hydrochloride promotes a greater release of TA. These results might

suggest that the higher (2%) amount of Orabase® in the formulations without lidocaine accounted for the higher retention of TA in the formulation, or that the ionic nature of lidocaine hydrochloride, which can undergo a faster solvation and diffusion in the medium, could have indirectly promoted a faster release of TA.

3.5. Permeation and Retention Studies

Ex vivo permeation studies of the four different formulations ($n = 5$) were carried out to test the ability of TA to permeate the buccal mucosa and be retained within the tissue upon application. The experiment setup was similar to the release studies, but the membrane was replaced with either porcine sublingual or buccal mucosa. The amount of TA (μg) permeated across either mucous tissue was plotted versus time (h) and a linear least squares regression was performed (Figure 5).

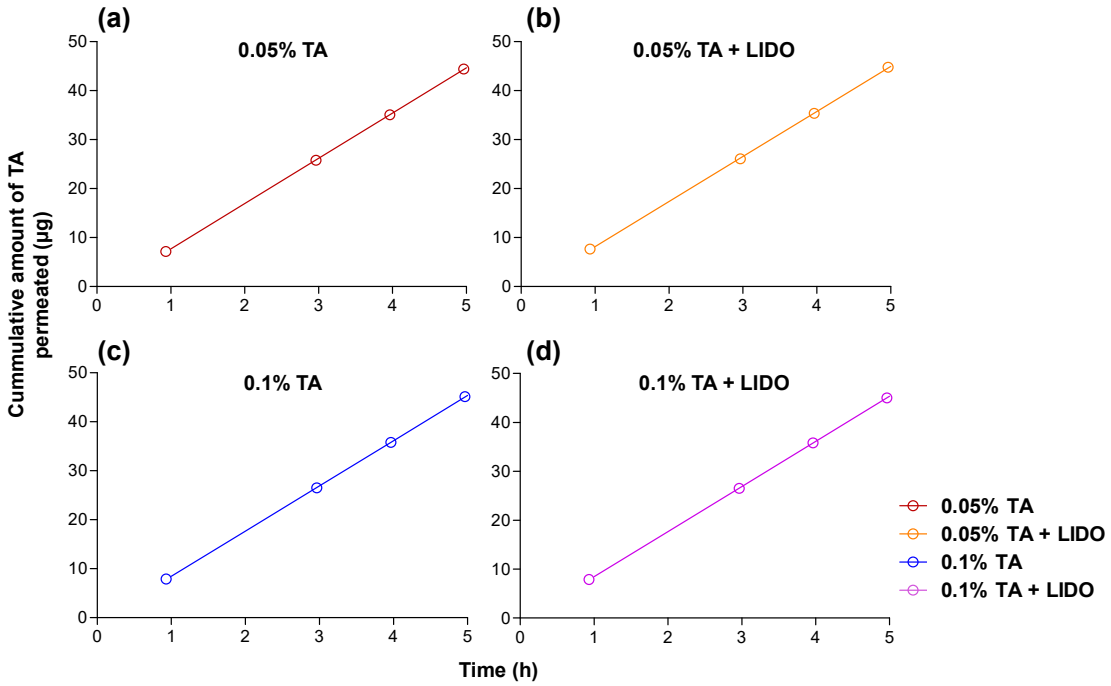


Figure 5. Buccal permeation kinetics of TA for the different formulations. (**a**) 0.05% TA; (**b**) 0.05% TA + LIDO; (**c**) 0.1% TA; (**d**) 0.1% TA + LIDO. Values represent means ± SD ($n = 5$).

The results show that TA can permeate buccal mucosa at approximately 9.2 μg/h regardless of the TA concentration (0.05% or 0.1%) or the presence or absence of lidocaine hydrochloride (Table 3), as no significant differences were observed (>0.05) according to the Student's *t*-tests.

Table 3. Amount of TA permeated in buccal mucosa per hour (flow). Values represent means ± SD ($n = 5$). No significant differences were observed ($p < 0.05$).

Formulations	Flow (μg/h)
0.05% TA	9.24 ± 0.03
0.05% TA + LIDO	9.19 ± 0.06
0.1% TA	9.24 ± 0.03
0.1% TA + LIDO	9.22 ± 0.02

Considering a possible systemic effect after application of the formulations, Argenti D et al. [17] determined the multiple-dose pharmacokinetics, pharmacodynamics, and tolerability of a newly developed formulation of inhaled TA. They found that the maximum

serum concentration (Cmax) at the steady state was 1.83 ng/mL. Further, they found that TA treatment reduced the basal serum cortisol concentrations by 20% relative to the placebo treatment.

For this reason, the concentration at steady state (Css) for each formulation was calculated according to the permeation parameters obtained and the reported pharmacokinetic parameters of TA. For instance, upon treatment with these formulations, Css values would oscillate between 1.54–1.57 ng/mL, which is 15% below the values reported (1.83 ng/mL), all formulations having similar systemic safety profiles.

The amount of TA retained within the buccal mucosa was calculated by extracting the drug from the tissue after permeation experiments with the four different formulations (Figure 6), finding that application of 0.05% TA led to 9.2 ± 2.4 mg TA retained per gram and centimeter squared of tissue, whereas application of 0.05% TA + LIDO led to 14.8 ± 2.7 mg g^{-1}·cm^{-2}, representing a 60% increase. Similarly, the application of 0.1% TA resulted in 8.0 ± 1.4 mg·g^{-1}·cm^{-2} while 0.1% TA + LIDO resulted in 15.6 ± 2.2 mg·g^{-1} cm^{-2}, representing a 95% increase in retained TA. Student's *t*-tests confirmed there was a significant increase ($p < 0.01$) in the amount of TA that could be retained in the tissue for performing its therapeutic activity when the formulations included lidocaine hydrochloride, suggesting that this drug also behaves as a penetration enhancer.

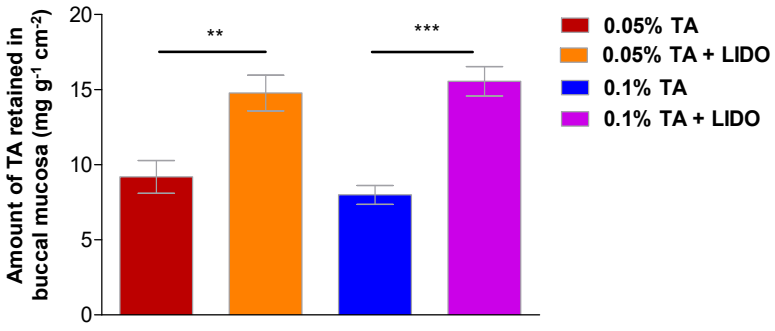


Figure 6. Amount of TA retained per gram and centimeter squared of buccal mucosa, 6 h after application of each formulation (0.05% TA or 0.05% TA + LIDO, or 0.1% TA or 0.1% TA + LIDO). Values represent means ± SEM ($n = 5$). Statistical differences ** ($p > 0.01$), *** ($p < 0.001$).

In addition, permeation studies were also performed in sublingual mucosa, considering the possibility that the tongue accidentally contacts the formula, revealing whether the applied TA could still permeate in the sublingual mucosa. The cumulative permeated amount of TA in sublingual mucosa for 6 h after application of each type of formulation ($n = 5$) was obtained (Figure 7).

Sublingual permeation also showed linear behavior, with fluxes slightly higher than those observed in buccal permeation, ranging between 10.1 µg/h and 12.4 µg/h, as observed in Table 4. Student's *t*-tests were performed for evaluating the influence of the presence of lidocaine hydrochloride in the formulations, revealing significantly higher fluxes both at the 0.05% TA concentration ($p < 0.001$) and the 0.1% TA concentration ($p < 0.05$), suggesting that lidocaine hydrochloride behaves as a permeation enhancer in sublingual mucosa through mechanisms of action that could include a reversible integrity loss of the skin and mucosa barriers, an increase in the partitioning of the drug into the tissue, or an increase in the solubility of the drug [18,19]. The effects of a permeation enhancer may differ when combined with one or more drugs [20].

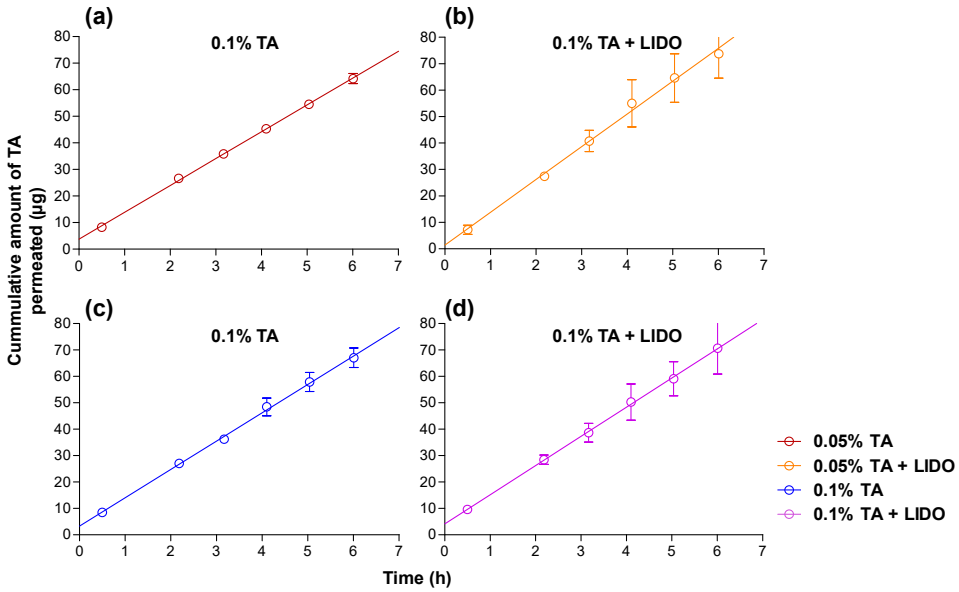


Figure 7. Sublingual permeation kinetics of TA for the different formulations. (**a**) 0.05% TA; (**b**) 0.05% TA + LIDO; (**c**) 0.1% TA; (**d**) 0.1% TA + LIDO. Values represent means ± SD (n = 5).

Table 4. Amount of TA permeated in sublingual tissue per hour (flow). Values represent means ± SD (n = 5). Significant differences * ($p < 0.05$), *** ($p < 0.001$).

Formulations	Flow (µg/h)
0.05% TA	10.10 ± 0.12
0.05% TA + LIDO	12.40 ± 0.42 ***
0.1% TA	10.74 ± 0.20
0.1% TA + LIDO	11.04 ± 0.14 *

For this reason, the concentration at steady state (Css) for each formulation was also calculated and resulted in a range of 1.67–2.06 ng/mL, similar to values previously reported (1.83 nm/mL) [17], and which could indicate some possible systemic effects in the semisolid formulations of TA.

Corticosteroids can affect keratinocytes and prevent the secretion of collagen and hyaluronic acid by fibroblasts in dermis, interfering with cell proliferation, and, with long-term glucocorticoid usage, skin thinning ensues. Topical administration could produce local side effects, which could include skin atrophy, ecchymosis, erosions, striae, delayed wound healing, purpura, easy bruising, acne, hirsutism, and hair loss [21]. Therefore, it is important to point out that these semisolid formulations should be used promptly and following doctor's instructions.

Overall, it seems that lidocaine hydrochloride is a promoter for the release of TA from the matrix and its retention in the buccal mucosa. This could mean that, besides the anesthetic effects of lidocaine, the presence of this API is important to reduce the dose of TA in the formulations. As seen in Figure 6, there was the same degree of TA retention upon application of either the 0.05% TA + LIDO or the 0.1% TA + LIDO formulation, and there was the same degree of permeation, as observed in Table 4. Thus, it would be unnecessary to use the formulation with a higher content of TA, and the best formulation for buccal administration would be the 0.05% TA + LIDO. On the other hand, if a sublingual administration is needed, it would be safer to use the formulations without lidocaine, since they show a lower rate of TA permeation.

4. Conclusions

Formulations containing 0.05% or 0.1% TA, and in the presence of absence of 2% lidocaine hydrochloride, were designed and developed for buccal application as potential treatments for important inflammatory processes in the buccal mucosa, such as those occurring upon buccal cancer radiotherapy, lichen planus, and canker sores, among others. The effect of TA concentration and the presence or absence of lidocaine hydrochloride on the mechanical or biopharmaceutical properties of the formulations was extensively studied. The four different formulations showed shear thinning and thixotropic behavior, ideal for topical application. On the other hand, TA could be released from the formulations following Boltzmann sigmoidal behavior, and we found that the presence of lidocaine hydrochloride promoted between 107% and 212% more TA released after 92 h ($p < 0.05$). The formulation of 0.05% TA + LIDO showed the highest amount of TA released (1330 μg).

Moreover, permeation studies showed that TA could successfully permeate buccal mucosa at rates ranging between 9.19 and 9.24 μg/h, with the rate not being influenced by either the concentration of TA or the presence of lidocaine hydrochloride. However, upon application, TA was successfully retained beneath the buccal mucosa to perform its anti-inflammatory activity, regardless of TA concentration, and the presence of lidocaine hydrochloride could increase the amount of TA retained by 60% or 95% ($p < 0.01$). Nonetheless, continuous contact of the tongue with the applied formula could also lead to TA permeation, especially in the presence of lidocaine hydrochloride, as observed in sublingual mucosa permeation experiments.

Besides the anesthetic activity lidocaine hydrochloride can provide, its inclusion may enable the lowering of the concentration of TA in the formulation with similar efficacy, and the reduction of the associated side effects of glucocorticoids, although the treatment should be used punctually due to the existence of permeation processes for TA.

Based on the results obtained, the formulation containing 0.05% TA and lidocaine hydrochloride seems to be the most suitable option for treating inflammatory processes in the buccal mucosa. Future studies would be useful to characterize the release and permeation processes for lidocaine hydrochloride, and it would be interesting to assess the stability of these topical formulations.

Author Contributions: Conception of the research: A.C.-C. Design of the experiments: Investigation: M.M.V., A.M.L., L.H.B., D.B.T. Data processing: M.M.V., A.M.L., L.H.B., P.C.S.G., D.B.T., D.L. Writing of fist draft: M.M.V. Revisions and final editing: D.L., L.H.B., A.C.-C. Funding acquisition: A.C.-C., L.H.B. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The study was carried out in accordance with the Declaration of secondary reuse of animal tissues for biopharmaceutical products, under the supervision of the Director of the Bellvitge Campus Establishment of the University of Barcelona and member of the Ethical Committee for Animal Experimentation (ECAE) of the University of Barcelona.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Gimeno, for his support and supervision as director of the Bellvitge Establishment for the extraction of biological samples.

Conflicts of Interest: None of the authors had any financial or personal relationships that could have inappropriately influenced the content of the paper. All are members of the Faculty of Pharmacy and Food Sciences of the University of Barcelona. The Institution played no role in the study design or in the decision to submit the manuscript for publication. The authors are freely responsible for the collection, analysis and interpretation of data.

Abbreviations

API	Active pharmaceutical ingredient
TA	Triamcinolone acetonide
LIDO	Lidocaine hydrochloride
HPLC	High-performance liquid chromatography
ACN	Acetonitrile
LOD	Limit of detection
LOQ	Limit of quantification
SD	Standard deviation
Css	Concentration at steady state
Cmax	Maximum concentration

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