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

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Abstract: The mouth can be affected by important inflammatory processes resulting from localized or systemic diseases such as diabetes, AIDS and leukemia among others, and 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 formula also contains Orabase® as an excipient which is a protective, hydrophobic, and anhydrous adhesive vehicle, used to retain or facilitate the application of active pharmaceutical ingredients (API) 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. As well, the amount of TA retained within the tissue was estimated, where it performs its anti-inflammatory activity, and showing no significant differences between the 0.05% TA + LIDO and 0.1% TA + LIDO formulations ($p > 0.05$). Therefore, results evidence the suitability of the administration of the lowest concentration of TA tested, achieving similar efficacy, and decreasing the potential systemic effects of corticoid administration. Besides, sublingual permeation studies were carried out to evaluate a scenario of a continuous contact of the tongue with the applied formulation. The four formulations studied show a pseudoplastic and thixotropic behaviour, ideal for topical application. These results evidence the potential of these topical formulations for the treatment of inflammatory processes in the buccal mucosa.

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

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, and can be manifested in various types of buccal sores, such as canker sores or lichen planus, conditions that typically present inflammation and pain [1]. As well, alghough 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 workshows the design and development of 4 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 anaesthetic 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 the evaluation of 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 can be released from the formulation was determined. As well the ability for permitting 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 reiable results from release, permeation, and retention studies, we designed and validated an analytical method using High-Performance Liquid Chromatography (HPLC), which showed to be linear and accurate in the rage of concentrations studied.

2. Experiments

2.1. Materials

Triamcinolone acetonide (TA), Lidocaine hydrochloride (LIDO), Orabase® and Liquid paraffin were purchased in Fagron. Transcutol P was purchased in Gatefossé. Acetonitrile (ACN) was purchased in Fisher Chemical. Ammonium acetate (≥98%) was purchased in Panreac.

2.2. Composition of the Formulations

Four different formulations containing TA for topical administration were developed for evaluating 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} during 3 min, followed 1 min at a constant rate of 100 s^{-1}, and from 100 s^{-1} to 0 s^{-1} during 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 analythical method of TA using High Performace Liquid Chromatography (HPLC) 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; 4.6mm and 5 μm) column. The data were collected and processed using the Empower Pro software (Waters, Milford, USA).The mobile phase consisted on 50:50 (*v*/*v*) water/methanol. 10 μL samples were injected and TA was detected at 232 nm according to a validated method for a different route of administration [4]. TA was initially dissolved in Transcutol P, and further diluted using a mixture of Acetonitrile (ACN):Ammonium acetate buffer pH 4.7 (10:90) [5]. 6 different calibration curves were done by preparing stock solutions of 205 μg/mL TA, and further dilutions to 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 to the experimental data and evaluating the the correlation coefficient (r) based on Equation (1):

$$\boldsymbol{y = SB \cdot x + a} \quad (1)$$

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, %) calculated using the Equation (2):

$$\%\boldsymbol{RE} = [(\boldsymbol{Cobs - Cnom})/\boldsymbol{Cnom}] \cdot \mathbf{100} \quad (2)$$

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 the Equation (3):

$$\%\boldsymbol{RSD} = (\boldsymbol{SD}/\boldsymbol{Cobs}) \cdot \mathbf{100} \quad (3)$$

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:

$$\boldsymbol{LOD\ or\ LOQ = K \cdot SDsa/Sb} \quad (4)$$

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].

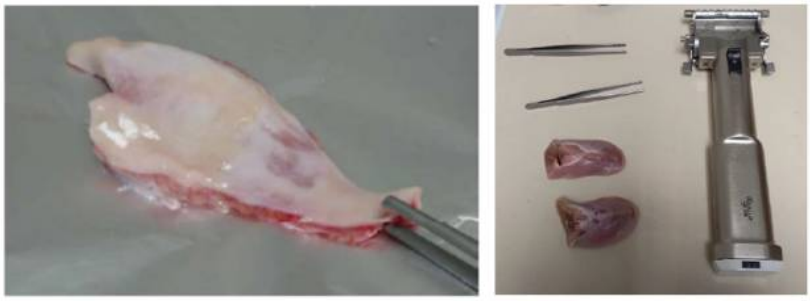
2.5. Release Studies

To assess the release of TA from the 4 different types of formulations, drug release experiments were performed in triplicate using Franz-type diffusion cells (FDC 400, Crown Glass, Somerville,

NY), being the donnor and receptor chambers separated by nylon synthetic membranes (Type NY41 41 μm). The receptor chambers were filled with a mixture of Acetonitrile (ACN):Ammonium acetate buffer pH 4.7 (10:90) and Transcutol, complying 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 is similar to that of release studies, but replacing the membrane for 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. 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 t-tests were performed using GraphPad prism 3 for comparing the different formulations.

3. Results and Discussion

3.1. Composition of the Formulations

Four different formulations containing TA for topical administration were developed for evaluating 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 consistendt 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 the formulations play an important role in physical stability, and are an important attribute in the development of topical drug products [9]. In order to know the mechanical properties of the formulations, rheology studies were performed, and revealed a

pseudoplastic and apparent thixotropic behaviour in all the formulations (Figure 2), both being desirable characteristics for topical application allowing the formation of a consistent film covering the application area that facilitates the diffusion of the drug through the matrix [10–13].

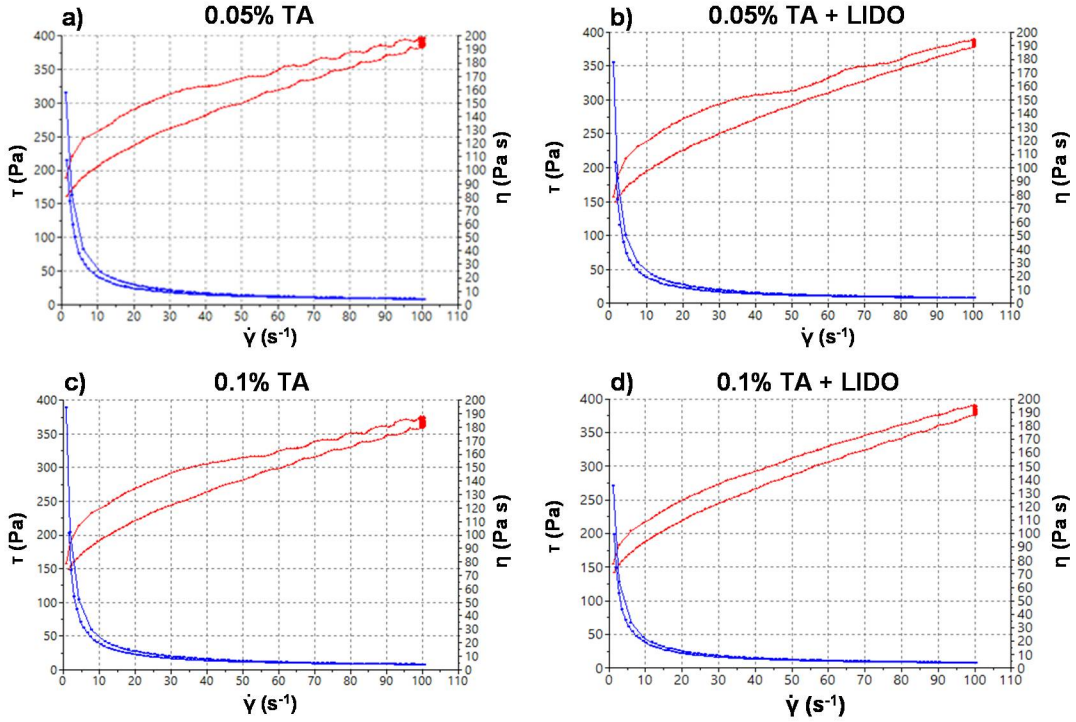


Figure 2. Rheology profile of the 4 formulations. (**a**): 0.05% TA. (**b**): 0.05% TA + Lidocaine. (**c**): 0.1% TA. (**d**): 0.15 TA + Lidocaine

On the other hand, Table 2 shows that the viscosity is similar in all formulations except for 0.1% TA which is 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); 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

3.2. Analytical Method Validation

The analythical method of TA using High Performace Liquid Chromatography (HPLC) was validated in order to achieve consistent, reliable, and accurate data [14,15] in formulations of topical administration, as analytical methods for TA have been validated only for other routes of administration [4,5]. Linearity is the ability within a defined range to obtain 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.

The results of the analytical method validation show that the 6 calibration lines are linear from 6.26 to 100.20 μg/mL, showing a correlation coefficient (r) in the range of 0.9993 – 0.9998 for each line. The method is accurate and precise in the range of 6.26 μg/mL and 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.

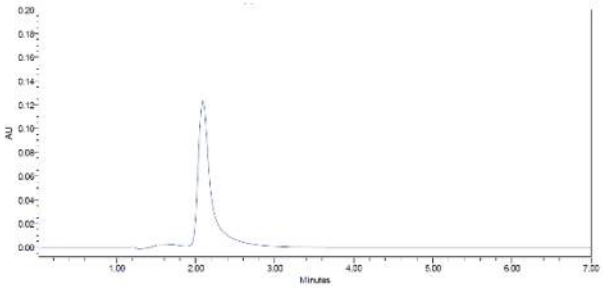


Figure 3. Chromatogram of the TA standard solution.

3.3. *Release Studies*

In order to assure that the API 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 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.

TA is released to a different extent depending on the formulation, after 76.2h being released 1154.33 μg from TA 0.1% + LIDO, 609.11 μg from TA 0.1%, 546.33 μg from TA 0.05% + LIDO, and 190.78 μg from TA 0.05% formulation. Therefore, the presence of lidocaine hydrochloride promotes a higher amount of TA released. These results might suggest that the higher (2%) amount of Orabase ® in the formulations without lidocaine might account for a 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 indirectly promote a faster release of TA.

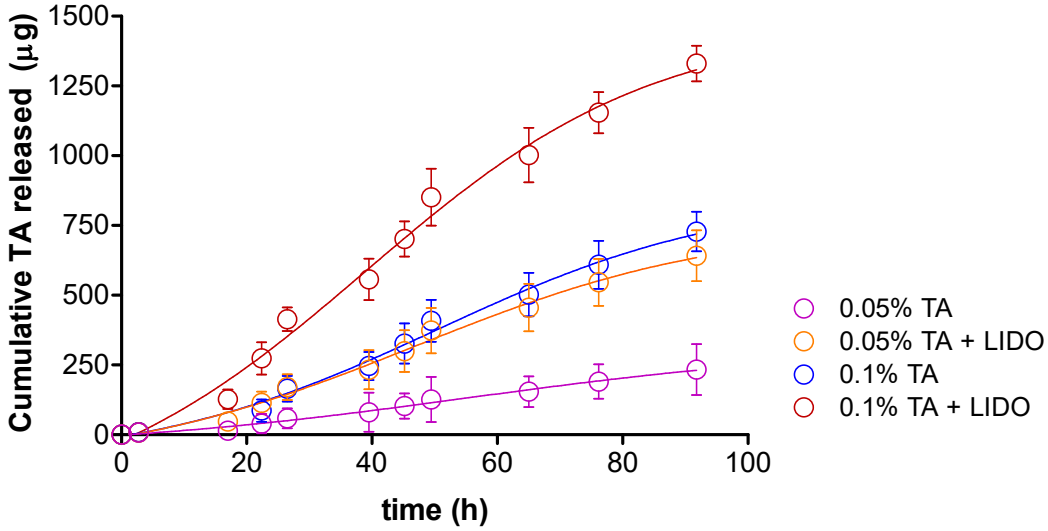


Figure 4. Cummulative amount (μg) of TA released versus time (h) from the four different formulations. Values represent Mean ± SD (n = 3).

3.3. *Permeation and Retention Studies*

Ex vivo permeation studies of the four different formulations (n = 5) were carried out to test the ability of TA for permeating the buccal mucosa and being retained within the tissue upon application. Experiment setup was similar to the release studies, but replacing the membrane for 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).

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 Student t-tests.

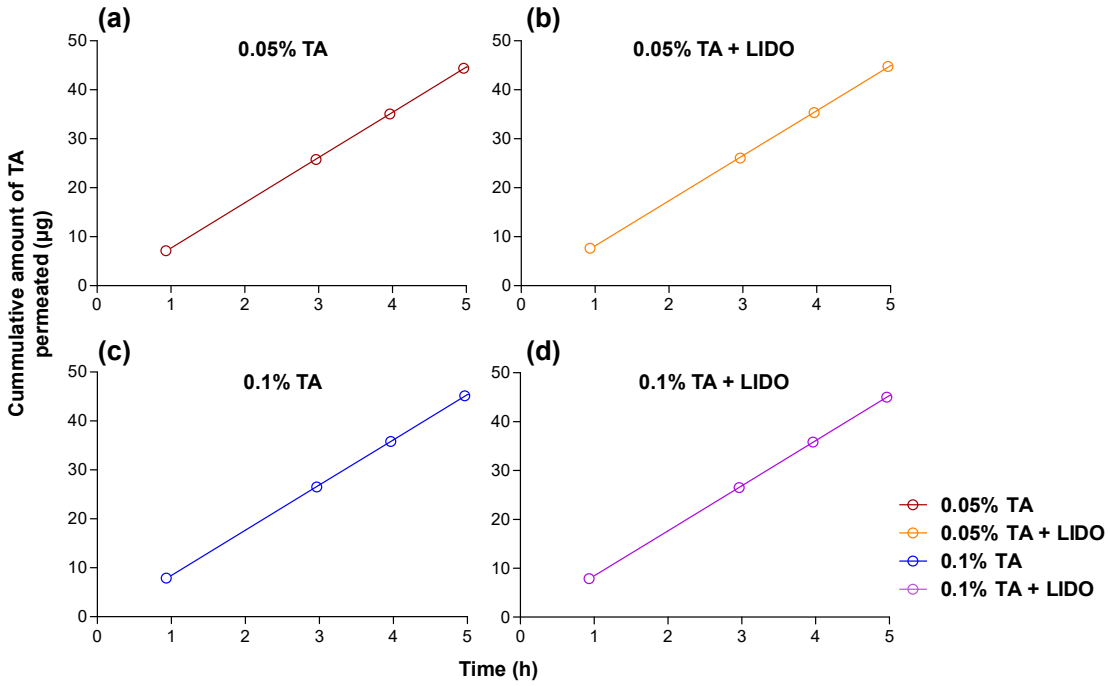


Figure 5. Buccal permeation kinetics of TA for the different formulations. (**a**): 0.05% TA. (**b**) 0.05% TA + LIDO. (**c**): 0.1% TA. (**d**): 0.1% TA +LIDO. Values represent Means±SD (n = 5).

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. Besides, they found that TA treatment reduced by 20% the basal serum cortisol concentrations 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 are 15% below those reported (1.83 ng/mL), having all formulations 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 leads to 9.2 ± 2.4 mg TA retained per gram and centimeter squared of tissue, whereas application of 0.05% TA + LIDO leads to 14.8 ± 2.7 mg g^{-1}·cm^{-2}, representing a 60% increase. Similarly, the application of 0.1% TA results in 8.0 ± 1.4 mg·g^{-1}·cm^{-2} while 0.1% TA + LIDO results in 15.6 ± 2.2 mg·g^{-1} cm^{-2} representing a 95% increase in retained TA. Student t-tests confirmed there is a significant increase ($p < 0.01$) in the amount of TA that can be retained in the tissue for performing its therapeutic activity when the formulations include lidocaine hydrochloride, suggesting this drug also behaves as a penetration enhancer.

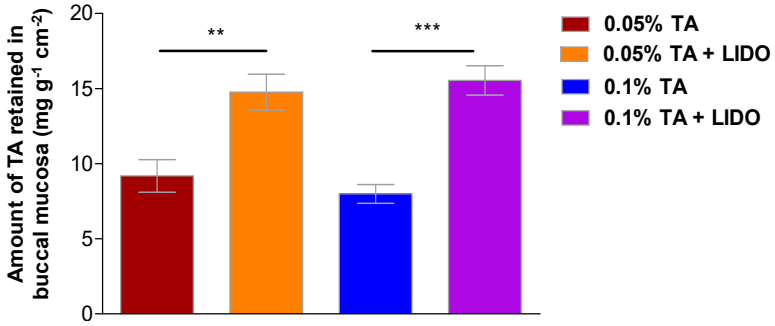


Figure 6. Amount of TA retained per gram and centimeter squared of buccal mucosa, 6 h after application of each formulation (0.05% TA or 0.05% TA + LIDO, or 0.1% TA or 0.1% TA + LIDO). Values represent Mean ± SEM ($n = 5$).Statistical differences ** ($p > 0.01$), *** ($p < 0.001$).

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

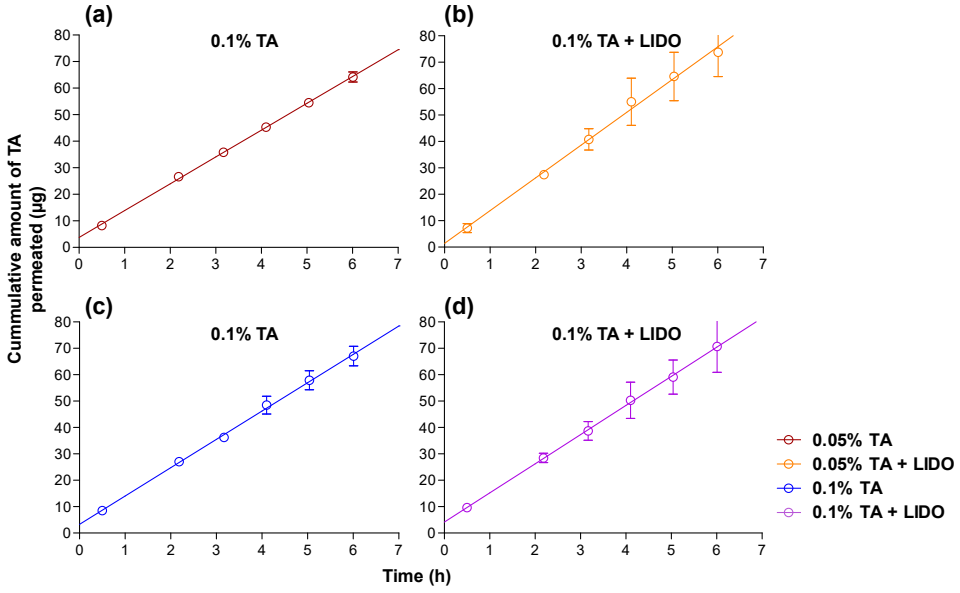


Figure 7. Sublingual permeation kinetics of TA for the different formulations. (**a**) 0.05% TA. (**b**) 0.05% TA + LIDO. (**c**) 0.1% TA. (**d**) 0.1% TA +LIDO. Values represent Means±SD (n = 5).

Sublingual permeation also shows a linear behaviour 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 t-tests were performed for evaluating the influence of the presence of lidocaine hydrochloride in the formulations, revealing significantly higher fluxes both at 0.05% TA concentration ($p < 0.001$) and 0.1% TA concentratiosn ($p < 0.05$), and suggesting that lidocaine hydrochloride behaves as a permeation enhancer in subligual mucosa, through mechanisms of action that could include the reversible integrity loss of the skin and mucosa barriers, the increase in the partitioning of the drug into the tissue, or the increase in the solubility of the drug [18,19]. The effects of a permeation enhancer may differ when combined with one or other drug [20].

Table 4. Amount of TA permeated in sublingual tissue per hour (flow). Values represent Mean ± SD (n = 5). Signifficant 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 the values 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 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. These could mean that, besides the anaesthetic 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 is the same degree of TA retention upon application of either the 0.05% TA + LIDO or the 0.1%TA + LIDO formulation, and there is the same degree of permeation as observed in Table 4. Thus, it would be unnecessary to use the formulation with 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.

5. Conclusions

Formulations containing 0.05% or 0.1% TA, and in 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, 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 a pseudoplastic and thixotropic behavior, ideal for topical application. On the other hand, TA can be released from the formulations following a Boltz Sigmoidal behaviour, finding that the presence of lidocaine hydrochloride promotes between 107% and 212% more TA released after 92 h ($p < 0.05$), where the formulation of 0.05% TA + LIDO showed the highest amount of TA released (1330 μg).

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

Besides the anesthetic activity lidocaine hydrochloride can provide, its inclusion may permit lowering the concentration of TA in the formulation with similar efficacy, and lowering 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, formultation containg 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. Funds acquisition: A.C.-C., L.H.B. All authors have read and agreed to the published version of the manuscript.

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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 serum concentration

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