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Electrophoretic bottom up design of chitosan patches for Topical Drug Delivery

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Abstract:	Clobetasol propionate (CP) is a high-potency corticosteroid, representing the standard of care for the symptomatic treatment of different skin disorders as well as oral cavity diseases. Several topical delivery systems are available in oral lesion care, but the ideal one is still lacking, being serious side-effects still reported. In this work, we propose a novel class of chitosan (CS) patches, loaded with CP, for the topical treatment of inflammatory chronic oral muco-cutaneous diseases. Chitosan patches have been fabricated via electrophoretic deposition (EPD), by using a one-pot approach in order to load controlled quantity of CP. Optimized structures showed a water uptake in the range of 200-360% and mechanical properties that allow the design of flexible patches in wet state (E = 0.6 MPa and δbr = 0.55 MPa). Ultraviolet-visible (UV-Vis) spectroscopy was used for the evaluation of both loading and release profile of CP in CS patches. The CP loading has been tuned by adjusting CP concentration in deposition bath -in the range 0.002 to 0.12 mg cm-² while releasing curves show an in vitro CP burst of about 80% in the first two hours. Overall, the obtained properties paved the way for the application of this new class of patches for the local oral release of CP. Keywords Electrophoretic deposition (EPD); Hierarchical patches; Chitosan; Clobetasol propionate; Topical oral drug delivery.
Response to Reviewers:	The PDF file has been attached to the "attach Files" section: Comments to reviewer 1. We would like to thank again the reviewer for her/his invaluable effort in reviewing the paper. Comments have been very useful in improving the overall quality of the text. Abstract

	-clarify "issue reduction and prevention" Abstract has been revised in this section as follow: “In this work, we propose a novel class of chitosan (CS) patches, loaded with CP, for the topical treatment of inflammatory chronic oral muco-cutaneous diseases.” -Reword significant water uptake to state amounts or ranges. avoid "significant" unless statistics are presented. Abstract has been revised in this section as follow: “…Optimized structures showed a water uptake in the range of 200-360% and mechanical properties that allow the design of flexible patches in wet state (E = 0.6 MPa and δbr = 0.55 MPa). -What are stripes? is this strips, or patches? Try to make wording uniform throughout. It is revised to patches and uniformed throughout the manuscript. -Recommend revising line 36 page 1 to remove completely. It is revised and removed. Introduction -Clarify why topical delivery systems are controversial. Reword this statement to include what the requirements for oral steroid delivery are vs. other applications. Introduction has been revised in this section as follow: “To date, CP aqueous solutions [5], adhesive denture pastes [6], ointments [7] and galenic preparations in form of gel have been proposed and tested over the last decade and are usually prescribed for oral lesions”. And in the next paragraph, in order to better respond to the reviewer comment, we modified the introduction section and explained this point by adding additional references, as follows: “The use of these novel systems results in a significant accumulation of CP in the target, resulting in an improvement of the risk–benefit ratio, due to the reduction of several cutaneous and systemic side-effects related to topical steroidal treatment. Although local administration of corticosteroid is generally accepted to be safe, serious side effects have been reported, either locally or secondary to the systemic absorption of these drugs [10,11], and new delivery systems are hence actively studied”. -Recommend line 7 page 4, revise "interesting" to "versatile" It is revised to “versatile”. Materials and Methods: -Give specifics on the chitosan and supplier. What is the molecular weight avg., what is the degree of deacetylation. These are very important for reproducibility The requested information has been added into the main manuscript: Medium molecular weight chitosan (MMWC) has a molecular weight of 190–310 kDa, deacetylation degree of 75–85%, and a viscosity of 200–800 cPs. -recommend removing redundant sentence in page 5 line 51. Next sentence covers this. It is removed. -Clarify the elution procedure. Were small samples taken and was media refreshed any? Elution procedure was described as follows:

	“Specimens were incubated in 7.5 ml of PBS/ethanol (1:1) solution. They were fixed vertically in 15 ml falcon tubes with conical end, to allow release of the CP from both sides of the specimen. The tubes were maintained at 37 °C and shaken at 100 rpm. At each time-points, the patches were taken out of the tubes and the solutions were collected and analyzed by UV-Vis spectrophotometer to determine the concentration of the CP released. The media was not refreshed at any time point. Tests were triplicated.”. (Section 2.2.2). Results: -Figure 1 a does not look "uniform and compact" as described in 3.1. Figures b and d could be adjusted in brightness to show more detail. The sentence is changed to: “Figure 1 shows the OM and SEM micrographs of the EPD optimization process in terms of microstructure and morphology of patches: selected processing parameters (pH, ethanol/water ratio in deposition bath, and EPD processing parameters) allow to modulate both thickness and morphology. A porous and discontinuous structure is evident in pure chitosan patches, processed in water based bath (c-d) [12], while a continuous and more uniform structure can be noticed in CP-loaded CS patches, processed in ethanol-based bath.”. -page 8 line 9. Clarify increase or decrease of pH. According to the reviewer comment, this section description is changed to: “Figure 2 reports the results of mass deposition vs. time of EPD process at different compositions of the deposition bath. Plots show a monotonic increase of deposited mass vs. time, in which a significant difference in deposition rate can be noticed between water-based vs. ethanol-based baths. However, the addition of Clobetasol Propionate in the ethanol-bath seems to have a negligible influence on the final deposited mass and deposition rate. Here, data related to [CP]/[CS]=10 are reported, being the maximum concentration of CP in bath and hence the situation in which the drug can influence the EPD process”. -page 9. Statistics aren't shown in table 1. Which two sets differ? The significantly different values have been highlighted in the table 1 and the table description sentence revised to: “As it is evident from table 1, there is a statistically significant difference ($p < 0.05$ in ANOVA) between two sets of data, zeta potential and conductivity, of CS patches fabricated from water-based deposition bath and those from the ethanol-based one (the values are highlighted).” -clarify whether CP was loaded into patches prepared with water or ethanol based solutions. Why were there not two sets of CP loaded in table 2 for instance? Ethanol-based bath used to fabricate CP loaded CS patches as it is mentioned in section 2.1.1, “Clobetasol propionate (Farmacia Comunale n.70, Milan, Italy) has been used in 30% water + 70% ethanol bath (pH = 5.0, [CS] = 1g L^{-1}, [CP] = 250-10000 mg L^{-1}) to produce CP loaded CS patches”, and also in table 1 and table 2, “CP loaded CS patches (ethanol-based)”. To address the comment rose by the reviewer, the table 2 description has been revised to: “Patches processed in ethanol-based baths show a lower Young modulus (E) ($p < 0.05$) and higher strain (ε) at break if compared to water-based bath. However, the incorporation of Clobetasol Propionate, at the higher concentration reported in this study, does not have a significant influence on the overall mechanical behavior”. -page 12 lines 40-46 contain information that should be in methods, not results. The information was moved to “Materials and methods”, section 2.2.2 Water uptake and drug release study. -page 12 line 20-52--this statement is not a result, but a discussion point. Avoid

	references in results. Remove sentence The sentence was moved to discussion section. Discussion: -Avoid references back to figures in the discussion; they have already been presented in the results The figure references have been removed from the discussion section. -page 15, line 7 remove "a enough" The sentence was revised to: “On the other hand, if the particles have the optimum surface charge, the packed and dense film has been occurred due to particles repulsion”. -page 15 line 31. revise "better" to explain why organic solvents improve electrophoretic deposition, or what quality makes this "better" The sentence was revised to: “Organic solvents are preferred to water as a suspension medium in electrophoretic process. While the generally lower dielectric constant of organic liquids (e.g., ethanol dielectric constant = 25) limits the charge on the particles because of the lower dissociating power, much higher field strengths can be used to solve the problem concerning the electrolytic gas. Hence, the electrolysis and gas evolution associated with aqueous EPD processing can be avoided by using solvents with extremely high oxidation–reduction potentials as ethanol. Moreover, the organic liquids are preferred due to their higher density, good chemical stability and low conductivity”. As it is mentioned above the organic solvents are preferred due to their higher density, good chemical stability and low conductivity compared to water as a suspension medium in electrophoretic process. -page 16 line 24. remove probably. It is removed. -According to results, only 80% is released in 24 hours. Is there some left in the patches? What happens as patches degrade? Are they swallowed or removed? A better sense of the clinical application would improve reader interest To address the reviewer comment and describe a better sense of the clinical application, the intro of the discussion section has been also revised as follows: “4. Discussion Topical steroid therapy is largely used in the management of a variety of skin diseases and it is considered first-line therapy in patients with chronic inflammatory oral mucosal diseases. The local administration of Clobetasol Propionate, as other topical corticosteroid, is in general safe, even if some works report local or systemic side-effects [11]. It is not yet clear if these side effects are correlated to the direct absorption of the drug from the skin or by an ingestion. An optimal delivery system, in this context, should localize the cargo bioavailability by preventing rapid dilution and systemic uptake, and facilitate the release at a physiologically relevant rate and duration [21]. Thin films have been identified as an alternative approach to conventional dosage forms: an efficient design of such delivery system requires a comprehensive knowledge of the pharmacological properties of drugs and polymers, along with an appropriate selection of manufacturing processes [22]. In this work, we proposed Electrophoretic Deposition as an efficient yet versatile technique to both realize chitosan-based thin film and incorporate clobetasol propionate drug. EPD has been optimized in this work by using ethanol as solvent, showing the feasibility of an optimal thin film preparation for patches and the incorporation of a hydrophobic molecule in a one-pot process. The obtained patches are intended for the local and selective delivery of the drugs in the oral mucosa: Needlemand and co-workers originally showed, in an in vivo study, that chitosan films have prolonged adhesion time on the oral mucosa [23] while Senel et al. [24] showed that chitosan prolongs the adhesion time of oral gels and drug release from them. The rationale of designing chitosan patches loaded with

	Clobetasol propionate is hence to have a platform for topical drug delivery from films showing bio-adhesion, that can have a fast-local release to avoid side-effects, and that can be easily removed. A residence time longer than the release should help to minimize side-effects due to swallowing. section 4.2 has been also added to the discussion to illustrate the delivery system better: “4.2. Drug incorporation and delivery from Chitosan patches Clobetasol propionate is a lipophilic drug with a negligible water solubility: in order to be processed via Electrophoretic Deposition, CP has been dissolved in ethanol. This approach allowed the preparation of stable EPD bath and the incorporation of the drug in the patches. The explored processing range resulted in a concentration of CP in Chitosan up to 1.2 x 10-1 mg cm-2, easily tuned by adjusting the drug concentration in ethanol/water EPD bath. Gels and films have been found suitable for oral mucosa drug delivery applications since they are able to cover a wider area of mucosa for the purposes of drug delivery and physical protection [36]. The rate of hydration and the rheological properties of the polymeric formulations have a major impact on bio-adhesion and consequently the duration of retention: burst releasing of Clobetasol Propionate from the patches (80% release in less than 2h) has been obtained for all the concentration, fulfilling the requirements for topical oral applications”. -Avoid the use of optimal (page 16 line 52). There are advantages, but more work needs to be done as suggested. To consider the reviewer comment, conclusions section has been rephrased as follows: “Electrophoretic Deposition has been exploited as a powerful technique to fabricate patches for oral mucosal disease, effectively incorporating Clobetasol Propionate, a potent corticosteroid widely used for topical administration. EPD fabrication method has been originally shown as an effective while simple one pot approach to support the easy incorporation of water insoluble agents into chitosan matrix. While solvent plays a major role in both morphological and mechanical properties of the obtained patch, the incorporation of the drug does not affect such properties. Further studies are required to evaluate pharmacokinetics and to demonstrate the effectiveness of both support topical administration and reduce local and systemic side-effects”.

Title

Electrophoretic bottom up design of chitosan patches for Topical Drug Delivery

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Abstract

Clobetasol propionate (CP) is a high-potency corticosteroid, representing the standard of care for the symptomatic treatment of different skin disorders as well as oral cavity diseases. Several topical delivery systems are available in oral lesion care, but the ideal one is still lacking, being serious side-effects still reported. In this work, we propose a novel class of chitosan (CS) patches, loaded with CP, for the topical treatment of inflammatory chronic oral muco-cutaneous diseases.

Chitosan patches have been fabricated via electrophoretic deposition (EPD), by using a one-pot approach in order to load controlled quantity of CP. Optimized structures showed a water uptake in the range of 200-360% and mechanical properties that allow the design of flexible patches in wet state (E = 0.6 MPa and σ_{br} = 0.55 MPa). Ultraviolet-visible (UV-Vis) spectroscopy was used for the evaluation of both loading and release profile of CP in CS patches. The CP loading has been tuned by adjusting CP concentration in deposition bath -in the range 0.002 to 0.12 mg cm^{-2}- while releasing curves show an *in vitro* CP burst of about 80% in the first two hours.

Overall, the obtained properties paved the way for the application of this new class of patches for the local oral release of CP.

Keywords

Electrophoretic deposition (EPD); Hierarchical patches; Chitosan; Clobetasol propionate; Topical oral drug delivery.

1. Introduction

Clobetasol-17-Propionate (CP) is the most potent, currently available, synthetic corticosteroid used in dermatological applications, as ointment and cream, because of its high lipophilicity. It is used as standard of care for the symptomatic treatment of several skin disorders, including eczema and psoriasis [1,2], as well as chronic, immune cell-mediated, and autoimmune diseases of the oral cavity [3,4]. In latter applications, the environment is even more challenging than dermatological applications, because the presence of salivary flow and muscular activity during speech, chewing, and swallowing. CP aqueous solutions [5], adhesive denture pastes [6], ointments [7] and galenic preparations in form of gel, have been proposed and tested over the last decade and are usually prescribed for oral lesions.

Several studies investigated different innovative drug delivery systems to improve topical efficacy by increasing CP transdermal and transmucosal absorption, including solid/lipid nanoparticles [2], lipid microspheres [1], nanostructured lipid carriers [8], and lecithin/chitosan nanoparticles [9]. The use of these novel systems shows a significant accumulation of CP in the target, resulting in an improvement of the risk–benefit ratio, due to the reduction of several related cutaneous and systemic side-effects. Although local administration of corticosteroid is generally accepted to be safe, serious side-effects have been reported, either locally or secondary to the systemic absorption of these drugs [10,11], and new delivery systems are hence actively studied.

Electrophoretic deposition (EPD) is an efficient bottom-up technology that enables the fabrication of self-standing structures based on biopolymers. Thin or thick films, deposited on working electrodes and under suitable processing parameters, replicate the substrate morphology: by peeling them off the substrates, structures with controlled three-dimensional morphologies can be obtained [12]. Moreover, the mild EPD conditions allow to process

sensitive organic molecules, such as drugs, without impairing both their structure and activity [13,14]. Previous studies have shown the feasibility of cationic electrophoretic deposition of chitosan [12–14] and its removal from the cathode to be used as self-standing structures.

Chitosan is a cationic polysaccharide widely studied for its properties, such as biodegradability, biocompatibility, non-toxicity, and bio functionality [15]. It is one of the most versatile natural polymers, for applications ranging from skin, bone, cartilage, and vascular grafts to substrates for cell culture and drug delivery systems [16].

In this work, we aim to develop a novel class of biopolymer-based patches in which anti-inflammatory corticosteroid (CP) is loaded in Chitosan via electrophoretic deposition. CP is insoluble in water; therefore, ethanol-based deposition bath was used to allow one-pot deposition process of the hydrophobic CP drug, together by modulating its concentration in chitosan patches, in order to design a platform for drug-delivery in oral mucosa disease.

2. Materials and Methods.

2.1. Preparation of CP-loaded chitosan patches.

2.1.1 Materials

Chitosan (CS, medium molecular weight, MMWC, Lot#STBG1894V) with 190–310 kDa molecular weight, 75–85% deacetylation degree, and 200–800 cPs viscosity, ethanol (99.5%), acetic acid (99.7%), water (CHROMASOLV® Plus, for HPLC), Dulbecco's Phosphate Buffered Saline (PBS) (Lot#RNBG5989) were all supplied by Sigma-Aldrich and used without further purification. Clobetasol propionate (Farmacia Comunale n.70, Milan, Italy) has been used in 30% water + 70% ethanol bath (pH = 5.0, [CS] = 1g L^{-1}, [CP] = 250-10000 mg L^{-1}) to manufacture CP loaded CS patches. EPD baths were characterized in terms of pH, conductivity (Crison, CM 35), and Zeta Potential [17,18]. Dynamic light scattering (DLS) (MALVERN,

Zetasizer nano) has been used to measure zeta potential of different suspensions. Deposition bath conductivity has been measured by a conductivity meter (Crison, CM 35).

2.1.2 Patches preparation

Titanium sheets (c.p. Ti, grade 2) were used as cathode in an electrophoretic deposition cell: electrodes were positioned at distance of 10 mm [19]. Electrophoretic deposition conditions have been optimized to obtain CP-loaded patches: pulse EPD was conducted at constant voltage mode by the application of a series of pulses of DC voltage (V_{min} = 75 V, V_{max}=100 V), using a system source meter (Keithley 2425 SourceMeter, Keithley Instruments Inc. Cleveland, Ohio). The duty cycle (DC) of the pulse (i.e., DC = $t_{max}/(t_{max}+t_{min})$) was set constant at a fixed value of DC = 0.17 [20].

The obtained patches were mechanically peeled-off the cathode substrate after freeze-drying (Freeze drier Lio 5Pascal) for 24h at -40 ºC and at pressure ≤ 0.5 mbar. The specimens were used without any further processing.

2.2. Patch characterization

2.2.1 Morphological and mechanical characterization

The morphology of the obtained patches has been evaluated via scanning electron microscope (SEM, Zeiss EVO 50), optical microscopy (OM) (Leica), and stereo microscope (Leica, WILD HEERBRUGG 439097). Samples were gold-coated via sputter coater (S150B, EDWARDS) for 60 s at 0.2 mbar and 1 KV for SEM observations.

Mechanical properties of wet patches (5 x 20 mm^2) (24h preconditioning in PBS) were evaluated by a dynamic mechanical analyzer (DMA) (TA, Q800). Tensile tests were performed at a strain rate (0.2 $min.^{-1}$), with preload of 1 mN, at room temperature. Tests were triplicated.

2.2.2 Water uptake and drug release study

In order to study swelling behavior of CP-loaded CS patches the water-uptake test was performed. The swelling properties of the patches were studied by immersing them in Phosphate Buffered Solution (PBS) (pH=7.4) at 37 °C. The dried patches (10 × 10 mm^2) were weighed (W_d). At considered time-points (up to 7 days), patches were removed from the solution and carefully dried using filter paper in order to remove free water. The specimens were then weighed (W_s) and put back into the test solution; this was repeated until the curves get plateau value. At each-time point, the percentage of water uptake (W.U. %) was calculated using the following equation:

$$W.U.\ (\%) = 100\ \left(\frac{Ws-Wd}{Wd}\right) \qquad \text{(Eq.1)}$$

Where Ws is the weight at each time point, Wd is the dry weight at time zero.

Loading and releasing curves of CP in CS patches were constructed by ultraviolet-visible spectrophotometer (UV-Vis, V-560, Jasco) by measuring absorption at λ = 248-250 nm. In order to study the presence of CP in CS patches, the fabricated materials were dissolved in acidic water (30%)/ethanol (70%) solutions and evaluated by UV-Vis spectrophotometer. Calibration curve was constructed by using CP in acidic water/ethanol solution with [CP]/[CS]=0.00155, 0.0031, 0.0062, 0.0125, 0.025, 0.05 (Supporting information).

CP release studies were performed in a (PBS)/ethanol (1:1) solution at different time points (t=1, 2, 6 and 24h). Specimens were incubated in 7.5 ml of PBS/ethanol (1:1) solution. They were fixed vertically in 15 ml falcon tubes with conical end, to allow release of the CP from both sides of the specimen. The tubes were maintained at 37 °C and shaken at 100 rpm. At each

time-points, the patches were taken out of the tubes and the solutions were collected and analyzed by UV-Vis spectrophotometer to determine the concentration of the CP released. The media was not refreshed at any time point. Tests were triplicated. Calibration curve with a linear trendline was constructed by using CP solution in CS/Ethanol (70%)/water (30%)/acetic acid (to adjust the pH=5) with concentration of [CP]/[CS]= 1.55×10^{-3} - 0.5×10^{-1} (see Supporting Information).

2.3. Statistical data analysis

All results are reported as mean ± standard deviation. Significant differences between two sets of data were determined by one-way ANOVA followed by Tukey post-hoc test for pairwise comparisons and $p < 0.05$ was considered statistically significant. The Statistical Package for Social Science was used for the calculations (Minitab Express™ Version 1.4.0).

3. Results

3.1. Physical and chemical properties of deposited patches

3.1.1 Morphological characterization of patches

Figure 1 shows the OM and SEM micrographs of the EPD optimization process in terms of microstructure and morphology of patches: selected processing parameters (pH, ethanol/water ratio in deposition bath, and EPD processing parameters) allow to modulate both thickness and morphology. A porous and discontinuous structure is evident in pure chitosan patches, processed in water-based EPD bath (c-d), while a continuous and more uniform structure can be noticed in CP-loaded CS patches, processed in ethanol-based EPD bath. At SEM observation, secondary random porosity is also evident, with spherical pores of diameter in the

10-100 µm range. Obtained patches showed a dry-state thickness in the range of 125 ± 25 µm for water based pure CS and 80 ± 15 µm for CP-loaded patches (ethanol based).

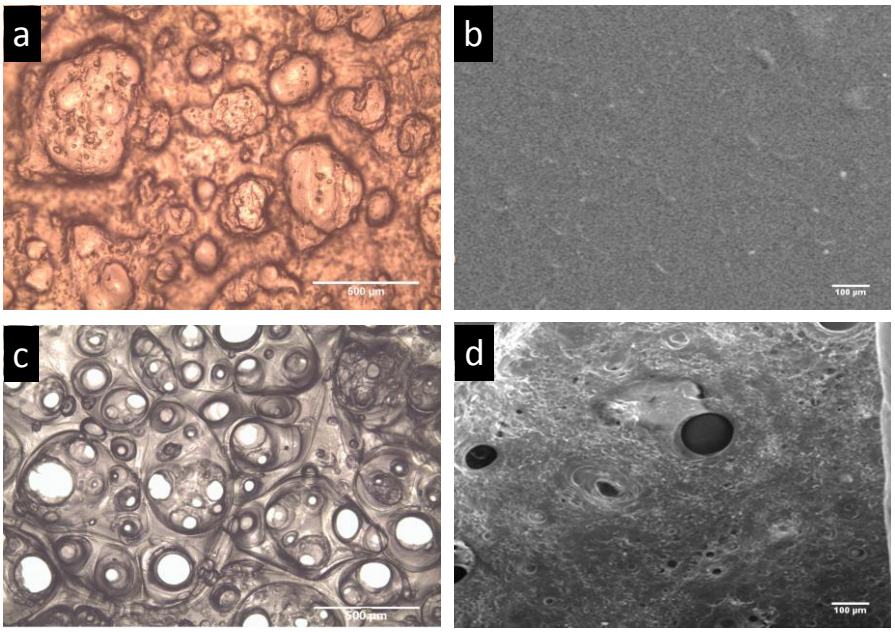

Fig. 1 (a)-(b) OM and SEM micrographs of EPD CS/CP-loaded patches (ethanol based), (c)-(d) OM and SEM images of EPD pure CS coating (water based)

3.1.2 EPD deposition rate

Figure 2 reports the results of mass deposition vs. time of EPD process for different compositions of the deposition bath. Plots show a monotonic increase of deposited mass vs. time, in which a significant difference in deposition rate can be noticed between water-based vs. ethanol-based baths. However, the addition of Clobetasol Propionate in the ethanol-bath seems to have a negligible influence on the final deposited mass and deposition rate. Here, data related to [CP]/[CS]=10 are reported, being the maximum concentration of CP in bath and hence the situation in which the drug can influence the EPD process.

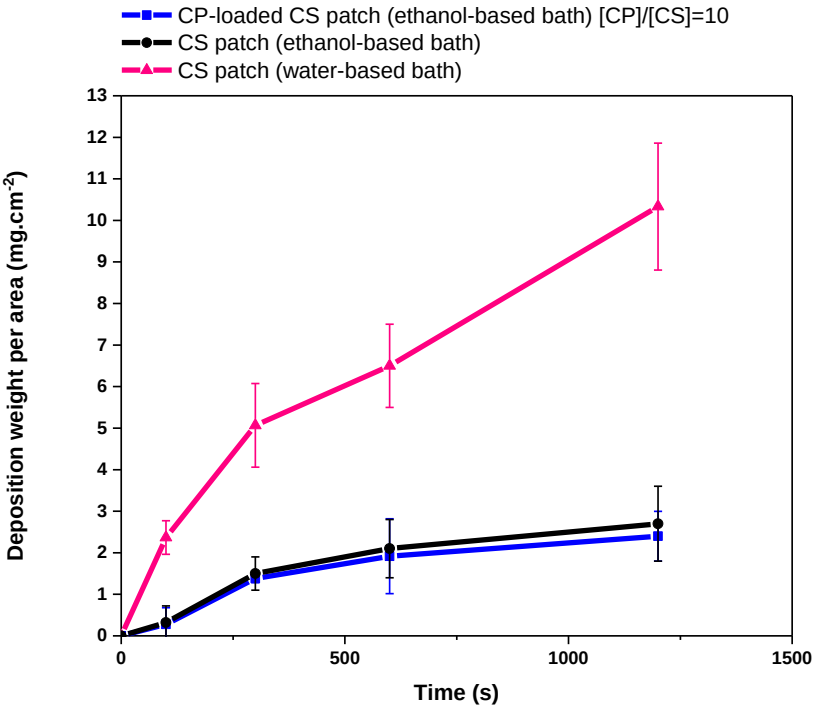

Fig. 2 CS deposition mass rate at different compositions of the deposition bath

Table 1 reports the pH, conductivity and zeta potential values of water-based deposition bath (CS/water/ acetic acid to adjust the pH), and CS/CP composite deposition bath (ethanol (70%)/ water (30%)/ acetic acid to adjust the pH, with [CP]/[CS]=10) respectively.

Table 1. Suspension parameters of different deposition bath.

	pH	Zeta potential (mV)	Conductivity (µs)
CS (water based)	3.4	**44.8±3**	**392±3**
CS (ethanol-based)	4.8	**12.4±3**	**54±2**
CP-loaded CS patches (ethanol-based) *	5.0	9.7±2	49±2

*[CP]/[CS]=10

As it is evident from table 1, there is a statistically significant difference ($p < 0.05$) between two sets of data, zeta potential and conductivity, of CS patches fabricated from water-based deposition bath and those from the ethanol-based one (the values are highlighted). (In all cases, $p < 0.05$ was considered statistically significant.)

Figure 3 illustrates the physical properties of CS and CP loaded CS patches fabricated of water-based and ethanol-based deposition baths.

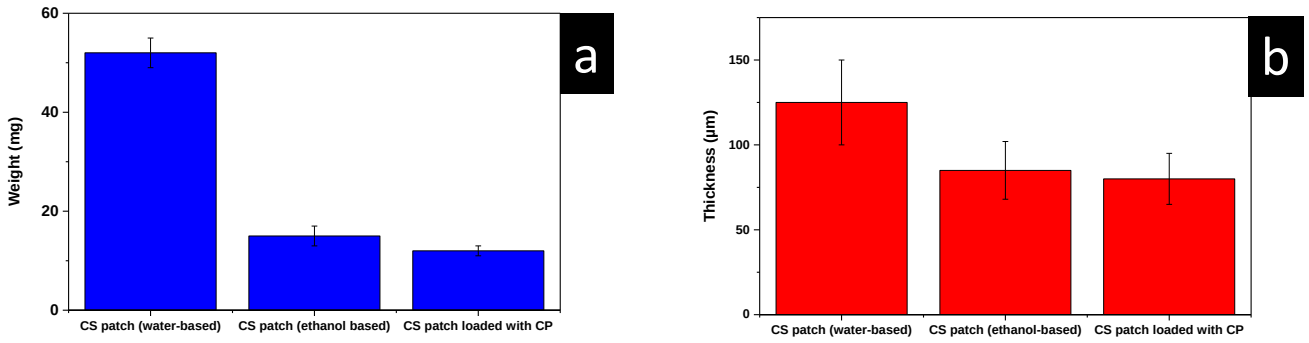

Fig. 3 Physical properties of water-based and ethanol-based ([CP]/[CS]=10) patches (a) weight; (b) thickness

3.1.3 Mechanical properties

Table 2 reports the mechanical properties of tensile stress/strain behavior of wet patches (24 h preconditioning in PBS). Patches processed in ethanol-based baths show a lower Young modulus (E) ($p < 0.05$) and higher strain (Ɛ) at break if compared to water-based bath. However, the incorporation of Clobetasol Propionate, even at the higher concentration r, does not have a significant influence on the overall mechanical behavior.

Table 2. Mechanical properties of patches obtained in tensile tests: Ɛ (strain at break), stress at break and Elastic Modulus of water-based CS, ethanol-based CS, and CP-loaded ethanol-based patches ([CP]/[CS]=10). *: $p < 0.05$.

	Ɛ (%)	Stress (MPa)	E (MPa)
CS patch (water-based)	44.6 ± 6.9	0.60 ± 0.18	1.6 ± 0.32*
CS patch (ethanol-based)	70.4 ± 20.2	0.55 ± 0.31	0.61 ± 0.2*
CP-loaded CS patches (ethanol-based)	71.2 ± 22.4	0.55 ± 0.23	0.63 ± 0.17*

3.2. CP Loading on EPD CS patches

Figure 4 shows loading diagram of CP in Chitosan patches vs. the different concentration of Clobetasol propionate in deposition bath.

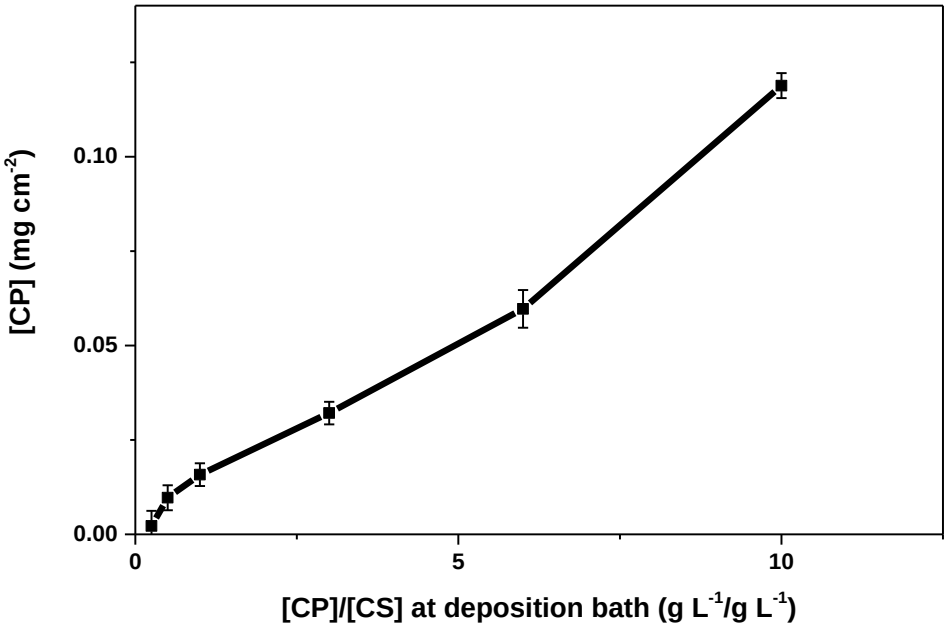

Fig. 4 The deposition yield versus different [CP]/[CS] concentration ratio in deposition bath

3.6. In vitro CP release study

3.6.1 Swelling test

Figure 5 describes swelling plots of water-based and ethanol-based CS patches both pristine and loaded with the maximum concentration of CP considered in this study. All the specimens showed strong water uptake (W.U. %) during the first 10 min; the curves then reach a plateau value.

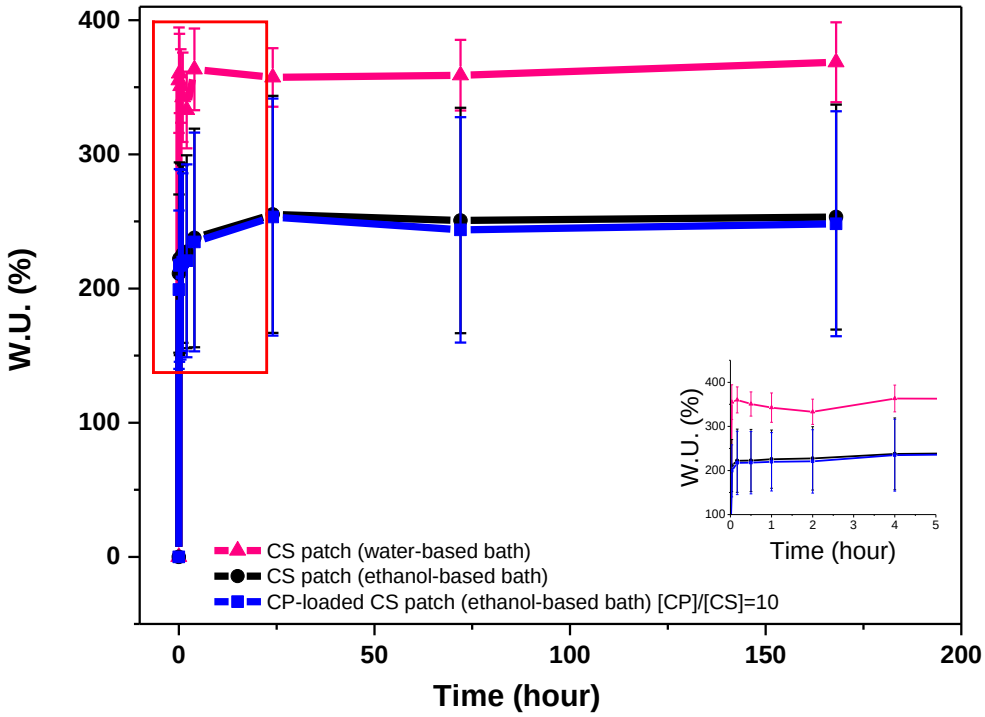

Fig. 5 Swelling degree comparison between water-based (pure CS) and ethanol-based CP-loaded patches ([CP]/[CS]=10)

3.6.2 Drug release

Figure 6 shows the releasing curve of CP in CS patches. The initial CP burst about 80% in first two hours was observed in the releasing curve.

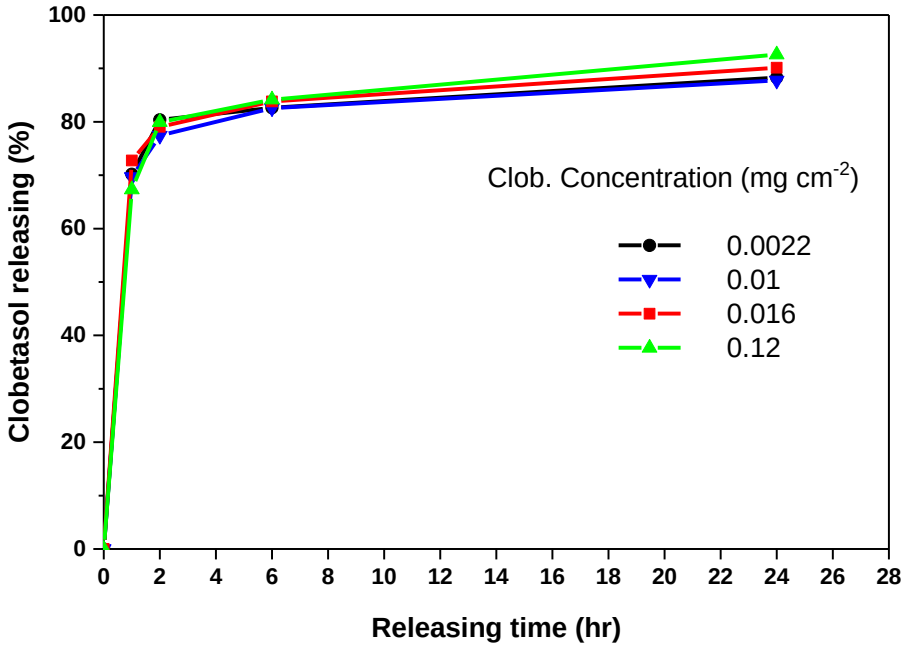

Fig. 6 Releasing curve of CP from CS patches

4. Discussion

Topical steroid therapy is largely used in the management of a variety of skin diseases and it is considered first-line therapy in patients with chronic inflammatory oral mucosal diseases. The local administration of Clobetasol Propionate, as other topical corticosteroid, is in general safe, even if some works report local or systemic side-effects [11]. It is not yet clear if these side effects are correlated to the direct absorption of the drug from the skin or by an ingestion. An optimal delivery system, in this context, should localize the cargo bioavailability by preventing rapid dilution and systemic uptake, and facilitate the release at a physiologically relevant rate and duration [21]. Thin films have been identified as an alternative approach to conventional dosage forms: an efficient design of such delivery system requires a comprehensive knowledge of the pharmacological properties of drugs and polymers, along with an appropriate selection of manufacturing processes [22]. In this work, we proposed Electrophoretic Deposition as an

efficient yet versatile technique to both realize chitosan-based thin film and incorporate clobetasol propionate drug. EPD has been optimized in this work by using ethanol as solvent, showing the feasibility of an optimal thin film preparation for patches and the incorporation of a hydrophobic molecule in a one-pot process. The obtained patches are intended for the local and selective delivery of the drugs in the oral mucosa: Needlemand and co-workers originally showed, in an in vivo study, that chitosan films have prolonged adhesion time on the oral mucosa [23] while Senel et al. [24] showed that chitosan prolongs the adhesion time of oral gels and drug release from them. The rationale of designing chitosan patches loaded with Clobetasol propionate is hence to have a platform for topical drug delivery from films showing bio-adhesion, that can have a fast-local release to avoid side-effects, and that can be easily removed. A residence time longer than the release should help to minimize side-effects due to swallowing.

4.1. Effects of bath composition on chitosan patches preparation

Electrophoretic deposition of chitosan has been widely proposed for the surface functionalization of metal prosthesis [14], largely based on the use water as a solvent. Electrochemical processes taking place at the cathode interface result in a significant production of gaseous H_2, leading to a porous structure [25]. The high oxidation–reduction potential in ethanol based suspension result in a lower production of hydrogen gas due to a decrease of water electrolysis processes [26]. As a consequence, more uniform, homogeneous and compact films were obtained in CP loaded CS patches, as observed via OM and SEM. A more homogeneous and continuous film characterized by a lower porosity can be correlated primarily to different water content (30%) in deposition bath.

Another possible explanation for this behavior should be related to the mass deposition rate dependence on the particle mobility [26]. According to Henry equation, the particle mobility μ in EPD is related to:

$$\mu = \frac{2}{3}\frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} f(ka) \qquad \text{(Eq. 2)}$$

where ζ is particle zeta potential, ε is dielectric constant, η is viscosity and f(ka) is Henry's function [15][27].

The zeta potential of particles is hence a key factor in the electrophoretic deposition process, being crucial to achieve a high and uniform surface charge of the suspended particles. If the particle charge is low, the particles would solidify even for relative large inter-particle distances, result in porous, spongy films [26]. On the other hand, if the particles have the optimum surface charge, the packed and dense film has been occurred due to particles repulsion [28].

The conductivity of suspension is also directly related to dielectric constant of the suspending medium and it increases with increase in dielectric constant [29]. According to Ferrari and Moreno [30], the conductivity of the suspension has a significant role in EPD procedure. It has been mentioned that if the suspension is too conductive, particle motion is very low, and if the suspension is too resistive, the particles charge electronically and the stability is lost [26].

As it is evident in table 1, significant differences between two sets of data (zeta potential and conductivity) have been detected. In all cases, $p < 0.05$ was considered statistically significant. As a consequence, there is descending trend in ethanol-based bath according to mass deposition.

Organic solvents are preferred to water as a suspension medium in electrophoretic process. While the generally lower dielectric constant of organic liquids (e.g., ethanol dielectric constant

= 25) limits the charge on the particles because of the lower dissociating power [26], much higher field strengths can be used to solve the problem concerning the electrolytic gas. Hence, the electrolysis and gas evolution associated with aqueous EPD processing can be avoided by using solvents with extremely high oxidation–reduction potentials as ethanol. Moreover, the organic liquids are preferred due to their higher density, good chemical stability and low conductivity [31].

The use of ethanol as the EPD bath solvent influenced also the mechanical properties, mainly, a reduction of the Young modulus and mechanical strength and an increase of the elongation at break. Previous studies similarly showed that the processing of both bio-derived and fossil-based polymers can result in their plasticization [32,33][34,35]. However, the incorporation of Clobetasol Propionate didn't affect the mechanical behavior of the patches significantly.

4.2. Drug incorporation and delivery from Chitosan patches

Clobetasol propionate is a lipophilic drug with a negligible water solubility: in order to be processed via Electrophoretic Deposition, CP has been dissolved in ethanol. This approach allowed the preparation of stable EPD bath and the incorporation of the drug in the patches. The explored processing range resulted in a concentration of CP in Chitosan up to 1.2 x 10^{-1} mg cm^{-2}, easily tuned by adjusting the drug concentration in ethanol/water EPD bath.

Gels and films have been found suitable for oral mucosa drug delivery applications since they are able to cover a wider area of mucosa for the purposes of drug delivery and physical protection [36]. The rate of hydration and the rheological properties of the polymeric formulations have a major impact on bio-adhesion and consequently the duration of retention: burst releasing of Clobetasol Propionate from the patches (80% release in less than 2h) has been obtained for all the concentration, fulfilling the requirements for topical oral applications.

5. Conclusions

Electrophoretic Deposition has been exploited as a powerful technique to fabricate patches for oral mucosal disease, effectively incorporating Clobetasol Propionate, a potent corticosteroid widely used for topical administration. EPD fabrication method has been originally shown as an effective while simple one pot approach to support the easy incorporation of water insoluble agents into chitosan matrix. While solvent plays a major role in both morphological and mechanical properties of the obtained patch, the incorporation of the drug does not affect such properties.

Further studies are required to evaluate pharmacokinetics and to demonstrate the effectiveness of both support topical administration and reduce local and systemic side effects.

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Figure captions:

Fig. 1 (a)-(b) OM and SEM micrographs of EPD CS/CP-loaded patches (ethanol based), (c)-(d) OM and SEM images of EPD pure CS coating (water based)

Fig. 2 CS deposition mass rate at different compositions of the deposition bath

Fig. 3 Physical properties of water-based and ethanol-based ([CP]/[CS]=10) patches (a) weight; (b) thickness

Fig. 4 The deposition yield versus different [CP]/[CS] concentration ratio in deposition bath

Fig. 5 Swelling degree comparison between water-based (pure CS) and ethanol-based CP-loaded patches ([CP]/[CS]=10)

Fig. 6 Releasing curve of CP from CS patches

Supporting information:

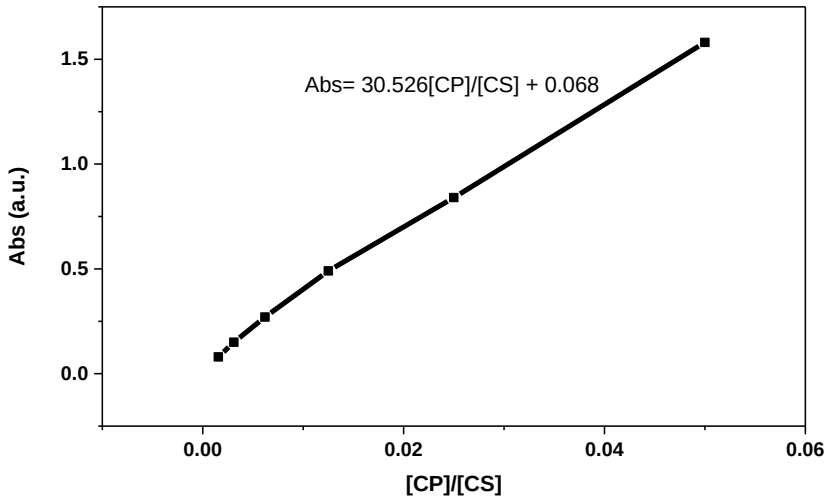

Fig.1 Relation between optical density and CP/CS concentration ratio in the deposition bath of EPD CS/CP composite patch

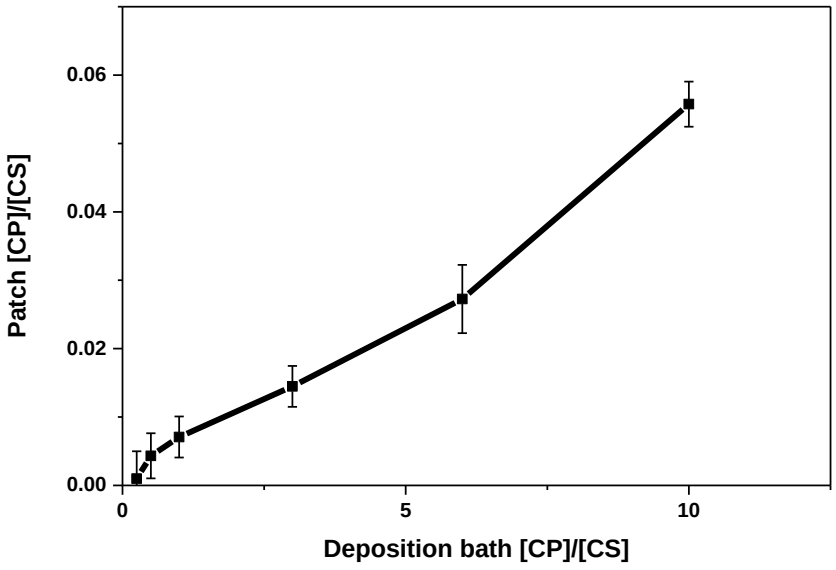

Fig. 2 The CP loading graphs versus different CP concentration in deposition bath

Figure 1 illustrates the relation between optical density and different CP/CS concentration ratios in the deposition bath.

As it is evident in Figure 2, the efficiency of EPD CS/CP composite patches is not as expected. It is rationalized that the electrolysis and gas evolution associated with aqueous EPD processing can be avoided by using solvents of extremely high oxidation–reduction potentials like ethanol. However, the electric charge on oxide particles in ethanol will be insufficient for EPD as very small amounts of free ions exist in this solvent. Consequently, a few hundreds of volts are required to provide proper driving force for EPD process [26][37].

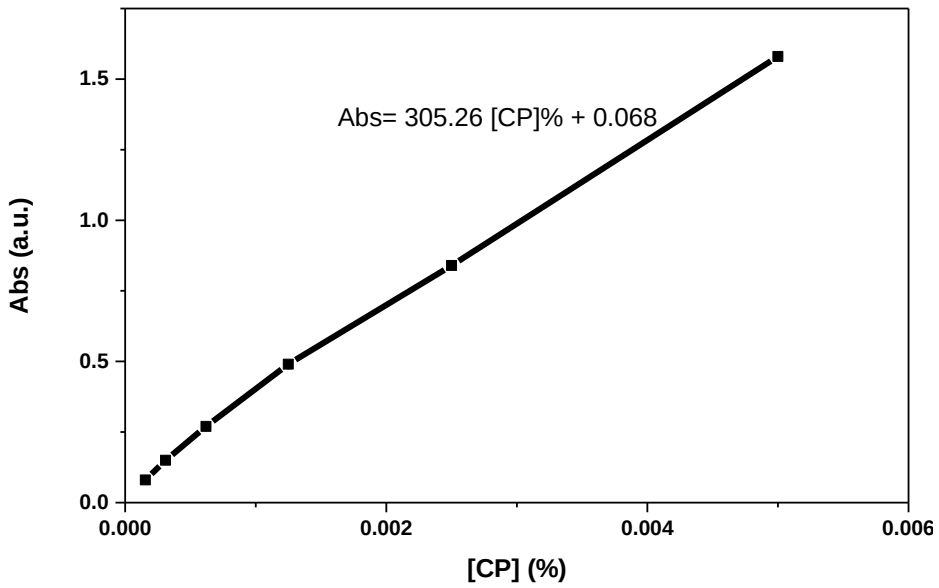

Fig. 3 Relation between optical density and CP concentration ratio in the similar deposition bath of EPD CP-loaded CS patch

The calibration curve about the [CP] was constructed by using CP in acidic water/ethanol solution with [CP]%= 0.000155, 0.00031, 0.00062, 0.00125, 0.0025, 0.005 (Figure 3.)

Table 1. Suspension parameters of different deposition bath.

	pH	Zeta potential (mV)	Conductivity (µs)
CS (water based)	3.4	**44.8±3**	**392±3**
CS (ethanol-based)	4.8	**12.4±3**	**54±2**
CP-loaded CS patches (ethanol-based) *	5.0	9.7±2	49±2

*[CP]/[CS]=10

Table 2. Mechanical properties of patches obtained in tensile tests: Ɛ (strain at break), stress at break and Elastic Modulus of water-based CS, ethanol-based CS, and CP-loaded ethanol-based patches ([CP]/[CS]=10). *: $p < 0.05$.

	Ɛ (%)	Stress (MPa)	E (MPa)
CS patch (water-based)	44.6 ± 6.9	0.60 ± 0.18	1.6 ± 0.32*
CS patch (ethanol-based)	70.4 ± 20.2	0.55 ± 0.31	0.61 ± 0.2*
CP-loaded CS patches (ethanol-based)	71.2 ± 22.4	0.55 ± 0.23	0.63 ± 0.17*

Abstract

Clobetasol propionate (CP) is a high-potency corticosteroid, representing the standard of care for the symptomatic treatment of different skin disorders as well as oral cavity diseases. Several topical delivery systems are available in oral lesion care, but the ideal one is still lacking, being serious side-effects still reported. In this work, we propose a novel class of chitosan (CS) patches, loaded with CP, for the topical treatment of inflammatory chronic oral muco-cutaneous diseases.

Chitosan patches have been fabricated via electrophoretic deposition (EPD), by using a one-pot approach in order to load controlled quantity of CP. Optimized structures showed a water uptake in the range of 200-360% and mechanical properties that allow the design of flexible patches in wet state (E = 0.6 MPa and σ_{br} = 0.55 MPa). Ultraviolet-visible (UV-Vis) spectroscopy was used for the evaluation of both loading and release profile of CP in CS patches. The CP loading has been tuned by adjusting CP concentration in deposition bath -in the range 0.002 to 0.12 mg cm^{-2}- while releasing curves show an *in vitro* CP burst of about 80% in the first two hours.

Overall, the obtained properties paved the way for the application of this new class of patches for the local oral release of CP.

Keywords

Electrophoretic deposition (EPD); Hierarchical patches; Chitosan; Clobetasol propionate; Topical oral drug delivery.

List of responses to the comments