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Hydrogel containing dexamethasone-loaded nanocapsules for cutaneous administration: preparation, characterization, and in vitro drug release study

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Abstract

Context: Our group previously reported the development of dexamethasone-loaded polymeric nanocapsules as an alternative for topical dermatological treatments. *Objective*: Our study aimed to prepare and characterize a hydrogel containing this system to improve the effectiveness of the glucocorticoid for cutaneous disorders. *Methods*: For the antiproliferative activity assay, a dexamethasone solution and D-NC were tested on *Allium cepa* root meristem model. D-NC were prepared by the interfacial deposition of preformed polymer. Hydrogels were prepared using Carbopol Ultrez® 10 NF, as polymer, and characterized according to the following characteristics: pH, drug content, spreadability, viscosity, and in vitro drug release. *Results and Discussion*: Nanocapsules showed mean particle size and zeta potential of 201 ± 6 and −5.73 ± 0.42 nm, respectively. They demonstrated a lower mitotic index (4.62%) compared to free dexamethasone (8.60%). Semisolid formulations presented acidic pH values and adequate drug content (between 5.4% and 6.1% and 100% and 105%, respectively). The presence of nanocapsules in hydrogels led to a decrease in their spreadability factor. Intact nanoparticles were demonstrated by TEM as well as by dynamic light scattering (mean particle size < 300 nm). In vitro studies showed a controlled dexamethasone release from hydrogels containing the drug associated to the nanocapsules following the Higuchi's squared root model (k = 20.21 ± 2.96 mg/cm^2/h$^{1/2}$) compared to the hydrogels containing the free drug (k = 26.65 ± 2.09 mg/cm^2/h$^{1/2}$). *Conclusion*: Taking all these results together, the hydrogel containing D-NC represent a promising approach to treat antiproliferative-related dermatological disorders.

Key words: *Dexamethasone; hydrogels; in vitro release; nanocapsules*

Introduction

Nanocarriers are materials or devices of nanoscale (below 1 μm) including a wide array of systems such as polymeric nanoparticles, dendrimers, liposomes, and solid lipid nanoparticles[1,2]. These nanocarriers have been widely studied in the pharmaceutical field in the development of nanomedicines, which are drug delivery systems developed to provide medical and pharmaceutical benefits[3]. Because of their small size, which allows them to permeate through biological barriers, these systems showed potential use following dermal administration[4,5]. Furthermore, they allow to alter some properties of active substances, such as aqueous solubility[6,7], photostability[6,8], distribution after topical administration[9,10], and efficacy[5,10]. Besides, they have been showed to be efficient carriers for drug delivery in hair follicles[11].

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Nanocapsules are nanoparticles composed of an oily core surrounded by a thin polymer wall[12,13] or by a membrane made from a mixture of lecithin and a pegylated surfactant[14] called polymeric or lipid nanocapsules. Although several studies have been reported in the literature for systemic, ocular, and oral administration of drug-loaded nanocapsules[4,13], few studies have aimed to study the behavior of these systems in semisolid formulations for dermal application[9,10,15–20]. Drug-loaded nanocapsules in semisolid dosage forms present several advantages compared to conventional semisolids for cutaneous application, such as to improve the in vitro sunscreen effectiveness[10,15] and photostability[10], as well as to promote the drug penetration in the stratum corneum and/or in the layer of viable skin[9]. In addition, these systems can provide a sustained release acting as a reservoir for active substances in the skin[18]. They can also modify the activity of the drug or its time of permanence in the skin, as well as decrease the cutaneous metabolism of drugs[5] improving the drug efficacy[16].

Dexamethasone is a poor water-soluble glucocorticoid used clinically as an anti-inflammatory and immunosuppressive agent. The cutaneous administration of dexamethasone is clinically applied for the treatment of some skin disorders, such as atopic dermatitis[21], allergic dermatitis, eczematous dermatitis[22], psoriasis, acne rosacea[22], and phimosis[23]. The effectiveness of glucocorticoids in the treatment of psoriasis and atopic dermatitis is related to their vasoconstrictive, anti-inflammatory, immunosuppressive, and antiproliferative effects[24,25]. However, prolonged topical therapy with glucocorticoid preparations can result in adverse effects, such as cutaneous reactivity, skin atrophy, and suppression of the hypothalamic–pituitary–adrenal axis[26].

Recently, we reported a study focused on the pharmacotechnical development of dexamethasone-loaded polymeric nanocapsules as an alternative to topical administration of dexamethasone[27]. However, that study demonstrated the preparation, physicochemical characterization, and stability studies of such nanocapsule formulations without studying their pharmacological benefits and their incorporation in semisolid dosage forms.

In this work, first, we evaluated the potential use of such formulations in the treatment of psoriasis studying their potential to improve the antiproliferative activity of dexamethasone by its nanoencapsulation. The antimitotic nature of glucocorticoids is the property on which their use in psoriasis is based[24]. Furthermore, dexamethasone-loaded polymeric nanocapsules were incorporated in semisolid formulations (hydrogels) to obtain a new alternative for the topical treatment of psoriasis and other skin disorders. This approach could promote a controlled release of dexamethasone and consequently a potential decrease in the immediate contact of the total applied drug to the skin. Hydrogels were characterized by means of pH, spreadability, viscosity, drug content, presence of intact nanoparticles, and in vitro drug release. To the best of our knowledge, there is no report in the literature that proposes the development of hydrogels containing dexamethasone-loaded nanocapsules (D-NC) aiming to improve the effectiveness of the glucocorticoid treatment for cutaneous disorders, as psoriasis.

Materials and methods

Materials

Dexamethasone and polysorbate 80 were obtained from Henrifarma (São Paulo, Brazil). Poly(ε-caprolactone) (PCL) and sorbitan monooleate (Span 80®) were acquired from Sigma (São Paulo, Brazil). Caprylic/capric triglyceride mixture was delivered from Brasquim (Porto Alegre, Brazil); Carbopol Ultrez® 10 NF was supplied by DEG (São Paulo, Brazil). Acetate cellulose membranes (0.45 μm pore size) were acquired from Millipore (Porto Alegre, Brazil). All other chemicals and solvents presented pharmaceutical grade and were used as received.

Preparation of nanocapsule suspensions

D-NC were prepared by interfacial deposition of preformed polymer[28] at a drug concentration of 0.5 mg/mL according to the quali-quantitative formula described in Table 1[27]. Briefly, an organic solution consisted of dexamethasone, medium-chain triglycerides mixture, a surfactant of low hydrophilic–lipophilic balance (Span 80®), the polymer (PCL), and acetone was added under moderate magnetic stirring to an aqueous solution containing a surfactant of high hydrophilic–lipophilic balance (polysorbate 80). The magnetic stirring was maintained for 10 minutes. Then, acetone was elimi-

Table 1. Quali-quantitative composition of dexamethasone-loaded nanocapsule suspensions.

Phase	Component	Amount
Organic	Poly(ε-caprolactone)	1000 mg
	Sorbitan monooleate	766 mg
	Caprylic/capric triglyceride mixture	3.3 mL
	Acetone	267 mL
	Dexamethasone	50 mg
Aqueous	Polysorbate 80	766 mg
	Distilled water	534 mL
	Final volume	100 mL

nated and the aqueous phase concentrated by evaporation under reduced pressure to a final volume of 100 mL (10 mg/mL of polymer). Blank NC (B-NC) suspensions were prepared, as control, using the same protocol described above but omitting the presence of the drug. All formulations were prepared protected from light and kept in the dark during all the time.

Physicochemical and morphological characterization of nanocapsule suspensions

After preparation, nanocapsule suspensions were characterized according to the following parameters: particle size and polydispersity index, zeta potential, pH, morphology by transmission electron microscopy (TEM), and drug content. Particle size and polydispersity index were determined by photon correlation spectroscopy at 25 ± 2°C after adequate dilution of an aliquot of the suspension in purified water (Zetasizer Nanoseries; Malvern Instruments, Worcestershire, UK) 48 hours after their preparation. Zeta potentials were measured using the same instrument at 25°C after the dilution of the samples in 10 mM NaCl aqueous solution. pH values were determined by immersion of the electrode directly in the suspension using a calibrated potentiometer (MPA-210 Model; MS-Tecnopon, São Paulo, Brazil). Morphological analyses were conducted at Centro de Microscopia (Universidade Federal do Rio Grande do Sul—UFRGS, Porto Alegre, Brazil) by TEM (Jeol, JEM 1200 Exll, Tokyo, Japan) operating at 80 kV. Diluted nanocapsules suspension was deposited on specimen grid (Formvar-Carbon support films, Electron Microscopy Sciences), negatively stained with uranyl acetate solution (2%, w/v), and observed at different magnifications.

The drug content (mg/mL) was determined after dissolution of nanocapsules in acetonitrile (1 mL of suspension to 25 mL of acetonitrile) and assayed by high-performance liquid chromatography (HPLC). The chromatographic system consisted of a Gemini RP-18 column (150 × 4.60 mm, 5 μm; Phenomenex, Torrance, CA, USA) and a Shimadzu instrument (LC-10AVP Pump, UV-VIS SPD-10AVP Module, Class-VP Software; Shimadzu, Tokyo, Japan). The mobile phase at a flow rate of 1.0 mL/min consisted of acetonitrile/water (45:55%, v/v). The volume injected was 20 μL and dexamethasone was detected at 254 nm[27].

Evaluation of the antiproliferative effects and genotoxicity potential of free and nanoencapsulated dexamethasone on **Allium cepa** *root meristem cells*

For the onion root meristem cell test, we used 20 *A. cepa* bulbs divided into five groups of four onion bulbs for each treatment (formaldehyde, positive control; distilled water, negative control; dexamethasone aqueous solution; D-NC; B-NC). Dexamethasone aqueous solution (D-S) and D-NC were tested at a concentration of 0.50 mg/mL. This concentration was chosen considering the stability of the systems, taking into account our previous study showing that dexamethasone crystallization occurs at higher concentration[27]. D-S was prepared using its sodium phosphate at an equimolar concentration. For each treatment, all bulbs were rooted in distilled water for 3 days. Afterward, they were placed in their respective treatment for 24 hours. The negative control group remained in distilled water. After 24 hours of treatment, root meristem of the control and experimental bulbs were collected and fixed in 3:1 (v/v) ethanol: acetic acid for 24 hours before being placed in 70% (v/v) ethanol and refrigerated (4 ± 2°C) until analysis. For each bulb, five slides were made using five-root meristem hydrolyzed in 1 N hydrochloric acid for 5 minutes and washed in distilled water. The fragmented meristematic regions were stained with 2% (w/v) acetic orcein. Five fields of each slide were assessed by bright-field optical microscopy at 500× magnification, and the number of interphase, prophase, metaphase, anaphase, and telophase cells was recorded. Four thousand cells for each treatment were scored (~200 cells/meristem or 1000 cells/bulb). Mean values for the different cell cycle phases, mitotic index (MI), and percentage (%) of division inhibition in relation to the control (distilled water) were calculated[29,30]. Chromosome aberrations were observed during cell division and counted as breaks (all mitotic phases), bridges, and laggards (telophase and anaphase).

Preparation of hydrogels

Hydrogels were prepared with a mortar and pestle. Carbopol Ultrez® (acrylic acid polymer) was used for the preparation of the hydrogels because of its better dispersion properties and potential wide range of applicability in the pharmaceutical and dermocosmetic fields[31]. Briefly, Carbopol Ultrez® was dispersed using D-NC suspension resulting in a concentration of 0.05% of the drug (w/w). D-NC were incorporated until 24 hours after their preparation although their physicochemical stability for 1 month was previously reported[27]. This dispersion was neutralized with triethanolamine, and imidazolidinyl urea was added as preservative. Using the same method, formulations were prepared containing B-NC (without the drug) and a control hydrogel. Additionally, we prepared a hydrogel containing free dexamethasone. In this case, an aqueous dispersion containing dexamethasone at a concentration of 0.5 mg/mL and polysorbate 80 were used instead of nanocapsule suspension during the

Table 2. Quali-quantitative composition of hydrogels containing dexamethasone-loaded nanocapsules (HG-D-NC), blank nanocapsules (HG-NC), free dexamethasone (HG-D), and control hydrogels (HG).

Component	HG-D-NC	HG-NC	HG-D	HG
Carbopol Ultrez®	1.0 g	1.0 g	1.0 g	1.0 g
Imidazolidinyl urea	0.6 g	0.6 g	0.6 g	0.6 g
Triethanolamine	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Dexamethasone	—	—	0.05 g	—
Polysorbate 80	—	—	0.76 g	—
D-NC	q.s. 100 g	—	—	—
B-NC	—	q.s. 100 g	—	—
Distilled water	—	—	q.s. 100 g	q.s. 100 g
D-NC, dexamethasone-loaded nanocapsules; B-NC, blank nanocapsules				

q.s., quantity sufficient

dispersion of the polymer. The formulations were called HG-D-NC, HG-NC, HG-D, and HG for hydrogels containing D-NC, B-NC, free dexamethasone, and control hydrogels, respectively. The composition of hydrogels is presented in Table 2. Three batches of each formulation were prepared.

Physicochemical characterization of hydrogels

All hydrogel formulations were characterized after preparation (first 48 hours) on the pH, dexamethasone content, viscosity, spreadability, and particle size after aqueous redispersion.

Determination of pH

pH values were determined in a dispersion of an aliquot of the formulation in distilled water (10%, w/v) using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil). The measurements were performed in triplicate for all batches.

Determination of dexamethasone content

Assay of dexamethasone in hydrogels was done by HPLC. Approximately, 1.0 g of each formulation was placed in a 50-mL volumetric flask. Acetonitrile was added and the flask was maintained in an ultrasonic bath for 30 minutes until the hydrogel was completely dissolved. The solution was properly diluted with further acetonitrile, followed by centrifugation at 2300 × *g* for 15 minutes, filtered through a 0.45-µm membrane, and injected in the HPLC system, according to the conditions described in the section 'Physicochemical and morphological characterization of nanocapsule suspensions.' The method was linear (r = 0.9998, in the range of 5–40 µg/mL), precise (relative standard deviation (RSD): < 3.0% for repeatability and intermediate precision), and specific. The specificity was tested in presence of the excipients of the formulations (nanocapsules and hydrogels) and demonstrated that these components did not alter the dexamethasone assay.

Evaluation of the viscosity of hydrogels

The viscosity of hydrogels was evaluated at 25 ± 2°C using a rotational viscosimeter (DV II+ Pro model; Brookfield, Middleboro, Massachusetts, USA), spindle SC4-25 with a small sample adapter, using a single point shear rate (0.10 s^{-1}). The data obtained were analyzed with the Rheocalc software (V3.1-1 version; Brookfield, Middleboro, Massachusetts). The aim of this characterization was to show the lack of influence of nanocapsules on the viscosity of the hydrogels and to attest the similar viscosity of formulations used for the in vitro release studies.

Determination of the spreadability

The spreadability of formulations was evaluated according to the methodology previously described by Borghetti and Knorst[32]. The sample was introduced in a central hole (1 cm) of a mold glass plate. The mold plate was carefully removed and the sample was pressed subsequently with glass plates of known weights, with intervals of 1 minute between each plate. Spreading areas reached by samples between every addition of a glass plate were measured in millimeters in vertical and the horizontal axes. Results were expressed in terms of the spreading area as a function of the applied mass according to Equation (1) and represent the mean of three determinations:

$$S_i = \frac{d^2 \cdot \pi}{4}, \quad (1)$$

in which S_i is the spreading area (mm^2) after the application of a determined mass i (g) and d is the mean diameter (mm) reached by each sample. The spreading area was plotted against the plate weights to obtain the spreading profiles.

The spreadability factor (S_f) was also calculated and represents the spread a formulation is able to expand on a smooth horizontal surface when a gram of weight is added on it, under the conditions described in the methodology above. The following Equation (2) is used to calculate the spreadability factor[33]:

$$S_f = \frac{A}{W}, \quad (2)$$

in which S_f (mm^2 g) is the spreadability factor resulting from the ratio between (A) the maximum spread area (mm^2) after the addition of the sequence of weights used in the experiment and (W) the total weight added (g).

Determination of the particle size and polydispersity index after aqueous redispersion of hydrogels

The particle size of intact nanoparticles in hydrogels was determined by photon correlation spectroscopy at 25 ± 2°C (Zetasizer Nanoseries; Malvern Instruments). Before the analysis, an aliquot of hydrogel was diluted in purified water (500×) and shaken (vortex) until its complete redispersion. Analyses were made in triplicate. In addition, the presence of intact nanocapsules in this redispersion was evaluated by TEM analysis. The preparation of the diluted sample for TEM analysis was carried out according to the methodology previously described (section 'Physicochemical and morphological characterization of nanocapsule suspensions').

In vitro release assay

In vitro release of dexamethasone from HG-D-NC and HG-D was studied using vertical Franz diffusion cells at 37 ± 0.5°C (n = 6). Two independent experiments (n = 3) were carried out for each formulation. The area for diffusion was 2.14 cm^2 and the receptor chamber volume was 5.0 mL. Acetate cellulose membrane (0.45 µm pore size) was fit between donor and receptor compartment. The receptor medium consisting of phosphate buffer (pH 7.4) containing 20% (v/v) of polysorbate 80 was continuously stirred. An amount of hydrogel containing 500 µg of dexamethasone (infinite dose) was evenly spread on the membrane surface. Half of a milliliter of the receptor medium was taken at predetermined time intervals of 2, 4, 6, 8, and 24 hours replaced by an equal volume of fresh medium. The amount of dexamethasone released was determined by HPLC, according to the conditions described in section 'Determination of dexamethasone content'. The method was linear (r = 0.9998), precise (RSD: <2.0% for repeatability and intermediate precision), and specific. The specificity was tested in the presence of the components of the release medium and demonstrated that these components did not alter the dexamethasone assay. Higuchi's model ($C = kt^{0.5}$) was used to evaluate the drug release profiles. C is the cumulative amount of drug released at time t and k is a constant reflecting the design variables of the system related to the diffusion area, diffusion coefficient, and drug's solubility in the system[34,35]. The mathematical modeling was performed using the software MicroMath® Scientist® for Windows™.

Statistical analysis

All formulations were prepared and analyzed in triplicate. Results are expressed as mean ± SD. One-way analysis of variance (ANOVA) was used for the comparison of the experimental data. Post hoc multiple comparisons were done by Tukey's test for significance at $P \leq 0.05$. For the antiproliferative studies, statistical analyses were performed using the Holm Sidak test at $P \leq 0.05$. All analyses were run using the SigmaStat Statistical Program (Version 3.0; Jandel Scientific, Erkrath, Germany).

Results and discussion

Characterization of nanocapsule suspensions

The aqueous colloidal formulations of nanocapsules used to prepare the hydrogels presented a macroscopic homogeneous appearance, similar to a milky bluish opalescent liquid. Physicochemical characteristics of the formulations after the preparation are presented in Table 3. All formulations showed mean particle size in the nanometric range and polydispersity indices below 0.25 indicating an adequate homogeneity of these systems[9]. Regarding the dexamethasone content and pH, all formulations used for the preparation of hydrogels were in accordance with the theoretical drug content (95–105%) and presented acid pH. The acidic pH values could be explained

Table 3. Physicochemical characteristics of nanocapsule suspensions: D-NC (dexamethasone-loaded nanocapsules) and B-NC (blank nanocapsules) (mean ± SD, $n = 3$).

Formulation	Particle size (nm)	Polydispersity index	Zeta potential (mV)
D-NC	201 ± 06	0.11 ± 0.01	−5.73 ± 0.42
B-NC	205 ± 03	0.10 ± 0.01	−5.95 ± 0.41

by the presence of terminal carboxylic groups in the polymeric chains (PCL). These results are in agreement with our previous work that focused on the development of D-NC[27]. In addition, the particle size and zeta potential were not influenced by the presence of the drug in the formulations ($P > 0.05$), as can be seen by comparing the results obtained for both formulations (D-NC and B-NC, Table 3). The negative zeta potential values (~−5 mV) are a consequence of the particle/emulsion coating with polysorbate 80, presenting a negative surface density of charge because of the presence of oxygen atoms in molecules. Despite the low potential values (in module), it must be pointed out that the physical colloidal stability of these nanocapsules is because of the steric effect of the surfactant (polysorbate 80) at the interface particle/water[12]. TEM analysis showed spherical-shaped nanocapsules with similar diameters as determined by photon correlation spectroscopy (Figure 1a).

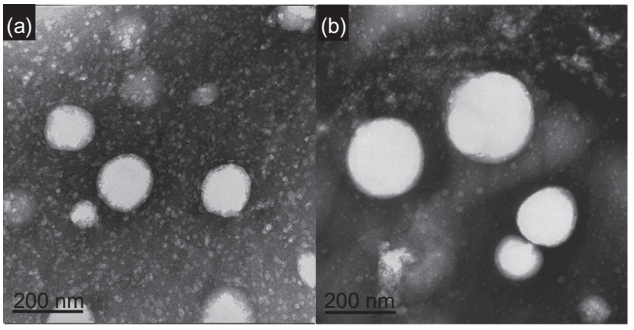

Figure 1. Transmission electron microscopy image of (a) dexamethasone-loaded nanocapsules [bar = 200 nm (150,000×)] and (b) hydrogel containing dexamethasone-loaded nanocapsules [bar = 200 nm (150,000×)].

Antiproliferative activity

Cytotoxicity tests, using a vegetal in vivo method as the *A. cepa* test, are previously validated with a good correlation to the in vitro method using animal cells[36,37]. Although the vegetal metabolism is different from the animal metabolism, the *A. cepa* test is an excellent tool of preliminary cytotoxic or antiproliferative analyses[36,38]. This test was carried out to preliminarily evaluate the potential of the dexamethasone associated with nanocapsules to improve the efficacy of the drug. This result could lead us to study its incorporation in semisolid formulations for the dermatological treatment of cell proliferative-related disorders, such as psoriasis. The antimitotic effect, which can be observed by this experimental model, is believed to confer the beneficial effects of topical glucocorticoids in the treatment of psoriasis, a disease characterized by a high cell turnover rate[24].

The number of interphase, prophase, metaphase, anaphase, and telophase as well as the mean MI (%) for all tested groups are shown in Table 4. It can be observed that no phase of the cellular cycle for all treatments was suppressed. The positive control (formaldehyde) depicted a high inhibition of the cell division (MI: 0.90%), showing that the experiment was able to demonstrate the antiproliferative activity of the treatments. Dexamethasone, in its free form in aqueous solution, presented an MI

Table 4. Number of cells in different phases of the cell cycle and the mitotic index of onion root-tip cells treated for 24 hours in formaldehyde (positive control), water (negative control), dexamethasone aqueous solution (D-S) (0.5 mg/mL), dexamethasone-loaded nanocapsules (D-NC) (0.5 mg/mL), and blank nanocapsules (B-NC). The total number of cells analyzed for each treatment was 4000.

	Number of cells in different phases of the cell cycle					
Treatment	Interphase	Prophase	Metaphase	Anaphase	Telophase	Mitotic index (%)*
Formaldehyde	3964	06	10	09	11	0.90[a]
Water	3771	114	48	24	43	5.91[b]
D-S	3656	217	52	39	36	8.60[c]
D-NC	3815	97	38	23	27	4.62[d]
B-NC	3749	156	38	19	38	6.27[b]

* Means, in column, with the same letter are not significantly different (ANOVA, Holm Sidak test, $P \leq 0.05$).

of 8.60%, which demonstrated a significant increase ($P \leq 0.05$) in relation to the control group-water (5.91%). This result showed that at this concentration, no antiproliferative activity on *A. cepa* root meristem cells could be observed for free dexamethasone. On the contrary, nanoencapsulated dexamethasone (D-NC) presented a significant decrease ($P \leq 0.05$) in the MI (4.62%) compared to free dexamethasone (8.60%), water (5.91%), and B-NC (6.27%). Considering that D-NC presented a significant difference in the MI compared to the control-water to the B-NC, we can suggest that the association of dexamethasone to nanocapsules improves its antiproliferative efficacy. Furthermore, B-NC showed no antiproliferative activity because no statistical difference in the MI was observed when compared to the treatment with water. Our previous report showed similar results for nanoencapsulated tretinoin, whose antiproliferative activity was improved compared with its free form[30].

However, to verify if this decrease in the MI (%) may be related to important chromosome aberrations, the presence of breaks, bridges, and laggards was studied in each slide by microscopic observation. Only four bridges in the group treated with dexamethasone solution were observed. This result showed that no treatment (dexamethasone solution, D-NC, and B-NC) induced to an increase in the frequency of chromosome alterations on onion root meristem cells. This result is also in agreement with our previous results for tretinoin-loaded nanocapsules[30] and showed the potential of such D-NC to be incorporated in topical formulations for further studies.

The improvement of the antiproliferative activity of D-NC could be explained by the higher penetration of the drug in onion root meristem cells promoted by its nanoencapsulation. This higher penetration of drug encapsulated in nanoparticles has been reported by several authors to explain the improvement of the drug efficacy in intracellular infection and anticancer therapy[1,39,40]. This effect is important for dermatological treatments as the glucocorticoid receptors are localized in the cytoplasm of cells and are distributed both in epidermis and dermis of human skin, suggesting that both are targeting tissues for the glucocorticoid activity[41].

Characterization of hydrogels

D-NC (0.5 mg/mL), freshly prepared, were incorporated in the Carbopol Ultrez® 10 NF hydrogels (HG-D-NC). Hydrogels containing free dexamethasone (HG-D) and blank nanocapsules (HG-NC) were also prepared. HG-D-NC and HG-D presented theoretical final dexamethasone concentration of 0.50 mg/g. This concentration was limited by the dexamethasone concentration in nanocapsules, which was used as aqueous phase in the preparation of hydrogels. All hydrogel formulations showed satisfactory organoleptic characteristics. When free dexamethasone was incorporated, the hydrogel presented a transparent and homogeneous aspect. On the contrary, hydrogels containing dexamethasone-loaded nanocapsules or B-NC presented a homogeneous white color, like a gel–cream formulation.

Properties of all hydrogels are shown in Table 5. pH values were in the range of 5.4–6.1 for all formulations. The pH of the skin is slightly acidic (4.6–5.8) and maintained by substances with buffer capacity present in skin secretions, which helps to protect the surface against microorganisms[42]. Therefore, all hydrogel formulations were developed with pH values compatible with topical application, and no influence of nanocapsules was observed on this parameter. Drug contents were between 100% and 105% for both drug-loaded formulations (HG-D-NC and HG-D). These results show the efficiency of the protocol for the preparation of such hydrogels.

Regarding the viscosity of hydrogels determined in a single point (shear rate of 0.10 s^{-1}), no differences ($P > 0.05$) were observed among the formulations containing nanocapsules with (HG-D-NC) or without drug (HG-NC) and hydrogels containing the free dexamethasone (HG-D). Also, no difference ($P > 0.05$) was observed between formulations containing nanocapsules (HG-D-NC and HG-NC) and control hydrogel (HG). These results showed that the presence of nanocapsules did not interfere in the viscosity of these hydrogels (at this shear rate) and are in agreement with the findings reported by Alves and coworkers[19], which showed similar consistency indexes for Carbopol 940® hydrogels with and without nanocarriers (nanocapsules, nanospheres, and nanoemulsions). The higher viscosity of HG compared to the HG-D ($P \leq 0.05$) could be explained by the presence of

Table 5. Properties of hydrogels containing dexamethasone-loaded nanocapsules (HG-D-NC), blank nanocapsules (HG-NC), free dexamethasone (HG-D), and control hydrogel (HG) (mean ± SD).

Formulation	pH	Drug content (%)	Viscosity (mPa s×10^3)	Spreadability factor (mm^2 g)
HG-NC	5.62 ± 0.14	—	893.54 ± 62.85	1.24 ± 0.06
HG-D-NC	5.74 ± 0.19	100.95 ± 0.42	766.68 ± 82.33	1.23 ± 0.03
HG-D	5.99 ± 0.03	104.85 ± 0.43	703.50 ± 61.60	1.46 ± 0.03
HG	5.96 ± 0.02	—	993.48 ± 47.49	1.60 ± 0.06

– not determined.

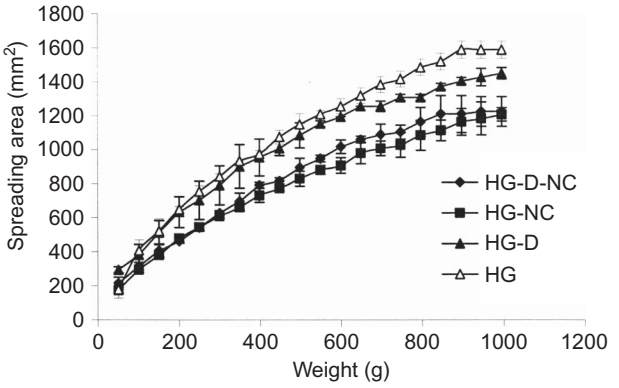

Figure 2. Graphical representation of the spreadability of hydrogels containing dexamethasone-loaded nanocapsules (HG-D-NC), blank nanocapsules (HG-NC), free dexamethasone (HG-D), and control hydrogels (HG).

polysorbate 80 in the latter. Barreiro-Iglesias and coworkers[43] demonstrated a decrease in viscosity of Carbopol 934® hydrogels in the presence of similar concentration of polysorbate 80 (0.75 mg/mL) at pH 5.0, explained by the formation of intrapolymeric micelles and breaking the interpolymer connections.

During the development of a semisolid formulation for cutaneous application, it is noteworthy to evaluate its spreadability because the efficacy of such a topical therapy depends also on how it spreads on the skin surface. Therefore, the spreadability is related to the correct dosage transference to the target tissue[44] and is directly related to the viscosity and composition of the formula[45]. In our study we used the parallel plate method, which is the most used to regulate and quantify the applicability of semisolid preparations[44]. Figure 2 shows the spreadability of hydrogels. The graphic representation of spreadability showed similar profile for hydrogels containing nanocapsules, regardless of the presence of the drug (HG-D-NC and HG-NC). On the contrary, the incorporation of the nanocapsules in the semisolid led to a slight decrease in its spreadability compared to HG-D and HG. To highlight this difference, the spreadability factor was calculated. In fact, hydrogels containing free dexamethasone (HG-D) and the hydrogel base (HG) showed a higher ($P \leq 0.05$) spreadability factor compared to hydrogels containing nanocapsules. This difference could be related to the presence of the polymer (1%) in nanocapsule suspensions. Alves and coworkers[19] did not detect differences of spreadability between formulations containing nanocarriers and the gel control (Carbopol 940® at 0.2%, w/w). Our different result may be related because of the higher concentration of the gel-forming polymer (1.0%, w/w) or because of the different type of polymer in our study (Carbopol Ultrez®). In addition, in this specific case, the slight lower spreadability of HG-D-NC could also be visualized as an advantage because of the limited area of treatment for psoriasis or other proliferative-related cutaneous diseases.

Table 6. Particle size and polydispersity index determined in water after the redispersion of hydrogels containing dexamethasone-loaded nanocapsules (HG-D-NC), blank nanocapsules (HG-NC), and free dexamethasone (HG-D) (mean ± SD).

Formulation	Particle size (nm)	Polydispersity index
HG-NC	280 ± 12	0.31 ± 0.03
HG-D-NC	282 ± 17	0.34 ± 0.03
HG-D	733 ± 449	0.74 ± 0.30

To evaluate the integrity of the nanocapsules after their incorporation in hydrogels, the particle size and polydispersity index of an aqueous redispersion of hydrogels were determined 48 hours after preparation (Table 6). The presence of nanostructures with a mean particle size compatible with the original suspension (about 200 nm) and a polydispersity index below 0.35 was observed for HG-D-NC and HG-NC. On the contrary, HG-D showed particles with a mean size higher than 700 nm and a wide particle size distribution (polydispersity index higher than 0.70). To detect the presence of nanocapsules in the hydrogels, a morphological observation by TEM was carried out for HG-D-NC (Figure 1b). TEM image showed the presence of intact nanostructures in the hydrogel similar in shape and diameter to those observed in the original nanocapsules suspension (Figure 1a).

In vitro dexamethasone release from hydrogels

Dexamethasone possesses low aqueous solubility (100 μg/mL)[46]. Thus, to provide sink conditions, a phosphate buffer (pH 7.4) containing 20% of polysorbate 80 (v/v) was chosen as receptor medium. Dexamethasone solubility in this medium was 1244.11 ± 16.02 μg/mL after 24 hours, whereas this value in a phosphate buffer (pH 7.4) without any adjuvant was 79.01 ± 2.16 μg/mL. In vitro release studies from hydrogels containing dexamethasone-loaded nanocapsules (HG-D-NC) as well as free dexamethasone (HG-D) were carried out using vertical diffusion Franz cells. Drug fluxes (release rate) of each experiment were determined by the slope of the curve obtained by plotting the amount of dexamethasone released per cm^2 against the square root of time[47,48]. This study aimed to evaluate if the nanoencapsulation of dexamethasone could impact in some modifications on its release behavior from hydrogels. In vitro release studies from both models HG-D-NC and HG-D demonstrated release profiles following the Higuchi's square root model (Figure 3). Statistical analyses showed a lower amount of drug release per cm^2 after 2 hours as well as after 24 hours for HG-D-NC. As the viscosity values at a low shear rate ($0.1\ s^{-1}$) were similar for both hydrogels as showed

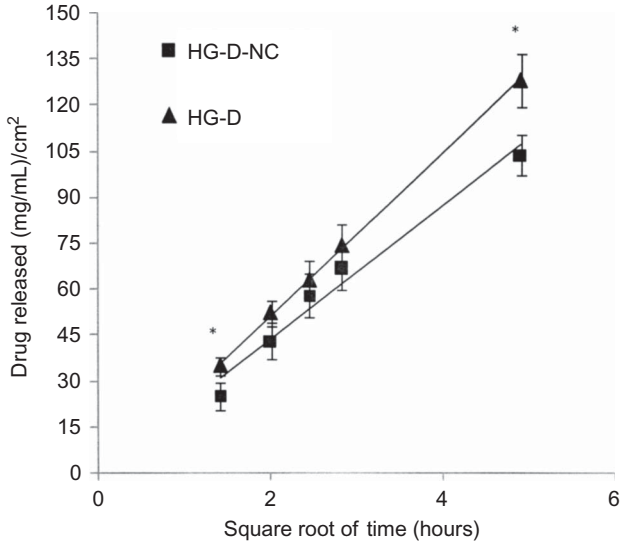

Figure 3. Release profile of dexamethasone from hydrogel containing dexamethasone-loaded nanocapsules (HG-D-NC) (n = 6) and hydrogel containing free dexamethasone (HG-D) (n = 6), according to Higuchi's square root model (r = 0.9991 and 0.9905, for HG-D-NC and HG-D, respectively). *Means statistically different (ANOVA, Tukey test, $P \leq 0.05$).

previously (Table 5), the lower release of dexamethasone at the first 2 hours and after 24 hours of the experiment could be explained by the highest controlled release of dexamethasone associated with nanocapsules from the semisolid vehicle. HG-D-NC presented a lower mean flux (release rate) of dexamethasone (20.21 ± 2.96 mg/cm^2/h$^{1/2}$, r = 0.9691) than HG-D (26.65 ± 2.09 mg/cm^2/h$^{1/2}$, r = 0.9924) ($P \leq 0.05$). These results were in agreement with those obtained by the modeling of drug release data, which showed Higuchi constant (k) of 0.0218 ± 0.0017 and 0.0260 ± 0.0017 for both formulations, respectively. Up to now, as far as we know, this is the first report showing the slower drug release rate through cellulose acetate membrane from hydrogels containing drug-loaded nanocapsules compared to hydrogels containing free drug using the Franz cells model, as a preliminary evaluation for further skin permeation studies. Furthermore, our study showed that this experimental method represents a useful tool to discriminate the in vitro behavior of semisolid formulations containing drug-free or drug-loaded polymeric nanocapsules.

Conclusion

Taking all the results together, this work showed the feasibility to prepare hydrogels containing D-NC for topical application in dermatological disorders. In vitro release studies showed a controlled dexamethasone release from hydrogels containing the nanoencapsulated drug following the Higuchi's squared root model compared to the hydrogels containing the free drug. In addition, the potential improvement of the in vivo antiproliferative activity of nanoencapsulated dexamethasone and the observation of intact nanocapsules in the formulation allow concluding that this formulation presented adequate profile for further studies, such as the evaluation of in vivo pharmacological behavior after topical application as well as the in vitro cutaneous permeation profile of dexamethasone.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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