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Applying the sensory analysis in the development of chitosan hydrogel containing polymeric nanocapsules for cutaneous use

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Synopsis

This work aimed to develop a chitosan hydrogel containing polymeric nanocapsules with optimized sensory properties, linking the advantages of the nanocarriers, such as controlled release and protection of the substances, to the chitosan properties, such as bioadherence, cicatrizing effect, and antimicrobial activity. Sixty untrained volunteers evaluated the sensory properties of chitosan hydrogels compared to hydroxyethyl cellulose gels (Phase I) and to optimized chitosan gels (Phase II). The volunteers' preference between formulations was also evaluated. The chitosan hydrogel, despite the presence of nanocapsules, presented higher immediate stickiness and film formation on the skin, and lower acceptance than the hydroxyethyl cellulose gels. Regarding the optimized gel, decrease on the film formation and increase on the homogeneity of the film was observed, compared to the prior chitosan gel. So, the optimization of the chitosan gel led to higher acceptance by the volunteers. The presence of nanocapsules, besides increasing the chitosan gel consistence, increased the perception of film formation. For the optimized chitosan gel, the nanocapsules increased the homogeneity of the film formed on the skin, without increasing the perception of film formation. In conclusion, through sensory analysis, the formulation was optimized presenting, at the final stage, adequate sensory properties for cutaneous use.

INTRODUCTION

Hydrogels are networks that retain a great amount of water while are maintained insoluble due to the crosslinks between the polymeric chains (1). It is a very common pharmaceutical dosage form in dermatology and cosmetology due to its nongreasy properties. The most usual hydrogel-forming polymers are derived from the polyacrylic acid, such as the carbomers, and from cellulose, such as hydroxyethyl cellulose (2). Chitosan, gelatin,

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and xanthan gum can be mentioned among the natural polymers used in the production of hydrogels (1).

Chitosan, a cationic biopolymer, presents applications in several distinct areas, such as the biomedical, cosmetics, food technology, and pharmaceutical technology (3). The chitosan hydrogels present interesting cutaneous properties including a great bioadherence and film formation (4), cicatrizing effect (5), and antimicrobial activity (6). It is also possible that these hydrogels interfere in the drug skin penetration due to the chitosan effect on the tight junctions between epithelial cells (2).

The incorporation of solid lipid nanoparticles (7) and liposomes (8) in chitosan hydrogels has already been proposed, but the incorporation of polymeric nanocapsules in such hydrogels represents an innovative proposal from our research group (9) aiming to link the advantages of both systems. The polymeric nanocapsules are characterized by the presence of a liquid core surrounded by a polymeric wall (10). Regarding the cutaneous use, the nanoencapsulation of active substances can increase the drug photostability (11) and change the sensory properties (12). Besides that, the control of the drug release can prolong the action (13) and modulate the skin penetration as well (14,15). In order to facilitate the cutaneous application of aqueous nanoparticles suspensions, several studies have been devoted to the development of semisolid vehicles containing polymeric nanocapsules (16,17).

The measurement and interpretation of human responses (senses) to the properties of products is called sensory analysis. It has been applied in the development of distinct products, including cosmetics (18–21). Among the different kinds of tests used in the sensory analysis, there are discriminative, descriptive, and affective tests. The discriminative and affective tests, which are the focus of this work, aim to detect if there is a difference between samples and which the preferred sample is, respectively (21).

This work explores the sensory properties as a tool in the pharmacotechnical development of a novel chitosan hydrogel containing nanoparticles linking the advantages of the chitosan and of the nanocapsules to obtain a cutaneous formulation of high skin permanence. Despite its interesting cutaneous properties, the sensory properties of a chitosan hydrogel for cutaneous use have never been described before, as far as it is known. Also, the influence of the nanocapsules to the sensory properties is of great importance, considering the great number of research papers and products in the market containing nanoparticles. The main questions to be addressed are: (i) Are there differences between the chitosan hydrogel and another hydrogel of common cosmetic use (hydroxyethyl cellulose) regarding the sensory properties? (ii) How is it possible, by means of the pharmacotechnical development, to improve the sensory acceptance of the chitosan hydrogel? (iii) Does the presence of nanocapsules interfere in the sensory properties of the chitosan hydrogels? The influence of the chitosan gel and of the polymeric nanocapsules on the sensory properties may impact the future development of dermal formulations based on chitosan and/or nanoparticles.

EXPERIMENTAL

MATERIALS

The hydrophilic surfactant polisorbate 80 was purchased from Labsynth (São Paulo, Brazil) and the capric/caprylic tryglicerides, used as nanocapsule core, was obtained from Brasquim (Porto Alegre, Brazil). EudragitRS 100®, used to form the nanoparticle shell,

was purchased from Degussa (Darmstadt, Germany). To obtain the hydrogels, chitosan (Sigma-Aldrich, São Paulo, Brazil) and hydroxyethyl cellulose (Embacaps, Porto Alegre, Brazil) were used. Also, lactic acid (85%), decamethylcyclopentasiloxane (volatile silicone fluid—DC 245) and carboxylic pirrolidon acid sodium salt (PCA-Na), obtained from Via Farma (São Paulo, Brazil) were applied. Acetone of analytical grade [Vetec, Rio de Janeiro, Brazil] and MilliQ® water were used in the preparation of the nanocapsules.

METHODS

Production of polymeric nanocapsule suspension. The nanocapsule aqueous suspension was prepared by means of the interfacial deposition of preformed polymer, through the preparation of two separate phases. An organic phase was obtained by dissolving the polymer Eudragit RS 100® (500 mg) and the capric/caprylic triglycerides (1.65 ml) in acetone (135 ml) at 40°C, under magnetic stirring. An aqueous phase was obtained by dissolving polisorbate 80 (380 mg) in ultrapure water (265 ml). The organic phase was then injected into the aqueous phase, by a controlled rate, and the nanocapsules were formed and maintained under stirring for 10 min. The total amount of organic solvent and a partial amount of water were eliminated under reduced pressure. The final volume was 50 ml and the suspension was then stored at room temperature protected from light.

Production of hydrogels containing, or not, polymeric nanocapsules. Hydrophilic gels based on chitosan or hydroxyethyl cellulose were prepared (Table I). For the hydrogels containing nanocapsules, the colloidal suspensions were incorporated in total substitution of the water.

The chitosan hydrogels were obtained through the dispersion of the polymer in water or in nanocapsule suspension. Then, lactic acid was added in order to allow the entanglement of the chitosan chains, leading to hydrogels. After incorporation of diazolidinyl urea, the hydrogels were well homogenized and stored under room temperature in plastic semisolid flasks. They were named CH and CH-NC.

Table I
Hydrogel Components

Component	CH	CH-NC	HEC	HEC-NC	CH-OPT	CH-NC-OPT
Hidroxyethyl cellulose	—	—	2%	2%	—	—
Chitosan	2.50%	2.50%	—	—	2.50%	2.50%
Lactic acid	1%	1%	—	—	1%	1%
Silicone DC 245®	—	—	—	—	3%	3%
PCA-Na	—	—	—	—	2%	2%
Diazolidinyl urea	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%
NC suspension	—	qsp	—	qsp	—	qsp
Ultrapure water	qsp	—	qsp	—	qsp	—

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized hydrogels, PCA-NA: carboxylic pirrolidon acid sodium salt.

The hydroxyethyl cellulose hydrogels were obtained through the dispersion of the polymer in water or in nanocapsule suspension. The dispersion was stored for 48 h. Then, the hydrogels were well homogenized, added of diazolidinyl urea, and stored under room temperature in plastic semisolid flasks. They were named HEC and HEC-NC.

On the basis of the results of the sensory analysis (phase I), which aimed to determine the attributes that differentiated the chitosan gels from the hydroxyethyl cellulose gels, the chitosan hydrogel was optimized as described on Table I. The hydrogels were prepared as for the prior chitosan gels, in except for the addition of PCA-Na in the water or nanocapsules suspension, before the gel formation, and the addition of silicone at the final homogenization process. They were named CH-OPT and CH-NC-OPT.

Presence of nanocapsules in the hydrogels. The presence of nanocapsules in the hydrogels obtained by addition of nanocapsule aqueous suspension was confirmed through size distribution analysis (laser diffraction) and electron microscopy analysis (transmission electron microscopy [TEM]). For the size distribution analysis, the hydrogels were diluted in ultrapure water in the sample container at room temperature. The refraction index applied was 1.38, related to Eudragit RS100®, the former of the nanocapsule shell. The results represent an average measurement from three different batches, in a way that the size distribution was measured by volume and by number of particles. For the electron microscopy analysis, the hydrogels were diluted in ultrapure water (1:100 w:v) and placed on formvar-carbon grids (400 mesh, Electron Microscopy Sciences, Hatfield, PA), which were stained with uranyl acetate (2% w:v). The TEM equipment (JEM 1200 Exll, Jeol, Tokyo, Japan) was operated at 80 kV.

Analysis of the hydrogels pH. The pH values of the formulations were measured in a calibrated potentiometer, after dilution of hydrogels in distilled water (1:10 w/v), immediately after hydrogels production. The results represent an average measurement from three different batches.

Analysis of the hydrogels consistence and flow. The rheological analysis was performed in a rotational viscosimeter (Brookfield® LV-DV-II+Pro, spindle SC4-25, Middleboro, MA), at 25°C. Hydroxyethyl cellulose is a nonionic hydrogel-forming polymer of common use, behaving as non-Newtonian fluid with pseudoplastic character (22). The chitosan hydrogel was previously described as presenting pseudoplastic behavior as well (9,23). So, the data obtained from rheological viscosimetry were treated according to the Ostwald flow model:

$$\tau = K\gamma n, \tag{1}$$

where τ represents the shear rate, γ represents the shear stress, K is the consistence index, and n is the Power Law index. On the basis of this flow model, it is possible to obtain the consistence values of the hydrogels, what enables a better comparison between formulations. The results represent an average measurement from three different batches.

Sensory analysis. The sensory study was conducted through the analysis of the samples by 60 untrained volunteers (pharmacy undergraduate and postgraduate students), aging between 18 and 35 years. The volunteers performed the analysis in rooms with constant temperature and lightning. This study was approved by the ethics on research national committee. After being introduced to the research and signing of the consent term, the volunteers washed their arms up to the elbows, with neutral soap, without any fragrance. Three circles of 51 mm of diameter were drawn. The samples (approximately 0.3 g) were

applied on the skin by the volunteers during 1 min, controlling the rate by a metronome (120 beats per min). The samples were presented in pairs and the volunteers answered about the difference between formulations regarding specific attributes (discriminative analysis) and about their general preference (affective analysis). All volunteers analyzed three pairs of samples identified with three-letter random codes. For the samples that appeared more than one time, in different pair of samples, the code was different for avoiding identification of the sample by the volunteer.

The sensory study was characterized as discriminative and affective and it was performed in two distinct phases. Each phase was composed of 60 volunteers analyzing distinct pairs of samples. The attributes analyzed in both phases were: spreadability, oiliness, immediate and residual stickiness, film formation, homogeneity of the film, and preference.

Phase I sensory study: Chitosan gel versus hydroxyethyl cellulose gel. The sample pairs analyzed were the following: CH versus HEC, CH-NC versus HEC-NC, and CH versus CH-NC. The first two pairs aimed to identify differences between the chitosan and hydroxylethyl cellulose gels containing or not containing nanocapsules, for the mentioned attributes. The preference between such formulations was analyzed, without relating the preference to any attribute. The last pair (CH vs. CH-NC) aimed to observe the differences that were brought to the formulation due to the incorporation of nanocapsules in the chitosan hydrogel, the focus of this study. The preference of the volunteers was determined also for this pair of sample.

Phase II sensory study: Chitosan gel versus optimized chitosan gel. The sample pairs analyzed were the following: CH versus CH-OPT, CH-NC versus CH-NC-OPT, and CH-OPT versus CH-NC-OPT. The first two pairs aimed to identify differences between the chitosan and optimized chitosan gels containing or not containing nanocapsules, for the mentioned attributes. The preference between such formulations was analyzed, without relating the preference to any attribute. The last pair (CH-OPT vs. CH-NC-OPT) aimed to observe the differences that were brought to the formulation due to the incorporation of nanocapsules in the optimized chitosan hydrogel. The preference of the volunteers was determined also for this pair of sample.

Statistical analysis. The statistical analysis of the characterization properties were performed by Student's *t*-test, while the statistical difference of the sensory studies was determined through the χ^2 test (proportions test). In both cases, differences were considered significant for $\alpha = 0.05$.

RESULTS AND DISCUSSION

CHARACTERIZATION OF HYDROGELS

All hydrogels presented slightly different aspects. The hydrogels containing nanocapsules presented opacity due to the nanocapsule aqueous suspension, while the hydrogels without nanocapsules presented transparency, in except for the optimized chitosan hydrogel, which presented some opacity due to the adjuvants addition. The pH values were around 4.5 for chitosan hydrogels (CH = 4.32 ± 0.21, CH-NC = 4.31 ± 0.16, CH-OPT = 4.50 ± 0.18, CH-NC-OPT = 4.42 ± 0.15) due to lactic acid used for chitosan solubilization and chains entanglement, in a way that neither the addition of adjuvants (silicone

and PCA-Na) nor the addition of nanocapsules led to significant changes in the pH values. The hydroxyethyl cellulose gels presented pH values around 6.5 (HEC = 6.68 ± 0.27, HEC-NC = 6.77 ± 0.13) and no differences were observed when the nanocapsules were added, as well. The values were considered suitable for skin application due to slight acidity of the stratum corneum (24).

Regarding the presence of nanocapsules in the hydrogels, CH-NC, HEC-NC, and CH-NC-OPT, it was observed, through analysis of the volume (Figure 1A) and number (%) (Figure 1B) distribution of particles sizes, that the nanocapsules are present in the gels, showing similar size when compared to the nanocapsule aqueous suspension. The presence of a micrometric peak in HEC-NC gel only appears when the volume of the particles are considered, disappearing when taking into consideration the number of the particles. This micrometric peak could be related to the HEC microdomains. TEM photomicrographs (Figure 2) confirmed that the nanocapsules were not damaged due to incorporation in the hydrogel formulations and that similar size is observed comparing both techniques for determination of particles sizes. These results demonstrate the applicability of the proposed hydrogels as suitable vehicles for polymeric nanocapsules.

The hydrogels were submitted to rheological analysis aiming to verify the viscosity of the products. Since non-Newtonian fluids present viscosity dependent on the shear rate, rheological profiles were obtained (shear stress vs. shear rate, Figure 3). When fitting such data by the Ostwald flow model of pseudoplasticity, the determination coefficients were above 0.99 for all hydrogels, indicating that the model is suitable for the data obtained.

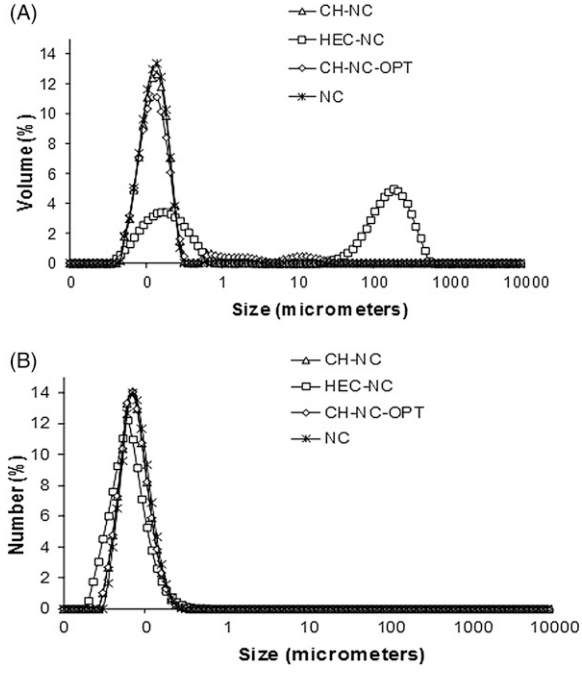

Figure 1. (A) Volume percentage and (B) number percentage distribution of particles sizes (CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized hydrogels).

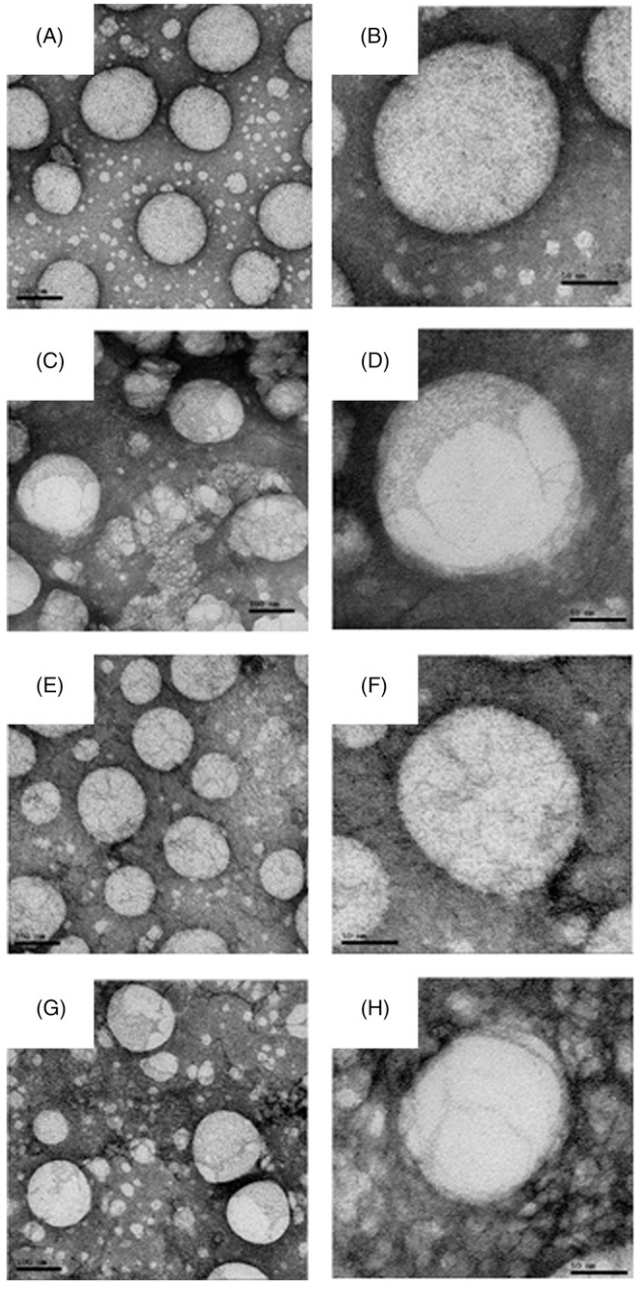

Figure 2. Transmission electron photomicrographs of formulations. (A) NC in aqueous suspension 200.000×, (B) NC in aqueous suspension 500,000×, (C) CH-NC 200,000×, (D) CH-NC 500,000×, (E) HEC-NC 200,000×, (F) HEC-NC 500,000×, (G) CH-OPT 200,000×, and (H) CH-OPT 500,000× (CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized gel) Size bars to the left corresponds to 100nm, while size bars to the right corresponds to 50nm.

The consistence indexes obtained for the formulations, through the Ostwald flow model, were 12.28 ± 1.45 (CH), 17.19 ± 0.55 (CH-NC), 13.93 ± 0.32 (HEC), 17.27 ± 0.18

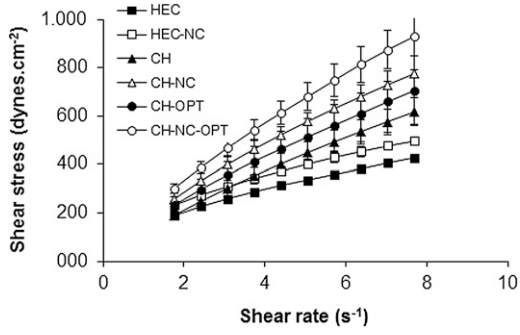

Figure 3. Rheological profiles of formulations (shear stress vs. shear rate) (CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized gel).

(HEC-NC), 14.84 ± 2.74 (CH-OPT), 19.70 ± 1.02 (CH-NC-OPT). All hydrogels showed similar consistence values, although the hydrogels containing nanocapsules presented significant slightly higher values than the respective hydrogels containing pure water ($p < 0.05$). Comparing the different hydrogels containing water (CH, HEC, CH-OPT), no differences were found but, on the other way, comparing the different hydrogels containing nanocapsules (CH-NC, HEC-NC, CH-NC-OPT), the CH-NC-OPT presented significant slightly higher consistence ($p < 0.05$), maybe due to an interaction of the nanocapsules and the adjuvants, increasing the consistence. The rheological properties are important to be determined since there might be a relation between them and the sensory properties (25).

SENSORY ANALYSIS

Phase I: Chitosan gels versus hydroxyethyl cellulose gels. The phase I sensory analysis comprised the comparison between the chitosan gel and a hydrogel commonly used in cutaneous formulations (hydroxyethyl cellulose gel) and also, between the chitosan hydrogel containing and not containing nanocapsules. Table II shows the responses from the volunteers, in percentages, when comparing the paired samples. It is interesting to notice that the percentage of volunteers who saw differences between samples was always higher than the percentage of volunteers who thought the samples were similar for the present attribute. Nevertheless, it was important to determine if the percentage of volunteers who pointed out that there were differences among samples were significantly higher than the volunteers who pointed out no differences were perceptible. When this requirement was achieved, the answers of the volunteers who choose one or other sample for the evaluated attributes were taken into account and another statistical test was performed only considering these volunteers.

Table III shows the p value obtained comparing the percentage of volunteers who saw differences among samples with the percentage of volunteers who could not see differences. For the attributes oiliness (comparison CH vs. CH-NC), immediate stickiness (all comparisons), film formation (all comparisons), homogeneity of the film (comparisons CH vs. HEC and CH vs. CH-NC), and preference (all comparisons), the percentage of volunteers who choose one or the other sample was significantly higher than the

Table II
Responses from the Volunteers, in Percentage, When Comparing the Paired Samples in the Phase I Sensory Study

		Paired analysis		
Attribute	Percentage of volunteers	CH vs. HEC	CH-NC vs. HEC-NC	CH vs. CH-NC
Spreadability	Did not see differences	45	48	40
	Saw differences	55	52	60
	Chose the first sample	13	2	35
	Chose the second sample	42	50	25
Oiliness	Did not see differences	48	50	35
	Saw differences	51	50	65
	Chose the first sample	33	37	38
	Chose the second sample	18	13	27
Immediate stickiness	Did not see differences	42	37	20
	Saw differences	69	63	80
	Chose the first sample	47	58	33
	Chose the second sample	22	5	47
Residual stickiness	Did not see differences	45	42	48
	Saw differences	55	58	52
	Chose the first sample	45	45	17
	Chose the second sample	10	13	35
Film formation	Did not see differences	28	35	28
	Saw differences	72	65	72
	Chose the first sample	62	53	22
	Chose the second sample	10	12	50
Homogeneity of the film	Did not see differences	35	45	22
	Saw differences	65	55	78
	Chose the first sample	28	20	48
	Chose the second sample	37	35	30
Preference	Did not see differences	18	27	10
	Saw differences	81	73	90
	Chose the first sample	13	20	52
	Chose the second sample	68	53	38

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules.

volunteers who did not see differences. Table IV shows the *p* value obtained comparing the percentage of volunteers who choose the first or the second sample in the pair, for the attributes mentioned above. Only for the attributes immediate stickiness (comparisons CH vs. HEC and CH-NC vs. HEC-NC), film formation (all comparisons), and preference (comparisons CH vs. HEC and CH-NC vs. HEC-NC), the percentage of volunteers who choose one sample in the pair was different from the percentage of volunteers who choose the other sample. The statistically significant results are shown in Figure 4.

Table III
p Values Obtained from χ^2 test for Phase I Sensory Study (Comparison between the Percentage of Volunteers Who Saw Differences with the Percentage of Volunteers Who Did Not See Differences)

	p Value		
Attribute	CH vs. HEC	CH-NC vs. HEC-NC	CH vs. CH-NC
Spreadability	0.439		
Oiliness	0.796	1	0.020*
Immediate stickiness	0.005*	0.039*	0.000*
Residual stickiness	0.439	0.197	0.796
Film formation	0.001*	0.020*	0.001*
Homogeneity of the film	0.020*	0.439	0.000*
Preference	0.000*	0.000*	0.000*

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules.
*Significant difference ($p < 0.05$).

Concerning the discriminative sensory analysis, it could be seen that there were no significant differences among CH and HEC gels for the attributes spreadability, oiliness, and residual stickiness, demonstrating that the innovative chitosan gel could be used without prejudice on some sensory properties. However, the chitosan hydrogel showed significant higher immediate stickiness and perception of film formation on the skin than the hydroxyethyl cellulose gel, despite the presence of nanocapsules. The higher stickiness is probably related to the bioadhesive properties of the polymer, and the film formation is widely described in the literature for this polymer (3). It is important to notice that the residual stickiness was not considered higher for the chitosan hydrogels, showing that this effect was temporary, limited to time of application.

Table IV
p Values Obtained from χ^2 test for Phase I Sensory Study (Comparison between the Percentage of Volunteers Who Chose One or Another Formulation from the Paired Analysis)

	p Value		
Attribute	CH vs. HEC	CH-NC vs. HEC-NC	CH vs. CH-NC
Spreadability	—[a]	—[a]	—[a]
Oiliness	—[a]	—[a]	0.262
Immediate stickiness	0.019*	0.000*	0.248
Residual stickiness	—[a]	—[a]	—[a]
Film formation	0.000*	0.000*	0.010*
Homogeneity of the film	0.423	—[a]	0.109
Preference	0.000*	0.003*	0.276

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules.
[a]The test was not applicable since the number of volunteers who saw difference was not statistically higher than the number of volunteers who did not see differences.
*Statistical difference ($p < 0.05$).

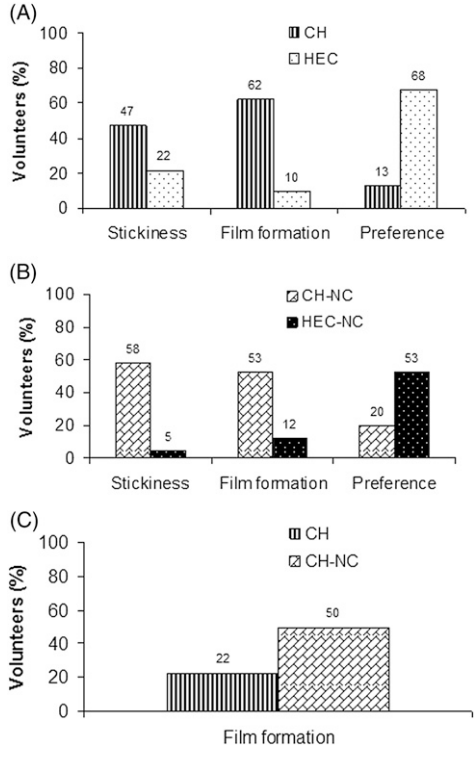

Figure 4. Attributes showing statistical differences in the phase I sensory study for the following sample comparisons: (A) CH vs. HEC, (B) CH-NC vs. HEC-NC, and (C) CH vs. CH-NC (CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules).

The incorporation of nanocapsules in the chitosan hydrogel only led to a higher perception of film formation on the skin, regarding the discriminative sensory analysis. This may be due to the nanocapsules skin adhesion and their high permanence time on the skin, or due to a higher consistence of this formulation. The increase on the film formation due to the nanocapsules is interesting for the cutaneous application because of the promotion of the interaction between skin and drug, and also the protection against drug degradation or drug washability. However, regarding the sensory, the volunteers probably considered the film formation as a bad attribute.

Considering the affective sensory analysis, the hydroxyethyl cellulose gels were preferred over the chitosan hydrogels, for the plain gels and gels containing nanocapsules. The presence of nanocapsules in the chitosan gel did not alter the acceptance of this formulation. The differences observed in the discriminative analysis (stickiness and film formation) probably influenced the result verified in the affective analysis, indicating the attributes to be improved for the development of a new formulation of improved sensory properties.

Phase II: Chitosan gels versus optimized chitosan gels. The phase II was devoted to the analysis of the improvements made on the chitosan hydrogel, after the results of phase I sensory analysis. The chitosan hydrogel was added of PCA-Na (carboxylic pirrolidon acid sodium salt) and volatile silicone fluid (decamethylcyclopentasiloxane [DC 245]) to decrease the

Table V
Responses from the Volunteers, in Percentage, When Comparing the Paired Samples in the Phase II Sensory Study

		Paired analysis		
Attribute	Response from volunteers	CH vs. CH-OPT	CH-NC vs. CH-NC-OPT	CH-OPT vs. CH-NC-OPT
Spreadability	Did not see differences	42	35	27
	Saw differences	59	65	74
	Chose the first sample	32	30	52
	Chose the second sample	27	35	22
Oiliness	Did not see differences	45	53	32
	Saw difference	55	47	68
	Chose the first sample	25	22	45
	Chose the second sample	30	25	23
Imediate stickiness	Did not see differences	37	45	23
	Saw differences	64	55	77
	Chose the first sample	27	28	30
	Chose the second sample	37	27	47
Residual stickiness	Did not see differences	47	48	33
	Saw differences	54	52	66
	Chose the first sample	17	22	23
	Chose the second sample	37	30	43
Film formation	Did not see differences	35	27	27
	Saw differences	65	73	74
	Chose the first sample	47	50	37
	Chose the second sample	18	23	37
Homogeneity of the film	Did not see differences	17	25	10
	Saw differences	83	75	90
	Chose the first sample	28	32	30
	Chose the second sample	55	43	60
Preference	Did not see differences	10	7	7
	Saw differences	90	94	93
	Chose the first sample	30	37	38
	Chose the second sample	60	57	55

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized hydrogels.

stickiness and perception of film formation on the skin. So, the prior chitosan hydrogel was compared to the optimized chitosan hydrogel, both of them containing and not containing nanocapsules. Also, in this phase, the presence of nanocapsules was evaluated regarding its influence on the sensory properties of the optimized chitosan hydrogel. Table V shows the answers of the volunteers, in percentage, when comparing the paired samples. As in phase I, the percentage of volunteers who saw differences among samples were always higher than the percentage of volunteers who thought the samples were similar for the present attribute. Table VI shows the *p* value obtained comparing the percentage of

Table VI
p Values Obtained from χ^2 Test for Phase II Sensory Study (Comparison between the Percentage of Volunteers Who Saw Differences with the Percentage of Volunteers Who Did Not See Differences)

	p Value		
Attribute	CH vs. CH-OPT	CH-NC vs. CH-NC-OPT	CH-OPT vs. CH-NC-OPT
Spreadability	0.197	0.020*	0.000*
Oiliness	0.439	0.606	0.005*
Immediate stickiness	0.039*	0.439	0.000*
Residual stickiness	0.606	0.796	0.010*
Film formation	0.020*	0.000*	0.000*
Homogeneity of the film	0.000*	0.000*	0.000*
Preference	0.000*	0.000*	0.000*

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized hydrogels.
*Statistical difference ($p < 0.05$).

volunteers who saw differences among samples with the percentage of volunteers who could not identify differences. For the attributes spreadability (comparisons CH-NC vs. CH-NC-OPT and CH-OPT vs. CH-NC-OPT), oiliness (comparison CH-OPT vs. CH-NC-OPT), immediate stickiness (comparisons CH vs. CH-OPT and CH-OPT vs. CH-NC-OPT), residual stickiness (comparison CH-OPT vs. CH-NC-OPT), film formation (all comparisons), homogeneity of the film (comparisons CH vs. CH-OPT and CH-OPT vs. CH-NC-OPT), and preference (all comparisons), the percentage of volunteers who choose one or another sample was significantly higher than the volunteers who did not see differences. Table VII shows the *p* value obtained when comparing the percentage of volunteers who choose the first or the second sample in the pair, for the attributes mentioned above. Analyzing those results, it is possible to see that, only for the attributes spreadability (comparison CH-OPT vs. CH-NC-OPT), oiliness (comparison CH-OPT vs.

Table VII
p Values Obtained from χ^2 Test for Phase II Sensory Study (Comparison between the Percentage of Volunteers Who Chose One or Another Formulation from the Paired Analysis)

	p Value		
Attribute	CH vs. CH-OPT	CH-NC vs. CH-NC-OPT	CH-OPT vs. CH-NC-OPT
Spreadability	—[a]	0.631	0.007*
Oiliness	—[a]	—[a]	0.042*
Immediate stickiness	0.330	0.862	0.140
Residual stickiness	0.340	0.369	0.058
Film formation	0.006*	0.016*	1.000
Homogeneity of the film	0.024*	0.180	0.014*
Preference	0.014*	0.109	0.181

CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized hydrogels.
[a]The test was not applicable since the number of volunteers who saw difference was not statistically higher than the number of volunteers who did not see differences.
*Statistical difference ($p < 0.05$).

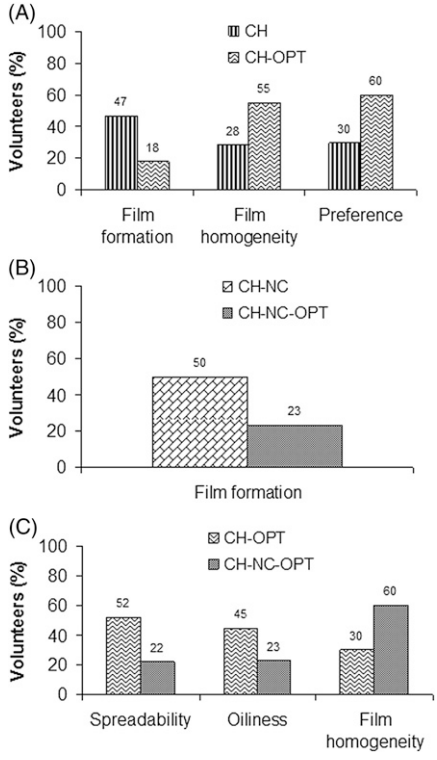

Figure 5. Attributes showing statistical differences in the phase II sensory study for the following sample comparisons: (A) CH vs. CH-OPT, (B) CH-NC vs. CH-NC-OPT, and (C) CH-OPT vs. CH-NC-OPT (CH: chitosan gel, HEC: hydroxyethyl cellulose gel, NC: nanocapsules, OPT: optimized gel).

CH-NC-OPT), film formation (comparisons CH vs. CH-OPT and CH-NC vs. CH-NC-OPT), homogeneity of the film (comparisons CH vs. CH-OPT and CH-OPT vs.CH-NC-OPT), and preference (comparisons CH vs. CH-OPT), the percentage of volunteers who choose one sample in the pair was different from the percentage of volunteers who choose the other sample. These results, showing significant difference, are shown in Figure 5.

Regarding the attributes considered to be improved, from the results of phase I (stickiness and film formation), it can be seen that the perception of residual film on the skin was decreased when the chitosan gel was optimized, despite the presence of nanocapsules. It is worth mentioning that the film is still formed on the skin since the chitosan is still present, but its perception has been reduced. Besides that, the homogeneity of the film was increased when the adjuvants were added in the gels without nanocapsules, leading to a higher preference for the CH-OPT gel comparing with CH. Although similar results were not statistically found for the gels with nanocapsules, there was a tendency for increasing the film homogeneity and the gel preference due to the modifications in the chitosan hydrogel containing nanocapsules (Table 5). The stickiness, which was the other attribute probably responsible for the low acceptance of chitosan gel in phase I, could not be decreased due to incorporation of PCA-Na and volatile silicone, for the gels with and without nanocapsules. Since the residual stickiness was not higher in the chitosan

hydrogel (when comparing to HEC gel in phase I) and no changes occurred after modifications in the gel, the stickiness was considered less important for improving the sensory than the film formation. Regarding the other attributes (spreadability, oiliness), no differences were found comparing to the basic chitosan hydrogel. Those attributes were not expected to change since the results were considered satisfactory when no differences were detected comparing to the hydroxyethyl cellulose gel.

The presence of nanocapsules in the optimized chitosan hydrogel did not lead to higher film formation on the skin, as observed in phase I. This is considered a satisfactory result since the film formation was probably responsible for the low acceptance during phase I, as already mentioned. The nanocapsules, on the other hand, improved the homogeneity of the film formed. Films with greater homogeneity formed on the skin probably induce a more uniform application and a more uniform skin permeation of actives besides a greater skin permanence of the film formed. Another interesting effect of the nanocapsules could be seen at this point of the study. The nanocapsules decreased the spreadability and the oiliness of the optimized chitosan gel. This result can be related to changes in the gel network when the adjuvants were added to the chitosan hydrogel.

CONCLUSION

The chitosan hydrogels were considered innovative and suitable formulations for cosmetic use, with adequate pH, pseudoplastic flow, and possibility to incorporate polymeric nanocapsules. Regarding the main questions asked in the introduction section, it was concluded that: (i) For the attributes spreadability, oiliness, and residual stickiness, no significant differences were detected between the chitosan gel and hydroxyethyl cellulose gel. However, the higher immediate stickiness and the perception of film formation on the skin, probably led to the low acceptance of this gel, indicating the attributes to be improved. (ii) The addition of adjuvants was essential to turn the gel into a formulation with better sensory properties, demonstrating that the sensory analysis is a suitable tool in the pharmacotechnical development of the novel chitosan hydrogel containing nanoparticles. (iii) For the basic chitosan gel, the nanoparticles increased the perception of residual film on the skin, while for the optimized chitosan gel, the nanocapsules increased the homogeneity of the film formed, without increasing its perception. The work performed showed for the first time the sensory attributes of a chitosan hydrogel for cutaneous use and confirmed that the nanoparticles may interfere on the sensory attributes of dermatological and cosmetic formulations.

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