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Vitamin A and vitamin E interaction behavior on chitosan microspheres

A calorimetric view

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Abstract Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, and non-toxicity. In this study, chitosan microspheres were successfully prepared by an adapted method of coagulation/dispersion. The degree of deacetylation of chitosan powder was obtained by NMR ^{1}H and FTIR techniques. Chitosan powder and chitosan microspheres were characterized by BET surface area and scanning electron microscopy (SEM). The interactions among the chitosan microspheres and the vitamins A and E were characterized by FTIR. In order to evaluate the ability of interaction of vitamin A and vitamin E with the chitosan microspheres, the thermodynamic parameters were followed by calorimetric titration. Different experimental approaches were applied, such as adsorption isotherms, kinetics and thermodynamics studies. The obtained results showed that the interactions of chitosan microspheres with the vitamins were spontaneous, enthalpically and entropically favorable, indicating that the chitosan microspheres can be used with success in the controlled release of these vitamins.

Keywords Calorimetry · Chitosan · Vitamins

Introduction

The beauty industry represents a major share in the global market, which has a large growth in recent years. The biggest market for cosmetics is the U.S. with 15.6% of the market followed by Japan with 10.1% and Brazil with 8.6% of the world. The Brazilian toiletry, perfumery, and cosmetics are an important industrial area corresponding to 5.4% of Brazilian GDP. Sales of cosmetics industry have grown exponentially in Brazil, reaching a turnover of 11.9 billion U.S. dollars in 2008, as shown in Fig. 1 [1]. Cosmetics also have an essential role in Brazilian trade balance. Brazil exported 160.6 million dollars and imported 120.6 million in personal care products and beauty, reaching a surplus of 40 million in 2007. Among the various types of cosmetics, skin creams have an important part of this growing market. Vitamins are among the main compounds used to develop cosmetics.

Vitamins are essential compounds for many functions of the human organism. The most important are vitamins A, B, C, D, E, and K, as well as folic acid. Besides their specific functions, certain vitamins are useful for prevention, being used as drugs or cosmetic products [2]. Vitamin A (also called retinol) has been shown to improve depigmentation of photodamaged skin [3]. Vitamin E contributes to the antioxidant defense of the skin, absorbing UV-light in the solar spectrum region that is responsible for most of the deleterious biologic effects of the sun [4, 5]. Despite the efficacy of retinoic acid and tocopherol, there are some limitations in their uses in cosmetics, such as low water solubility, instability in the presence of light and oxygen, and the occurrence of reactions of local irritation such as erythema, xerosis, and mild scaling [6, 7]. To avoid this problem, these vitamins can be immobilized in chitosan microspheres in order to reduce its degradation by the heat and light. Then, the chitosan microspheres can be a viable alternative channel for retinoic acid and tocopherol in the skin to minimize irritation produced when used topically, because of their

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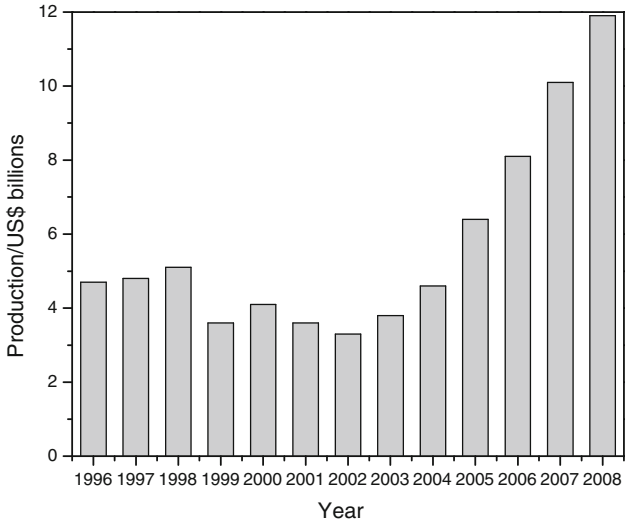

Fig. 1 Billing of Brazilian toiletry, perfumery, and cosmetics industries

extended release, and give more stability to the molecule [8, 9].

In this way, the aim of the present study is to investigate the interaction between chitosan microspheres and the vitamin A and B using calorimetric titration as well as to assess the relation of vitamin–polymer interaction.

Experimental

Materials

The chitosan was obtained from Genix Pharmaceutical Industry (Brazil) and Retinoic acid (vitamin A) and Tocopherol (Vitamin E) were obtained from Aldrich. The reagents such as acetic acid, sodium hydroxide, and glutaraldehyde were obtained from Vetec.

Preparation of chitosan microspheres

The chitosan microspheres were prepared according to the method proposed by Rorrer and Hsien (split-coating) [10], with some modifications from Prado et al. [11], as represented in Fig. 2. Chitosan 10% (w/v) was dissolved in 5% (v/v) acetic acid at room temperature. The solution was homogenized for 2 h and after dripped in a coagulant solution of sodium hydroxide 10% (w/v) kept under stirring. Then, the microspheres were washed with deionized water until reaching pH 7.0. Once neutralized, the gelled beads were crosslinked in a 25% glutaraldehyde solution, without stirring for 2 h. After that, microspheres were filtered, washed with deionized water and then with acetone and dried [11].

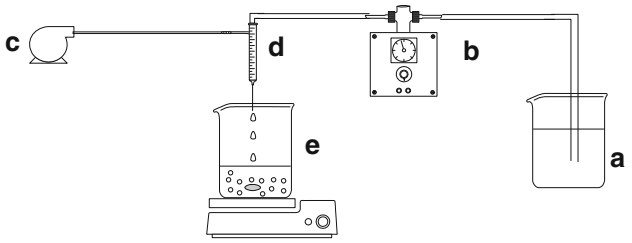

Fig. 2 Scheme of the system to produce chitosan microspheres: Chitosan solution (**a**), peristaltic pump (**b**), air compressor (**c**), drip system (**d**), coagulation solution (**e**)

Characterization

The chitosan was characterized by spectroscopy of NMR ^{1}H was obtained by a Varian Mercury Plus 7.05 T, 300 MHz to ^{1}H, was disbanded chitosan in D_2O/HCl (100:1 v/v) at room temperature.

The degree of deacetylation of the chitosan was calculated by Eq. 1 from data obtained from NMR ^{1}H.

$$\overline{\mathrm{GD}} = 100 - \left(\frac{\frac{I_{\mathrm{met}}}{3}}{\frac{I_{\mathrm{H2-6}}}{6}} \times 100 \right) \quad (1)$$

where, I_{met} is the integral intensity of the signal from the methyl protons of acetamide groups, $I_{\mathrm{H2\text{-}6}}$ is the sum of integral intensities of the signals from the H atoms bonded to carbons 2, 3, 4, 5, and 6 of the glycosidic ring, in the region between 1 and 4 ppm and GD is the degree of deacetylation [12].

The infrared spectra were analyzed in a spectrophotometer JASCO 4100, with resolution of 4 cm^{-1} by accumulation 250 scans.

The sample surface images were morphologically observed on Optical Video-microscope Bell Optics and on a Scanning Electron Microscope Zeis EVO 050. For SEM analysis, the samples were coated with gold plating for 100 s in a Baltec SCD 050 spray. The SEM was operated with an electron beam of 20 keV.

The chitosan and microspheres surface areas were determined through isotherm adsorption–desorption, employing a Quantachrome Nova 2200 analyzer. It was performed a preliminary drying at 100 °C under reduced pressure for 12 h.

Interaction between vitamins and microspheres of chitosan

The interactions of the microspheres with the vitamins were given by suspension of 100 mg of microspheres in a 2 g L^{-1} ethanolic solution of retinoic acid or tocopherol. The reaction mixture was maintained for 24 h at room temperature and the amount of vitamins adsorbed microspheres was determined by a UV–Visible Spectrophotometer Cary-50.

For interaction infrared data, 100 mg of chitosan microspheres were suspended in a 2 g L^{-1} ethanolic solution of retinoic acid or tocopherol. The reaction mixture was maintained for 24 h at room temperature, and the ethanol was removed by vacuum line at 50 °C during 3 h. Infrared spectra of the mixtures were analyzed in a spectrophotometer JASCO 4100, with resolution of 4 cm^{-1} and by accumulation 250 scans.

Calorimetric analyses

The thermodynamic parameters of the interaction between the vitamins and the microspheres were followed by calorimetric titration in an adiabatic calorimeter PAR 6755. In this experiment, 500 mg of the microspheres were suspended in 100 mL of water, equilibrated at 25.00 °C (thermostatically controlled), and titrated with 2 g L^{-1} ethanolic solution of retinoic acid or tocopherol.

Results and discussion

Characterization

NMR spectrum confirms the structure of chitosan, as according to the labeled hydrogen atoms in Fig. 3. The application of Eq. 1 in the proton NMR of chitosan resulted in the deacetylation degree of 83.50%.

The surface area values of materials were calculated by applying the BET equation in N_2 isotherms, which gave 4.2, 14.1, and 9.6 m^2 g^{-1} for start chitosan, chitosan microspheres and reticulated chitosan microspheres, respectively. This fact evidences that the formation of microspheres from start chitosan causes a significant increase on the surface area of the material by a morphological organization of the material, which is one of the new adsorbent qualities of this material when compared with the start material. On the other hand, the reticulation causes the decrease of the surface area because of the introduction of glutaraldehyde frameworks between linear polymeric chains of chitosan, which reduces the surface area after incorporation of organic groups. This fact can be easily explained due to the fact that these groups block partially the adsorption of nitrogen molecules on the surface, resulting in a decrease of the surface area.

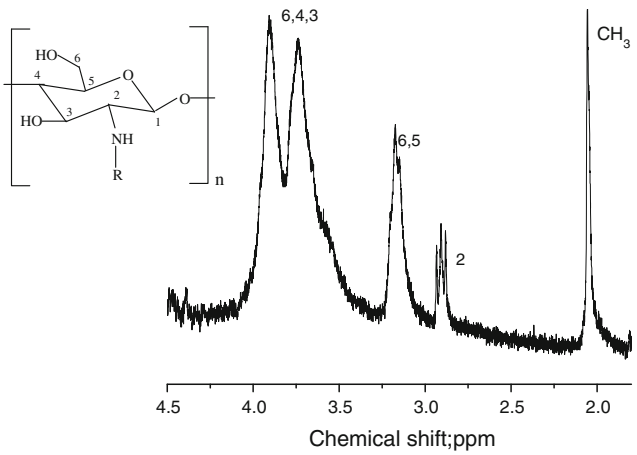

Fig. 3 ^{1}H-NMR spectrum of chitosan. *Inset* the labeled hydrogen atoms of chitosan

The morphology of the chitosan and microspheres were obtained by using optical microscopy and scanning electron microscopy (SEM), and the two images were represented in Fig. 4a and b, respectively. As shown in Fig. 4a, the morphology of chitosan is homogeneously dispersed presenting a particle diameter of 100 μm. This figure also shows that the particles have a soft candy-like morphology.

Vitamins–microsphere interaction

The FTIR spectra of microspheres, vitamins, and microsphere–vitamin interactions are shown in Fig. 5. The main features of chitosan microspheres spectra are associated with the organic backbone such as: a large broad band between 3400 and 3200 cm^{-1}, which is assigned to the O–H and N–H stretching modes; two peaks at 2950 and 2870 cm^{-1} assigned to C–H of sp^3 carbon; an intense band at 1655 cm^{-1}, related to deformation mode C=O from amide, attributed to an imine bond (N=C) of reticulation reaction; one peak at 1562 cm^{-1}, which is associated with an ethylenic bond (C=C); a peak at 1420 cm^{-1} assigned to angular deformation of N–H; and one shoulder at 1220 cm^{-1} related to C–OH stretching mode; and polysaccharides vibrations between 1153 and 890 cm^{-1} [13]. After the interaction between chitosan and retinoic acid, it can be observed the dislocation of N–H peak at 1417–1458 cm^{-1}. This fact suggests the formation of ammonium ion by the interaction between OH groups of retinoic acid and NH_2 groups of chitosan. It can also be observed in this interaction that the peak at 1220 cm^{-1} of retinoic acid spectrum of C–OH was dislocated to 1155 cm^{-1} due to the interaction with chitosan, which corroborates with the interaction of OH of retinoic acid and NH_2 of chitosan, as represented in Fig. 7a. It was not observed any changes in the spectra of the interaction between tocopherol and chitosan. This fact suggests that this interaction occurred because of the hydrogen bonds between OH of tocopherol and NH_2 of chitosan, as represented in Fig. 7b. Changes observed in retinoic acid–chitosan spectrum can be explained by the fact that the carboxylic groups can stabilize the ionic pair whereas the alcohol group of tocopherol does not allow this pair. Thus, the interaction of chitosan and tocopherol must have occurred due to hydrogen bonds.

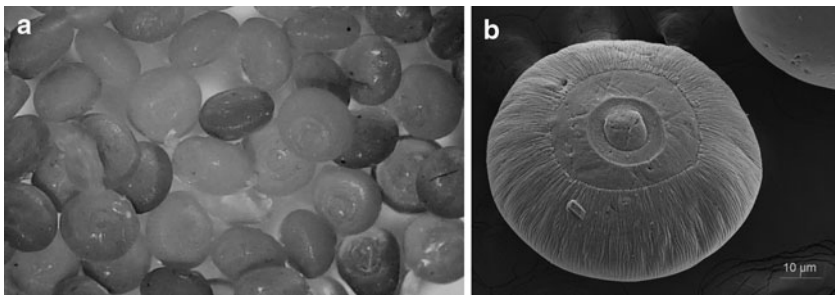

Fig. 4 Scanning electron micrograph of chitosan (**a**) and microspheres (**b**)

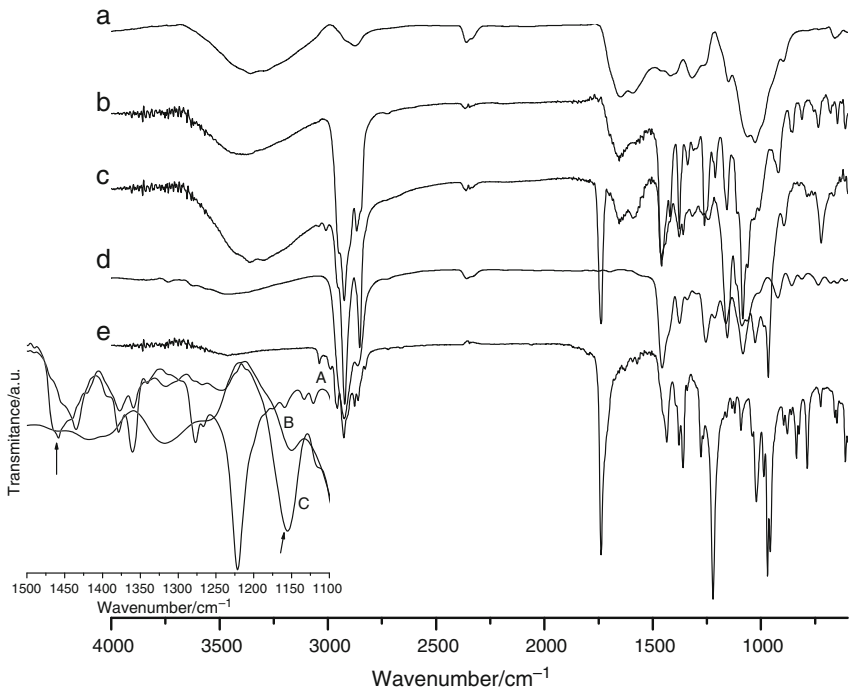

Fig. 5 Infrared spectra of chitosan microspheres (*a*), chitosan-tocopherol (*b*), chitosan-retinoic acid (*c*), tocopherol (*d*), retinoic acid (*e*). *Inset* the zoom of the corresponding spectrum section containing the interaction bands between chitosan–retinoic acid (retinoic acid (*A*), chitosan (*B*), and chitosan-retinoic acid (*C*) spectra)

Calorimetric analyses

The interaction of microspheres with the vitamin from water was followed by sorption isotherms. The number of moles adsorbed (N_f) per gram of solid material was calculated from the initial number of moles of vitamin (n_i) and those at the equilibrium (N_s) condition for a given mass (m) of the adsorbent in grams by applying the Eq. 2:

$$N_f = \frac{n_i - N_s}{m} \tag{2}$$

These experimental data are applied in the general equation for the modified Langmuir model:

$$\frac{C_s}{N_f} = \frac{C_s}{N_s} + \frac{1}{N_s \cdot K} \tag{3}$$

where C_s is the concentration of solution at equilibrium (mol L^{-1}), N_f and N_s are concentration of vitamin adsorbent and the maximum amount of vitamin adsorbed per gram in microspheres (mol g^{-1}), respectively, and K is the equilibrium constant. All these interactions studies were based on the linearized form of the adsorption isotherm, i.e., from plots of C_s/N_f as a function of C_s [13]. Maximum adsorbed number of moles of vitamin A or vitamin E, N_s, obtained from the application of modified Langmuir model, is listed in Table 1. The results show that retinoic acid has a more effective interaction with the microspheres than tocopherol. More information was obtained through the calorimetric titration according to Fig. 6, which shows the enthalpy effect of addition of the retinoic acid and tocopherol in chitosan microspheres, through the Eq. 4.

$$\Sigma\Delta_r Q = \Sigma\Delta_{tit} Q - \Sigma\Delta_{dil} Q \tag{4}$$

where the dilution heat of the vitamin ($\Delta_{dil}Q$) is expressed by the heat of vitamin titration onto these materials, the interaction heat effect values ($\sum\Delta_r Q$) are obtained for all interactions between vitamin and chitosan microspheres [11, 14, 15].

Using the net resultant heat output from reaction, which was adjusted to a modified Langmuir equation, ΔH was calculated through Eq. 5

Table 1 The maximum number of moles adsorbed, N_s, equilibrium constant, K, and the thermodynamic data, ΔH, ΔG, and ΔS for the interaction of retinoic acid and tocopherol with chitosan microspheres

	Retinoic acid	Tocopherol
N_s/mg g^{-1}	19.3 ± 0.5	13.4 ± 0.4
ΔH/kJ mol^{-1}	−18.59 ± 0.34	−15.17 ± 0.50
ΔG/kJ mol^{-1}	−10.36 ± 72	−9.42 ± 0.75
ΔS/J mol^{-1} K^{-1}	+19 ± 2	+26 ± 2
K	65.4 ± 4.58	44.7 ± 5.12

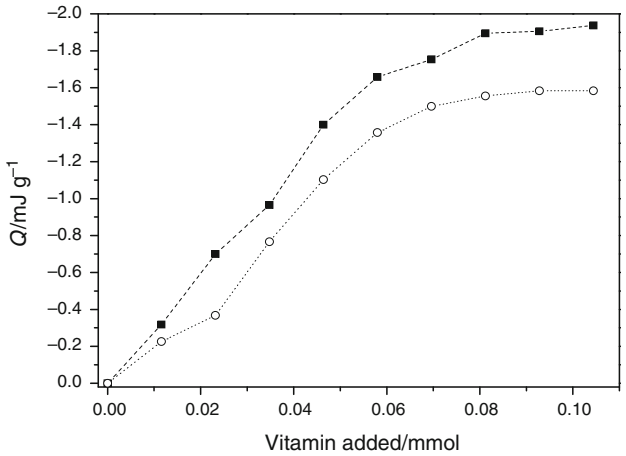

Fig. 6 Net heat changes of calorimetric titration of retinol (*filled square*) and tocopherol (*open circle*) against chitosan microspheres

$$\frac{\Sigma X}{\Sigma \Delta_R H} = \frac{1}{(K-1)\Delta_{mono} H} + \frac{\Sigma X}{\Delta L_{mono} H} \quad (5)$$

where $\sum X$ is the total moles fraction of vitamin in solution after adsorption and X is the value obtained for each addition of titrant. The equations $\Delta G = -RT \ln K$ and $\Delta G = \Delta H - T\Delta S$ were used to determine ΔG and ΔS values, respectively. All thermodynamic of interaction data are listed in Table 1.

The results show that the interactions of chitosan microspheres with retinoic acid and tocopherol are spontaneous, enthalpically and entropically favorable. Thus, the results suggest that chitosan microspheres can be applied in the development of controlled release of retinoic acid and tocopherol. N_s and enthalpy values are presented in Table 1 show that the interaction with chitosan was more effective with retinoic acid than tocopherol, which corroborates with FTIR analysis. This effect in the interactions can be explained by the energy of interaction of the carboxylic groups of retinoic acid with the amine groups of chitosan is higher than the energy of the interaction of phenolic groups of tocopherol as shown in Fig. 7.

Conclusions

In this research, chitosan microspheres have been successfully prepared. Clearly, the chitosan microspheres

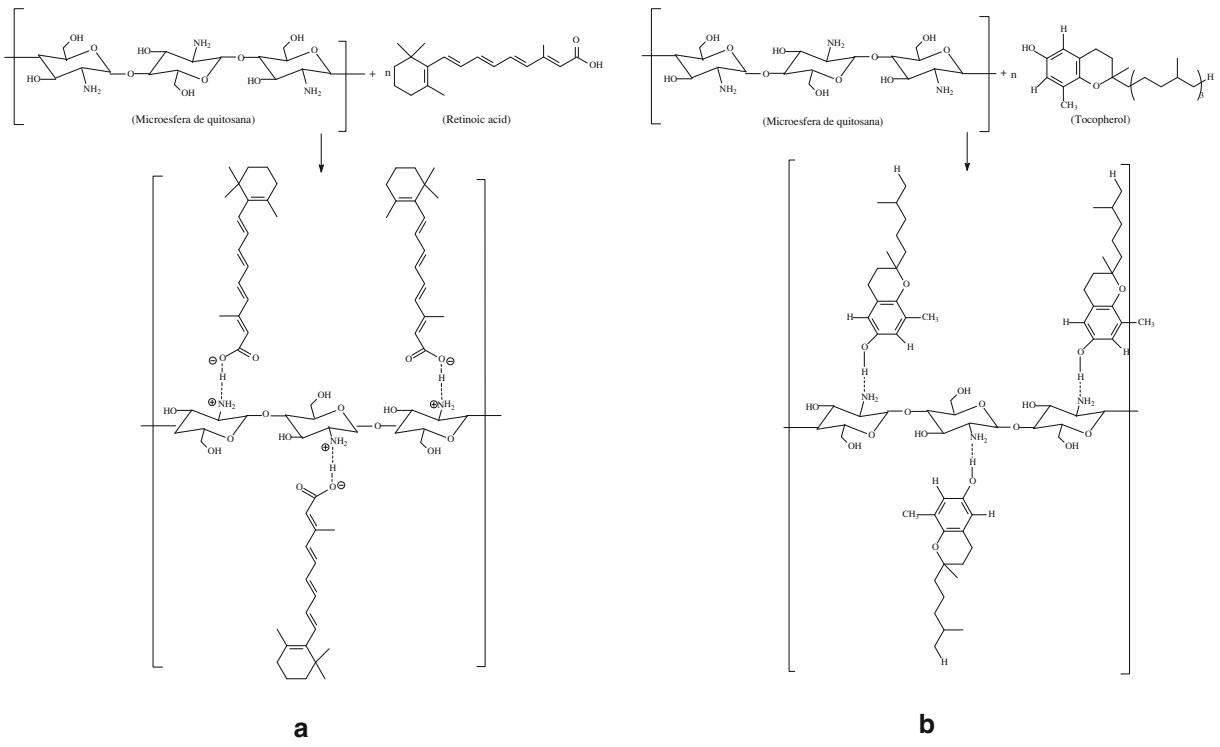

Fig. 7 Scheme of the interaction between retinoic acid and chitosan microspheres (**a**) and tocopherol with chitosan microspheres (**b**)

presented an excellent interaction with retinoic acid and tocopherol, once the interaction with these compounds is spontaneous, enthalpically and entropically favorable. Moreover, the FTIR analysis corroborate with thermodynamic data, confirm the success of the interaction between these vitamins and chitosan microspheres. Indeed, these interactions suggest that this material can be applied in the development of controlled release of retinoic acid and tocopherol, which represents a promising material in cosmetics industries.

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