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DRUG–POLYMER INTERACTION IN THE ALL-*trans* RETINOIC ACID RELEASE FROM CHITOSAN MICROPARTICLES

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Chitosan microparticles were prepared with the purpose of incorporating all-*trans* retinoic acid (ATRA). Morphology, drug content, release behavior and the interaction between chitosan and ATRA were investigated. Chitosan microparticles presented irregular and rough surface and drug content of 47±3%. The results of DSC and IR spectroscopy demonstrated interaction between drug and polymer resulting from retinoate or retinoamide formation. The drug release study showed that approximately 90% of drug was not released from microparticles until the end of experiment (48 h). That release behavior was probably due to the strong drug–polymer interaction and the more compact network of microparticles formed.

Keywords: *all-trans retinoic acid, chitosan microparticles, drug–polymer interaction*

Introduction

Chitosan, a cationic natural biopolymer produced from deacetylation of chitin, has been widely used for drug carrying devices in controlled drug delivery systems [1, 2]. It is essentially natural and biodegradable and has both reactive amino and hydroxyl groups that can be used to chemically alter its structure and properties under mild conditions [3–6].

Several interactions between chitosan (a polyamine) and carboxylic acids or anhydrides of carboxylic acids have been investigated with the purpose to obtain a composition with different characteristics from the original chitosan molecule, particularly for increasing its solubility, improving mucoadhesive properties and preparing micro/nanoparticles [6–11].

Chitosan micro/nanoparticles present many advantages such as its ability to control the release of active agents, its solubility in aqueous acidic solution that prevents the use of hazardous organic solvents, its linear polyamine structure containing a number of free amine groups that are readily available for cross-linking with multivalent anions, its mucoadhesive character, among others. Different methods have been used to prepare chitosan particulate systems [1, 2, 12–16].

As a basic polysaccharide, it is expected that chitosan interacts with acidic drugs rather than basic drugs [17–20]. Imai *et al*. found the interaction of indomethacin (acidic model molecule) with chitosan [17]. Puttipipatkhachorn *et al*. incorporated salicylic acid and theophylline into cast chitosan films as model acidic and basic drugs, respectively. The drug–polymer interaction between salicylic acid and chitosan was demonstrated, resulting in salicylate formation. It was suggested that the swelling property, the dissolution characteristics of the polymer, the pKa of the drug and the drug–polymer interaction were important factors governing drug release patterns from chitosan formulations [18]. Later, Drebushchak *et al.* also found an interaction between another drug acidic, piroxicam, and chitosan, where chitosan increased ten times the drug solubility [20].

The aim of the present study is to investigate the interaction between chitosan and ATRA (acidic drug) using differential scanning calorimetry (DSC) and infrared spectroscopy as well as to assess the relation of that drug–polymer interaction to drug release behavior from chitosan microparticles.

Experimental

Materials

ATRA acid was acquired from RJR Nutrientes e Farmoquímicos LTDA (Brazil). Chitosan (Hydagen® HCMF Type, *MW* 50.000–1.000.000 g mol^{-1}) with min. 80% deacetylation degree was donated by Cognis, Brazil. Solvents were obtained from HPLC grade (Merck & Co. Inc., USA). TTP and other reagents were all analytical grade reagents.

Preparation of drug-loaded chitosan microparticles

Microparticles were prepared according to the modified method earlier described by Ko *et al*. [21].

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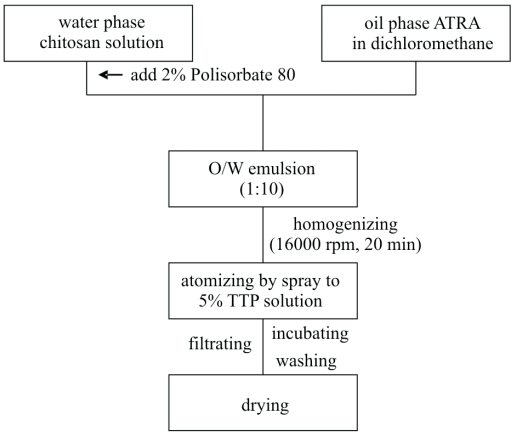

Scheme 1 Schematic representation of preparation procedures of chitosan microparticles

In general, 15 mg of ATRA were dissolved in 1.5 mL of dichloromethane (DCM). Chitosan solution (150 mg) was prepared by dissolving it in 1% acetic acid, and Tween 80 (2% mass/v) was added into the solution as a surfactant. Then, the oil phase (drug solution) was mixed with the aqueous phase (chitosan solution) using an Ultraturrax® at 16000 rpm for 20 min. The ratio of the oil and aqueous phases was 1:10. *O/W* emulsion was pumped (5 mL min^{-1} pump speed) to a 0.7 mm nozzle (air flow rate, 20 mL min^{-1}; pressure, 3 kgf cm^{-2}), sprayed downward into 100 mL of 5% TTP solution pH 5.0 and stirred for 1 h. After the cross-linking time, microparticles were filtered, washed with distilled water repeatedly and then freeze-dried.

Methods

Microparticles characterization

The surface morphology of microparticles was observed under a scanning electron microscope (Leica Model Stereoscan 440). The samples were attached to the slab surfaces with double-side adhesive tapes and then coated with gold. Scanning electron photomicrographs were taken at appropriate magnification.

To study the drug content, 5 mg of microparticles were dissolved in 10 mL of 0.1 N HCl. Then, the polymer was precipitated by the addition of 15 mL of ethanol. The supernatant was filtered and analyzed by HPLC. The experiments were performed in triplicates.

Differential scanning calorimetry

DSC curves of the pure drug, chitosan, binary mixtures (1:1) and microparticles loaded with ATRA were obtained using a Netzsch, model 200, differencial scanning calorimeter under a nitrogen flow of 50 mL min^{-1}, at a heating rate of 10°C min^{-1}, up to 300ºC. Samples (20 mg) were weighed to opened aluminium pans.

Infrared (IR) spectroscopy

IR spectra of microparticles loaded with ATRA, pure drug, isolated polymer, (1:1) drug–polymer physical mixture were recorded using KBr pellets by using Nicolet FTIR spectrophotometer (Model PROTEGÉ™ 460).

In vitro drug release study

The release rate of the microparticles was determined using a Hanson dissolutor, model SR-8 Plus, with paddle assembly (USP Apparatus 2 or BP Apparatus II). 10 mg of microparticles were suspended in 300 μL of 30% ethanol in pH 7.4 phosphate buffer solution (PBS), and then placed within an osmosis membrane with closed extremities. The bags were poured into 100 mL of 30% ethanol in PBS with a pH 7.4 solution as a dissolution medium and shaken at a rate of 100 rpm at 37°C. 1 mL of dissolution medium was periodically drawn for analyzing all-*trans* retinoic acid by HPLC. The same volume of initial dissolution medium was simultaneously replaced to the vials. The samples were analyzed by HPLC (n=5).

HPLC assay

The experiments were carried out by using HPLC Shimadzu® model SPD 10AVP equipped with a LC-10 ADVP pump, Reodyne injector, integrator model CR6-A and UV detector (λ=348), model SPD-10A VP. A column RP-18 shim-pack (125×4 mm, 5 μm) provided by Merck® and coupled to a guard column was used in controlled room temperature (25ºC). The mobile phase consisted of methanol: 1.2% solution of acetic acid:acetonitrile:isopropyl alcohol (10:30:30:30, *v*/*v*), 1.2 mL min^{-1} of flow.

Results and discussion

Microparticles characterization

The scanning electron micrographs of chitosan microparticles loaded with ATRA are shown in Fig. 1.

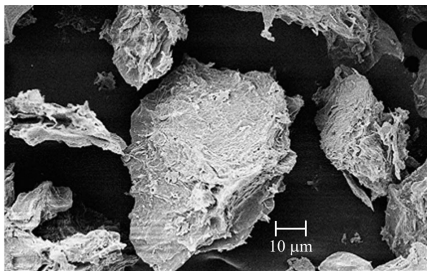

Fig. 1 Scanning electronic photomicrographs of chitosan microparticles loaded with ATRA; mag=2.00 kV, WD=24 mm, EHT=15.00 kV, detector=SE1

Microparticles presented irregular and rough surface. Their morphological characteristics were similar to those reported in [21–23]. The drug content of microparticles obtained from the used method was of 47±3%.

Drug–polymer interaction

To examine the interaction of chitosan with ATRA, differential scanning calorimetry (DSC) and IR spectroscopy were employed.

Figure 2 shows the DSC curves of pure drug, isolated chitosan, the physical mixtures and chitosan microparticles loaded with ATRA. Chitosan exhibited broad endothermic peak around 60°C probably due to water loss [20, 24]. In the case of ATRA and physical mixture, an endothermic peak due to the melting of ATRA was observed at 185 and 180ºC, respectively. That slight shift observed in the physical mixture may be due to an interaction between drug and polymer or drug solubilization in the polymer; however, further investigation using complementary techniques, such as FTIR spectrometry, should be carried out. Another endothermic peak observed in the DSC curves of ATRA and physical mixture around 160°C is probably due to some degradation of drug present in the ATRA sample.

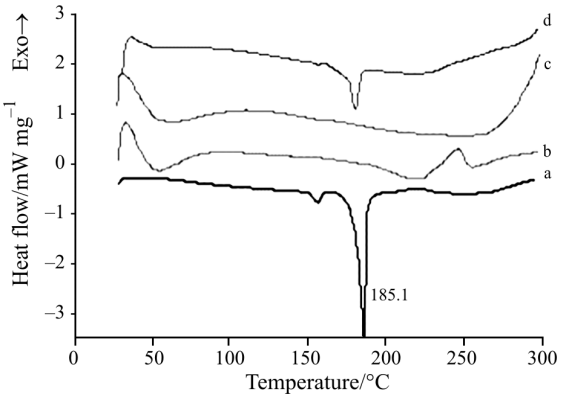

Fig. 2 DSC curves of a – pure ATRA, b – chitosan microparticles loaded with ATRA, c – chitosan and d – (1:1) drug–polymer physical mixture

On the other hand, the DSC curve of microparticles showed broad endothermic peaks around 219ºC that may be the result of a shift of the melting peak of ATRA, suggesting the formation of an ion pair between the carboxylic acid group of ATRA and the amino group of chitosan.

IR spectra of chitosan microparticles, ATRA and physical mixture are shown in Figs 3–6.

Changes in the IR spectrum of microparticles were observed at 1170–1030 cm^{-1} (C–O–N group vibrations) and 1600 cm^{-1} (NH bending absorptions). These changes may be attributed to C–N and O=C–N

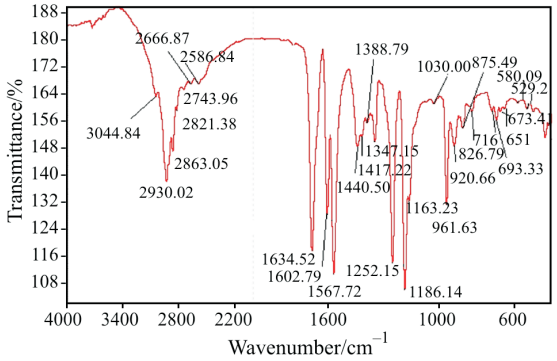

Fig. 3 IR spectrum of pure ATRA

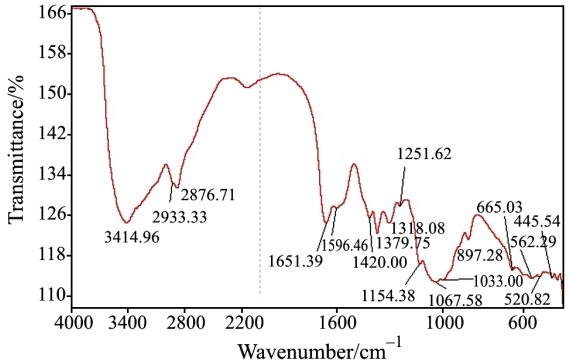

Fig. 4 IR spectrum of chitosan

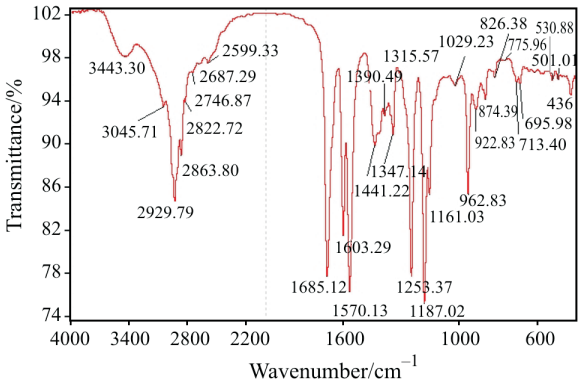

Fig. 5 IR spectrum of (1:1) drug–polymer physical mixture

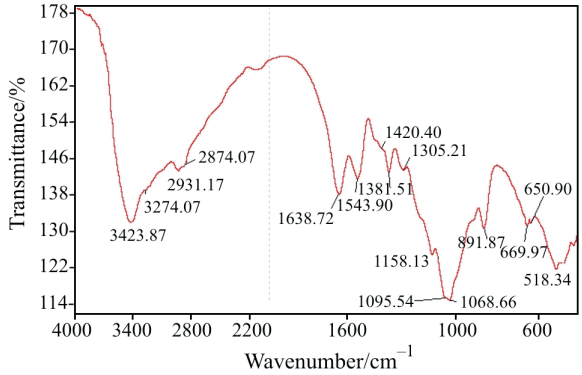

Fig. 6 IR spectrum of chitosan microparticles loaded with ATRA

bonds, indicating strong interaction between the carboxylate group of ATRA and the amino group of chitosan. The presence of ammonium salts is not favored, which is evidenced by the absence of 2540–2500 cm^{-1} bands (protonated amine of ammonium salts), since the bonds formed between drug and polymer were more likely to be amide bonds.

The changes observed in the spectrum of microparticles were not verified in the physical mixture, denying the drug–polymer interaction. Then, it can be stated that the slight shift observed in the DSC curve of the physical mixture was probably due to the solubilization of drug into polymer.

Chitosan has amino and hydroxyl groups in the polymeric chain leading to the possibility of several chemical modifications through the interaction with some reactive compounds, such as aldehydes, ketones and carboxylic acids [6–11, 25, 26]. These groups can be also specifically modified to prepare alkylated derivatives [27] or more complex macrostructures [28]. Although the reaction between ATRA and chitosan, contrarily to some formulations of chitosan gels loaded with ATRA [29], is not known so far, there are many cases where ammonium salts or amide bonds are formed due to the complexation between the amino groups of chitosan and the carboxylate groups of severals kinds of dicarboxylic acids and anhydrides of dicarboxylic acids [6–11]. Even reactions between chitin or chitosan and carboxylic acids, such as acetic acid, are known [11].

It was also found that chitosan can interact with acidic drugs leading to changes in the characteristics of the drug and the polymer. The interaction of indomethacin (acidic model molecule) with low-molecular-mass chitosan (MM 3800–25.000) was reported. Improved solubility of the complex led to the improved release of the drug from the drug–polymer complex [17]. It was demonstrated that salicylic acid (model acidic drug) and theophiline (model basic drugs) were incorporated into cast chitosan films. The drug–polymer interaction between salicylic acid and chitosan resulting in salicylate formation was demonstrated whereas no drug–polymer interaction was observed in theophylline-loaded chitosan films [18].

Other factors have also contributed to suggest a possible interaction between ATRA and chitosan such as:

- ATRA combined with chitosan exhibited a synergistic effect on the cell differentiation of myeloid leukemia [30].
- According to IR and UV spectra of ATRA, it is possible to observe that ATRA can form complexes even with molecules of lower molecular polarity [31–34]. Therefore, it could form these complexes more easily with molecules of higher polarity.

It was confirmed that ATRA might interact with chitosan at the position of amino groups to form retinoate salts or amides.

In vitro drug release study

Figure 7 shows the release behavior of ATRA from chitosan microparticles. Approximately 10% of drug was released rapidly within 2 h. Then, there was no drug release from microparticles until the end of experiment. The drug seemed to be seizured into microparticles, probably due to the strong drug–polymer interaction and the more compact network of microparticles formed that do not allowed the remaining of drug (85%) was dissolved and/or released. These results are according to the findings for salicylic acid by Puttipipatkhachorn *et al*. (2001) in which they found that the release profile of salicylic acid from the high viscosity chitosan films was more sustained due to the drug–polymer interaction and the high viscosity of the films [18].

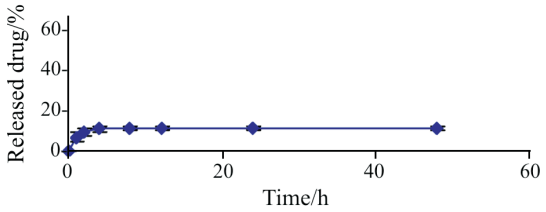

Fig. 7 Release profile of ATRA from chitosan microparticles. Mean values and their standard deviations (bars), n=5 determinations

Conclusions

DSC and IR spectroscopy results demonstrated the drug–polymer interaction between ATRA and chitosan, resulting in retinoate or retinoamide formation. The drug–polymer interaction affected the behavior of ATRA release from microparticles and resulted in the seizure of drug into microparticles.

Hence, it can be concluded that chitosan could interact with negatively charged (acidic) drugs and affect drug release characteristics.

Acknowledgements

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