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THERMOANALYTICAL CHARACTERIZATION OF POTENTIALLY SCHISTOSOMICIDE POLYMERIC DERIVATIVES

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Abstract

Oxamniquine (OXA) is a schistosomicide agent that causes some adverse effects in central nervous system. Intending to improve OXA therapeutic properties, a polymeric prodrug was designed. Currently, there is an increasing interest of thermal analytical techniques in the pharmaceutical area, so differential thermal analysis (DTA) and thermogravimetry (TG) were carried out to evaluate the thermal behavior of OXA, polymethacrylic acid (PMA), [poly(methacrylic-co-oxamniquine methacrylate)acid] (PMOXA) and physical mixture (OXA+PMA). The thermoanalytical profile of the physical mixture showed characteristic events of the thermal decomposition of OXA and PMA. Distinctly, PMOXA DTA curve did not show an endothermic peak at 148.5ºC indicating that the drug was incorporated into the polymeric system. These results were corroborated by the IR spectroscopy and X-ray diffraction data.

Keywords: DTA, OXA, polymeric prodrugs, schistosomiasis, TG

Introduction

Oxamniquine (OXA) is a schistosomicide agent widely used in Brazilian therapeutics *vs. S. mansoni* due to its low cost and good efficiency. Although the drug is relatively well tolerated, some adverse effects in the central nervous system, as convulsions and hallucinations, have been described. It constitutes a relevant risk factor to improve its therapeutic properties. In that case, alternative drugs that have good action spectra, better tolerability and less toxicity, must be designed to achieve the definitive cure [1].

A major approach to increase the therapeutic efficiency of bioactive agents while decreasing their toxicity has involved their chemical attachment to synthetic or naturally occurring macromolecules. Thus, various agents have been bound via degradable linkages to many different polymeric systems. The original argument behind this approach

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was that systems could be designed that would undergo hydrolysis or enzyme catalyzed cleavages when placed in the body so as to release the agent at a predetermined rate. This kind of polymers are structures that anchor drugs intending to obtain derivatives with improved bioavailability, prolonged action and reduced adverse effects [2].

Modern thermal analysis and new emerging combined techniques delivering calorimetric, microscopic and spectroscopic data offer a powerful analytical battery for the study of pharmaceuticals [3–7]. These analyses are relevant in all steps involving drug design, representing a critical role in pharmaceutical analyses [8]. Following this principle and evaluating the great utility of the thermal analytical methods, this concept was extended to the characterization of the biopolymers or polymeric prodrugs [9]. Methacrylic based homopolymers and its copolymers have received considerable attention owing to their versatile applications. These types of polymers have been used as hydrogels and particularly as support matrices for controlled drug release [10, 11]. There are many papers that evaluate certain properties of polymer-drug conjugates as a function of temperature [12–17], but just a few, like Davaran [2], used thermoanalytical techniques to characterize polymeric prodrugs.

Due to the increasing interest on thermal analytical techniques in the pharmaceutical area, the present paper deals with the synthesis and characterization of polymethacrylic based copolymers for the delivery of schistosomicide agent in the treatment of Schistosomiasis. Therefore, this work intends to evaluate the thermal behavior of OXA, polymethacrylic acid (PMA), poly(methacrylic acid-co-oxamniquine methacrylate)acid (PMOXA) and physical mixture (OXA+PMA).

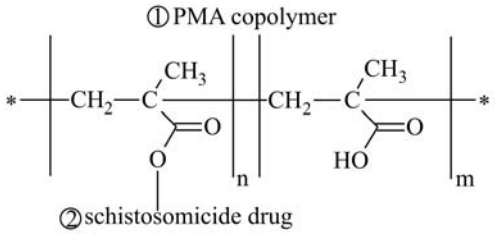

Fig. 1 Molecular structure of poly(methacrylic acid-co-oxamniquine methacrylate) acid (PMOXA)

The latentiation method was carried out to develop an OXA polymeric prodrug. It was obtained by the reaction between the drug hydroxyl group and the methacrylic acid carboxylic group. In that way, the radical copolymerization was carried out (Fig. 1).

Experimental

Materials

OXA was purchased from Pfizer Laboratories. Methacrylic acid was obtained from Merck and was distilled before use. Dicyclohexylcarbodiimide (DCC), dimetylaminopyridine (DMAP) and azo-bis-isobutyronitrile (AIBN) were purchased from Merck.

Synthesis of the PMOXA

Initially, PMA, was synthesized via radical polymerization using AIBN as initiator over the temperature range of 40–60ºC, in toluene. Diethylic ether was added to precipitate the polymer. The resultant polymer was dissolved in methanol and was precipitated from diethylic ether.

In a round bound flask, OXA (Fig. 2a) (1 mmol) was dissolved in dichloromethane (60 mL). Methacrylic acid (Fig. 2b) (3 mmol) was dropped slowly. After that, DMAP (0.5 mmol) and DCC (6 mmol) was added. The reactional mixture was stirred for 8 h at room temperature. The solution was filtered and evaporated to dryness under reduced pressure. The residue (Fig. 2c) was purified by column chromatographic and was widely characterized by RMN.

In a glass vial, OXA methacrylate (Fig. 2c) (1 mmol), methacrylic acid (Fig. 2b) (2 mmol) was dissolved in dimethylformamide (1.0 mL). The mixture was stirred and AIBN 0.5% p/p was added. The glass vial was sealed under nitrogen atmosphere. The copolymerization was carried out over the temperature range of 50–60ºC for 16 h. The copolymer (Fig. 2d) was precipitated by an excess of diethylic ether. The residue was washed three times with diethylic ether and dried under reduce pressure. The degree of substitution was determined by UV spectroscopy.

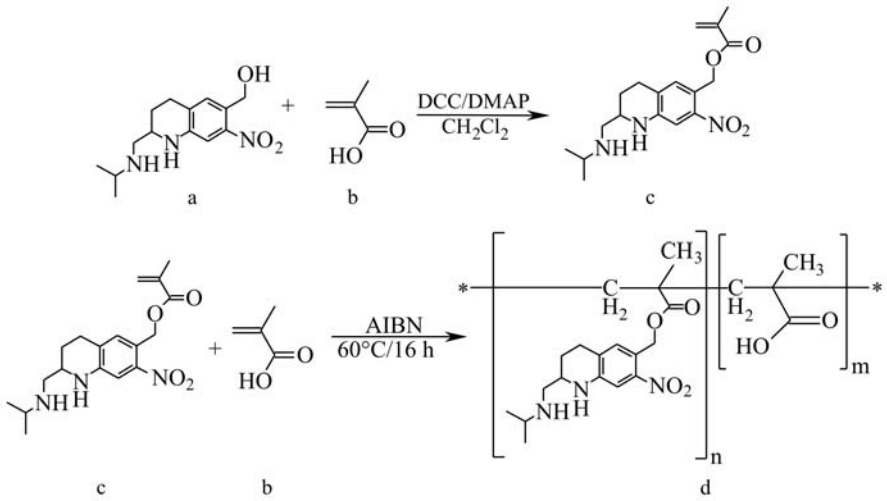

Fig. 2 Synthesis of poly(methacrylic acid-co-oxamniquine methacrylate) acid (PMOXA)

Intending to evaluate if the polymeric prodrug was obtained or not, a sample of the drug was blended mechanically with the polymer matrice (PMA), in the molar ratio of 1:2, physical mixture, OXA/PMA.

Methods

TG and DTA curves were recorded in simultaneous Seiko equipment, model TG/DTA 6300, in the temperature range of 25 to 1100ºC, heating rate of 10°C min^{-1},

dynamic nitrogen atmosphere (50 mL min^{-1}) and using platinum crucibles with ~4.0 mg of samples and 4 mg of α-Al_2O_3, which is the reference material. DTA was calibrated with indium (*m.p.*=156.6°C; ΔH_{fus}=28.54 J g^{-1}) and thermogravimetric system was calibrated using a $CaC_2O_4 \cdot H_2O$ standard substance in conformity to ASTM pattern [18]. The infrared absorption data were obtained in the range 4000–400 cm^{-1} in KBr pellets using a FTIR-Bomen spectrophotometer, model MB-120, at room temperature. The X-ray diffraction patterns were obtained on a Siemens, model D5000, with tube of CuK_α, in the interval of 3 to 65° (2θ) and 1 s of pass time, using the powder X-ray diffraction method.

Results and discussion

Figure 3 shows DTA(a1)/TG(a2) curves of PMA. TG curve shows four mass loss events at the following temperature ranges and mass loss percentages: 25–150°C (Δm=3.4%), 150–315°C (Δm=20.5%), 315–485°C (Δm=70.0%) and 485–630°C (Δm=6.0%). The first event is related to the superficial water releasing. The second and third events correspond to thermal decomposition process followed by carbonization. The last event is related to the carbon material elimination. DTA curve shows an endothermic peak between 200 and 245ºC, which corresponds to the first step of thermal degradation of PMA and can be related to the decarboxylation process. In the temperature range of 420 and 480ºC, TG curve shows only one mass loss event, however DTA curve evidences that the thermal process is very complex, since an addition of endothermic and exothermic events were observed. The last event, exothermic, is due to the burning of carbon material formed in the previous event.

According to Ho *et al*., 1992 and Polacco *et al*., 2000, the first event, corresponding to the water elimination due to the opening of anhydride group formed between adjacent carboxylic groups of the monomers [19, 20]. This event is also related to the decarboxylation of COOH, followed by CO_2 elimination. The second event of thermal degradation occurs due to the rupture of the polymeric chain monomers and the forma-

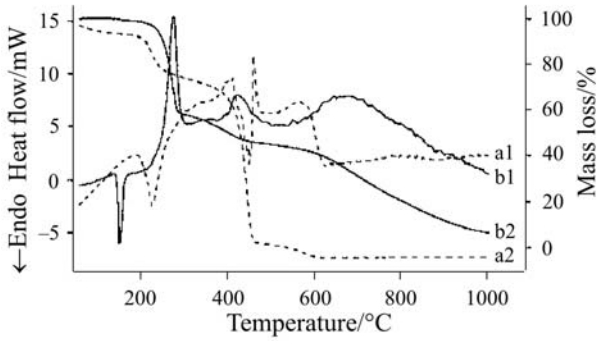

Fig. 3 DTA(a1)/TG(a2) curves of polymethacrylic acid (PMA) and DTA(b1)/TG(b2) curves of oxamniquine (OXA) obtained in heating rate of 10°C min^{-1} under dynamic nitrogen atmosphere (50 mL min^{-1})

tion of a small percentage of double bonds, followed by carbon material releasing. Later, a slow decomposition process was observed due to the burning of this carbon material.

The DTA(b1)/TG(b2) curves of OXA (Fig. 3) show thermal stability in the temperature range between 25 and 135ºC. At 148°C, an endothermic peak can be seen in the DTA curve, corresponding to the melting of OXA. This event is followed by thermal decomposition of the drug, which is characterized by an exothermic peak at 276ºC and mass loss approximately of 40% observed in the TG curve (Fig. 3). Sequential events, also exothermic, are observed in other stages of the thermal decomposition. According to TG curve, three events of thermal decomposition of the drug can be evidenced. These events occur in the following temperature ranges and mass loss percentages: 150–295°C (Δm=42%), 295–442°C (Δm=12%) and 442–1050°C (Δm=41%).

DTA(m1)/TG(m2) curves of the PMA/OXA physical mixture (Fig. 4) show characteristic events of the individual substances, mainly for PMA, because it has the major proportion in the mixture (2:1 M/M). The comparison of the thermoanalytical profiles of the mixture and individual compounds did not evidence interactions. The DTA curve shows an endothermic event in the temperature at 147ºC, characteristic of the melting of OXA. A second endothermic event at 221°C is observed and characterizes the start of PMA and OXA thermal decomposition. Other two exothermic events are observed in the temperatures at 400 and 480ºC that are related to release of water from anhydride links and decarboxylation of PMA, and thermal decomposition of PMA and OXA, respectively. These results showed that in the physical mixture the formation of a new species does not occur.

Investigations in DTA(c1)/TG(c2) curves of the PMOXA (Fig. 4) revealed four stages of thermal decomposition. The first event represents the water molecules releasing. Observing the mass loss events shown in the TG curve, no great distinctions can be seen between this thermoanalytical profile and that showed in Fig. 3 (m2), for the physical mixture. In this case, it is important to show that the kinetic of thermal decomposition of the PMOXA was slower and stages were not distinguished. Otherwise, the TG curve of the physical mixture shows well-defined events and a thermal decomposition similar to PMA and OXA, individually. According to DTA curve

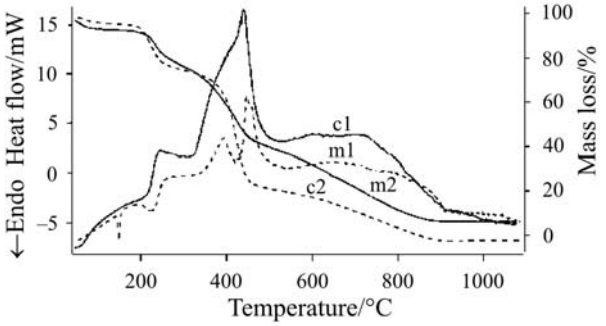

Fig. 4 DTA(m1)/TG(m2) curves of physical mixture (OXA/PMA) and DTA(c1)/TG(c2) curves of poly(methacrylic acid-co-oxamniquine methacrylate) acid (PMOXA) obtained in heating rate of 10°C min^{-1} under dynamic nitrogen atmosphere (50 mL min^{-1})

(Fig. 4), significant differences can be seen between physical mixture (m1) and PMOXA (c1). While in the physical mixture an endothermic peak at 148ºC is observed, indicative of the melting of OXA, this event is not observed in the DTA curve of PMOXA. This suggests the formation of a new species (polymeric prodrug).

The copolymer formation was also confirmed by infrared spectroscopy (FTIR). Figure 5 shows all information. Analyzing the OXA spectrum (Fig. 5a), characteristic bands are observed in 3324 cm^{-1} (N–H), 3093 cm^{-1} (C–H aromatic), 1508 and 1329 cm^{-1} (NO_2 asymmetric and symmetric) and 1050 cm^{-1} (C–O primary alcohol). Figure 5b shows the absorption bands of PMA: 3500 to 3220 cm^{-1} (OH), 1706 cm^{-1} (C=O stretch), 1391 and 948 cm^{-1} (C=O, stretch in and out of the plan). Figure 5c shows the bands of the physical mixture where it is possible to observe characteristics from free PMA and OXA. Peaks in 3500 and 3324 cm^{-1} are concerned to the secondary amine and the hydroxyl group. In 1706 cm^{-1} the carbonyl band is observed. In 1525 and 1343 cm^{-1}, asymmetric and symmetric bands of the NO_2 group, appear, respectively. In 1050 cm^{-1}, the C–O primary alcohol stretch is observed. In the spectrum of PMOXA (Fig. 5d), characteristic bands of the compounds PMA and OXA are observed indicating that the polymerization did not affect the stability of the drug. In Fig. 5d, some bands can be shown, like OH stretching in 3500 cm^{-1}, C=C aromatic stretch in 1625 cm^{-1} and NO_2 asymmetric and symmetric in 1525 and 1343 cm^{-1}. On the other hand, the absorption band in 1050 cm^{-1}, concerned to the OXA primary hydroxyl group, was not observed indicating that a new chemical entity was formed by an ester linkage. The utilization of different techniques, such as thermal analysis and spectroscopy, enabled to characterize and identify the studied compounds.

Table 1 IR wavenumbers/(cm^{-1}) of OXA, PMA and PMOXA and its assignments

OXA	PMA	PMOXA
3324 – (N–H) secondary amine	3500–3200 – (OH) carboxylic acid (due to hydrogen bond	3500, 3000 – (OH) carboxylic acid
2880 – (C–H) aliphatic	1706 – (C=O) carboxylic acid	3000, 2900 – (C–H) aliphatic
1620, 1580 – (C=C) aromatic	1391 – (C=O) acid carbonyl, stretch in the plan	1712 – (C=O) carboxylic acid
1508 – combination (NO_2 asymmetric and insaturation C=C)	1174 – (C–O) carbonyl	1625 – (C=O) aromatic
1329 – (NO_2 symmetric)	948 – (C=O) acid carbonyl, stretch out of the plan	1525, 1343 – (NO_2) asymmetric and symmetric
1385, 1362 – (CH_3 geminals)		1160 – (C–O) acid
1050 – (C–O) primary alcohol		

X-ray powder diffraction (XRD) (Fig. 6) helped the characterization of OXA, PMA, physical mixture and PMOXA. As can be observed in Fig. 6a, the OXA has well-defined reflections confirming its crystallinity. In the case of the PMA low

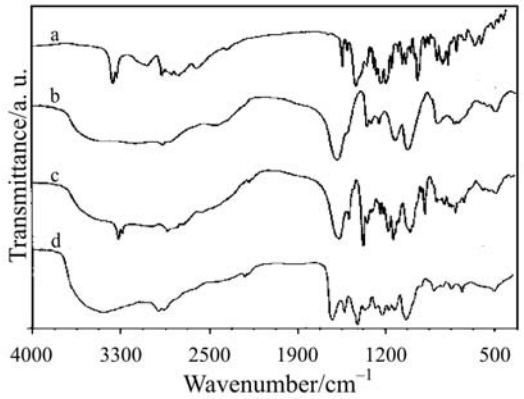

Fig. 5 IR spectra of a – OXA, b – PMA, c – physical mixture and d – PMOXA

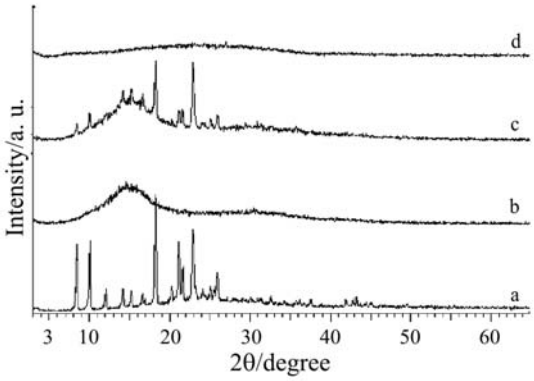

Fig. 6 X-ray powder diffraction of a – OXA, b – PMA, c – physical mixture and d – PMOXA

crystallinity is observed. The XRD of the physical mixture (Fig. 6c) shows the characteristic peaks arising from free OXA and PMA. However, the XRD of the PMOXA (Fig. 6d) no Bragg reflections are observed for the PMOXA indicating that this new chemical entity is not crystalline.

Conclusions

This work reveals that thermal analysis has great importance in the evaluation of the thermal behavior of polymeric and pharmaceuticals systems. This method was important because it indicated the formation of a new chemical entity. TG and DTA enabled to characterize the polymeric prodrug, mainly, when assisted by the infrared spectroscopy and X-ray powder diffraction. New experiments are running aiming at evaluating the OXA thermal degradation process due to the lack of a wide thermoanalytical study on this drug in literature. Preliminary in vivo tests were carried out to evaluate the hydrolysis and activity of the system. The drug was released from polymeric system indicating that the system behaved as a prodrug.

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