Research paper

Evaluation of pH-sensitive polyurethane/2-diethylaminoethyl methacrylate hybrids potentially useful for drug delivery developments

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ABSTRACT

Smart sensitive polymers have been used to improve processes in drug delivery. In this article, we evaluate the behavior of polyurethane/N,N-diethylaminoethyl methacrylate hybrids (PU/DEA) as pH-responsive polymers potentially useful for drug delivery systems development, using Rhodamine 6G (Rh6G) as a model drug. A detailed pH responsive characterization was performed by swelling studies and scanning electron microscopy (SEM). Two drug loading methods on drug release-immersion and direct loading were evaluated. The interaction between Rh6G and the polymer matrix was studied by Fourier Transform Infrared (FTIR) spectroscopy and contact angle determination. The kinetic study of Rh6G release was performed at basic and acidic pH; the mechanism of drug delivery was analyzed using Ritger-Peppas' equation. We discuss about polymer's active sites and drug's distribution through the matrix in relation to both loading methods. Results showed a pH-responsive behavior and morphological changes when pH solution varied from 9.0 to 4.0. In the immersion loading method, results indicated a higher Rh6G molecule concentration at the surface as well as ionic interaction between the drug and polymer's carboxylic groups. Release studies confirmed the pH-sensitive hybrid systems' behavior and kinetic exponent values indicated different mechanism's transport types depending on loading method and polymer composition.

1. Introduction

Stimuli-responsive polymers, also called 'intelligent' or 'smart' polymers, have the property to swell, shrink, bend, or degradation in response to changes in the environmental conditions such as pH, ionic strength, temperature or in the presence of specific chemical compounds [1,2]. Due to its properties, in recent years they have gained considerable attention and they have been proposed for a number of applications like drug delivery, biomedical therapies, industrial coatings separation techniques and sensors, oil exploration, biological and membrane science, colloid stabilization, and water remediation [3–8].

Particularly, in drug delivery application, stimuli-sensitive polymers allow not only a spatial control, but also a temporal one, i.e., during the period of time when the stimuli are present. This is why responsive polymers can improve the tissue bioavailability and reduce the appearance of side effects [9]. For that reason responsive polymers have been proposed, for example, as smart polymeric nano-devices for anti-cancer therapies and they have found wide application in controlled release of drugs, DNA or gene delivery, protein separation, coating thickeners and applications where triggering by external stimuli (like pH) is necessary [10].

Stimuli-responsive polymers are normally prepared by adding monomers with functional groups in the polymer backbone. The incorporation of these monomers in the polymer chain can improve the performance of these materials by increasing responsiveness in a particular medium or environment [11]. In particular, pH-sensitive polymers are normally prepared by including pendant acidic or basic functional groups during the polymerization.

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Polyurethanes are versatile polymers; its properties can be tailored by choosing the appropriated polyisocyanate, polyol and eventually functional monomers. Polyurethanes have also many uses in biomedical applications [12] especially in drug delivery systems [13,14]. A method to obtain a combined system between a functional monomer and PU is by blending polymer dispersions in a specific proportion [15]. This method is generally used to create blends with the desired properties. However, in most cases, incompatibility between polymers causes their phase separation. Another way to combine polymers is by chemical interactions. The creation of covalent bonds between both phases [16–18] can overcome the incompatibility between polyurethane and acrylic polymers leading to hybrid systems [19,20].

In this manner, pH-sensitive polymers can be obtained by combining polyurethane with an acidic or basic functional monomer, like N-(3-aminopropyl)methacrylamide, 2-(dimethylamino) ethyl methacrylate, methacrylic acid, 2-amino-ethyl methacrylate and 2-hydroxyethyl methacrylate [21–23]. We have recently reported the synthesis and characterization of news pH-sensitive hybrid polymers of polyurethane (PU) and (2-(diethyl amino) ethylmethacrylate) (DEA) [24] with adequate film and mechanical properties depending on the percentage of each polymer, and with potential applications as delivery systems.

In this contribution, we propose to evaluate the properties of these pH-sensitive hybrid polymers of PU and DEA with 10 wt. % and 30 wt. % of DEA content as potential materials for pH control drug delivery systems.

The performance of drug delivery systems depends on different factors, including loaded method process, strength of interactions between the drug and polymer, and the drug's molecular weight, among others. Therefore the drug's liberation from films can vary from fast release to slow release. For example, the way in which the incorporation of the active pharmaceutical ingredient (API) is carried out plays an important role in a drug's delivery performance [25,26]. The incorporation of any API in the polymer matrix may be performed in different ways, such as by dipping the polymer in a concentrated solution of the API (immersion load) or by adding the API in polymer dispersion before film formation (dispersion load). Wang et al. [25] have found that the drug loading methods have a significant effect on drug's load and encapsulation efficiency. More recently Sriamornsak [26] performed a similar study in gel beads of calcium pectinate and found that the mixing method provided a faster drug release and lower T_{50} than the absorption method and swelling method, respectively. Other authors have also reported progress on this subject for better understanding of these systems [27–30]. However, despite the number of recent studies incorporating different active pharmaceutical ingredients into responsive polymers, the interaction between the matrix and the drug has received limited attention in the literature and it is often overlooked.

The purpose of this work is to reveal the performance in vitro of the newly synthesized pH-sensitive hybrid polymers of PU and DEA as drug release system using Rhodamine 6G Chloride (Rh6G, MW = 479.01) as an API model [31]. Due to the possibility of preparing loaded-films either by immersion or dispersion method, we performed such comparison to evaluate not only the influence of each method in kinetic release behaviors but the interaction between the API and the polymer matrix as well. Pure PU was also included as reference material. No suitable films of pure 2-(diethylamino)ethyl methacrylate (pDEA) were obtained; therefore no release studies were performed using this material. Loaded-films' characterization was performed by FTIR spectroscopy and contact angle [32]. Pure systems were analyzed and compared to loaded-films in order to determine the influence and interaction between the matrix and Rh6G molecules [33].

In addition, we studied the swelling behavior and morphological changes using Scanning Electron Microscopy (SEM) of hybrid systems at different pH values, in order to characterize physical or chemical modifications can undergo in response to changes in environmental conditions, and to understand and predict the drug's release rate of them.

2. Experimental section

2.1. Materials

2-(diethylamino)ethyl methacrylate (DEA) was purchased from Scientific Polymers Products, isophorone diisocyanate (IPDI, Aldrich), 2-hydroxy ethylmethacrylate (HEMA, Aldrich), poly(-propylene glycol) diacrylate (PPGDA, Aldrich), ammonium persulfate (APS, Fisher Scientific), hydrazine monohydrate (HZM, Aldrich), dibutyltin dilaurate (DBTDL, Aldrich) and sodium dodecyl sulfate (SDS, Anedra) were of analytical grade and were used as received. Monomethoxy-capped poly(ethylene glycol) methacrylate (PEGMA) macromonomer (M_n = 2000; M_w/M_n = 1.10) was supplied by Cognis Performance Chemicals (Hythe, U.K.) as a 50 wt. % aqueous solution. Polypropylene glycol 1000 (PPG1000, Voranol 2110) was of technical grade and triethylamine (TEA) was provided by ADELFA S.A. The DEA monomer was treated with basic alumina to remove the inhibitor. PPG1000 was dried and degassed at 80 °C at 1–2 mm Hg before used. Dimethylol propionic acid (DMPA, Aldrich) was dried at 100 °C for 2 h in an oven. TEA was also dried before use. The buffer solutions for fixing the medium pHs were prepared from standard chemicals.

Rhodamine 6G Chloride (Rh6G, Sigma–Aldrich, MW = 479.01) was used as a model drug [34] and it was used without additional purification.

2.2. Methods

2.2.1. Polymers synthesis

In a 1000 mL six-neck separable flask PPG1000 and DMPA were charged and the mixture heated to 90 °C and bubbled dried air for approximately 60 min, followed by increasing the temperature to 98 °C and adding a mixture of IPDI and DBTDL catalyst. After 2 h of reaction the prepolymer was cooled to 45 °C and HEMA dissolved in acetone was added slowly and allowed to react for approximately 90 min. Then, the temperature was raised to 60 °C and kept constant until the isocyanate (NCO) content reached the desired value (approximately for 90 min).

Upon obtaining the theoretical NCO value (ca. 4.7%) the mixture was cooled to 55 °C and TEA (in acetone) was fed in slowly over 50 min. After neutralization the temperature was lowered to room temperature. An aqueous dispersion of PU was obtained by adding the PU prepolymer to water containing the appropriate amount of HZM to perform the chain extension reaction. The dispersion was performed at about 300 rpm in an ordinary glass reactor at 30 °C over a period of 45 min. The resulting product was a stable dispersion with solid content of about 30 wt. % and it was divided into several parts and added different amounts of DEA, PPGDA as crosslinking agent and PEGMA as steric stabilizer. The polymerization of PU-DEA/PPGDA/PEGMA mixtures was performed in batch mode using a glass reactor (1000 ml) with a water jacket for temperature control. The mixture was degassed with nitrogen gas and then dispersion polymerized at 80 °C using APS (0.015 wt. % on DEA monomer base) as initiator. The polymerization leads to the formation of PU/DEA hybrids having a chemical bond between polyurethane and DEA moieties. The resulting product was a stable dispersion with a solid content of approximately 30 wt. %. A shorthand notation is used in this paper to describe the hybrid

systems. Thus, "H90/10" denotes a hybrid system containing 90 wt. % of PU and 10 wt. % of DEA monomer. Fig. 1 shows the chemical structure of PU/DEA hybrid systems.

2.2.2. Film formation

Films were prepared by casting the aqueous dispersions on Petri dish. After drying at 30 °C during 24 h, the films were cut into circular pieces (about 22 mm diameter) with a cork borer, and cured at 60 °C for 48 h; and finally stored in desiccators with silica gel until they were ready to be used for the experiments.

2.2.3. Swelling degree

The equilibrium swelling degree of the films were determined by immersing the samples (22.0 mm diameter disk and thickness of 292 ± 16 μm) in a phosphate buffer solution (PBS, ca. 0.1 M) for pH 7 and 9 and at a temperature of 37 °C until reaching the swelling equilibrium. Buffer solution sodium dihydrogen phosphate/phosphoric acid (ca. 0.1 M) was used for pH 4.

The equilibrium swelling degree (Q_∞) was calculated using the following equation:

$$Q_\infty = \frac{(W_{s,\infty} - W_d) \times 100}{W_d} \quad (1)$$

where $W_{s,\infty}$ is the weight of swollen film at equilibrium and W_d is the weight of dry film. The experiments were performed in triplicate.

2.2.4. Scanning electron microscopy (SEM)

The changes in the morphology of hydrogels were observed by Scanning Electron Microscopy (SEM) with an FEI – Quanta 200 (The Netherlands) instrument, in high vacuum mode and operated at 15–20 kV acceleration voltage. PU and PU/DEA films were equilibrated during 24 h in different buffer solutions and then were frozen at −40 °C in an alcoholic solution followed by lyophilization under vacuum for 24 h. In order to prevent sample-charging effects during the observation, fractured pieces of samples were mounted onto the surface of an aluminum SEM specimen holder and sputter-coated with a thin overlayer of gold before observation.

2.2.5. Preparation of drug loaded films

Fig. 2 shows the schematic preparation of drug loaded film by the two methods.

2.2.5.1. Immersion load method (IL). In this method the drug was loaded into films by immersing them in an aqueous solution of Rh6G (50 mg/L), until the maximum load of Rh6G was reached. For practical purposes the loading time was unified at 72 h for all samples. Drug uptake values were followed by measuring the absorbance (concentration) of the solution by UV–Visible spectroscopy at 526 nm using a Nicolet Genesys 10 spectrophotometer, USA. The efficiency of incorporation depends on the pH and composition of the film. The amount of Rh6G loaded at three different pHs (8.0, 7.0 and 4.0) was tested and results indicated that the loading of Rh6G was more efficient at pH 8.0 for all the systems. At these conditions, the efficiency is 90%; 84% and 75% for pure PU and for hybrids H90/10 and H70/30 respectively. The soaked films were gently washed with distilled water to eliminate any superficial drops of Rh6G solution. The films were dried at 30 °C for 24 h before performing the release and characterization experiments.

2.2.5.2. Mixing (Dispersion) load method (DL). In this method an appropriate amount of Rh6G (similar to the drug incorporated into the film by the immersion method) dissolved in buffer pH 8.0 was added to the hybrid dispersion before forming the film. The resultant loaded dispersion was left for casting in the same manner as mentioned above (see film formation section). The efficiency of incorporation of Rh6G in the dispersion load method is 100% because the dye is incorporated directly in the dispersion before film formation.

2.2.6. FTIR spectroscopy

FTIR spectra were run using a MIRacle™ Single Reflection, attenuated total reflection (ATR) accessory from Pike Technologies, USA, in a FTIR Nicolet 380 spectrometer, Thermo Scientific, USA. FTIR spectra were obtained by recording 64 scans between 4000 and 500 cm^{-1}, with a resolution of 4 cm^{-1}; and they were processed and corrected using the Advanced ATR Correction facility of the EZ Omnic software.

2.2.7. Contact angle test

Contact angle tests were performed using the sessile drop method which involves the shedding of a droplet of distilled water (8 μL) on the polymeric films. Measurements were carried out with Ramé-Hart goniometer (model 500, USA) using an Automated Dispensing System (Ramé-Hart Instrument co., USA). All the tests were performed every 1 s during 30 s at 25 °C on five different

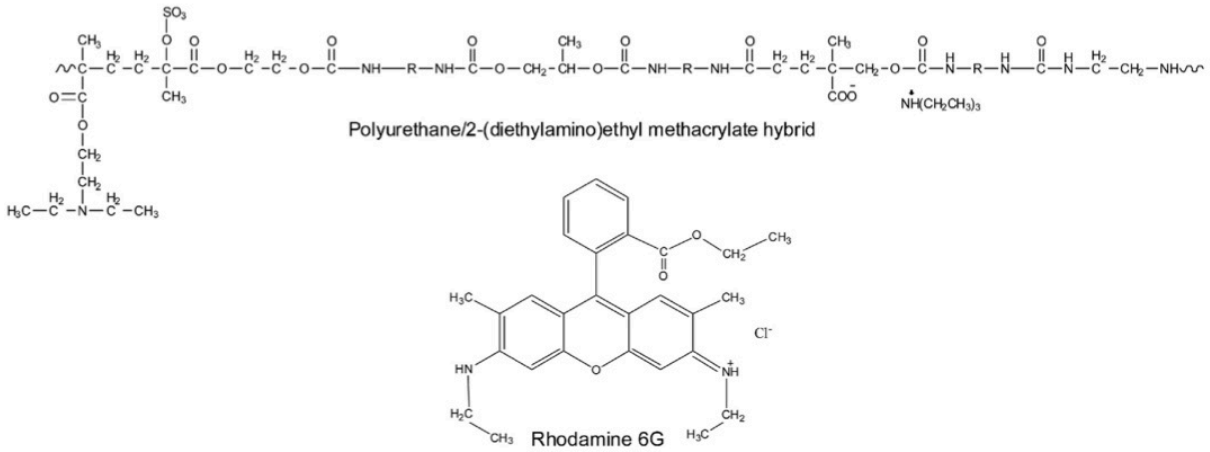

Fig. 1. Chemical structure of PU/DEA hybrid and Rh6G.

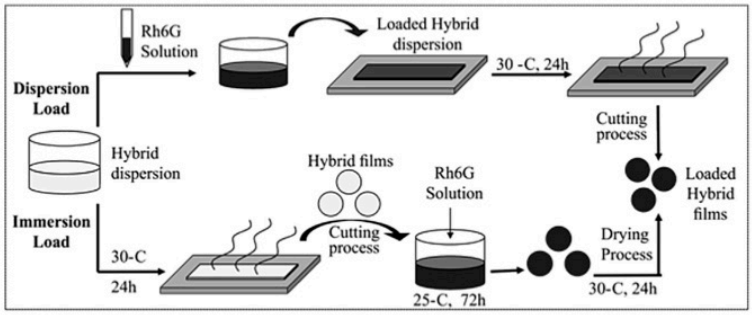

Fig. 2. Schematic drawing of drug loading methods.

points per sample to calculate the mean static contact angle, using the Drop Image software. The experiments were performed by triplicate.

2.2.8. Drug release studies

Drug release experiments of Rh6G loaded films were conducted in two different pH mediums (pH 9.0 and 4.0). Drug release experiments were performed by immersing polymer films into 80 ml of buffer solution (0.1 M) at 37.0 °C with magnetic stirring (400 rpm). First, films were placed in buffer solution at pH 9.0 until they reach a constant release drug value. Then, they were removed and after being gently washed, films were immersed in pH 4.0 buffer solution to continue drug release experiments. The dynamic drug concentration was monitored by measuring the absorbance at 526 nm [35] at several interval times. The experiments were performed in triplicate. The Rh6G concentration released as a function of time (t) was adjusted to a power-law type relationship [36,37] using the equation of Ritger-Peppas:

$$\frac{M_t}{M_e} = kt^n \tag{2}$$

Here M_t and M_e are the cumulative amount of drug released after a time t and at infinite time, respectively, while k is a constant related to kinetic behavior and experimental conditions and n is the exponent depending on the release process. Data were fitted only up to 60% of drug release in order to apply Eq. (2).

Parameters k and n were calculated from the intercept and the slope of the following equation:

$$ln(M_t/M_e) = ln\, k + n\, ln\, t \tag{3}$$

3. Results and discussion

3.1. Swelling degree

In pH-sensitive systems the release rate of the drug is regulated by several factors as swelling degree, drug–matrix interaction, water content and the initial PA concentration [38]. However the swelling behavior as a function of pH has a principal role in drug release regulation, which makes this technique to be an important tool to predict the drug's rate release.

The swelling degree (SD) of pure PU, H90/10 and H70/30 hybrids polymers is shown in Fig. 3.

At acidic pH for hybrids systems, an increase in the DEA proportion caused an increment in SD. This effect was attributed to the protonation of amino groups of the DEA moiety and to an increase of the electrostatic repulsive force between ionized groups [24]. The increase in the network space, in turn, allowed water to get into the matrix [39,40]. A similar behavior is also observed in other polymer systems with cationic groups [41,42]. For the PU system, the SD at pH 4 was lower than in hybrids systems because the carboxylic groups of the matrix are protonated.

At neutral pH the SD values for hybrids systems decrease due to the decreasing of the amount of protonated amino groups. However the SD for H90/10 hybrid follows a different behavior. The equilibrium swelling degree at pH 4.0 and 7.0 for pure PU is similar (~15 wt. %) and increases when increasing the pH to 9.0 due to the ionization of carboxylic groups −C(O)OH form the DMPA moiety (pK_a 4.41, Table 1. http://www.6chem.com/07.asp?id=1113, accessed June 3, 2014). When incorporating DEA monomer to the polymer chain a new ionizable group is introduced. The pK_a for the swollen-to-non swollen transition in linear PDEA homopolymer is around 7.0–7.3 [43] and in the hybrid polymers the critical pH for this transition is close to that value and around pH 6.5 [24]. When the pH of the solution is around 7.0 about half of $-NR_2$ groups are therefore in non-ionized state. In the H90/10 hybrid the $-NR_2$/−C(O)OH molar ratio (where R = $-CH_2CH_3$) is close to 2 and the swelling is mainly due to the partially ionized −C(O)OH groups with a low contribution from the DEA moiety. When increasing DEA content the $-NR_2$/−C(O)OH ratio is about 7 and the total ionic groups increases and also increases the SD. At pH 9 the $-NR_2$ groups do not contribute to the total ionic content but the −C(O)OH groups are totally ionized. In this way the SD decreases as the DEA content increases or the DMPA decreases.

Finally at alkaline pH all the DEA monomer is in neutral state but the carboxylic groups of the DMPA moiety of the PU chain are non-

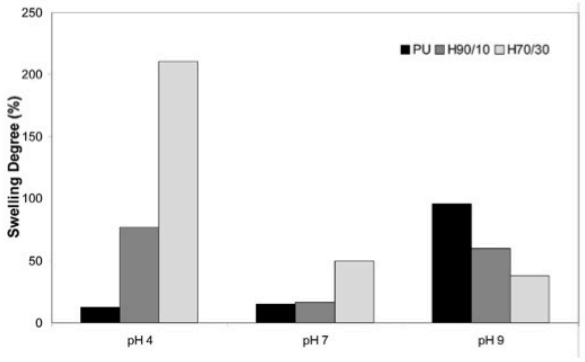

Fig. 3. Swelling degree (%) of pure polyurethane and hybrid systems films at different pH values.

Table 1
Swelling degree and percentage of Rh6G released at pH 9 and 4, and results of kinetic analysis of Rh6G release according to Eq. (2) at pH 4.

System	Swelling degree		% Rh6G released				Kinetic analysis (at pH 4.0)					
			DL		IL		*n* coefficient		Transport mechanism		Type of release	
	pH 9.0	pH 4.0	pH 9.0	pH 4.0	pH 9.0	pH 4.0	DL	IL	DL	IL	DL	IL
H90/10	59.94	76.92	5.46	27.08	1.47	54.95	0.45	1.06	Fickian	Case II	Time dependent $f(t^{-1/2})$	Time independent
H70/30	37.87	210.5	4.71	33.78	1.86	97.72	0.58	0.82	Anomalous	Anomalous	Time dependent $f(t^{n-1})$	Time dependent $f(t^{n-1})$

DL: Mixing (Dispersion) Load Method.
IL: Immersion Load Method.

protonated. This causes an increment of SD value for pure PU, about four times than for pH 4, and in a lesser extension for the H90/10 system.

3.2. SEM characterization

SEM is one of the best methods for characterizing the hydrogel structure, especially in drug delivery systems because it offers information of surface porosity, amorphous and crystalline regions, particle size and phase separation [44]. Morphologic changes of lyophilized pH-responsive films, after exposure them to aqueous solutions of different pH values (4.0 and 9.0), have been examined by SEM technique and the images are shown in Fig. 4.

The surface of the PU at pH 9.0 shows an open morphology state as a consequence of ionization of carboxylic groups (from DMPA) which produces the matrix expansion. At pH 4.0 a collapsed state is observed with almost a featureless structure. In hybrid systems (H90/10 and H70/30) at pH 9.0 a non-porous and compact surface is appreciated due to lower swelling degree and a more hydrophobic polymer characteristic. For H90/10 system at pH 4.0, the surface shows a morphologic change and an increment in the rugosity can be observed. However, despite of presence of DEA component, no changes in the polymer structure is observed, probably due to the low percentage of this component in the systems (10 wt. %). On the contrary, at pH 4.0 for H70/30 system, the surface shows an open morphology state with a porous structure, thin walls and a predominant free space as a consequence of the matrix expansion. When DEA content is higher, at pH 4.0, the equilibrium swelling degree increases and it results in an open morphology state as a consequence of amine group's ionization. The incorporation of DEA confers pH-responsive properties to the polymer, as noted in swelling studies; therefore varying pH medium, not only film's volume changes but also its morphology.

3.3. FTIR spectroscopy

The ATR-FTIR spectrum of Rh6G is shown in Fig. 5. The main band with high wavenumbers at 3229 cm^{-1} is assigned to the N–H stretching and the weak bands observed at 3000 cm^{-1} region are assigned to the C–H stretching. In the low wavenumbers region below 2000 cm^{-1} the main bands are observed at 1717 cm^{-1} (C=O stretching vibration); and at 1647, 1606 cm^{-1} (vibrational modes of xhantene ring). The bands at 1564, 1528, 1501, 1443 and 1367 cm^{-1} are vibrational modes of xhantene ring coupled with ethylamine, methyl and phenyl groups [45,46].

By comparing the spectra of the hybrid 70/30 IL and 70/30 DL with the hybrid 70/30 (pristine) in Fig. 6, it can be appreciated that the intensity of corresponding bands of Rh6G at 1605 cm^{-1}, assigned to the stretching vibration of C=O; and those at 1528 and 1498 cm^{-1}, assigned to the xanthene ring movements (Fig. 6) are different. The intensity of these bands is greater in the immersion loaded films, indicating a higher concentration of Rh6G at the surface, compared to the dispersion loaded films. This results in a faster and effective release of Rh6G for IL systems as it is shown in Figs. 9 and 10. The C=O band at 1714 cm^{-1} of the H70/30 shifts to lower wavenumbers (1705 cm^{-1}) in the IL film indicating

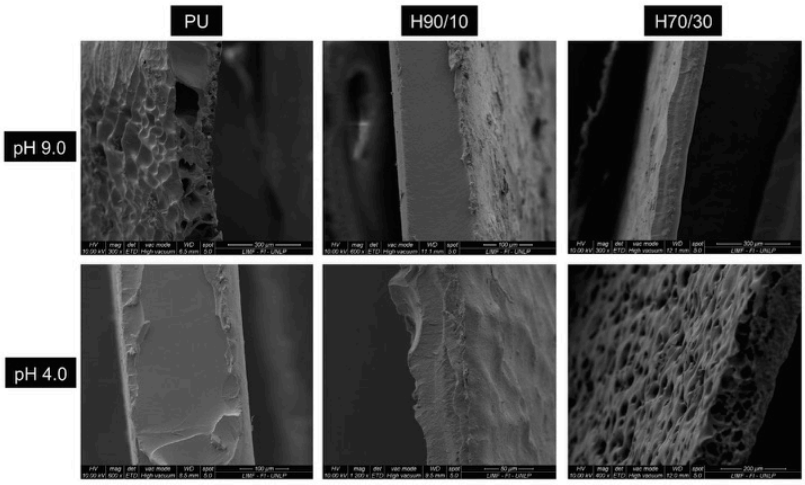

Fig. 4. SEM images of PU and hybrid systems at two pH values (9.0 and 4.0).

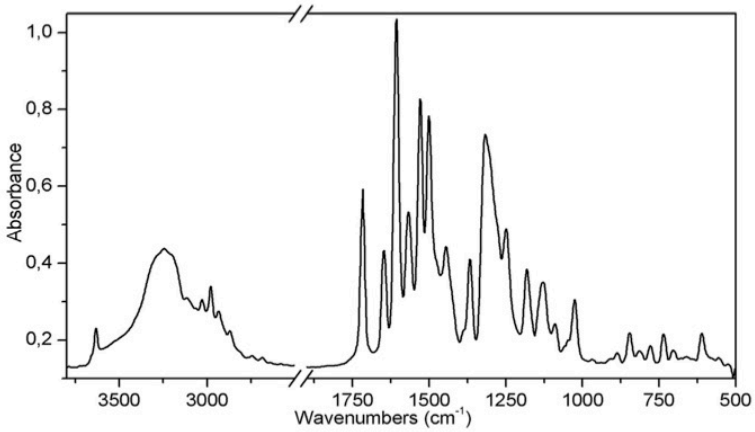

Fig. 5. ATR-FTIR spectrum of Rh6G.

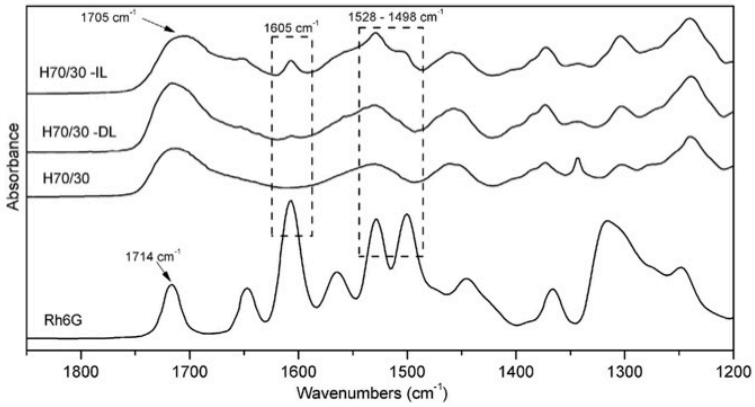

Fig. 6. ATR-FTIR spectra in the range 1900–1200 cm^{-1} of Rh6G and pristine H70/30 and loaded H70/30 by immersion (IL) and dispersion (DL) films.

interaction between the Rh6G and the polymer matrix through the carboxylic groups of PU [47]. However in the DL systems the C=O band of the PU/PDEA is located at the same wavenumbers that the pure matrix, indicating a different environment of these systems.

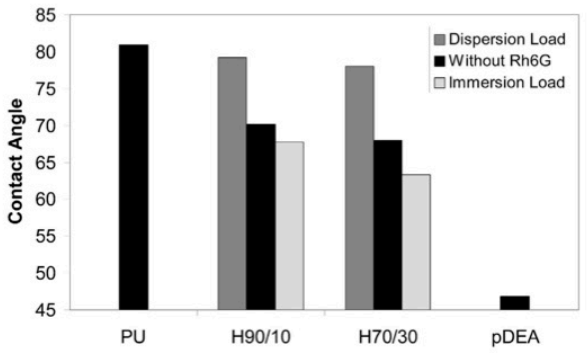

Fig. 7. Water contact angle of PU, pDEA and hybrid systems.

3.4. Contact angle

Contact angle measurement is a useful tool for evaluating the drug distribution on the surface and also give some insights on the

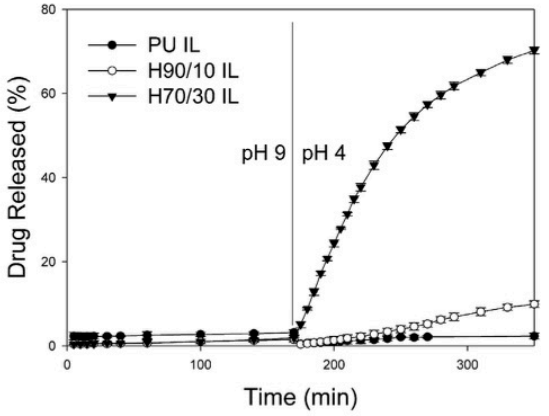

Fig. 8. Kinetics of Rh6G released from the polyurethane (PU) and the hybrids.

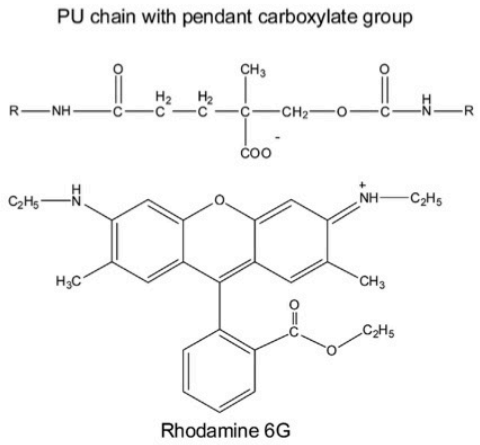

Fig. 9. Ionic interaction between cationic Rh6G molecule and the carboxylate group from DMPA of the hybrid PU/DEA system.

biocompatibility of the polymeric system [48]. In our case as Rh6G is a hydrophilic molecule, by increasing the drug surface concentration an increasing of the contact angle is expected.

The water contact angle (CA) of PU and hybrids systems loaded by both methods is shown in Fig. 7. The CAs measurements for pDEA loaded-films were not performed because they were mechanically difficult to handle for drug loading.

The pure PU film is more hydrophobic than the pure PDEA as expected by the presence of the hydrophilic PEGMA component in this case. The CA of the hybrid systems is between those of pure components. By increasing the DEA component the CA decreases because of the increasing amount of PEGMA in the formulation.

A clear difference in the CAs is observed for DL compared to IL samples. It is noteworthy that, the CA of DL samples is higher than that of pure hybrids, indicating a more hydrophobic (or less hydrophilic) surface and suggesting a different drug distribution at the surface of the polymer matrix. Contact angle measurements were used by other authors to study the migration, orientation of molecules or functional groups at the surface [49,50]. A possible explanation is as follows. In the DL case the ionic Rh6G molecules, trapped in non-specific sites of the polymeric matrix, renders it more hydrophilic reducing the migration to the surface of hydrophilic groups during film formation and modifying the distribution of functional groups. On the other hand in the IL process (at pH 8.0) the Rh6G molecules are incorporated through the natural channels formed during the film formation process in the matrix without changing the functional groups distribution at the surface. Additionally the higher amounts of the ionic Rh6G molecules at the surface (as observed by FTIR) made it more hydrophilic, decreasing in that way the water contact angle.

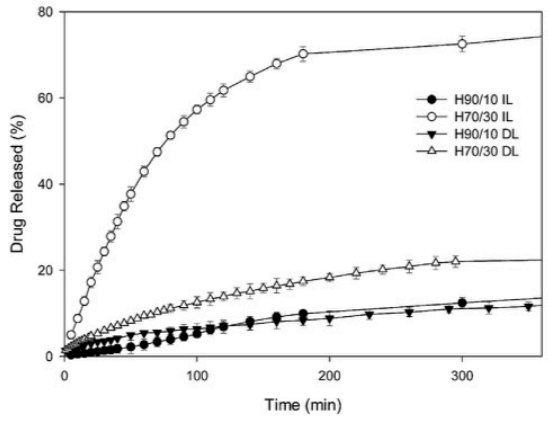

Fig. 10. Drug released for the first 6 h from H90/10 and H70/30 loaded films by immersion and dispersion methods at pH 4.0.

3.5. Drug release studies

In this section the results of the *in-vitro* studies of pH-sensitive hybrid polymers PU/DEA are discussed in extreme pH values (9 and 4) in order to characterize their drug release behavior and to show the sensitiveness of polymers at those pHs.

The amount of drug release with time at pH 9.0 and 4.0 for the PU and hybrids films (loaded by the immersion method) is shown in Fig. 8.

At this pH the Rh6G molecules are tightly retained in the matrix as a consequence of the carboxylate group – protonated ethylamine group interaction as illustrated in Fig. 9.

The PU film releases slightly more Rh6G than the hybrids systems at pH 9.0. Both hybrids systems (H90/10 and H70/30) behave in a similar way at this pH. After 170 min in solution at pH 9.0, a plateau was reached for all samples and the films were soaked in a solution at pH 4.0 leading to an important change in the delivery behavior. The change in delivery behavior is more important for the H70/30 than for the H90/10. On the contrary the release from pure polyurethane film is very low. The observed differences are related to the amino and carboxylic group contents when increasing the DEA monomer. The highest release rate is exhibited by the highest DEA-containing copolymer sample. The incorporation of DEA in the PU matrix interferes with the above-mentioned ionic interaction between Rh6G and PU. By lowering the pH to 4.0 the carboxylate group becomes protonated as well as the amine groups of the DEA moiety. At low pH the protonation of carboxylate group reduces the ionic interactions allowing more Rh6G to be delivered to the aqueous phase. At the same time the cationic Rh6G molecules are repelled by the protonated ethylamine group interaction with the protonated diethyl group of the DEA moiety ($-N^{+}(CH_2CH_3)_2$). Two driving-forces are acting in this case, the solubility of Rh6G during the water swelling of the matrix and the ionic repulsive interaction between Rh6G and the matrix.

The change in the amount of Rh6G released illustrates the pH dependent behavior of the hybrid systems. Fig. 8 also shows that the response to change in pH is very fast. The fast response behavior could also be useful for development of pH sensor devices. For the DL systems a similar behavior is observed when changing the pH from 9.0 to 4.0.

The behavior of the release of Rh6G for IL and DL systems at pH 4.0 is shown in Fig. 10.

For films with the same proportion of DEA, the amount of Rh6G released (%) vs. time at pH 4, was higher in the hybrids loaded by immersion than in the dispersion method. These results agree with those discussed in the spectroscopic and contact angle analysis.

During the first 6 h the H70/30 IL loaded system released more than 70% of Rh6G and the H70/30 DL loaded about 20%, suggesting that in the first case the Rh6G molecules are expelled from the matrix as a consequence of the protonated ethylamine group interaction with the protonated diethyl group of the DEA moiety ($-N^{+}(CH_2CH_3)_2$).

The release behavior of ionic drugs found in this work has also been observed by Bettini et al. [1] when using metoclopramide monohydrochloride in pH-sensitive systems.

The relative amounts of Rh6G released (%) in H90/10 and H70/30

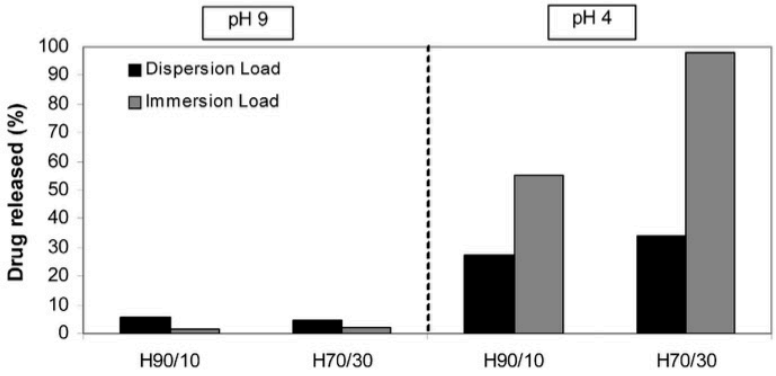

Fig. 11. Relative amounts of Rh6G released (%) in H90/10 and H70/30 loaded by both methods at different pH values.

loaded by both methods at different pH values and after reaching the equilibrium are shown in Fig. 11.

It is possible to see that the relative amounts of released Rh6G for both methods depend on the pH value. At pH 9.0 the release amount is low in both cases, but in DL systems the release of Rh6G is higher than in IL systems. In the DL systems the Rh6G molecules are located in unspecific sites but in the IL systems the molecules are bonded mainly to the carboxylic groups as suggested by the shift of the C=O band in the FTIR spectroscopy analysis. This specific interaction is responsible for the observed lower release of Rh6G. At pH 4.0 the opposite behavior is observed as it has been explained before in the text.

Table 1 shows the SD and the total Rh6G released at pH 9.0 and 4.0, and the results of the kinetic analysis of Rh6G release at pH 4.0 according to Eqs. (2) and (3).

At pH 9.0 the amount of Rh6G released follows the SD for DL systems. However for IL systems the reverse is true. The amount of Rh6G released for the 90/10 system is lower because of the higher amount of carboxylic groups as compared to 70/30 systems. Anyway the amount of Rh6G released is similar in both cases, and less than 2%.

At pH 4.0 the amount of released Rh6G for all systems follows the SD tendency, but not in the same way. Increasing of DEA monomer leads to an increase in SD of 274%. For DL systems the released Rh6G increased 125% and for IL systems 178%. This difference indicates again that the interactions of Rh6G molecules with the matrices are different. For the H70/30 IL the release is almost complete. In the DL case the Rh6G molecules after film formation are entrapped within the matrix and are not free for releasing. In the IL process (performed at pH 8.0) the Rh6G molecules are incorporated through the natural channels formed during the film formation process in the matrix having more freedom to release.

Comparing the H90/10 systems the amount of Rh6G delivered at pH 4.0 is twice as much for the IL system, but for the H70/30 is almost three times as much. As the SD is the same, this result indicates an additional contribution. The repulsion interaction of the cationic Rh6G molecules with the protonated diethyl group of the DEA moiety ($-N^{+}(CH_2CH_3)_2$) is responsible for those results.

The release curves at pH 4 were fitted with the classical power-law type relationship (see Table 1). When the ratio PU/DEA is 90/10, n values indicate a different type of transport: Fickian for dispersion loaded systems ($n = 0.45$) and type II for immersion loaded systems ($n = 1.06$). In this case the hybrid film is useful as a time-independent controlled release system [51].

With a PU/DEA ratio 70/30 both systems exhibit anomalous behavior (non-Fickian) with values of $n = 0.58$ (dispersion) and $n = 0.82$ (immersion). For IL systems the n values are close to 1, indicating that the polymer relaxation during drug release contributes to the process.

Similar results were obtained by Wang et al. [25] in the study of the effect of drug loading methods on the drug released mechanism. They have found that drug release is a zero-order kinetics process for the alginate/poly-L-arginine/chitosan ternary complex microcapsules made by mixing method and a first-order kinetics for the complex microcapsules made by absorption.

The films are slightly cross-linked and no erosion (weight loss) was detected during tests and inclusive after several months of immersion.

4. Conclusions

Hybrids of PU/DEA, having good film forming and physicochemical properties, were tested as drug delivery systems using Rh6G as model drug. Swelling studies indicate an increment in SD not only when pH varied from 9.0 to 4.0, but also when DEA is in higher proportion. SEM images at pH 4.0 show morphological changes and an open state structure as a consequence of matrix's expansion in H70/30 system.

ATR-FTIR and AC analysis indicate stronger drug–polymer interaction in DL systems and different distribution of Rh6G in the matrix, with a higher surface drug concentration for IL systems.

Futhermore release experiments confirm the pH-sensitive behavior of hybrids. The relative amounts of released Rh6G for IL systems are higher than those observed for DL ones, indicating a better performance (in terms of the releasing amount) of IL films.

Finally different ways of API's incorporation modify the subsequent release. When the ratio of PU/DEA was 90/10, n values at pH 4.0 indicate a different type of transport: Fickian for DL systems and type II for IL systems. With a 70/30 ratio both systems exhibit anomalous behavior (non-Fickian).

pH-sensitive hybrid films of PU/DEA are potentially drug delivery systems in which API's release depends not only on each loading method but also on polymer's composition.

Author contributions

All listed authors contributed to the research work.

Conflicts of interest

The authors declare no conflict of interest.

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