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Evaluation of mucoadhesive properties of α,β-poly(*N*-hydroxyethyl)-DL-aspartamide and α,β-poly(aspartylhydrazide) using ATR–FTIR spectroscopy

Filippo Saiano[a], Giovanna Pitarresi[b], Gennara Cavallaro[b], Mariano Licciardi[b], Gaetano Giammona[b,*]

[a]*Dipartimento di Ingegneria e Tecnologie Agro-Forestali, Università degli Studi di Palermo, Viale delle Scienze 13, 90128 Palermo, Italy*
[b]*Dipartimento di Chimica e Tecnologie Farmaceutiche, Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy*

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Abstract

The mucoadhesive properties of α,β poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) and α,β-polyaspartylhydrazide (PAHy) have been investigated using attenuated total reflection infrared (ATR–FTIR) spectroscopy. In particular, films based on these polymers have been contacted with a mucin solution at pH 7 and, the interfacial interaction and interpenetration between the glycoprotein and PHEA or PAHy have been studied by analysing the ATR–FTIR spectra. A diffusion model using a solution of Ficks' second law has been employed to determine the diffusion coefficient of water into polymeric films as a consequence of interdiffusion which occurs at the polymer film/mucin solution interface. © 2002 Published by Elsevier Science Ltd.

Keywords: α,β-Poly(*N*-hydroxyethyl)-DL-aspartamide; α,β-Polyasparthylhydrazide; Mucin

1. Introduction

The development of prolonged and/or controlled drug delivery release systems has often utilized the bioadhesive process [1]. In general, adhesion is defined as the state in which two bodies are in intimate contact for a prolonged time by interfacial forces. When one of the adherends or both are of a biological nature (such cellular secretions, mucus, extracellular matrix, cells or tissues), the phenomenon is defined as 'bioadhesion' and it almost always occurs in the presence of water [2,3].

The considerable interest for bioadhesive pharmaceutical systems is due to several advantages derived from their use, such as a prolonged residence time at the site of drug adsorption, a localization of the delivery system at a given target site and an increase in the drug concentration gradient due to the intense contact of the system with the mucosal surface. This allows bioadhesive devices to optimize local or systemic drug delivery in ocular, nasal, buccal, respiratory, gastrointestinal, rectal and vaginal routes [4–7].

Many authors define bioadhesive materials as 'muco-adhesive' when the interaction occurs with the mucus secreted by the epithelial globet cells [8,9]. In particular, these systems contain polymers capable to interact with glycoprotein chains that are the major components of mucus. These glycoproteins consist of a protein core with covalently attached carbohydrate side chains. Each of the side chains contains from 2 to 20 sugars and terminates with either L-fucose or sialic acid. Disulfide, electrostatic and hydrophobic interactions are involved in the entanglement of mucin chains responsible for the gel-like properties of mucus [10,11].

First, during the mucoadhesion process, the polymer establishes an intimate contact with mucus, therefore mucoadhesion can be described by interaction occurring at the interface glycoprotein/mucoadhesive polymer. After this absorption phase, segments of the mucoadhesive polymer and glycoproteins diffuse across the interface. During this interpenetration, the formation of physical bonds and entanglements between mucin and flexible polymer chains occurs [12,13]. The rate of penetration of polymer chains into the mucin layer is dependent on chain flexibility and diffusion coefficient of each. The strength of

* Corresponding author. Tel.: +39-91-623-6128; fax: +39-91-623-6150.
E-mail address: gaegiamm@unipa.it (G. Giammona).

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the adhesive bond is directly proportional to the depth of penetration of polymer chains, in particular, the maximum achievable bioadhesive bond for a given polymer is believed to occur when the depth of penetration is approximately equal to the end-to-end distance of the polymer chains [14]. Other parameters influencing the strength of adhesion are the presence of water, the time of contact between the materials and the length and flexibility of the polymer chains [15]. Macromolecules with high molecular weights and great amounts of polar groups tend to develop more intensive mucoadhesive bonds. For these reasons, polymers such as poly(acrylic acid), hydroxyalkylcellulose, hyaluronic acid, chitosan, polyalkylcyanoacrylate and collagen have relevant mucoadhesive properties [16–18]. In addition, for diffusion to occur, it is important that the bioadhesive polymer and mucus have a similar chemical structure; the more structurally similar to a bioadhesive is to mucus, the greater the mucoadhesive bond will be [19]. The chain interdiffusion represents the principal among various mechanisms involved in the mucoadhesion process. In effect, the bioadhesion is not a phenomenon which can be explained by a single model or theory but the operative mechanism which is observed is probably a combination of several possibilities such as electrostatic interaction, adsorption and wetting processes, besides chain interpenetration across the biointerface [13]. Besides the lack of an appropriate theoretical model, the major problem in the field of bioadhesion is the absence of a standard experimental technique to characterize bioadhesive systems. Most researchers have developed their own techniques to evaluate the interaction between a bioadhesive polymer and biological substrates [20–22]. Each technique has its own set of experimental conditions and, therefore, it is difficult to compare experimental data among investigators. Even for a given method, a small variation in experimental parameters such as contact time, speed of testing, preparation of biological substrates, applied force, rate of removal of bioadhesives and presence of impurities, results in very different values so that it is not possible to assign an absolute value representing the bioadhesive properties for a particular system [23]. However, among the several techniques employed, the spectroscopic analysis resulted useful to investigate the interaction between bioadhesive polymers and mucus. In particular, attenuated total reflection infrared spectroscopy (ATR–FTIR) has been applied successfully to study the chain interpenetration occurring between polyacrylic acid and mucin [24]. The aim of this work is the use of ATR–FTIR for the spectroscopic investigation of diffusion of water and chain interpenetration at a bioadhesive interface consisting of two new polymeric films based on α,β-poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) and α,β-polyaspartylhydrazide (PAHy) and a mucin solution.

PHEA and PAHy, two macromolecules with a protein-like structure, show interesting properties for applications in the pharmaceutical field. In particular, both polymers are highly water-soluble, non-toxic and non-antigenic and have been proposed as plasma expanders, drug carriers for macromolecular prodrugs and starting materials to prepare hydrogel systems [25–32]. From a structural point of view, PHEA and PAHy possess several polar groups (hydroxyl and hydrazide functions, respectively) available to interact with glycoprotein chains. Therefore, the evaluation of a potential mucoadhesive behaviour appears to be useful in the understanding of the properties and applications of these macromolecules.

2. Experimental

2.1. Materials

DL-aspartic acid, ethanolamine, hydrazine hydrate and *N*,*N*-dimethylformamide (DMF), were from Fluka (Milano, Italy). Disodium hydrogen phosphate, citric acid, hydrochloric acid, sodium hydroxide and mucin Type I-S from bovine submaxillary glands, were from Sigma-Aldrich (Milano, Italy). Water was freshly distilled (Milli-Q). All reagents were of the best available commercial grades.

α,β-polyaspartylhydrazide (PAHy) was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of DL-aspartic acid, with hydrazine in DMF solution and purified as reported elsewhere [25]. Analytical and spectral data (FT-IR and ^{1}H NMR) were in agreement with the literature values [25]. PAHy weight-average molecular weight was 23 500 g/mol ($M_w/M_n = 1.78$).

α,β poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) was synthesized by reaction of PSI with ethanolamine in DMF solution and purified as already reported [33]. Analytical and spectral data agreed with the values reported elsewhere [32,33]. The batch of PHEA used in the present study had a weight-average molecular weight of 56 900 g/mol ($M_w/M_n = 1.89$).

2.2. Methods

2.2.1. ATR–FTIR spectroscopy

A FTIR spectrometer (Vector 22 Bruker) with an ATR accessory (Specac), with a cover to prevent solution evaporation, was used for the interdiffusion studies in the configuration shown in Fig. 1.

This arrangement permits the IR beam to enter the film to

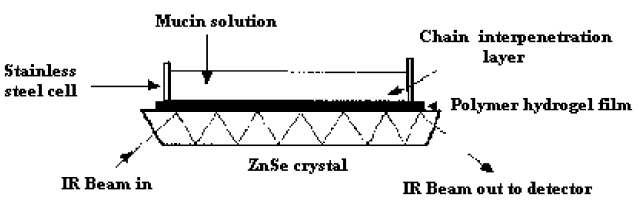

Fig. 1. Scheme of the ATR–FTIR experimental arrangement.

a small fixed depth and be specifically attenuated according to the molecules present in this region. The ATR crystal was ZnSe 50 mm long, 10 mm wide and 2 mm thick. A 500 μl aliquot of 20% (w/w) solution of polymer (buffer 7 phosphate/citrate) was placed on the crystal and then was dried in vacuo at 25 °C for 24 h. The resulting film thickness, measured at different points using a digital micrometer, was found to be ~50 and ~60 μm thick for PHEA and PAHy, respectively, and was contacted with 500 μl of a 1% buffer 7 (phosphate/citrate) mucin solution. The measurement range was 4000–700 cm^{-1} and the spectra were collected in situ, every 5 s, with four averaged scans and a resolution of 4 cm^{-1}. The spectrometer was linked to a PC equipped with Bruker Opus 2 software which allows for the continuous automated collection and subsequent manipulation of spectra, including the deconvolution and fit routines. The experiments were designed to study the diffusion of water through a polymeric film from a reservoir of mucin solution, until the formation of a homogenous and clear solution and this arrangement ensures an essentially constant concentration of the penetrant (water) in the polymeric film, to determine the diffusion coefficient of water in the polymers PHEA and PAHy and to study the interfacial interaction or interpenetration between mucin and both polymers. After different experimental trials, it has been necessary to employ a very short pause between two consecutive measurements (5 s), a low number of averaged scans (four) and a spectra resolution of 4 cm^{-1}, in order to obtain the best information to describe the diffusional process.

3. Results and discussion

The extent of chain interpenetration at a polymer–polymer interface depends on compatibility, i.e. the full miscibility, and then the possibility to prepare clear solutions, between the two polymers [34,35]. Taking into account that PHEA and PAHy are non-ionic polymers, we performed our experiments at pH 7, chosen as an example of a physiological medium. Since 1 wt% mucin aqueous solution at pH 7 was clear, indicating that mucin and water were compatible at this pH value [34], to verify the compatibility between mucin solution and PHEA or PAHy solution we prepared two 20 wt% aqueous solutions with each polymer that were kept in contact with a mucin solution. The solutions containing mucin and PHEA (or PAHy) resulted to be clear thus indicating the compatibility, and then the full miscibility, between mucin and the polymers investigated. However, after the addition of PHEA or PAHy, mucin solutions became viscous due to entanglement and establishment of hydrogen bonding between the polymeric chains.

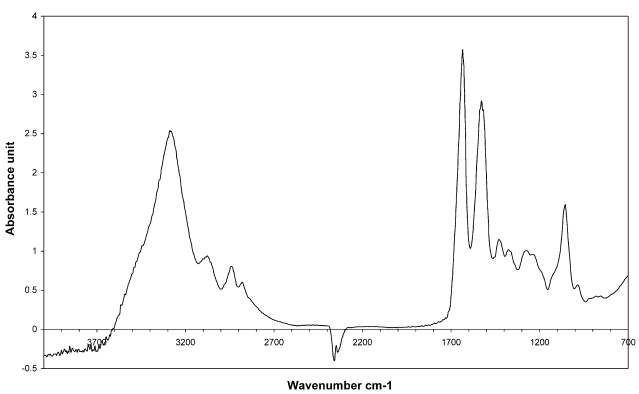

Fig. 2. Infrared spectrum of PHEA film.

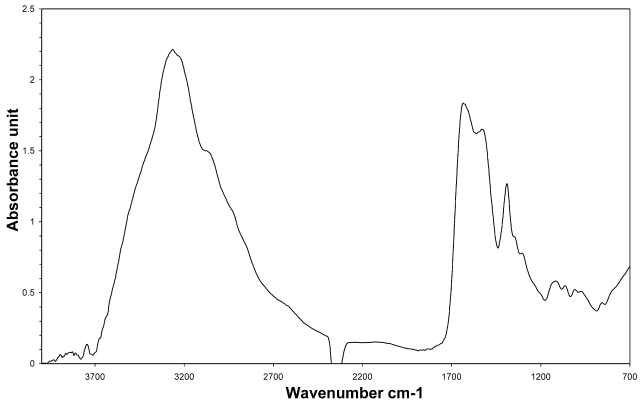

Fig. 3. Infrared spectrum of PAHy film.

Figs. 2 and 3 show the ATR–FTIR spectra of the PHEA and PAHy polymeric films, respectively, in the frequency region from 4000 to 700 cm^{-1}.

The broad and strong bands in the range 3500–3000 cm^{-1} are due to N–H and O–H stretching extensively involved in hydrogen bonding. The peaks centred around 2850 cm^{-1} arise from the C–H stretching. The bands at 1650 and 1530 cm^{-1}, the so-called Amide I and Amide II bands, are due, respectively, to C=O stretching and N–H bending of amide groups. The bands at 1400 cm^{-1} are due to C–O stretching [36]. In Fig. 4 is reported the ATR spectrum of a mucin solution in the buffer solution at pH 7.

The only band present is at 1550 cm^{-1}, due to the C=O stretching vibration of sialic acid of mucin. For quantitative analysis, the polymers/mucin spectra were deconvoluted to

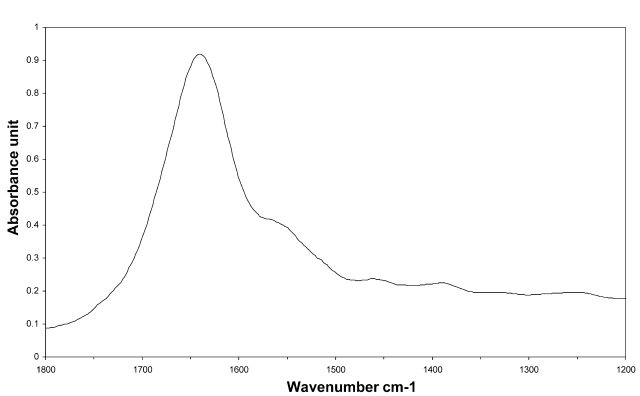

Fig. 4. Infrared spectrum of 1% mucin solution.

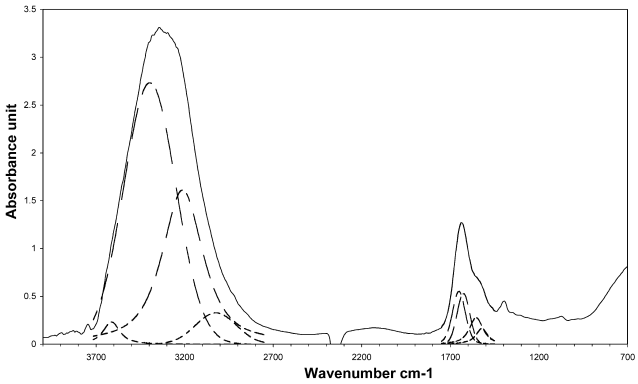

Fig. 5. Deconvolution of ATR–FTIR spectrum for PAHy in contact with 1% buffer 7 mucin solution at equilibrium time. The solid line represents the original spectrum, whereas the dashed curves are the deconvoluted peaks.

relate the area under the peaks to the polymers, mucin and water concentrations. Figs. 5 and 6 show the actual and the deconvoluted peak for polymers in contact with a 1 wt% mucin solution at pH 7 at the equilibrium time in the range 3700–3000 and 1750–1450 cm^{-1}.

Figs. 7 and 8 show the time evolution of the ATR–FTIR spectra for polymeric films in contact with a 1 wt% pH 7 aqueous mucin solution, for the PHEA and PAHy with film thickness of 50 and 60 μm, respectively.

The time evolution of the spectra show shifts of about 10 cm^{-1} in the principal bands, Amide I and Amide II of the two polymers, and C=O of mucin, shifts that are not present when the spectra are collected on the solutions of the polymers and mucin separately. These shifts are due to interactions by hydrogen bonding between mucin and PHEA or PAHy.

The integrated area of OH stretch band centred at 3400 cm^{-1} was used to monitor the diffusion of water as an indirect measure of any changes resulting from interpenetration of polymer–mucin chains at the aqueous solution/polymer film interface.

As diffusion of water into the film occurs, there will

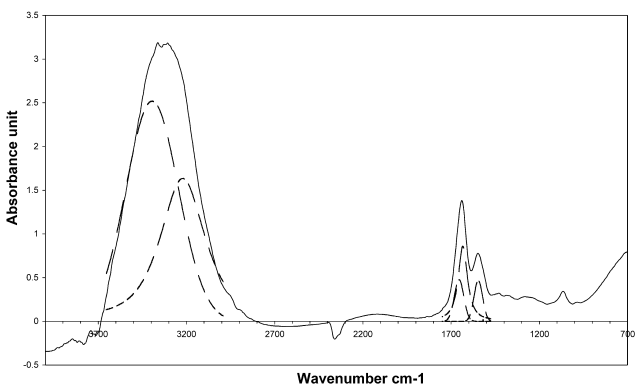

Fig. 6. Deconvolution of ATR–FTIR spectrum for PHEA in contact with 1% buffer 7 mucin solution at equilibrium time. The solid line represents the original spectrum, whereas the dashed curves are the deconvoluted peaks.

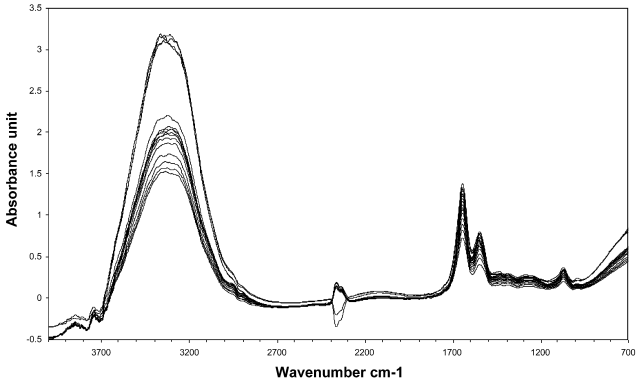

Fig. 7. Time evolution of the spectrum of PHEA film in contact with a 1% mucin solution.

be a steady concentration build-up of the water at the crystal/polymer film interface. As the water wets the PAHy and PHEA film, the intensities of the Amide I and Amide II band of both polymers around 1650 and 1530 cm^{-1} decrease, the intensity of the water peaks around 3400 and 1640 cm^{-1} increases and the mucin peak around 1550 cm^{-1} shows a slight increase with time.

The spectra were deconvoluted and the area relative to above bands was calculated. In Figs. 9 and 10 are represented the integrated areas under the band centred around 3400 cm^{-1} plotted against the evolution time of PAHy and PHEA polymer films contacted with the mucin solution.

A diffusion model using a solution of Ficks' second law that satisfies both initial and subsequent boundary conditions [34,37], has been employed to compare the experimental results of the evolution with time of water and other infrared bands at the polymer/mucin interface. Therefore, diffusion can be defined by the equation

$$C/C_0 = A/A_0 = 1 - 4/\pi \sum_{n=0}^{\infty} \{(-1)^n/2n+1\} \times \exp\{(-D(2n+1)^2\pi^2 t)/4h^2\} \quad (1)$$

where C is the water concentration at the interface at time t; C_0, the solubility of the water in the film; D, the water

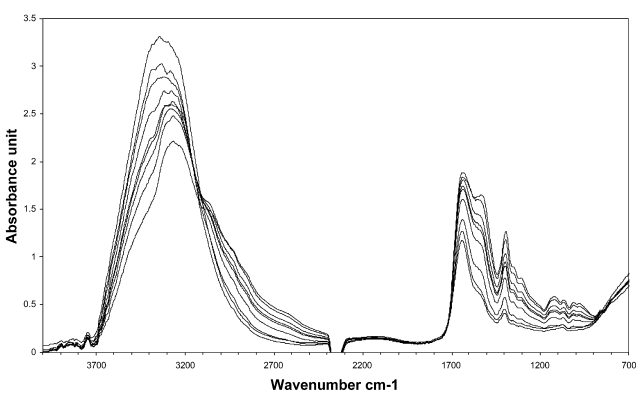

Fig. 8. Time evolution of the spectrum of PAHy film in contact with a 1% mucin solution.

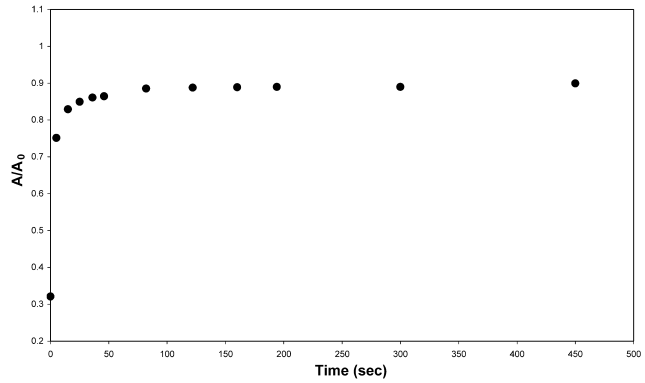

Fig. 9. Relative absorbance of water as a function of time for PAHy in contact with a 1% buffer 7 mucin solution.

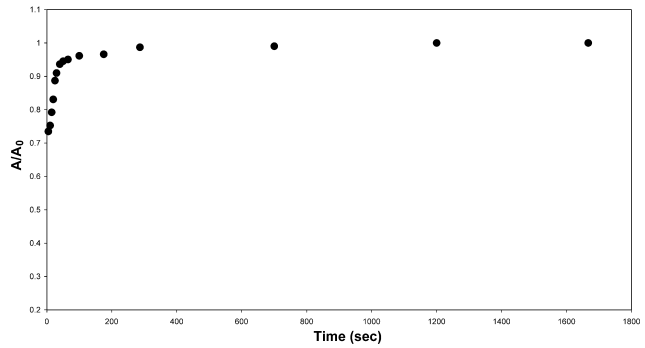

Fig. 10. Relative absorbance of water as a function of time for PHEA in contact with a 1% buffer 7 mucin solution.

diffusion coefficient; h is the film thickness. Concentration terms can be replaced with experimental absorbances, i.e. $C/C_0 = A/A_0$, where A is the area under the water peak curve and A_0 is the area under the water peak curve corresponding to film saturation with the water [38]. Diffusion coefficients were calculated by employing a non-linear curve fitting package in order to fit the experimental data to Eq. (1). Figs. 11 and 12 show the time evolution of the relative integrated areas of the deconvoluted peaks of polymers, water and mucin for PHEA and PAHy in contact with a 1 wt% pH 7 aqueous mucin solution at 25 °C.

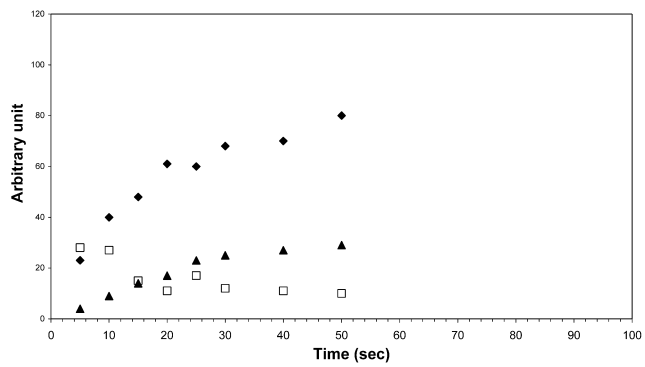

Fig. 11. Time evolution of integrated area of deconvoluted peaks of mucin (▲), water (◆) and PHEA (□).

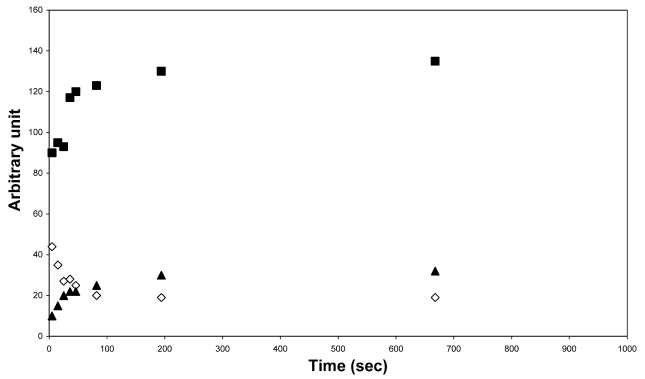

Fig. 12. Time evolution of integrated area of deconvoluted peaks of mucin (▲), water (■) and PAHy (◇).

The relative areas of polymers decrease with wetting while the same for water and mucine increase indicating that water and mucin wet and penetrate the polymeric matrix simultaneously. The mucin wets the polymeric matrix because polymers and mucin are miscible and compatible for pH 7.

The best fit with the experimental data gave a mean diffusion coefficient of $7.1 \times 10^{-4}\,\mathrm{cm^2\,s^{-1}}$ for water in PHEA polymer film with thickness of 50 μm and of $8.5 \times 10^{-5}\,\mathrm{cm^2\,s^{-1}}$ for water in PAHy polymer film thickness of 60 μm. The better according to the experimental data was observed at shorter time periods that however are more critical to determination of diffusion coefficients.

4. Conclusions

The results obtained offer an unequivocal evidence that a chain interdiffusion occurs at the interface consisting of PHEA or PAHy films and a mucin solution. The fit of the experimental data allowed the calculation of the diffusion coefficients of water molecules into the polymeric films, consistent with the very fast solubilization rate of both polymers. The ability of PHEA and PAHy to interact with mucin allows the use of these macromolecules in the preparation of mucoadhesive devices to prolong the contact time at the site of drug absorption (e.g. in the gastrointestinal tract or ocular region) or to achieve the localization and release of a drug to a specific region, thus improving pharmacological effectiveness.

Acknowledgements

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