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An investigation of the interfacial interaction between poly(acrylic acid) and glycoprotein

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Abstract

The swelling behaviour of poly(acrylic acid) microspheres, produced from poly(acrylic acid) crosslinked with maltose, was investigated as a function of time by using a laser diffraction spectrometer. Swelling was also studied in various pH glycoprotein solutions. Microscopy revealed confirmatory evidence of interfacial film formation when microsphere hydration occurred in a pH 5 glycoprotein solution. ATR-FTIR spectroscopy was used to determine the diffusion coefficient of water through the interfacial film existing at the poly(acrylic acid) glycoprotein solution interface. Both processes exhibited a pH dependency with rates decreasing in the sequence pH $7 > 6 > 5 = 4$.

Keywords: Mucoadhesion; Microsphere; Swelling poly(acrylic acid); Glycoprotein; ATR-FTIR spectroscopy

1. Introduction

The development of controlled release systems for targeted drug delivery to defined areas of the body has often utilized the bioadhesive process (Robinson, 1990). Increased interest by pharmaceutical scientists in bioadhesive dosage forms (Park and Robinson, 1987; Kerr et al., 1989; Mikos et al., 1991) has resulted in applications for buccal, nasal, ocular, vaginal, urinary and oral drug delivery (Peppas and Buri, 1985; Mikos and Peppas, 1990). In general, the mucoadhesive polymer is brought into intimate contact with mucus secreted by the epithelial goblet cells. Following this adsorption phase, the polymer hydrates by extraction of water from the glycoprotein gel resulting in the opportunity for interfacial diffusional mixing of the polymer and glycoprotein chains. The latter are the major component molecules of high molecular weight, responsible for the viscoelastic gel-like properties of the mucus.

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Mucoadhesion between a bioadhesive polymer and mucus can, therefore, be described initially by interactions occurring at the glycoprotein and the hydrating mucoadhesive polymer interface. This paper examines the effect of pH on the interfacial reaction as it influences both hydration rates of the polymer and the diffusion of water into the polymer film. The hydration rates were examined by determining size changes of polymeric microspheres formed from a mucoadhesive polymer as they hydrated under pH controlled conditions in the presence of glycoprotein. Water diffusion was determined by ATR-FTIR spectroscopy.

2. Materials and methods

2.1. Materials

Poly(acrylic acid) (MW 250.000), maltose, palmitic acid (Grade 2) and olive oil (purified) were supplied by Sigma-Aldrich (Poole, UK). Disodium hydrogen phosphate, citric acid, acetone, hydrochloric acid and sodium hydroxide, were obtained from Fisher Scientific (Loughborough, UK). Water was freshly distilled (Mili Q). Sepharose 4B was obtained from Pharmacia (Uppsala, Sweden).

Glycoprotein was extracted and purified from the stomachs of pigs obtained from an abattoir shortly after slaughter (Brown et al., 1981). The stomachs were emptied, washed and the mucus lightly scraped from the mucosal surface and immediately dispersed in isotonic pH 7.4 phosphate buffer, containing 0.2 M thiocyanate using a laboratory blender for 1 min. The mixture was centrifuged at $40000 \times g$ for 1 h at 4°C to remove cell debris. The supernatant was filtered through glass wool previously washed with dilute nitric acid, followed by washing with distilled deionised water to remove any large contaminants. Fractionation was carried out on a Sepharose 4B column and the glycoprotein fraction (void volume) identified by UV monitoring at 280 nm. The excluded glycoprotein was dialysed exhaustively against distilled deionised water at 4°C before concentrated to a gel using isotonic pH 7.4 phosphate/citrate buffer in an ultrafiltration cell (Amicon, Lexington, MA). The gel was frozen and stored at −20°C until required. Solutions were prepared from the gel by dilution with an appropriate pH phosphate/citrate buffer with final pH adjustment (HCl).

2.2. Methods

2.2.1. Preparation of microspheres

The aqueous phase was prepared by dissolving poly(acrylic acid) (0.25 g) and maltose (0.05 g) in 50 ml of deionised water by mechanical shaking overnight thereby ensuring thorough mixing. The organic phase consisted of olive oil (150 ml) and palmitic acid (3 g), present as a droplet stabiliser. This phase was heated to 110–115°C by an electrothermal isomantle whilst stirring at 1400 rpm (Janke and Kunkel Ika-werk RW20 DZM) with an homogeniser rod in a 250-ml wide-necked round-bottomed flask. Once the organic phase had attained a stable temperature, the aqueous phase was added dropwise to the flask. Stirring and heating were maintained until the oil appeared clear, indicating the evaporation of all the water derived from the polymer solution and therefore onset of the polycondensation reaction and for a further period of 2.5 h to ensure curing of the particles. The microspheres were harvested from the oil by repeatedly washing with acetone and collected by centrifugation. The supernatant was discarded after each washing procedure. The microspheres were dried at 30°C under vacuum and stored in a vacuum desiccator.

2.2.2. Microsphere sizing by laser diffraction

A Malvern laser diffraction spectrophotometer (2600 Malvern, UK) was used with a 300 mm lens. Samples of the microspheres were introduced into the laser beam in a magnetically stirred optical glass cell filled with acetone. Readings were taken for 5 s (200 sweeps). Microspheres were also suspended in acetone for up to 72 h to ensure that swelling was negligible. The batch of microspheres selected for hydration studies had a mean size of 24.4 μm (range: 16.7–50.2 μm).

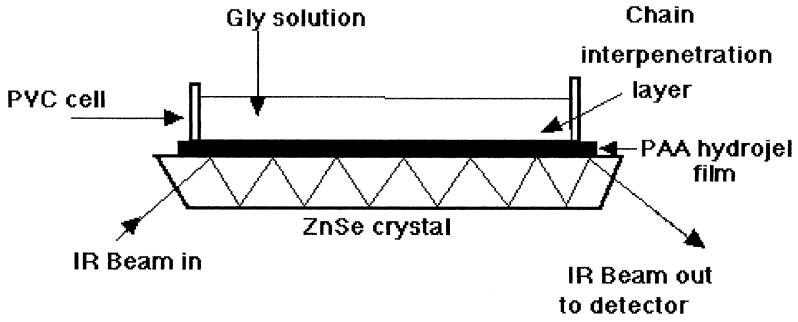

Fig. 1. Schematic of the ATR-FTIR experimental arrangement.

2.2.3. Microsphere hydration in glycoprotein solutions

Microspheres (100 mg) were introduced into the stirred laser diffraction cell containing 10 ml of water or glycoprotein solutions (0.3 and 3.0 mg/ml) at pH 4, 5, 6 and 7 and at 20°C. The mean size of the microspheres was measured at 0.5, 1, 3 and 5–8 h. Each hydration experiment was conducted in triplicate.

2.2.4. Light microscopy

Samples of the microspheres were examined by light microscopy (Olympus BH2) after 4 h hydration in 3 mg/ml glycoprotein pH 5 solution and in water. The latter acted as a control when visualising the interfacial regions of the hydrating microspheres. Magnification was $\times 100$.

2.2.5. ATR-FTIR spectroscopy

The interpenetration of poly(acrylic acid) and glycoprotein chains was investigated using ATR-FTIR spectroscopy. A thin film of poly(acrylic acid) was prepared by crosslinking 1 g of poly(-acrylic acid) with 0.2 g of maltose dissolved in 25 ml of water. The solution was poured into a Teflon mould and dried at 90°C for 24 h. The resulting film thickness was measured at multiple points using a digital micrometer and was found to be ≈ 300 μm thick. The film was carefully cut to overlie and be in direct contact with the ZnSe attenuated total reflectance crystal mounted on a Nicolet 710 ATR-FTIR spectrophotomer (Fig. 1). Once the film was placed on the crystal, a PVC donor cell was placed on top. This arrangement permits the IR beam to enter the film to a small fixed depth and be specifically attenuated according to the molecules present in this region. The cell and film were sealed together using a petroleum gel and the joints monitored for leaks throughout the experiment. Water or a glycoprotein solution (3 mg/ml and pH adjusted) was then placed in the cell to cover the upper surface of the poly(acrylic acid) film. The top of the cell was sealed with a plastic cover to prevent evaporation. The spectrophotometer was linked to a PC equipped with Nicolet Omnic software which allows for continuous automated collection and subsequent manipulation of spectra.

3. Results and discussion

3.1. Microsphere swelling

The swelling of the microspheres over 8 h is illustrated in Fig. 2. In the absence of glycoprotein, the microsphere mean size increased continuously over this period, with a linear increase observed from 3 to 8 h. The presence of glycoprotein in the swelling medium reduced the rate of increase in size. This was particularly noticeable for the higher glycoprotein concentration of 3 mg ml^{-1} at pH 4 and 5. At both concentrations of 0.3 mg ml^{-1} (Fig. 2a) and 3.0 mg ml^{-1} (Fig. 2b) the pH effect observed on swelling rate reduction was in the order pH $5 = 4 > 6 > 7$. These results suggest that glycoprotein is adsorbed at the solid/liquid interface to form an interfacial film exhibiting a pH dependent resistance to the diffusion of water. Observation by light microscopy of micro-

spheres after 4 h hydration in 3 mg ml^{-1} glycoprotein solution at pH 5 confirmed the existence of such film formation (Fig. 3). The polydisperse nature of the microspheres was a factor limiting the extent and quality of information on pH-dependent swelling and hence the move to a better defined boundary at the polymer film–liquid interface and the subsequent studies employing ATR-FTIR.

3.2. ATR-FTIR spectroscopy

The experiments were designed to determine the pH-dependent diffusion of water through a hydrating polymer film from a reservoir of water or glycoprotein solution. This arrangement ensures an essentially constant concentration of the penetrant (water) in the upper levels of the poly(acrylic acid) film. The OH stretch frequency of water at 3316 cm^{-1} was used to monitor the diffusion of water as an indirect measure of any resistance changes resulting from glycoprotein deposition and/or interpenetration of polymer-glycoprotein chains at the aqueous/polymer film interface.

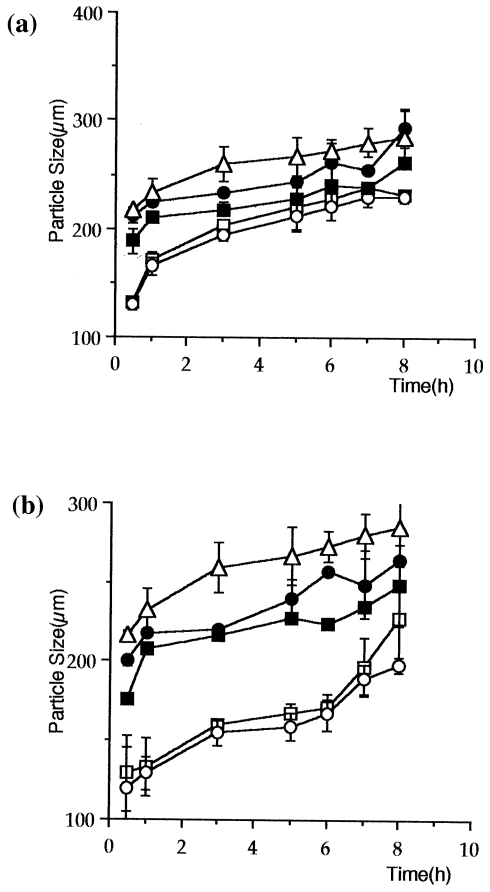

Fig. 2. Particle size of poly(acrylic acid) microspheres in (a) 0.3 mg/ml and (b) 3 mg/ml glycoprotein solutions as a function of hydration time. (Error bars represent S.D., $n=3$) (—△—: Water; —□—: Gly at pH 4; —○—: Gly at pH 5; —■—: Gly at pH 6; —●—: Gly at pH 7).

As diffusion of water into the film occurs, there will be a steady concentration build-up of the penetrant at the crystal/polymer film interface. Using a solution of Ficks' second law, that satisfies both initial and subsequent boundary conditions (Jabbari et al., 1993; Crank, 1975), 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 permeant concentration at the interface at time t, C_0, the solubility of the penetrant in the film, D, the permeant diffusion coefficient and h 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 penetrant (water) peak curve and A_0 the area under the penetrant peak curve corresponding to film saturation with the penetrant. The pH dependent spectra are illustrated in Fig. 4a and b which represent $t = 32.9$ and 165.7 min, respectively.

As the polymer film swells with time, it was necessary to apply a correction for the change in the dimension h. The peak at 1450 cm^{-1} (C–O–H bending vibrations of the poly(acrylic acid)) was used to monitor film swelling. We have, therefore, normalised the area of the water peak at 3316 cm^{-1} with reference to the poly(acrylic acid) peak at 1450 cm^{-1} to obtain corrected areas (Farinas et al., 1994; Watkinson et al., 1994). The normalised peaks plotted as a function of time are shown in Fig. 5 which illustrates the considerable lag observed at pH 5 (and, to a lesser extent, pH

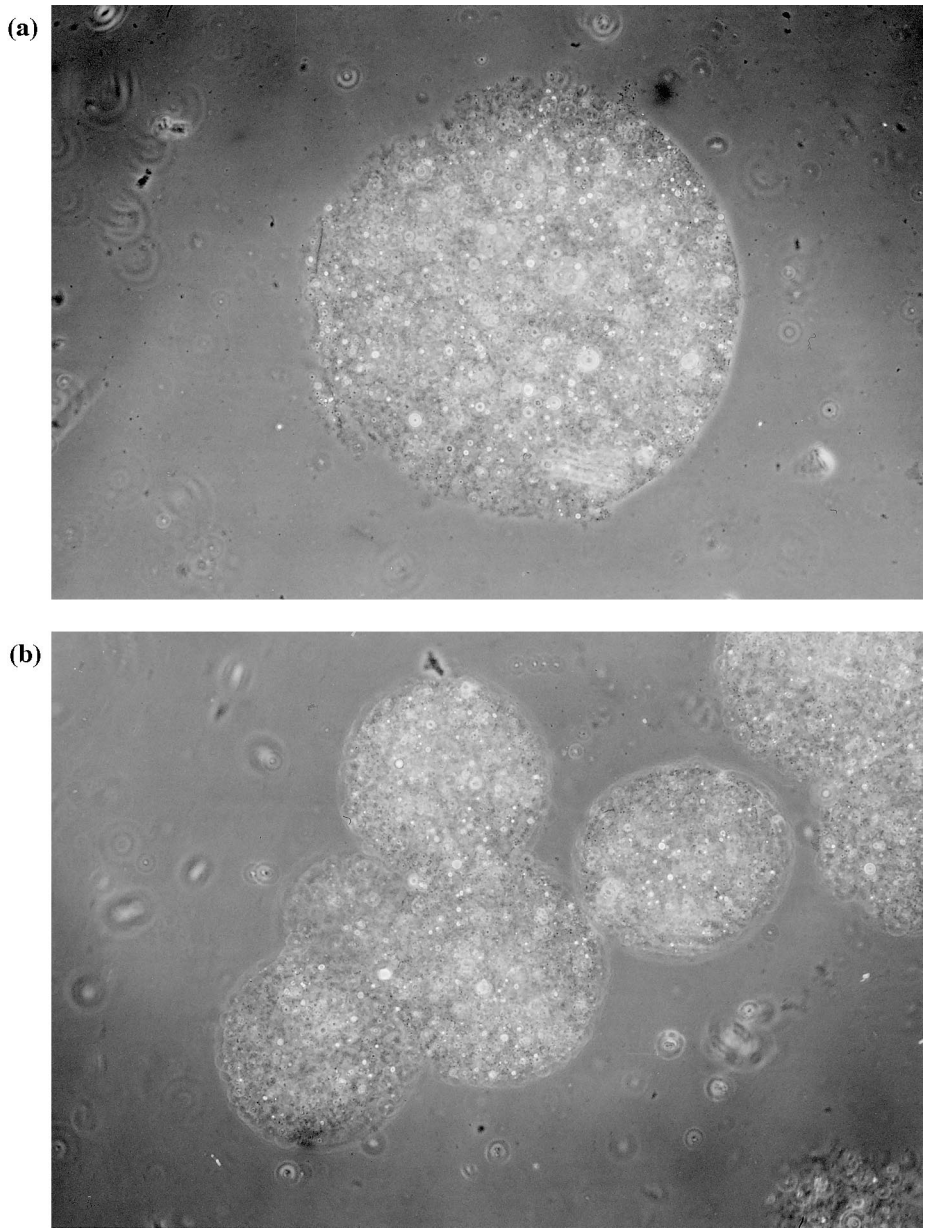

Fig. 3. Micrographs of microspheres after 4 h of hydration in (a) water and (b) glycoprotein 3 mg/ml at pH 5, illustrating the formation of an interfacial film.

4) for the OH peak when glycoprotein solution replaces water in the reservoir. At pH 7, there is a negligible lag observed indicating that glycoprotein is having minimal effects on the penetration of the water into the hydrogel film, in marked contrast to the interfacial film resistance evident at pH 5. To what extent this resistance is due to adsorption of glycoprotein and/or interpenetration of glycoprotein chains into the surface layers of the hydrating PAA film is unclear.

Diffusion coefficients were calculated by employing a non-linear curve fitting package (Ultra-

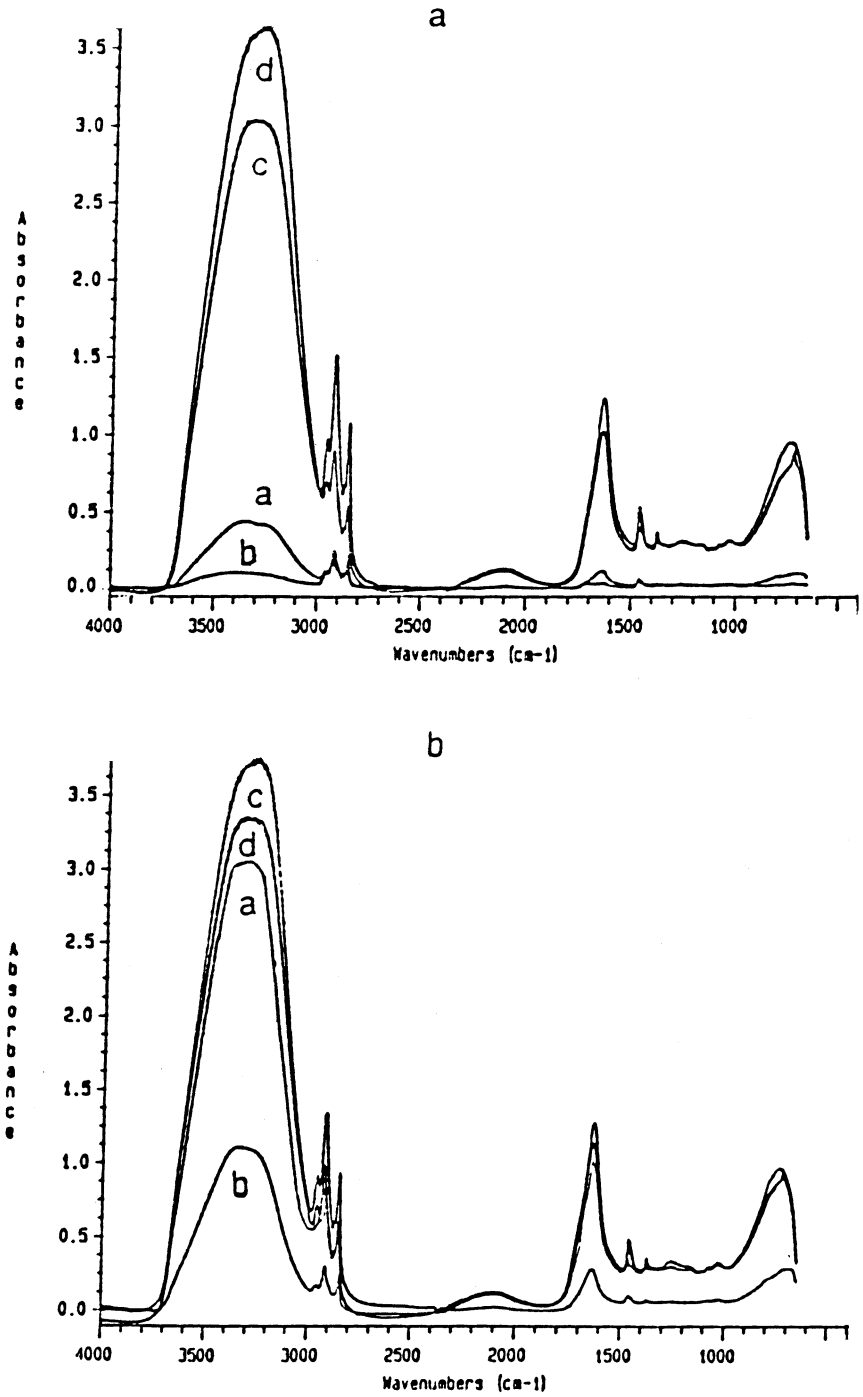

Fig. 4. The IR spectrum of poly(acrylic acid) hydrogel film at various pH after 32.9 min (a) and after 165.7 min (b). a = pH 4; b = pH 5; c = pH 6; d = pH 7.

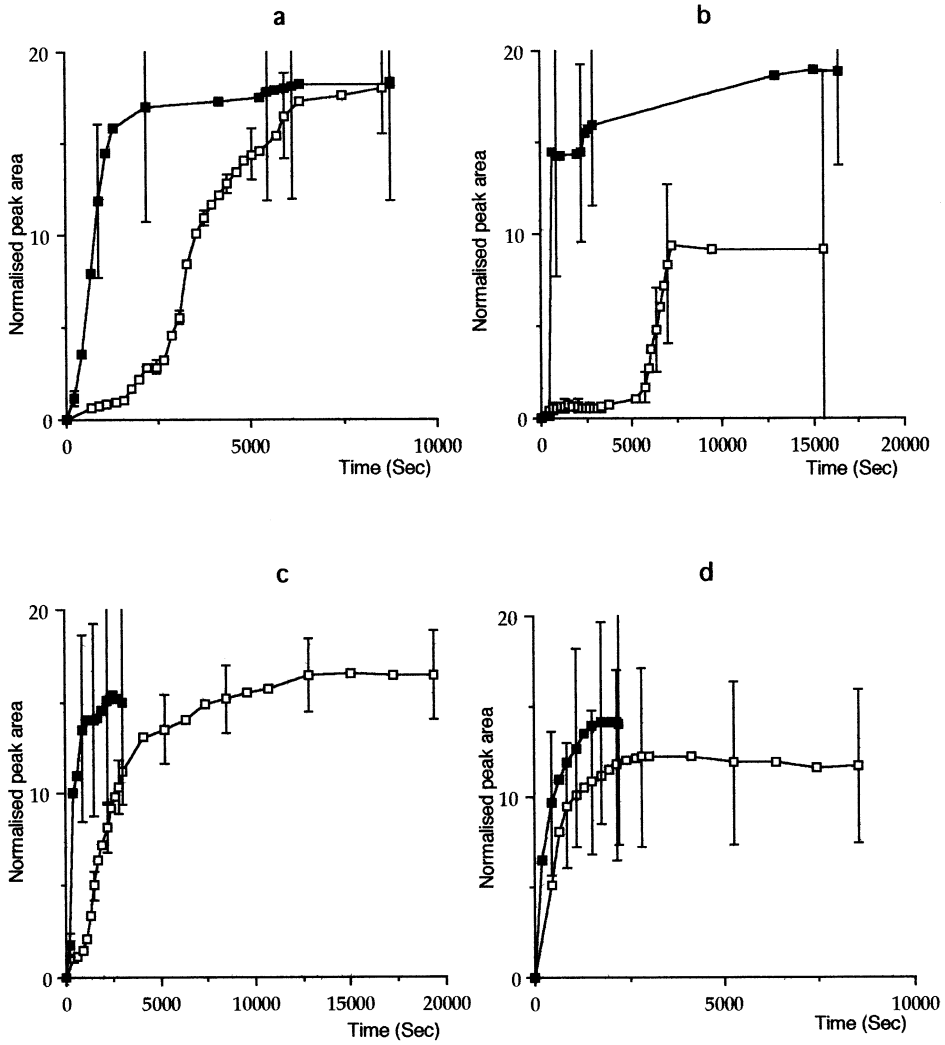

Fig. 5. Penetration of water from glycoprotein solutions (3 mg/ml) through poly(acrylic acid) films at various pH according to their peaks (peak area: 3316 cm^{-1}), (Error bars represent S.D., $n = 3$), —□—: OH peak arising from water diffusion when glycoprotein solution was placed in the cell. —■—: OH peak arising from water diffusion when water was placed in the cell. a = pH 4; b = pH 5; c = pH 6; d = pH 7.

Fit V.1.03, Biosoft, Cambridge, 1990) in order to fit the experimental data (Fig. 5) to Eq. (1). Although there was considerable variability in data generated at long time points, a greater consistency was observed at shorter time periods, which are more critical to the calculation of diffusion coefficients. The values of D/h^2 and C_0 were allowed to vary until the best fit was achieved, as determined by the minimisation of X^2, where $D = h^2.X^2$. The pH dependency of water diffusion followed the sequence pH $7 > 6 > 4 = 5$ (Fig. 6) which is the same as observed for the hydration of the microspheres.

We have therefore demonstrated that the swelling rates of mucoadhesive microspheres of poly(acrylic acid) are determined by the presence of glycoprotein and the pH of the swelling media. This may be expected to have a direct effect on drug availability from such carrier particles. The existence of interfacial films showing resistance to

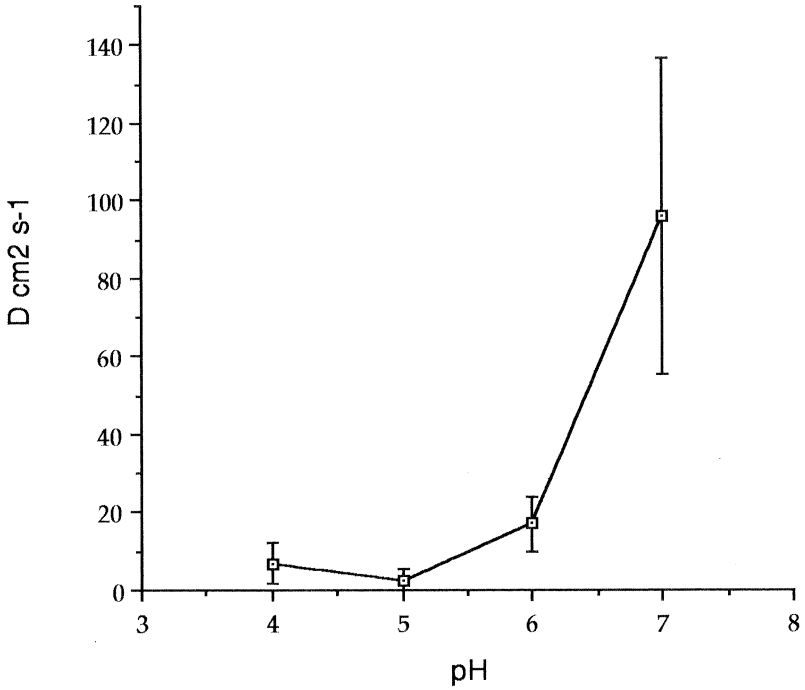

Fig. 6. The diffusion coefficients of water through poly(acrylic acid) hydrogels as a function of the glycoprotein solution (Error bars represent S.D., $n = 3$).

the transport of water may well arise from the pH dependent interaction of glycoprotein molecules with the hydrating polymer chains at the surface of the microspheres.

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