Analysis of Drug Distribution in Hydrogels Using Fourier Transform Infrared Microscopy

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Purpose. The purpose of this work was to study solute (drug and protein)/polymer interactions that affect solute diffusion in and subsequent release from swellable dosage forms based on environmentally responsive, pH-sensitive polymer networks.
Methods. Ionizable pH-sensitive hydrogels were synthesized by free-radical polymerization of acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) with ethylene glycol dimethacrylate as crosslinking agent. The degree of crosslinking and degree of hydrophilicity were controlled by varying the relative concentration of crosslinking agent and comonomer, respectively. The role of solute distribution within the hydrogels as related to the transport behavior was investigated by Fourier transform infrared (FTIR) microscopy.
Results. The solute-loaded hydrogels were cryotomed into 4 μm thin sections. The concentration profile of the solute was constructed from the two dimensional intensities measured by monitoring the infrared vibrational band indicative of that compound in the x and y direction. These studies indicated that the model solute, oxprenolol HCl, was evenly distributed throughout the bulk and surface of the hydrogel samples. Solute/polymer interactions were investigated as a function of the polymer composition and swelling media pH. The concentration profiles of oxprenolol HCl solution loaded into PAA and P(AA-co-HEMA) hydrogels were analyzed by scanning electron microscopy by FTIR microscopy. For hydrophilic polymers (hydrogels) containing ionizable pendant groups, the molecular weight between crosslinks, the degree of swelling, and the degree of ionization were altered by local changes in pH and ionic strength.
Conclusions. The data demonstrated that it is possible to evaluate the polymer/solute interactions by using FTIR microscopy.

KEY WORDS: Fourier transform infrared; scanning electron microscopy; hydrogel; distribution; oxprenolol HCl; microscopy.

INTRODUCTION

Hydrophilic networks (hydrogels) have been employed in a wide variety of biomedical applications, since Wichterle and Lim (1) first proposed the use of poly(2-hydroxyethyl methacrylate) (PHEMA) in contact lenses. Hydrogels mimic natural tissue best of any synthetic material due to the high water content and flexibility (2,3), which promotes their biocompatibility (4). Creative developments have utilized the favorable characteristics of these materials to control the release of incorporated solutes as delivery devices (5). The use of hydrogels as controlled release materials has led to a greater emphasis on the properties that influence their diffusive characteristics. Although there exists a great abundance of release studies for various solutes from hydrogels, very little has been reported on solute distribution in the hydrogel prior to its release into the solution medium. In order to effectively interpret diffusion and release data, the solute distribution in the hydrogel must be known, especially in the hydrated state. Major contributions to this area have been by Lee and his collaborators (6,7), who evaluated the distribution of oxprenolol HCl across the surface of the glassy PHEMA hydrogen matrices using a technique which requires a dehydrated sample, scanning electron microscopy (SEM).

SEM is a powerful technique capable of detecting compounds that emit x-rays, making it possible to scan for that compound across the surface of the polymer sample. Energy dispersive spectroscopy is utilized to detect and quantify the number of x-rays for a particular atom. Important requirements of SEM as an elemental analysis tool include that the polymer sample must be dehydrated since the system is under vacuum, and that elements containing x-ray emitting atoms, such as chlorine, be present. Currently SEM is the most frequently used characterization technique for biomaterials. In the present work SEM and FTIR-microscopy were used to study the drug distribution in ionizable hydrogels.

MATERIALS AND METHODS

Acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin), with 200–300 ppm methyl ether hydroquinone as an inhibitor. The inhibitor as well as dimerized monomer that was formed during storage were removed from AA by vacuum distillation at 47°C and 7 mm Hg. Removal of dimerized acrylic acid is important as the rate of its formation is 1.0–1.5% per month at 30°C (8). Purification of HEMA was performed by vacuum distillation at 65°C and 4 mm Hg. The crosslinking agent ethylene glycol dimethacrylate (EGDMA, Aldrich Chemical Co., Milwaukee, Wisconsin) and the initiator azobis isobutyronitrile (AIBN, Aldrich Chemical Co., Milwaukee, Wisconsin), were used without further purification. Oxprenolol HCl was purchased from Sigma Chemical Co. (St. Louis, Missouri) and was used as received.

Polymer Synthesis

Poly(acrylic acid), henceforth designated as PAA, hydrogels were produced by free radical solution polymerization of a 10 mol % aqueous solution of AA. The EGDMA concentration was maintained at 0.01 mol/mol of total monomer, and the initiator concentration was maintained constant at 0.1 wt % AIBN. Solution polymerization was carried out in 6 ml cylindrical vials with a 14 mm inner diameter. Appropriate amounts of initiator, inhibitor-free AA, crosslinking agent and solvent were transferred into the vials and purged with nitrogen for 10 min to remove oxygen as a free radical scavenger. The temperature program for the reactions was 50°C for 1 hour, 60°C for 2 hours, and 70°C for 24 hours. This temperature program provided the highest con-

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version (e.g. minimized the amounts of unreacted components that were extractable from the hydrogel).

Copolymerization of AA and HEMA was carried out in ethanol at a total monomer concentration of 60 mol% monomer to 40 mol% ethanol. The monomer portion was composed of 40 mol AA to 60 mol HEMA, and the crosslinking ratio was constant at 0.01 mol EGDMA/mol total monomer. The temperature program used to synthesize the copolymers was 50°C for 2 hours followed by 60°C for 45 hours.

Upon completion of the synthesis procedures, the cylindrical polymer samples were cut into thin disks (0.03 cm) with a diamond edge rotary saw (Buehler Ltd., Lake Bluff, Illinois), using Isocut®, a light ends hydrocarbon, as a cutting fluid.

Solute Loading

In order to compare the results of SEM and FTIR microscopy as techniques for evaluating solute distribution in hydrogels, the solute must contain an x-ray emitting atom and a unique IR absorption peak relative to the hydrogel. Oxprenolol HCl was selected as a model drug for this investigation. Oxprenolol HCl was loaded into the PAA and P-(AA-co-HEMA) hydrogels by immersing the polymers disks for a week in an aqueous drug solution at a concentration of 20 mg/ml at 37°C. This solution loading technique is commonly used to incorporate solutes at concentrations below the maximum solubility limit, as described by Carelli and collaborators (9).

Procedure of Determination of Solute Distribution Using SEM

A scanning electron microscope (SEM, JEOL JSM-35CF scanning microscope) with a Tencor Northern TN55 x-ray analyzer at an electron accelerating voltage of 15 kV was used to determine the distribution of a drug in a dehydrated hydrogel sample. The hydrated sample containing the solution-loaded drug was dehydrated, fractured, and placed in the SEM apparatus. A critical point drying apparatus (Polaron, model E3000 Series II, Watford Hertfordshire, U.K.) was used to dehydrate the hydrogel samples, by replacing the water with liquid carbon dioxide, CO_2 (*l*). Since CO_2 is essentially insoluble in water, the water was first replaced by ethanol. The substitution was carried out in several stages by placing the sample into several solutions containing increasing increments of ethanol relative to water in each successive solution. The sample was placed in the specimen holder of the critical point drying apparatus, where CO_2 (*l*) was introduced to displace the ethanol. The pressure was increased to 7.58 MPa while the temperature was increased to 32°C, at which point the CO_2 sublimed without formation of a meniscus to alter the surface or interior of the sample due to pressure effects.

SEM was employed in this study to determine the distribution of oxprenolol · HCl both on the surface and interior of PAA by scanning for the chlorine label along the centerline of the polymer and through the bulk. When the electron beam contacted the polymer, the sample emitted an array of electrons, of which the low energy secondary electrons were collected at the detector and used to distinguish x-ray emitting atoms within the sample. The electron beam was also focused at specific distances along the fractured surface, and evaluated for the presence of chlorine contained in the model drug substance.

Procedure for Determination of Solute Distribution by FTIR-Microscopy

An FTIR spectrophotometer (model 800, Nicolet Analytic Instruments, Madison, WI) was used for these studies. The mercury cadmium telluride (MCT) detector was cooled with liquid nitrogen prior to use of the microscope (model IR Plan connected to a motorized micropositioning stage, Spectra Tech, Stamford, CT), and the proper parameter file was selected from the FTIR spectrophotometer.

The optical layout of the FTIR microscope consists of an optical path which is traversed by both visible and IR radiation in the same direction (10). The solute-loaded hydrogel sample was cryotomed and collected on a barium fluoride crystal, which was placed on the microscope motorized stage. The microscope contained a dual aperture system to reduce the diffraction effects. In this study, the lower aperture was a rectangular area which delineated the sample section of 40 μm by 160 μm. Each area analyzed produced a single beam IR spectrum that was ratioed to the barium fluoride crystal background spectrum. One important limiting factor of this technique is the diffraction effects that translate to the absorption of energy from neighboring sections of the sample, causing an overlap of information. With a resolution of 10–20 μm (11) the sample areas analyzed were separated by 40 μm in an effort to reduce the probability of secondary information (i.e. microscope stage movements of 200 μm for an analysis area of 160 μm).

When the system was properly aligned and tuned, the stage controller was used to collect a spectrum of the sample at 200 μm intervals in the x-direction and 40 μm in the y-direction. After the spectra were collected at all locations, the area under the C-H in-ring in-plane bending peak from 1270 to 1230 cm^{-1} was integrated as a function of position using the program written for this purpose (12). This program was written to integrate the area under the peak, with a line drawn between the lowest extremes defining the baseline.

A three-dimensional map was created by monitoring the absorbance intensity of a functional group indicative of the solute, as a function of x,y distance. For oxprenolol HCl, the C-H in-ring bending mode located at 1251 cm^{-1} in the spectrum is distinctive from the PAA hydrogel. The intensity of the C-H in-ring bending mode was ratioed to the intensity of the CH_2 stretching mode located at 2940 cm^{-1} (integrated from 2900 to 2980 cm^{-1}) to maintain an independent reference.

RESULTS AND DISCUSSION

Distribution of Oxprenolol HCl Using SEM

The focus of our research was to evaluate the drug distribution within hydrogels in order to more effectively interpret drug transport studies. The drug distribution results from FTIR microscopy were compared to the standard method of SEM to evaluate the relative accuracy and effectiveness of each technique for this application.

The results from the analysis of oxyprenolol HCl on the surface of crosslinked PAA are summarized in Table I. The positions analyzed across the PAA surface (denoted by x) were located 50, 150, 250, 450, and 650 mm from the polymer edge, respectively. The chlorine concentration varied with position, as noted from the change from 4 to 67 wt % across the surface. This variability was attributed to salt formation resulting from dehydration of the hydrogel.

The two major disadvantages of SEM as an elemental analysis technique to evaluate solute distribution in hydrogels are the necessity of a dehydrated sample and the presence of an x-ray emitting atom in the drug. The collapsed polymer structure following the dehydration step does not represent the swollen sample, through which drug transport occurs. One important aspect of the distribution not discussed by Lee (6,7) was the solute concentration profile of the polymer interior, which verifies drug migration upon drying that causes a burst effect upon release. This technique also imparts radiation damage to the polymer (or charging), as indicated by the dark spots along the centerline of the micrograph (14). Furthermore, the dissociation of the chlorine label from the parent drug molecule prevented detection of the drug substance by SEM.

These results clearly demonstrated that SEM was not a reliable technique for evaluation of drug or protein distribution within hydrogels. Most importantly, the requirement for a dry specimen further deviated from the intended purpose of this investigation. Therefore, SEM was deemed an insufficient technique for evaluating solute distribution in hydrogels. These problems do not appear when using FTIR microscopy, which is a technique capable of detecting a variety of functional groups within the drug substance that do not dissociate.

Distribution of Oxprenolol HCl Using FTIR Microscopy

Three-dimensional surface plots were created by monitoring the intensity of the C-H in-ring bending band (1251 cm^{-1}) representing oxprenolol HCl across the two-dimensional cryotomed section. Each area that was mapped across the polymer section provided an IR spectrum of that sample (Figure 1). To separate the solute information from the polymer, it was essential that the solute contain a distinctive functional group.

The peaks located within the region from 1000 to 2000 cm^{-1} are listed in Table II by the wavenumber and functional group vibration. The vibrational frequencies of PAA in the region from 1000 to 2000 cm^{-1} include the carboxyl stretch (1738, 1718, and 1652 cm^{-1}), the carboxylate anion stretch (1550, 1400, and 1209 cm^{-1}), C-O dimer stretch (1272 cm^{-1}), and the C-C(=O)-C stretch (1172 cm^{-1}). The remaining IR vibrations are associated with the structure of oxprenolol HCl, which provide many options for choosing the characteristic peak.

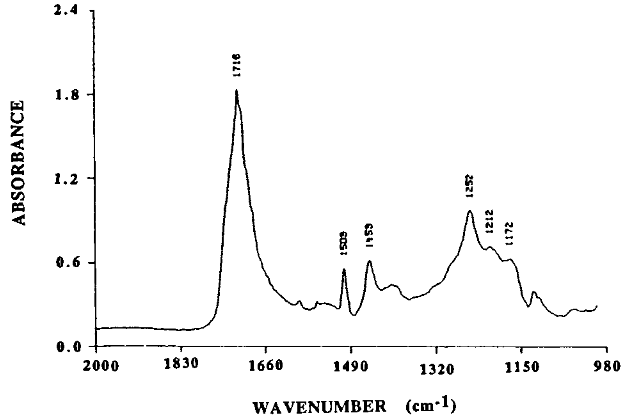

Fig. 1. Expanded FTIR spectrum of an apertured 4 μm PAA section containing oxprenolol HCl placed on a BaF$_2$ crystal. Aperture: 160 by 40 μm; 64 scans at 4 cm^{-1} resolution.

The selection of the C-H in-ring in-plane stretch at 1251 cm^{-1} was based on the ease of integration of this peak, necessary to determine the absorption intensity as a function of position within the hydrogel section. The spectral region from 2000 to 1000 cm^{-1} is shown in Figure 2 as a function of position for PAA solution loaded with oxprenolol HCl at pH 5.3. The x position was rastered 200 μm for each spectrum taken, and the y coordinate remained constant. There was no observable change in the spectrum as a function of location within the section.

Effect of Varying pH on Solute Distribution

The solute loading pH was varied in order to study its affect on the distribution of oxprenolol HCl loaded into PAA hydrogels. The pH values selected spanned from the non-ionized state to the completely ionized form of PAA, i.e. from 3.2 to 3.7, 4.3, 4.7, 5.3, 5.8 and 7.0.

The two-dimensional concentration profile (or map) consists of the integrated C-H in-ring bending peak intensity at 1251 cm^{-1} ratioed to the CH$_2$ stretching peak representing the polymer located consistently at 2940 cm^{-1} as a function

Table I. SEM Elemental Analysis for Oxprenolol HCl Solution Loaded into PAA Samples Containing 0.01 mol EGDMA/mol AA with pH 5 Buffered Solution, at Positions across the Polymer Surface of 5 (x_1), 10 (x_2), 50 (x_3), 150 (x_4), 150 (x_5), 200 (x_6), 290 (x_7), and 295 μm (x_8) from the Left Edge

Element	Weight percentage of element analyzed							
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8
P	36.64	38.52	45.61	45.19	6.13	41.78	8.54	6.12
Cl	6.72	7.31	8.80	10.08	67.37	3.77	49.17	64.61
Al	2.90	3.63	0	1.66	0.78	0	0.53	1.26
Ca	0	0.36	0	0	1.32	0.15	0	0.81
Na	53.74	50.18	45.49	43.08	24.42	54.30	41.76	27.20

Table II. IR Wavenumber of Vibrational Mode for Functional Groups Present in Oxprenolol HCl and PAA

Wavenumber (cm^{-1})	Functional vibration
1738	C = O stretch
1718	C = Ostretch
1695	C = Oconjugated stretch
1652	C = Ostretch, internal H-bonded
1550	$C(=O)-O^-$ asymmetric stretch
1503	C = C conjugated ring stretch
1453	C = C conjugated ring stretch
1453	$O-CH_2$ deformation
1400	$C(=O)-O^-$ symmetric stretch
1272	C = O stretch, dimer
1251	C–H in-ring in-plane bending
1209	$C-C(=O)-O^-$ stretch
1209	O–H bend
1183	O–C = C asymmetric stretch
1172	C–C(= O)–C stretch and bend
997	C–H out of plane stretch

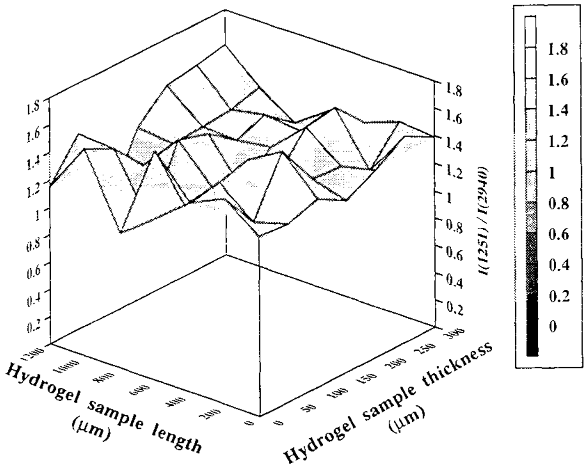

Fig. 3. Intensity map of C-H in-ring in-plane bending mode at 1251 cm^1 ratioed to the CH_2 stretching vibration at 2940 cm^{-1} for the cryotomed PAA section crosslinked with 0.01 mol EGDMA/mol AA containing oxprenolol HCl solution loaded in pH 3.7 at 37°C.

of the hydrogel sample thickness (x-axis) and the hydrogel sample length (y-axis) across a PAA cryotomed section. The effect of a porous hydrogel on the IR spectrum is a large variation and reduction in the peak intensity values obtained by integration of the peak area. Elimination of the porous effect was accomplished by ratioing the drug peak intensity to an independent polymer peak. The concentration profile for oxprenolol HCl in PAA solution loaded with pH 3.7 media is shown in Figure 3. The drug concentration was found to be essentially constant throughout the thickness of the polymer. The relative variation in absorption intensity through the interior of the hydrogel, was determined to be 12.5 % for this particular sample section. Clearly, FTIR microscopy is a more sensitive technique than SEM for evaluating the distribution of solutes in hydrated polymer samples. Using this same procedure, oxprenolol HCl was distributed evenly throughout the polymer interior following solution loading at pH 3.2 and 7.0 (Figure 4), respectively.

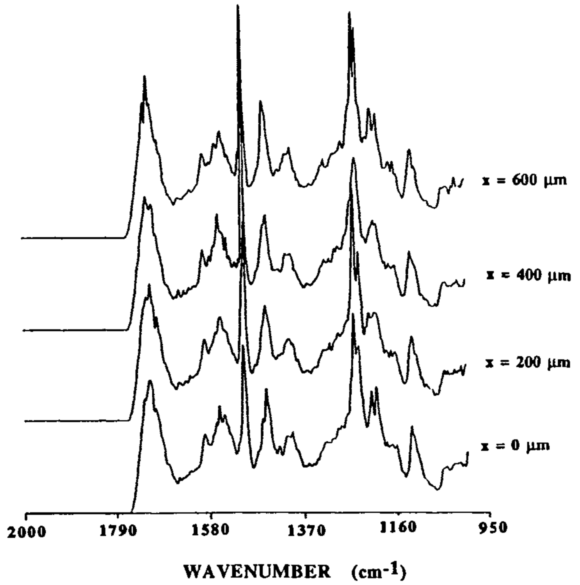

Fig. 2. FTIR spectrum as a function of position in microscope that was rastered by 200 µm increments for PAA crosslinked with 0.01 mol EGMDA/mol AA solution loaded in oxprenolol HCl at pH 5.3 placed on a BaF_4 crystal. Aperture: 160 by 40 µm; 64 scans at 4 cm^{-1} resolution.

Effect of pH on Structural Characteristics

The pH gradient studies were insightful for the interpretation of structural changes that occurred in the drug/ polymer system as they affected the solute distribution within the hydrogel. Several distinguishable peaks located in the 1718 and 1251 cm^{-1} regions are observed as the pH increased from 3.2 to 3.7 and 4.3. This feature caused difficulty in the integration procedure for evaluation of the peak intensities, which required two minima to define the baseline of the integrated region. Figure 5 was designed as a progressive plot of the IR spectrum from 1800 to 950 cm^{-1} for the pH values of 4.7, 5.3 and 7.0. It is quite evident that the

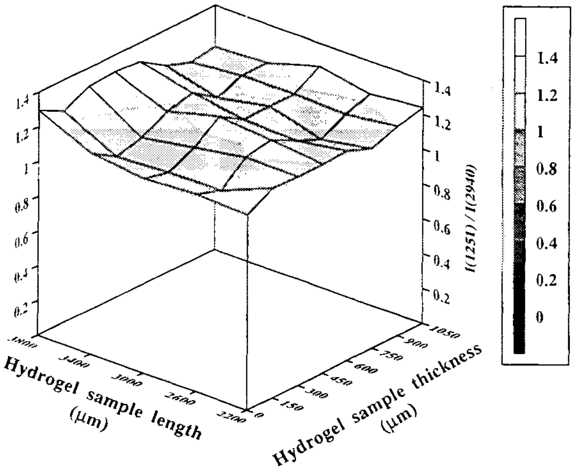

Fig. 4. Intensity map of C-H in-ring in-plane bending mode at 1251 cm^1 ratioed to the CH_2 stretching vibration at 2940 cm^{-1} for the cryotomed PAA section crosslinked with 0.1 mol EGDMA/mol AA containing oxprenolol HCl solution loaded in pH 7.0 at 37°C.

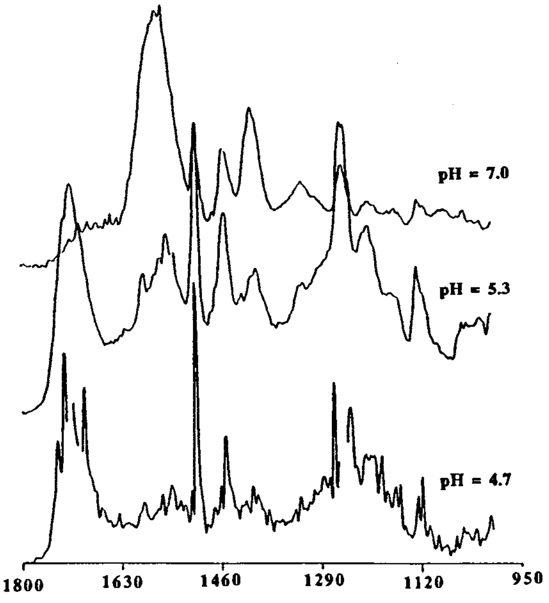

Fig. 5. FTIR spectrum as a function of pH for PAA crosslinked with 0.01 mol EGDMA/mol AA that was solution loaded in oxprenolol HCl at pH 4.7, 5.3, and 7.0 placed on a BaF_2 crystal. Aperture: 160 by 40 μm; 64 scans at 4 cm^{-1} resolution.

carboxyl groups in PAA were completely ionized by the solution pH of 7.0. This conclusion was established from the spectral evidence, which included the disappearance of the carboxyl stretching vibration at 1718 cm^{-1}, and the increased intensities of the asymmetric and symmetric carboxylate anionic form at 1558 and 1407 cm^{-1}.

Further analysis of the IR peak maxima summarized in Table III revealed that the number of separately distinguishable peaks in the carboxyl region increased from one (1716 cm^{-1}) to two (1720, 1735 cm^{-1}), (1712, 1731 cm^{-1}) and three (1718, 1726, 1739 cm^{-1}) as the solution pH increased from 3.2 to 3.7 to 4.3 and 4.7. This same region contained only one peak at pH 5.8 and no carbonyl peaks at pH 7.0. The asymmetric stretching vibration of the carboxylate anion appeared at a solution loading pH of 4.7, located at 1545 cm^{-1}. The symmetric stretching peak of the carboxylate anion appeared in the spectrum of solution pH 5.3 (1399 cm^{-1}). The presence of both the asymmetric and symmetric stretching vibrations at 1550 and 1400 cm^{-1}, respectively, specifically identifies the $C(=O)\text{-}O^-$ functional group. A full analysis of the carbonyl region of the mapping spectra indicated the presence of several small peaks, and therefore PAA was partially ionized under these conditions.

Effect of Copolymer Composition on Drug Distribution

The effect of copolymer composition on the solute distribution was investigated by comparing the results obtained for PAA homopolymer with P(AA-co-HEMA) copolymer containing 40 mol % AA and 60 mol % HEMA and 0.010 mol EGDMA/mol monomer, both loaded with oxprenolol · HCl in pH 7 buffer. The concentration profile of oxprenolol · HCl determined from the C-H in-ring bending peak was affected by the decreased hydrophilicity of the copolymer, as evidenced by the lower intensity value. Analysis of the IR peak maxima summary for oxprenolol HCl distributed in PAA and P(AA-co-HEMA) indicated the major difference between the spectra was due to the presence of the carboxyl stretching vibration in the copolymer spectrum, located at 1724 cm^{-1} (Table III).

CONCLUSIONS

The results from this investigation demonstrated that FTIR microscopy accurately determined the drug distributions in hydrogels, requiring only that the drug contain a distinctive IR vibrational or rotational functional group from the polymer. These studies verified that oxprenolol HCl was evenly distributed within PAA and P(AA-co-HEMA) hydro-

Table III. FTIR Peak Summary of Oxprenolol HCl Loaded in PAA and P(AA-co-HEMA) Hydrogels as a Function of pH

	IR peak locations (cm^{-1}) as a function of pH						
	PAA hydrogels						P(AA-co-HEMA) hydrogel
Functional vibration	3.2	3.7	4.3	4.7	5.3	7.0	7.0
C=O stretch				1739			
		1735	1731	1726			
	1716	1720	1712	1718	1716		
C=O conjugated stretch	1697	1695	1694	1692			
				1592	1590		1569
					1563	1568	1724
$C(=O)\text{-}O^-$ asymmetric				1545	1553	1558	1558
C=C conjugated ring stretch	1504	1503	1503	1503	1502	1503	1503
$O\text{-}CH_2$ deformation	1453	1453	1453	1453	1453	1456	1451
$C(=O)\text{-}O^-$ symmetric stretch					1399	1407	1406
					1319	1322	
				1287			
C-O stretch (dimer)				1278			
C-H in-ring in-plane bending	1253	1257	1253	1253[a]	1259	1255	1254
$C\text{-}C(=O)\text{-}O^-$ stretch		1209		1212	1214	1209	1207
				overabsorbed			peak

gels upon solution loading at pH values ranging from 3.2 to 7.0. An added feature of FTIR microscopy for evaluation of solute (drug, protein, peptide, or hormone) distribution is that a unique spectrum is obtained at each mapping location, allowing interpretation of structural changes within the solute/polymer system.

A comparison of the solute distribution results obtained both from FTIR microscopy and SEM proved the former was more sensitive to concentration variations, provided a reduction in the error, increased reliability, and most importantly, did not alter the hydrogen state by exchanging the solvent as was necessary for SEM in the critical point drying process.

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