Characterization of Hydroxypropylcellulose–Indomethacin Grafts as a Function of Molecular Weight

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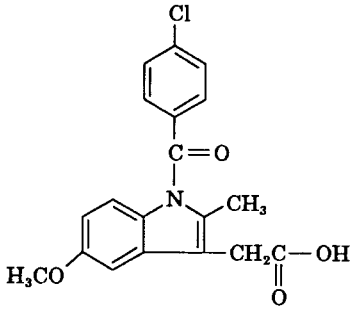
SYNOPSIS

The physical characterization of hydroxypropylcellulose–indomethacin grafts (HPC–IND) is described. Using size-exclusion chromatography (SEC) and serial differential refractive index (dRI) and ultraviolet spectrophotometric (UV) detection methods, it was possible to characterize the chemical substitution of the HPC–IND graft. Using the acid chloride of IND as the reactive intermediate, the HPC–IND graft was synthesized. The amount of IND grafted onto the polymer as a function of the HPC molecular weight (MW) was then quantitatively estimated. It was found that the substitution of IND onto the HPC was not uniform, representing chemical heterogeneity of the first kind. Increasingly higher substitution (of IND to HPC) was observed as the MW decreased (< 250,000 Daltons) for the HPC backbone. Lower, more uniform substitution was observed for the higher molecular weight regions of the HPC (> 250,000 Daltons). Possible contributing factors affecting the observed nonuniform substitution include (1) the chain coiling of the HPC in solution, thus limiting the availability of the pendant hydroxyl groups, (2) nonuniform substitution of the hydroxypropyl moiety onto the cellulose backbone, and (3) steric effects resulting from the incorporation of the bulky aromatic drug molecule onto the backbone HPC.

INTRODUCTION

The study of polymeric-based prodrugs to control or target the release of drugs has been under investigation for some time.[1-7] Drug–polymer grafts, whereby the polymer backbone serves to carry the drug and potentially target it to a specific site or control the release rate of the drug, represent areas of interest. Numerous reviews in these areas demonstrate the desire to deliver drugs to the body in this manner.[8-12] However, most approach the problem empirically, ignoring possible variations within specific molecular weight components. Although this may be sufficient for the animal models used to date,[13] thorough characterizations of the polymer–drug grafts would be necessary if applications in humans were proposed. If polymeric carriers or prodrug systems are to be rationally developed and exploited, the physical chemical characterization of these systems must be considered in more depth. This has prompted our investigation with a system utilizing indomethacin (IND), grafted onto a model polymer, hydroxypropylcellulose (HPC). IND is a nonsteroidal anti-inflammatory drug with the following structure:



Our interest was in characterizing the substitution of IND onto the polymer backbone, HPC. Using size-exclusion chromatography (SEC) and multiple means of detection in series (differential refractive index [dRI] and spectrophotometric [UV]), the goals of the study were to assess the success of the reaction and estimate the degree of IND substitution

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onto the backbone polymer as a function of the molecular weight of the HPC polymer.

EXPERIMENTAL

Materials

Thionyl chloride (Sigma) was distilled upon receipt and stored under argon. Indomethacin (Sigma) was dried upon receipt *in vacuo* at room temperature over P_2O_5 and stored under the same conditions. Hydroxypropylcellulose (Klucel EF, $\bar{M}_n$ = 57,000) was supplied courtesy of Hercules Inc. and was dried upon receipt *in vacuo* at room temperature over P_2O_5, and stored under the same conditions. Triethylamine (Sigma) was distilled upon receipt and stored under argon. Anhydrous reagent grade ether (J. T. Baker Co.) was used as received. Tetrahydrofuran (EM Science) used for synthesis was distilled over calcium anhydride and stored under argon. Tetrahydrofuran (LC/CC grade) used for size-exclusion chromatography (EM Science) contains 250 ppm butylated hydroxytoluene (BHT) as a stabilizer and was used as received.

Methods

The synthesis of the HPC–IND polymeric prodrug utilized the acid chloride intermediate of IND. The IND, 0.980 g, was reacted with an excess (25 mL) of $SOCl_2$ in a 50 mL round-bottom flask fitted with a condenser and drying tube (using $CaSO_4$) with stirring for 24 h. The excess $SOCl_2$, SO_2, and HCl were removed under partial vacuum at 0°C. The indomethacin acid chloride remaining was then reconstituted in anhydrous distilled tetrahydrofuran (THF). This solution was added dropwise to a solution of 400 mL anhydrous distilled THF containing 5.000 g HPC at 0°C to produce a final molar ratio of 1 : 6 (IND : HPC monomer) in 425 mL anhydrous distilled THF. Triethylamine (1.06 mL) was used as a sequestering agent, and an argon purge was used throughout the duration of the reaction. After the acid chloride addition was complete, the reaction mixture was allowed to come to room temperature and additionally stirred for 24 h. Aliquots were removed from the bulk reaction vessel for later analysis. The product was then decanted from the triethylamine HCl in the reaction vessel and precipitated from solution with anhydrous ether, filtered, and washed with additional anhydrous ether. The structure was verified using SEC and Fourier transform infrared spectroscopy.

IR (KBr) Results

Polymer graft:	ester carbonyl (1730 cm^{-1}) amide carbonyl (1690 cm^{-1}, 1540 cm^{-1}) C—Cl (745 cm^{-1})
Physical mixture:	acid carbonyl (1700 cm^{-1}) amide carbonyl (1690 cm^{-1}, 1540 cm^{-1}) C—Cl (745 cm^{-1})

Characterization

Characterization was performed using nonaqueous SEC with BHT-stabilized THF as the mobile phase. All chromatography utilized a Waters 510 pump and a dual detector apparatus consisting of a Waters 410 differential refractive index detector, a Waters 490 programmable ultraviolet detector in series, and five Ultrastyragel columns (10^4, 10^3, 500, and two 100 Å). Analysis for relative molecular weights and distribution reports for calculation of the degree of substitution of IND on HPC was performed using a Waters 840 Chromatography Station (Version 6.0). The calibration curve for the dRI detector was fit using a third-order polynomial, and the coefficients of the fitted curve and the correlation coefficient is shown in Figure 1. Spectrophotometric (UV) analysis was conducted at 265 nm, generating a similar third-order calibration curve that is depicted in Figure 2.

Calibration of the column set for response to the HPC was performed using dRI detection and for IND was performed using both dRI and UV detection using physical mixtures of them in the same molar ratio as that of the reaction vessel (1 : 6 molar,

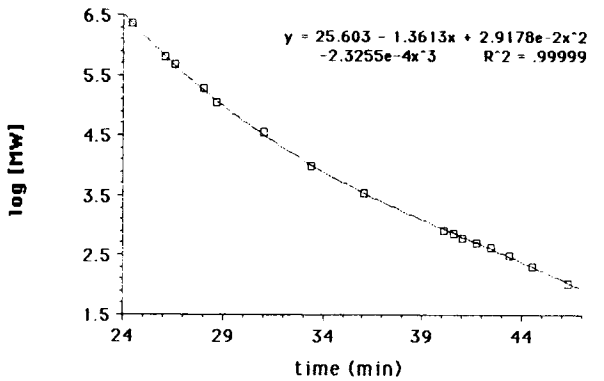

Figure 1 Polystyrene calibration curve for dRI detector; 16 polystyrene standards molecular weight 2,300,000 to 106.

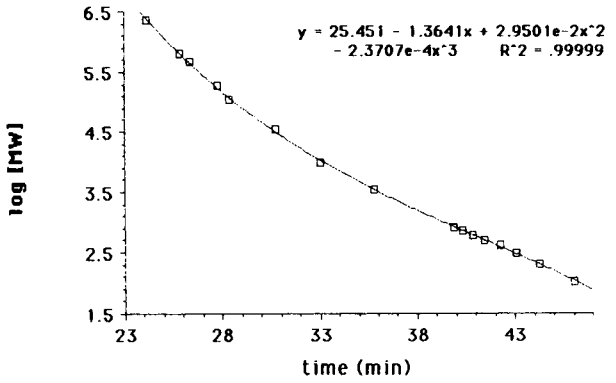

Figure 2 Polystyrene calibration curve for UV detector (λ = 265 nm); 16 polystyrene standards molecular weight 2,300,000 to 106.

IND : HPC). A 0.300% solution was made, filtered through 0.45 μ nylon 6,6 filters, and injections of varying amounts (50–300 μL) were used to obtain a calibration curve for the individual IND and HPC components on the dRI detector and a Beer's law curve for the IND using the UV detector (λ = 265 nm). Table I shows the calibration constants for the HPC and IND components used and their correlation coefficients.

DATA ANALYSIS METHOD

Since the area under the curve represents the total mass of material constituting that peak, any fractional slice of this area represents a fraction corresponding to that slice's percent of the total peak area. In mathematical form, this may be represented as

$$^{T}AUC \propto \sum_{i=1}^{N} A_i \qquad (1)$$

where ^{T}AUC = total area under the curve for dRI detector (mV s); N = number of slices used over sample elution profile (= 25); and A_i = area under the slice, i (mV s). This may then be related to the total amount under the slice by

$$Q_i^T = A_i(r) \qquad (2)$$

where Q_i^T = total amount for slice, i(g) and r = calibration constant for the differential refractive index (g/mV s). A similar equation can be developed for the UV response for the IND present in the eluent

$$^{I}AUC \propto \sum_{i=1}^{N} A_i \qquad (3)$$

where ^{I}AUC = total area under the curve for UV detector (λ = 265 nm, mV s); N = number of slices used over sample elution curve (= 25); A_i = area under the slice, i (mV s); and, similar to the above equation, the amount of IND for that slice may be determined by

$$Q_i^I = A_i(\epsilon) \qquad (4)$$

where Q_i^I = amount of IND at slice, i(g) and ϵ = corrected extinction coefficient for IND (λ = 265 nm, in g/mV s). This equation is specific for the IND since the HPC is UV transparent at the 265 nm wavelength used. The amount of IND per polymer chain is a function of the molecular weight (or slice number) of that chain. For a given slice i, the mass of HPC (Q_i^P) that the IND in the slice is bound to may be expressed as

$$Q_i^P = Q_i^T - Q_i^I \qquad (5)$$

The rationale for the use of eq. (5) is as follows. The dRI detector is a universal detector and therefore responds to the total mass of material at a given elution volume (or slice). The UV response, however, is specific for the mass of IND present at that same elution volume (or slice) as shown in Figure 3. Using the quantities for the IND obtained from the calibration curve, the average number of mol bound at each relative molecular weight slice may be calculated using

$$\frac{Q_i^I}{(357.8\ \text{g/mol})} = n_i^I \qquad (6)$$

where 357.8 g/mol = molecular weight of IND and n_i^I = moles of IND at slice i. Similarly, the quantity

Table I Calibration Constants and the Correlation Coefficients for Starting Materials

Starting Material	Calibration Constant (g/mV s)	Correlation Coefficient r^2
HPC	3.6483×10^{-8}	0.999
IND	5.3557×10^{-10}	0.992

The characterization of the polymer graft solutions were done using concentrations of 0.515 and 1.40%, filtered through 0.45 μ nylon 6,6 filters, and 100 μL injections were made for all solutions.

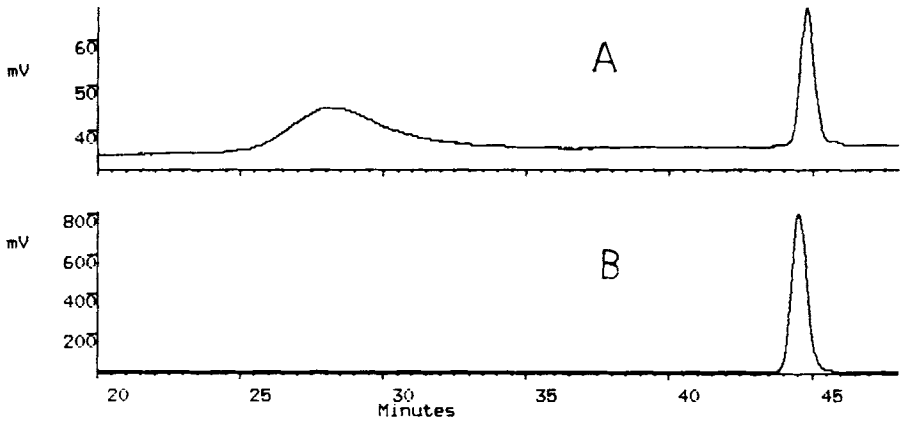

Figure 3 Chromatogram of **physical mixture** (1 : 6 molar ratio). (a) Differential refractive index, (b) UV spectroscopy, λ = 265 nm.

of HPC to which the IND is bound at relative molecular weight slice may be converted to the number of moles of HPC (expressed as monomer) using

$$\frac{Q_i^P}{(336.0\ \text{g/mol})} = n_i^P \tag{7}$$

where 336.0 g/mol = molecular weight of HPC monomer and n_i^P = mol of HPC monomer at slice i. Having these molar quantities, the ratio was taken and the level of IND substitution determined for specific molecular weight slices of the HPC distribution shown by

$$\frac{n_i^P}{n_i^I} = \frac{\#\ \text{HPC monomers}}{\text{IND}} = L \tag{8}$$

This parameter, L, is the average number of repeat units between each IND molecule and technically represents the **inverse** of the level of substitution. This method of representation was chosen since fractional substitution of the drug molecule to each monomer is not physically possible and, therefore, it represents a more intuitively acceptable characterization parameter.

RESULTS AND DISCUSSION

After the final reaction of the acid chloride intermediate of IND with HPC, aliquots were removed from the bulk reaction vessel solution and SEC chromatograms generated (Fig. 4). This was done prior to precipitation, thus maintaining an overall mass balance for the IND and HPC components and permitting an evaluation of the extent of reaction. Using the calibration curves for IND, the amount of free IND (or that which reacted with the residual monomer), ${}^{\text{IND}}Q_{\text{Free}}$, was determined. Knowing the total amount of IND introduced, ${}^{\text{IND}}Q_{\text{Total}}$, the amount that was successfully esterified to the HPC ${}^{\text{IND}}$, Q_{Bound} could be evaluated using

$${}^{\text{IND}}Q_{\text{Bound}} = {}^{\text{IND}}Q_{\text{Total}} - {}^{\text{IND}}Q_{\text{Free}} \tag{9}$$

In addition to allowing the extent of reaction to be evaluated, since mass balance was maintained, any changes in the extinction coefficient for the graft (ϵ) could also be determined. Using

$$A = \epsilon b C_{\text{molar}} \tag{10}$$

for calculation of the extinction coefficient, and using mV s as an electronic response for absorbance area, A, the cells path length (1 cm), b, and molar concentration (as IND), C_{molar} permits the calculation of the extinction coefficient ϵ for the IND bound to the graft:

$$\epsilon_{\text{graft}} = \frac{A(\mu\text{V s})}{b(\text{cm})C(\text{mol L}^{-1})} \tag{11}$$

which yields

$$\epsilon_{\text{graft}} = \frac{5.78 \times 10^7\ \text{mV s}}{(5.85 \times 10^{-3}\ \text{mol L}^{-1})(1\ \text{cm})}$$

$$= 9.89 \times 10^9\ \frac{\text{mV s L}}{\text{mol cm}} \tag{12}$$

Similarly, the extinction coefficient for the pure IND was calculated to be

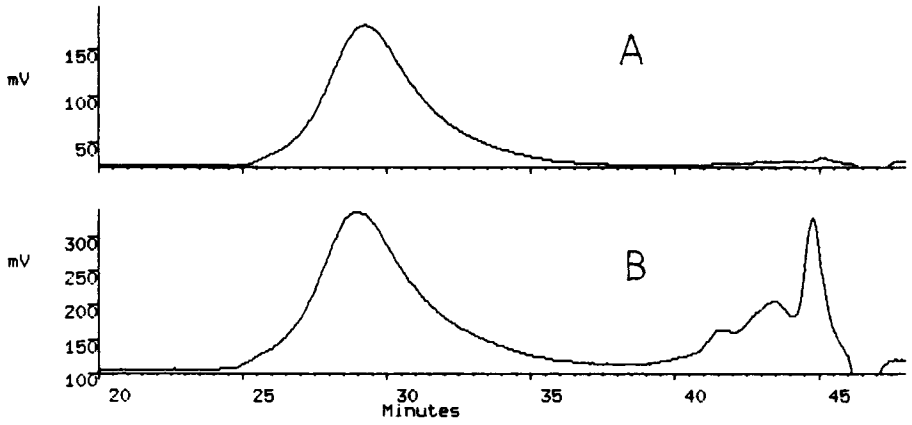

Figure 4 Chromatogram of **unprecipitated graft** (1 : 6 molar ratio). (a) Differential refractive index, (b) UV spectroscopy, λ = 265 nm.

$$\epsilon_{free} = 6.34 \times 10^{10} \frac{\text{mV s L}}{\text{mol cm}} \qquad (13)$$

Taking the ratio of the free and bound extinction coefficients for IND gives the factor by which the extinction coefficient has apparently changed after the reaction with the HPC polymer:

$$\frac{\epsilon_{free}}{\epsilon_{graft}} = 6.41 \pm 0.28 (\pm SD, N = 4) \qquad (14)$$

This change is substantial, and although changes in absorptivity are known to occur due to conformational changes during polymerization with amino acids,[14] they have not been observed in other drug carrier systems using drugs grafted to a polymer backbone.[15]

Using this change as a correction factor, the substitution of IND as a function of the molecular weight of the HPC backbone could be determined accurately. This factor also appears to corroborate results[16] for the hydrolysis of this graft in an aqueous environment. For a given decrease in the area under the curve (AUC) over time, from hydrolysis of the graft, a much larger AUC correspondingly appears in the low molecular weight (free indomethacin) region of the SEC chromatogram. This was ultimately attributed to the change in extinction coefficient as described herein. Because of the low level of IND substitution and the nonspecific nature of the refractive index response, the dRI response sensitivity for the HPC polymer was assumed to be the same as that of the HPC–IND graft.

After the IND–HPC polymer graft was precipitated from solution, solutions in THF were again prepared and filtered and SEC chromatograms collected (Fig. 5). Utilizing the polystyrene calibration curve for relative molecular weights, molecular weight distributions were calculated and slice reports generated for chromatograms produced from the grafts using both the dRI and UV detectors (λ = 265 nm). Using the method outlined under Data Analysis, the parameter L, (the average number of HPC monomer units between each IND molecule) was determined for the precipitated product. This parameter, L, is of particular interest since it can be used to characterize the graft's chemical heterogeneity of the first kind (using the terminology of Barth[17]) for the addition of IND onto HPC polymer of relative chain lengths, i. This parameter is expected to be polymer, solvent, and process specific and will infer information on the polymer conformation for the solutions in which the graft reaction is carried out. Using this parameter, substitution as a function of log molecular weight (Fig. 6) was found to be nonuniform over the molecular weight distribution of the backbone polymer, HPC. Converting the log molecular weight axis to molecular weight illustrates two distinct regions (Fig. 7) with a break at the relative molecular weight of approximately 250,000 Daltons. Region I shows a steadily decreasing load as the molecular weight increases, and region II shows a lower and relatively more uniform substitution for the IND onto the HPC backbone. These results suggest that the degree of IND loading directly onto the HPC polymer backbone is sensitive to the polymer molecular weight.

For small oligomers where substantial coiling or self-association is absent, greater availability of the reactive hydroxyl groups is possible. As molecular weight increases, chain coiling may inhibit hydroxyl reactivity in direct proportion to the backbone size

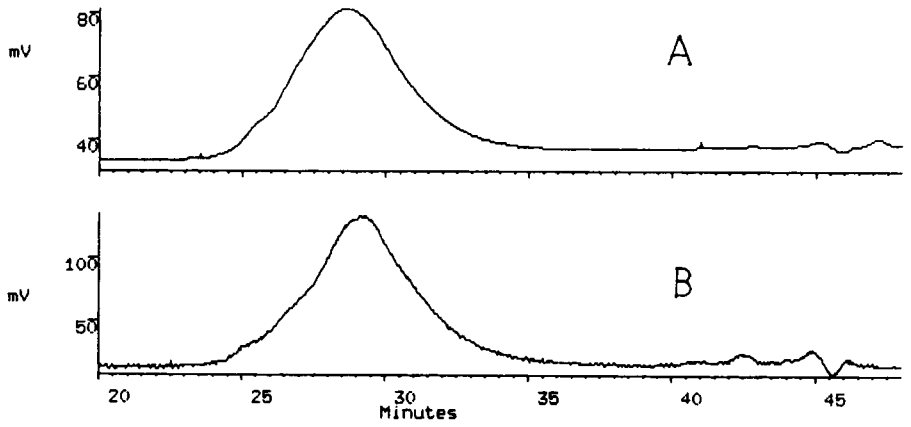

Figure 5 Chromatogram of **precipitated graft.** (a) Differential refractive index; (b) UV spectroscopy, λ = 265 nm.

(Region I, Fig. 7). For very large polymer chains, the degree of coiling is maximized and further coiling is offset by the greater number of total hydroxyl groups present. This last scenario would result in a more apparently uniform substitution pattern (Region II, Fig. 7). Another contributing factor lies in the hydroxypropyl derivatization process that is used to form the HPC. One report by Wirick and Waldeman[18] indicates that the hydroxypropylation as a function of the molecular weight of the backbone cellulose material is itself not a uniform process. They indicated that the derivatization process placing hydroxypropyl groups on the cellulose backbone preferentially occurred on the lower molecular weight portions of the distribution and that gradually lower molar degrees of substitution occurred as

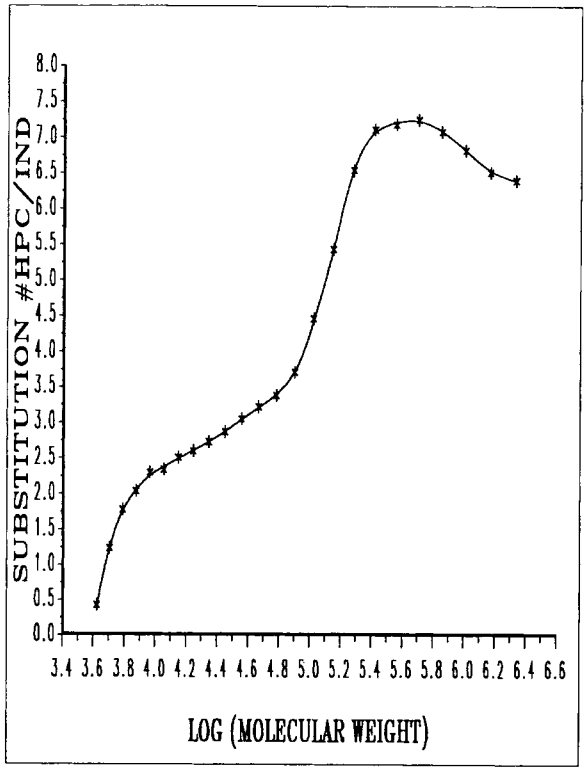

Figure 6 Degree of substitution (avg. no HPC/IND) vs. Log(molecular weight).

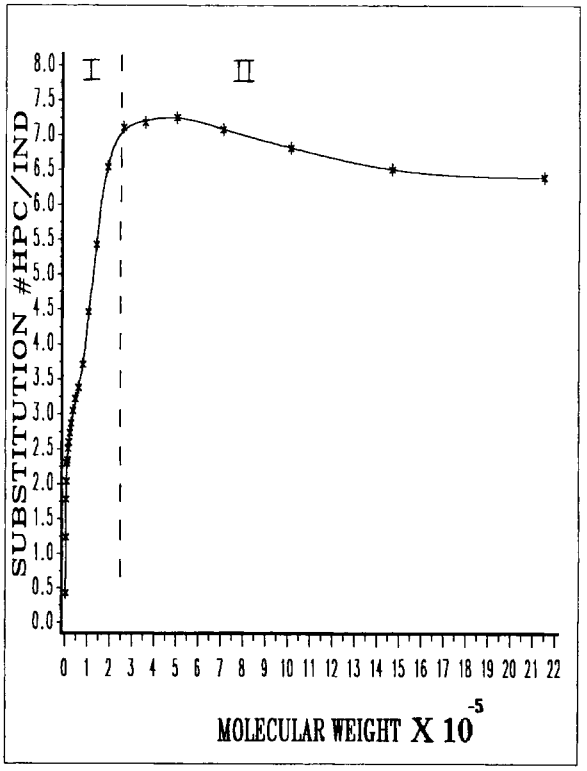

Figure 7 Degree of substitution (avg. no HPC/IND) vs. molecular weight.

the molecular weight increased. This could easily contribute to decrease the accessibility of the HPC hydroxyl groups as the molecular weight increases. In addition to those already mentioned, the short distance between the site for the esterification (acetyl carboxy) and the rather bulky structure of the drug molecule may greatly influence the ability of the drug to have uniform accessibility to substitute onto the backbone of the HPC. It should be pointed out that this graft was produced in the absence of any spacer moiety between the drug molecule and the backbone polymer that might serve to facilitate the uniformity of the IND substitution.

CONCLUSIONS

From our results, the nonuniform substitution of indomethacin as a function of the molecular weight of the HPC backbone indicates chemical heterogeneity of the first kind for this polymer graft. The cause for this behavior is hypothesized to be due to a combination of factors elucidated in the discussion. These include (1) chain coiling as the molecular weight of the backbone polymer, HPC increases; (2) nonuniform substitution of the hydroxypropyl groups as a function of the molecular weight of the polymer backbone; and (3) the short distance between the polymer backbone and the bulky structure of the IND molecule. The coiling of the polymer molecule may inhibit the drug molecule from penetrating the chains of the HPC backbone. This would lead to a decrease in the level of substitution as the molecular weight of the chains increases substantially. The use of spacer arms was not undertaken in this study but would allow for this last point to be addressed. Through the use of straight chain spacers in future work, more uniform access to the higher molecular weight components of the polymer may facilitate better uniformity of substitution.

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