Evaluation of a Statistical Model for the Formation of Poly [Acryloyl Hydroxyethyl Starch] Microspheres

L. K. Huang,[1] R. C. Mehta,[2] and P. P. DeLuca[3,4]

Received January 24, 1997; accepted February 4, 1997

Purpose. To characterize the network structure of Poly(Acryloyl Hydroxyethyl Starch) (Ac-HES) microspheres and test the theoretical model and the hypothesis that the rate of swelling of microspheres is inversely related to the extent of crosslinking.

Methods. Microspheres were prepared with varying degrees of derivatization (DD) and molar ratios (MR) and subjected to the characterization of matrix structure by dynamic and equlilibrium swelling studies utilizing direct microscopic observation and the Flory-Rehner equation. The dependence of average molecular weight between crosslinking Mc, on DD and MR were compared to test the validity of the model.

Results. Study of the dependence of Mc on the microspheres preparation parameters, DD and MR, showed that at constant MR, the Mc decreased with DD, while at constant DD, the Mc initially decreased with MR to a minimum, and then increased with MR, complying with the model prediction. Dynamic swelling of microspheres showed a monotonical increase to equilibrium size featured by two time variables, Tp and Teq, that were dependent on Mc; this permitted a conceptual view of the general structure of the Ac-HES microspheres. The Mc, which was more accurately determined by the weight method (as opposed to volume method), was independent of the size of microspheres although there was evidence of variation among particles within a batch.

Conclusions. The results validated the model in describing the polymerization/crosslinking reaction of the Ac-HES microsphere system and suggested that Mc is the principal factor in controlling release.

KEY WORDS: Flory-Rehner model; dynamic and equilibrium swelling; molecular weight between crosslinks (Mc); degree of derivatization(DD); molar ratio (MR).

INTRODUCTION

Crosslinking polymerization of acryloyl hydroxyethyl starch with vinyl monomers provide biodegradable poly(acryloyl hydroxyethyl starch) (Ac-HES) microspheres with porous, hydrophilic matrix (1,2). The pores and channels in these microspheres are well suited for the incorporation and sustained release of hydrophilic macromolecules such as proteins and oligonucleotides. Release of drugs from Ac-HES microspheres depend on the rate of swelling and the pore size of the hydrated matrix (2) which are in-turn dependent on the extent of matrix crosslinking; thus matrix with high crosslinking would have smaller pores than that with low crosslinking. The extent of crosslinking can be characterized by the molecular weight between the points of crosslinks (Mc). Therefore, Mc is an important parameter that governs the performance of the microspheres as a drug delivery system.

Crosslinking polymerization reactions are complicated due to a multitude of parameters that affect the properties of the microspheres. A mathematical model describing the effects of a number of parameters was developed and the effects of processing variables on the value of Mc was predicted (1). Variables predicted to have the greatest effect on Mc were the degree of derivatization, (DD), of the hydroxyethyl starch polymer, i.e. the number of vinyl groups attached to the polymer, and the molar ratio, (MR), of the polymerizing agents, Ac-HES and acrylamide. According to the model, Mc decreased with an increase in MR, reached a minimum and then increased with further increase in MR, thereby giving a concave-up function of MR in the 10^{-3} to 10^{4} range. At MR smaller than that which gave minimum Mc (MRmMc), Mc was inversely dependent on the DD and at MR larger than MRmMc, Mc was independent of DD.

The objective of this work was to evaluate the mathematical model for the effect of MR and DD on Mc. The preparation method of microspheres was that described by DeLuca and Rypacek (2). The characterization included the determination of Mc, by equilibrium and dynamic swelling techniques and the assessment of crosslinking as a function of DD. This evaluation was performed to test the hypothesis that the rate of swelling of microspheres is inversely related to the extent of crosslinking.

METHODS

Derivatization of HES Polymer and Determination of the Degree of Derivatization

5 g of hydroxyethyl starch (HES, "Hespan", Wilmington, DE) were dissolved in 15 ml of dimethylacetamide (DMAA) at room temperature and transferred to a jacketed reactor cooled to 0–5°C and stirred at 400 rpm with a stainless steel mixer. Distilled acryloyl chloride and an equimolar amount of triethylamine (TEA) were added at a rate of 1.0 ml/min by an automatic syringe pump. The reaction mixture was precipitated in 200 ml acetone (0–5°C). The precipitate was dissolved in 15 ml of DMAA and again precipitated in cooled acetone. The dissolution and precipitation steps were repeated three times. The final precipitate was dissolved in double-distilled water and transferred to Spectrapor dialysis tubing with molecular weight cutoff of 12,000 to 14,000 daltons and dialyzed against double-distilled water with frequent change of water for 48 hours. The dialysed solution was concentrated using a rotary evaporator and freeze-dried. The Ac-HES polymer recovered from freeze-drying was stored in a dessicator at <5°C. Ten batches of Ac-HES polymer were derivatized by adding various amounts of acryloyl chloride together with equal moles of tertiary amine to the HES solution in DMAA. The DD was determined by proton-NMR spectroscopy as described by Lepisto *et al.* (3).

Preparation of Microspheres

The procedure for preparation of microspheres was divided into four steps.

[1] Present Address: 202, Her-Ping St., East District, Taichung City 401, Taiwan, R.O.C.

[2] ISIS Pharmaceuticals, 2292 Faraday Ave., Carlsbad, California 92008.

[3] College of Pharmacy, University of Kentucky, 907 Rose Street, Lexington, Kentucky 40536-0082.

[4] To whom correspondence should be addressed.

0724–8741/97/0400–0475$12.50/0

Preparation of Dispersed Phase

Ac-HES polymer and acrylamide monomer in the desired molar ratio were dissolved in 0.1 M phosphate buffer solution (pH 7.4) to make a 30% (w/v) solution and degassed using nitrogen at 0–5°C. The initiator, ammonium peroxidisulfate, in the amount of 3% mmole of acrylamide was added to the solution to form the dispersed phase (DP).

Preparation of Continuous Phase

A jacketed polymerization reactor was charged with 50 ml of mineral oil or heptane containing 0.4% of surfactant SO-15 (Sorbitan Sesquioleate, Arlacel 83) as the continuous phase(CP). The CP was degassed with nitrogen and cooled to 0–5°C.

Emulsification and Crosslinking Reaction

The DP was added dropwise to the CP while stirring to form an emulsion. 300 μl of accelerator, N,N,N′,N′,tetramethylethylenediamine (TEMED), was added to the emulsion to initiate the polymerization which was carried out for 30 minutes.

Washing and Collection of the Microspheres

The microsphere suspension from step 3 was poured slowly into pre-cooled hexane while sonicating to remove the CP. The microspheres were collected by centrifugation at 1000 rpm for 5 minutes, washed twice with hexane, transferred to a petri-dish and dried in a vacuum oven at room temperature until constant weight.

Characterization of the Matrix Structure of Microspheres by Mc

The estimation of Mc was based on the Flory-Rehner equation (4,5),

$$\frac{1}{Mc} = \frac{2}{Mn} - \frac{(v/v_1)[\ln(v_{2,S}) + v_{2,S} + \chi(v_{2,S})^2]}{\left[(v_{2,S})^{1/3} - \frac{v_{2,S}}{2}\right]} \quad (1)$$

where Mn is the number-average molecular weight of polymer before copolymerization; v is the specific volume of the polymer network; v_1 is the molar volume of the swelling solvent; $v_{2,S}$ is the polymer volume fraction in equilibrium swelling; and χ is the Flory-Rehner polymer-solvent interaction parameter. The estimation consists of a serial determination of $v_{2,S}$, v, and χ.

Determination of $v_{2,S}$ by Equilibrium Swelling

The polymer volume fraction at swollen state, $v_{2,S}$, could be mathematically represented by,

$$v_{2,S} = \frac{V_p}{V_s} = \frac{W_d/\rho_p}{[W_d/\rho_p + (W_s - W_d)/\rho_1]} \quad (2)$$

where V_p is the volume of polymeric matrix alone, V_s is the volume of the swollen gel, i.e., the total volume of the matrix plus the swelling solvent. W_d is the dried polymer weight, W_s the swollen gel weight, and ρ_p and ρ_1 are the density of polymer and solvent, respectively. ρ_1 is 1.0 when water is used as the swelling solvent.

$v_{2,S}$ can be determined by measuring either the volume change (volume method) or weight change (weight method) due to swelling. The volume method measures the volume of each single microsphere while the weight method measures the weight of a group of microspheres before and after swelling . The former method would present information on the variability of the degree of swelling among particles, while the latter treats the degree of swelling as a bulk property of microsphere population. To ensure the accuracy of the method, all samples of microspheres before swelling measurement were subjected to repeated extraction in water to remove any soluble fractions from the microspheres (6,7).

Volume Method

The $v_{2,S}$ of a single microsphere was determined microscopically by observing the swelling capability of the particle in water. For easy observation and to enhance the accuracy of monitoring the swelling, microspheres were prepared in a size range of 100 to 700 μm. Although there are obvious surface area differences, it was anticipated that the results from these particles could be extrapolated to smaller sized particles. The swelling capability of microspheres was expressed as a swelling ratio, SR, and was the reciprocal of the polymer volume in the swollen microspheres, $v_{2,S}$,

$$SR = 1/v_{2,S} = V_s/V_p = (D_s/D_p)^3 \quad (3)$$

where D_p and D_s are the diameter of the microspheres before and after swelling, respectively.

Weight Method

The $v_{2,S}$ as a bulk property of a group of microspheres was determined by measuring W_s, W_d, and ρ_p of a certain amount of microspheres, from which $v_{2,S}$ was calculated from equation 2. The procedure for determining the value of W_s and W_d of a group of microspheres was as follows.

1. Approximately 0.4 g of the microspheres after reaction were placed in a glass jar containing deionized water and subjected to repeated sonication and change of de-ionized water to remove any soluble material residues from the microspheres.
2. The glass jar was then maintained at 25°C.
3. After equilibrium was reached, the swollen microspheres were removed, filtered, and quickly weighed. This was the swollen weight, W_s.
4. The same amount of microspheres as that from step 3 was then dried in a vacuum oven until constant weight. This was the dry polymer weight, W_d.

Determination of $v_{2,S}$ and χ

The density of water was taken as 1.0 g/cm^3 at 25°C and the density of polymer ρ_p (or the specific volume v) was determined for each sample by volume displacement method using a specific gravity bottle. The $v_{2,S}$ obtained from equation 2 was then used in equation 1 in conjunction with the interaction parameter, χ, and specific volume, v, to calculate Mc. The interaction parameter χ of microspheres with water was estimated as described in appendix A.

The Dynamic Swelling of Microspheres

A single dried microsphere was placed into the depression of a specially designed glass slide. The microsphere was observed microscopically and the diameter recorded. At time zero, water was added to totally immerse the microsphere. The increase in diameter of the microsphere in water as it swelled was recorded with time.

Verification of the Statistical Model

To test the validity and reliability of the model a cross-over study was developed to determine Mc (by weight method) of serial batches of microspheres which differed in MR from 10^{-2} to 10^{+2} and DD from 0.054 to 0.378 during the preparation of microspheres.

RESULTS AND DISCUSSIONS

The Degree of Derivatization

Table 1 lists the respective DD as determined by NMR and their relations to the reaction conditions. The DD increased linearly with the amount of acryloyl chloride added. In addition, comparison of batches 8 and 9 show that pyridine had a higher catalytic activity than triethylamine (TEA) which is consistent with the proposed order of reactivity (8).

Characterization of the Matrix Structure of Microspheres by Mc

Determination of $v_{2,S}$

Volume Method. Figure 1 shows the values of $v_{2,S}$ (or 1/SR) of 6 batches of microspheres prepared from different DD of Ac-HES as a function of the particle size. The MR of polymer to monomer for these batches of microspheres was 1.0. Apparently, there are variations of $v_{2,S}$ among particles within each batch. The variation existed even for particles of exactly the same size. The horizontal lines represent the means of $v_{2,S}$ for the number of particles measured within each batch. However,

Table 1. The Degree of Derivatization in Relation to the Reaction Conditions of Derivatization

Batch	DD	Ac-Cl (ml)	Tertiary Amine
1	0.044	0.30	TEA
2	0.056	0.50	TEA
3	0.054	0.50	TEA
4	0.127	1.00	TEA
5	0.126	1.00	TEA
6	0.166	2.00	TEA
7	0.289	3.00	TEA
8	0.292	3.00	TEA
9	0.378	3.00	Pyridine
10	0.679	5.00	TEA

Note: DD, Degree of Derivatization (number of double bonds per glucose unit); Ac-Cl, Acryloyl Chloride; TEA, Triethylamine. The tertiary amine was added in equimole of Ac-Cl.

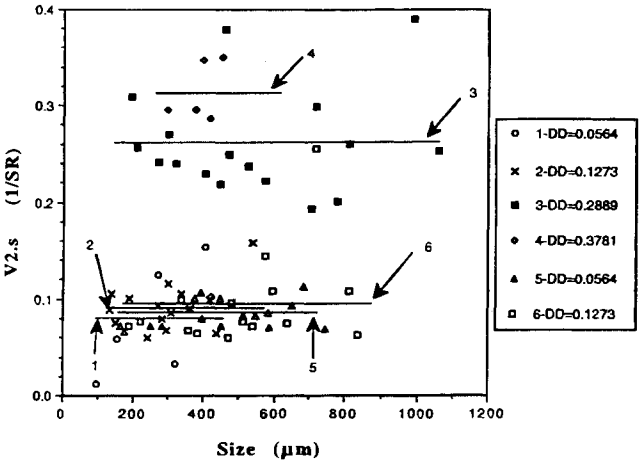

Fig. 1. Illustration of the independence of $v_{2,S}$ on particle size of microspheres.

there is no obvious trend of dependence of $v_{2,S}$ on the particle size. This is different from other systems of crosslinked microparticles such as polystyene (9) and poly (HEMA) (10) in which $v_{2,S}$ decreased as the particle size increased. The independence of $v_{2,S}$ on particle size suggested that it is valid to measure $v_{2,S}$, and thus Mc as a bulk property of a group of microspheres for preparations which differ in particle size. In other words, it is reasonable to compare Mc of different batches of microspheres regardless of the possible differences in particle size from batch to batch. There is a trend that $v_{2,S}$ increases with increasing DD of the Ac-HES, suggesting that the Mc of the microspheres is decreasing with DD, provided the specific volume of the microspheres, v, is constant in equation 1. There was good reproducibility of the microsphere preparation in terms of the $v_{2,S}$ measurement. This can be seen in Figure 1 by comparing the $v_{2,S}$ of batch 2 with that of 6; and batch 1 with 5, where the same DD and reaction conditions were used.

The results suggest that the crosslinked structure of Ac-HES microspheres is not dependent on the particle size. However, variation does exist from particle to particle within the microspheres population. This means that particles within a batch could release incorporated drug at different rates if Mc is the dominant factor for controlling the release.

Weight Method. With the finding that the crosslinked structure of Ac-HES microsphere was independent of particle size, it was reasonable to determine Mc as a bulk property of a group of microspheres. Comparison of Mc for different batches of microspheres could then be made regardless of size differences. Therefore, the $v_{2,S}$ of each batch of microspheres was determined by the weight method. Table 2 lists the measured values of hydration (H_2O%), polymer density (ρ_p), $v_{2,S}$, and the estimated Mc from equation 1 for the various batches of microspheres. The MR of Ac-HES to acrylamide of these microspheres was maintained at 1.0 for all batches. The interaction parameter, χ, was estimated from equation A. 6.

With the exception of group D, as the DD of polymer increased, the H_2O% decreased such that $v_{2,S}$ increased, and Mc decreased, i.e., the crosslinking density increased. Therefore, an increase of DD of Ac-HES polymer resulted in increase of crosslinking density. This was consistent with the theoretical

Table 2. The Molecular Weight Between Crosslinks (Mc) of Various Batches of Microspheres (MR = 1.0)

Group	DD	r_p	$H_2O\%$	$v_{2,S}$	c	Mc
A	0.054	1.163 ± 0.051	95.7 ± 0.27	0.037	0.0578	9879. ± 721
B	0.126	1.448 ± 0.037	85.0 ± 0.56	0.109	0.0743	1980. ± 377
C	0.289	1.648 ± 0.026	71.3 ± 1.61	0.197	0.0945	765.7 ± 37
D	0.378	1.548 ± 0.016	77.8 ± 1.80	0.155	0.0850	1116. ± 167

Note: r_p, The density of the microspheres (g/ml); $H_2O\%$, The water content of microspheres at equilibrium swelling; $V_{2,S}$, The polymer volume fraction of microspheres at equilibrium swelling; c, $c = 0.0492 + 0.231\ v_{2,S}$, eqt.A.6; Mc, The molecular weight between crosslinks, calculated by eqt. 1.

prediction (1). The degree of crosslinking also influenced the density of microspheres, ρ_p. As the crosslinking density increased (Mc decreased), ρ_p also increased. This was reasonable since as degree of crosslinking increased, more monomer was reacted and aggregated into the network such that the mass per unit volume within the microsphere increased, therefore, ρ_p increased. The higher Mc of group D than C can probably be attributed to the intramolecular reaction of Ac-HES itself at high DD. As the DD was high, the possibility of intramolecular reaction increased, and the effective crosslinking decreased.

Comparison of the $v_{2,S}$ values in Table 2 with those determined by the volume method in Figure 1 showed that with exception of batch B, $v_{2,S}$ obtained from the weight method was considerably smaller than that from the volume method. This difference could be attributed to the fact that microspheres in the dry state might not be 100% solid density. Instead, they contained pores and inner space that could also contribute to the total volume of the microspheres. When observed microscopically, the measured volume of dry microsphere was larger than it actually was, which led to a higher value of $v_{2,S}$ than it should have. On the other hand, when determining $v_{2,S}$ by the weight method, the inner space and pores within the dry particle were excluded from the weight since only the solid portion of the microsphere could contribute to the weight of microsphere, Thus, a more accurate dry volume and $v_{2,S}$ were obtained. For determination of Mc, it appears that the weight method is more accurate than volume method.

The Dynamic Swelling of Microspheres

During swelling it was observed microscopically that the water penetration moved inward to the center of microsphere; and a swelling front, i.e., the contour of microsphere, expanded outward to the water. Figure 2 illustrates the typical profile of swelling due to water uptake by the Ac-HES microspheres. The water uptake was always a monotonical increase to equilibrium, as was shown by Fukuda *et al.* (11), on the swelling phenomena of HEMA and PGMA spherical beads. However, it was different from the studies of Lee (12) and Scranton (13) on beads and cylindrical discs of poly (HEMA/EGDMA) where a swelling overshoot was observed whereby the dimension of the gel goes through a maximum followed by a gradual decrease to an equilibrium value. These researchers attributed the occurrence of the overshoot to the existence of either soluble fraction or high percentage of drug load within the microspheres. Therefore, the absence of swelling overshoot in the Ac-HES microspheres might suggest that the microspheres contained a very low soluble fraction.

Figure 2 shows the time at which the water penetration front reached and disappeared at the center of microsphere (Tp) and the final equilibrium time at which the microsphere reached it's maximum equilibrium dimension (Teq). For all batches of Ac-HES microspheres studied, Tp was smaller than Teq. Tp and Teq are characteristic parameters of the microsphere swelling process, which could be correlated with the Mc of the microsphere. Naturally, the two parameters were dependent on the size of microspheres. As the size increased, the time required for the microsphere to reach equilibrium swelling also increased.

To correlate the degree of crosslinking (reciprocal of Mc) with Tp and Teq, recognizing the difficulty in isolating microspheres of the same size from microspheres of different crosslinking density, dynamic swelling of several sizes of microspheres for each crosslinking density was performed. Figures 3a and 3b show respectively the two parameters, Tp and Teq, as a function of particle size and Mc. As shown, both Tp and Teq were best correlated with the square of the particle diameter. This is consistent with the finding that the water uptake rate is proportional to the surface area of the microsphere. More interestingly, when considering the same size of microspheres with different Mc, Tp increased, while Teq decreased with Mc. In other words, as the microsphere was more crosslinked (smaller Mc), the initial penetration rate of water was slower,

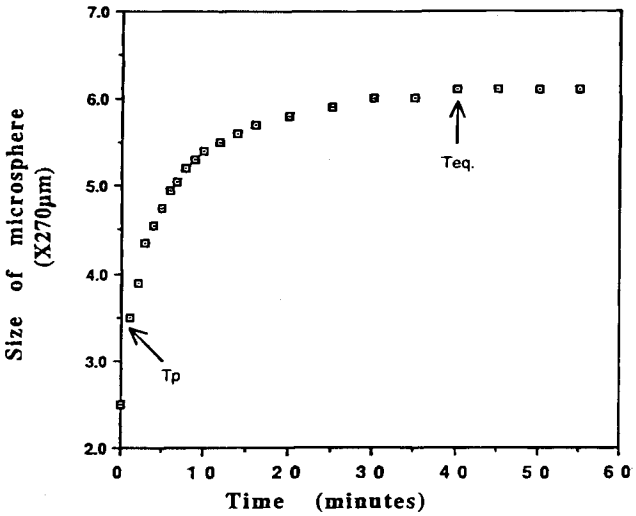

Fig. 2. A typical swelling profile of microsphere with time. T_p is the time the water penetration front disappeared at the center of the microsphere. T_{eq} is the time microsphere reached its equilibrium swelling size.

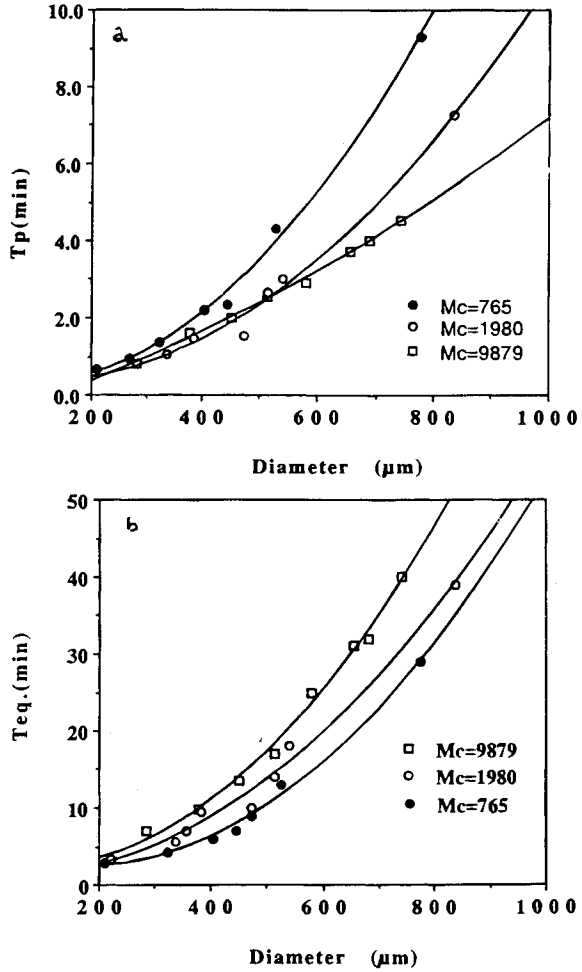

Fig. 3. Effect of microsphere molecular weight between crosslinks (Mc) on penetration time (a) and swelling time (b).

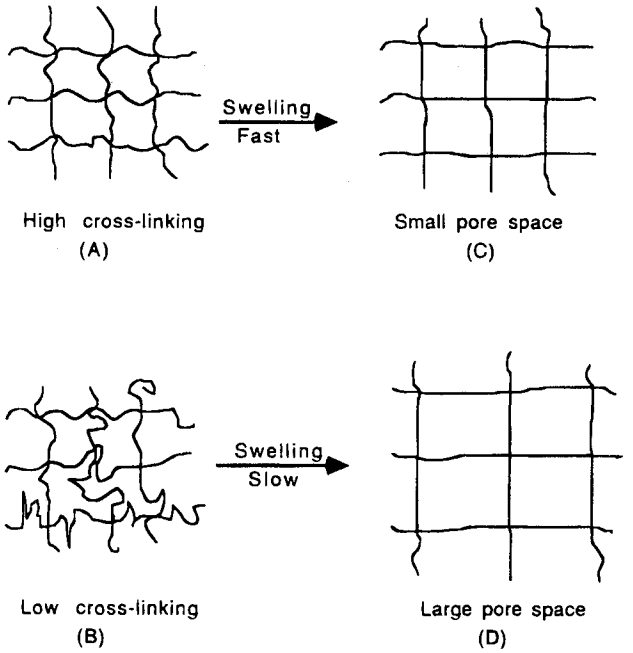

Fig. 4. Conceptual view of the structure of poly(Ac-HES) microspheres as affected by the crosslinking density.

while the expansion rate to equilibrium of the network structure was faster. The correlation produced a conceptual view into the structure of Ac-HES microspheres with respect to crosslinking density.

Considering a crosslinked structure as that shown in Figure 4. In the unswollen state a highly crosslinked structure has smaller pore size (space) than a lowly crosslinked structure due to the latter having more chain folding between crosslinks which results in irregular stacking and arrangement of the chains. Therefore, water penetrates and moves faster in a lowly crosslinked structure. Hence, as crosslinking density increased, Tp increased. However, for water to expand the structure into a fully swollen state, it must occupy every possible inner space within the microsphere. In a highly crosslinked structure, the swelling to equilibrium is rapid due to the limited space. In contrast, in a lowly crosslinked structure, the swelling process might require water to first interact with folded chains to cause unfolding, rearrangement, and rotational movement, etc., of the folded chains, and then to occupy the place initially taken by the folded chains as the microsphere swelled. Therefore, the swelling process to equilibrium was slow for lowly crosslinked structure. The difference in inner space could also be understood by noting that a low crosslinking density usually resulted in a higher degree of swelling.

Verification of the Statistical Model

Table 3 lists the results of the cross-over study on Mc as a function of DD and MR. During the preparation of these microspheres, it was found that the stable formation of microspheres depended on MR and DD of the polymerization condition. As DD decreased, the range of MR over which stable microspheres could be formed decreased. This was because the minimum MR required to form stable microspheres increased with decreasing DD (i.e. The amount of Ac-HES polymer relative to acrylamide monomer had to be increased). "Stable" microspheres describe those that retained their integrity and shape before and after swelling. An unstable microsphere gradually lost its content and spherical shape when immersed in water. This was also the reason, as shown in Table 3, that as DD decreased to 0.0542, only three of the batches prepared were stable at MR between 1.0 to 10^2 and could be characterized.

Figures 5a, b, c show the dependence of Mc on MR for two DDs. For all DD, Mc initially decreased with MR to a minimum and then increased in a manner predicted by the statistical model (1). The result justified the validity of the developed model in describing the polymerization/crosslinking reaction of Ac-HES system. Quantitatively, there was fairly good agreement between the experimental results and the theoretical values. Therefore, for providing a preliminary estimation and guidance to the experimental design before experiment, the developed model appears to be satisfactory.

The trend showing Mc as the concave-up function of MR might be unique to the type of matrix system such as Ac-HES, where a mixture of high molecular weight polymer and low molecular weight monomer was polymerized. Such behavior has not been reported in a polymer system where both monomer and crosslinking agent were small molecules. A somewhat similar trend was discussed from a study by Ramis *et al.* (14), on

Table 3. Summary of the Data from Cross-Over Study of Mc and Mesh Size as a Function of DD and MR

DD	MR	H_2O%	r_p	$v_{2,S}$	c	Mc
0.378	0.01	98.0	1.130	0.018	−0.411	16250 ± 5180
	0.10	89.3	1.414	0.078	−0.447	1654 ± 209
	1.00	77.8	1.549	0.155	0.085	1116 ± 167
	5.00	75.6	1.728	0.157	0.418	4030 ± 526
	10^2	72.6	1.585	0.192	0.547	12320 ± 1130
0.289	0.01	97.8	1.078	0.020	−0.411	12670 ± 1790
	0.10	89.9	1.534	0.068	−0.447	2253 ± 118
	0.62	78.0	1.568	0.153	−0.069	880 ± 155
	1.00	71.3	1.648	0.197	0.094	766 ± 37
	5.00	74.9	1.703	0.164	0.421	3666 ± 544
0.126	0.10	90.4	1.395	0.070	−0.447	1952 ± 137
	1.00	85.0	1.448	0.109	0.0743	1980 ± 377
	10.0	82.0	1.483	0.129	0.467	8432 ± 1106
	10^2	81.8	1.481	0.131	0.524	24920 ± 3790
0.0542	1.0	95.7	1.163	0.037	0.0578	9879 ± 721
	10.0	83.7	1.433	0.119	0.463	9316 ± 912
	10^2	90.0	1.473	0.070	0.502	62360 ± 2050

Note: r_p, the density of the microspheres (g/ml); H_2O%, the water content of microspheres at equilibrium swelling; $v_{2,S}$, the polymer volume fraction of microspheres at equilibrium swelling; c, the interaction parameter, eqt. A.6; Mc, the molecular weight between crosslinks, calculated by eqt. 1.

the heat of crosslinking reaction as a function of molar ratio (MR) of styrene monomer to polyester polymer. Polyesters of different degrees of unsaturation were crosslinked with styrene monomer at different MR. The measured heat of reaction increased with MR to a maximum and then decreased with MR. As the degree of unsaturation of polyester increased, this effect became more significant and the maxima shifted toward lower MR. The occurrence of the maximum heat of reaction was explained as due to a change in the morphology of the sample

Mateescu *et al.* (15), showed a reversed trend in their study on the system of amylose (also polyglucose polymer) crosslinked with epichlorohydrin (small molecule) as a new matrix for drug controlled release. The matrix was also characterized by equilibrium swelling method using the Flory-Rehner model. Data were reported for $v_{2,S}$, instead of the crosslinking degree (R). R was the amount of epichlorohydrin per 100 gm of amylose. The result showed that $v_{2,S}$ was initially decreasing with R to a minimum and then increasing with R. At the same time, the release time of theophylline from the matrix initially increased with R to a maximum and then decreased with R. In the Ac-HES system, $v_{2,S}$ only monotonically increased with MR.

CONCLUSIONS

The swelling of microspheres involves a monotonical increase to equilibrium size, featured by the occurrence of solvent-penetration and swelling fronts. The time period (Tp and Teq, respectively) during which the above two features are observed are dependent on the Mc of the microspheres. As Mc decreased, the solvent penetration rate decreased, and the expansion rate to equilibrium increased. At constant MR, the Mc decreased with increase in DD between 0.054 and 0.378 while at constant DD, the Mc initially decreased as MR increased, went through a minimum, and then increased with further increase in MR. The results support the model in describing the polymerization/crosslinking reaction of the Ac-HES system.

APPENDIX A

Estimation of the Interaction Parameter, c, by Group Contribution Method

The poly (Ac-HES) system is actually a copolymer of acryloyl hydroxyethyl starch and acrylamide. The interaction parameter of water with copolymer was expressed as (16),

$$^{i(jk)}c = {}^{ij}c\,{}^{j}f + {}^{ik}c\,{}^{k}f - {}^{jk}c\,{}^{j}f\,{}^{k}f \qquad (A.1)$$

where ^{ij}c and ^{ik}c are the interaction parameters of homopolymers with the liquid i, and ^{jk}c characterized the interaction of j and k component in the coploymer. Here, i designates water, j, Ac-NH_2, and k, Ac-HES polymer. f is the volume fraction of the copolymer components.

^{ij}c had a value of −0.39 at 25°C as determined by Ahad (17). In determining ^{ik}c by group contribution method, it was found that HES had nearly the same number of functional group and atoms as hydroxypropyl cellulose (HPC) and the interaction parameter of HPC with water was experimentally available. Thererfore, ^{ik}c is considered to have a value, adopted from HPC (18), dependent on the polymer concertration at 25°C as,

$$^{ik}c = 0.482 + 0.371j \qquad (A.2)$$

where j is the polymer volume fraction in the water solution. The interaction parameter ^{jk}c could be estimated based on the Flory-Huggins theory which defined the interaction parameter

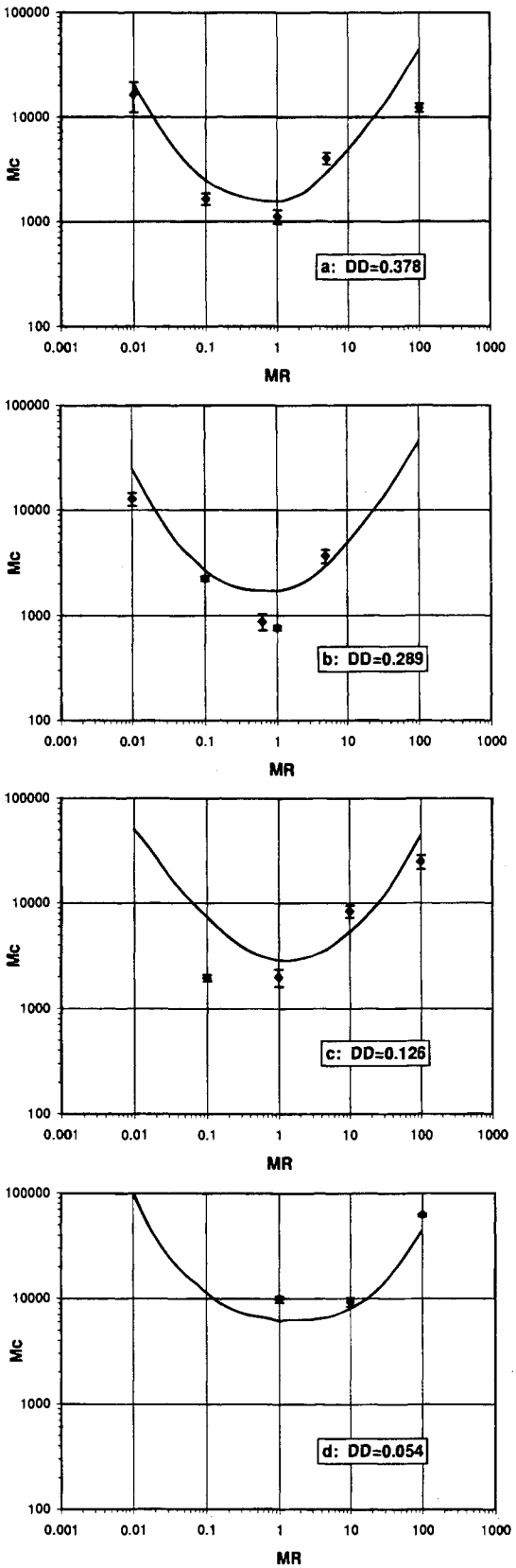

Fig. 5. Correlation between calculated (solid lines) and experimental values (points) of molecular Mc at various degree of derivatiztions. (a) DD = 0.378; (b) DD = 0.289; (c) DD = 0.126; (d) DD = 0.0542. The solid lines were generated with 21 values between 10^{-2} and 10^{2} MR.

as a measure of both excess entropic and enthalpic interactions (19),

$$^{jk}c = {}^{jk}c_s + {}^{jk}c_H$$
$$= 0.34 + {}^{jk}c_H \quad \text{(A.3)}$$

where $^{jk}c_H$ was evaluated from Van Laar-Hildebrand equation (20),

$$^{jk}c_H = V_u(d_j - d_k)^2 \quad \text{(A.4)}$$

where V_u is the molar volume of the smallest repeat unit between the two components; d_j and d_k are the solubility parameters of Ac-NH_2 and Ac-HES, respectively. Applying the group contribution method (21,22), it was found that V_u was 50.3 cm^3/mole for Ac-NH_2, d_j was 32.76 $J^{1/2}$ $cm^{-3/2}$, and d_k was 25.22 $J^{1/2}$ $cm^{-3/2}$. $^{jk}c_H$ was estimated from equation A.4 to be 1.157. Therefore, ^{jk}c was given as 1.497 by equation A.3.

With values of all cs substituted, equation A.1 became,

$$^{i(jk)}c = -.039^{j}f + (0.482 + 0.371j)^{k}f - 1.497^{j}f\,{}^{k}f \quad \text{(A.5)}$$

It should be mentioned that f is different from j. While f designates the volume fraction of components in the copolymer, j is the volume fraction of the Ac-HES component in the copolymer at swollen state. Therefore, j is actually equal to ^{k}f $v_{2,S}$, where $v_{2,S}$ is the polymer volume fraction in the swelling state.

In the case of equal molar ratio of Ac-HES to Ac-NH_2 during polymerization reaction, ^{j}f and ^{k}f were estimated as 0.211 and 0.789, respectively. After substitution of these values, equation A.5 becomes,

$$^{j(jk)}c = 0.0492 + 0.231v_{2,S} \quad \text{(A.6)}$$

The final equation for the estimation of the interaction parameter between water and the Ac-HES system, as expressed by equation A.6, was thus dependent on the polymer volume fraction in the swelling state $v_{2,S}$. A similar type of equation could be derived for other cases where unequal molar ratio of Ac-HES to Ac-NH_2 was incorporated into the microsphere structure.

REFERENCES

1. L. K. Huang, R. C. Mehta and P. P. DeLuca. *Pharm. Res.* **14**:469–474 (1997).
2. P. P. DeLuca and F. Rypacek. Preparation of biodegradable microspheres useful as carriers for macromolecules. US patent 4741872. (1988).
3. M. Lepisto, P. Artursson, P. Edman, T. Laakso, and I. Sjoholm. *Ana. Biochem.* **133**:132–135 (1983).
4. P. J. Flory and R. Rehner, Jr. *J. Chem. Phys.* **11**:521–526 (1943).
5. N. A. Peppas and E. W. Merrill. *J. Polym. Sci. Polym. Chem.* **14**:441 (1976).
6. J. P. Queslel and J. E. Mark. In A.R. Cooper (Ed.) *Determination of Molecular Weight.*, Chap. 16, pp. 487–504, Wiley, NY. (1989).
7. E. A. Collins and F. W. Billmeyer, *Experiments in Polymer Science.*, pp. 481–484, Wiley, NY. (1973).
8. R. L. Whistler (Ed.) *Starch: Chemistry and Technology I.*, pp. 458–460, Academic Press, NY. (1965).
9. B. D. Barr-Howell and N. A. Peppas. *J. Appl. Polym. Sci.* **30**:4583–4589 (1985).
10. B. D. Barr-Howell and N. A. Peppas. *Eur. Polymer J.* **23**:591–594 (1987).

11. T. Fukuda, N. Kohara, Y. Onogi and H. Inagaki. *J. Appl. Polym. Sci.* **43**:2201–2205 (1991).
12. P. I. Lee and C. J. Kim. *J. Controlled Rel.* **16**:229–236 (1991).
13. A. B. Scranton, J. Klier and N. A. Peppas. *Polymer.* **31**:1288–1293 (1990).
14. X. Ramis and J. M. Salla. *J. Appl. Polym. Sci.* **45**:227–236 (1992).
15. M. A. Mateescu, Y. Dumoulin, G. Delmas, V. Lenaerts, and L. Cartilier. *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.* **20**:290–291 (1993).
16. A. F. M. Barton. *CRC Handbook of Polymer-Liquid Interaction Parameters and Solubility Parameters.*, pp. 7–10, CRC Press, Boca Raton, FL. (1990).
17. E. Ahad. *J. Appl. Polym. Sci.* **18**:1587–1602 (1974).
18. A. F. M. Barton. *CRC Handbook of Polymer-Liquid Interaction Parameters and Solubility Parameters.*, pp. 219–221, CRC Press, Boca Raton, FL. (1990).
19. F. Gundert and B. A. Wolf. In J. Brandrup and E.H. Immergut (Eds.) *Polymer Handbook.* 3rd. ed., pp. VII/173-VII/182, Wiley, NY. (1989).
20. A. F. M. Barton. *CRC Handbook of Solubility Parameters and Other Cohesion Parameters.*, pp. 543–545, CRC Press, Boca Raton, FL. (1991).
21. A. F. M. Barton. *CRC Handbook of Solubility Parameters and Other Cohesion Parameters.* pp. 438, CRC Press, Boca Raton, FL. (1991).
22. A. F. M. Barton. *CRC Handbook of Solubility Parameters and Other Cohesion Parameters,* pp. 373, CRC Press, Boca Raton, FL. (1991).