Hydrophobically Graded Polyester Polyol Acrylate Polymers: Synthesis, Characterization, and Microencapsulation of Sulfamethoxazole for Controlled Release Application

M. Venkata Nivasu, Shekharam Tammishetti

Organic Coatings and Polymers Division, Indian Institute of Chemical Technology, Hyderabad 500 007, Andhra Pradesh, India

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ABSTRACT: Polyester polyol macromers were prepared by using diacid-diol condensation reaction using succinic acid as the acid component and polyethylene glycol 200 (PEG 200) as the diol component. Replacing PEG 200 with increasing amounts of butanediol resulted in macromers, which upon acrylation of end hydroxy groups and polymerization resulted in polymers with graded hydrophobicity depending on the amount of butanediol present in the polymer. These polymers showed expected trends in water equilibrium swells, equilibrium water contact angles, and *in vitro* degradation times depending on the amount of modification with butanediol. These polymers were used to microencapsulate sulfamethoxazole as a model drug and the *in vitro* delivery of the drug also followed the expected trend depending on the polymer hydrophobicity. Thus, it was shown that it is possible to prepare polyesters of graded properties by judicious selection of diacids and diols. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 4058–4065, 2006

Key words: biodegradable; drug delivery systems; hydrophilic polymers; polyesters

INTRODUCTION

Polyesters show great promise as biomaterials because of their ready degradation into acid and alcohol components by hydrolysis of the ester linkages. Several polyesters have been synthesized and used for different applications such as sutures, surgical meshes, sealants, drug delivery matrices, etc. Most popular of these polymers are unquestionably lactide and glycolide polymers, which have been shown to be safe biomaterials and are increasingly investigated by groups across the world for different applications. The semicrystalline morphology of these polymers leads to heterogeneous degradation rates, whereby the amorphous regions degrade faster than the crystalline regions.[1,2] For this reason, several amorphous biodegradable polymers were investigated.

Bulk properties of any polymer depend to a great extent on the properties of the monomers it is made of. Thus in case of polyesters, it is possible to control the properties by appropriate selection of the acid and alcohol components. Hydrophilicty/hydrophobicity of a polyester is an important property, since it determines its ability to attract and retain water, thus dictating its degradation rates. Danprasert et al. have synthesized a series of polyesters based on polyethylene glycol and substituted isophthalic acid.[3] They have shown that by adjusting the hydrophobic and hydrophilic segments in the polymer, it is possible to change the polymer solution properties. Kissel et al. studied ABA block copolymers containing hydrophilic polyethylene oxide as B blocks and lacctide/glycolide as hydrophobic A blocks.[4] AB block copolymer micelles containing polyethylene oxide as hydrophilic block and poly(L-amino acid) as the hydrophobic block have been investigated as long circulating drug vehicles.[5] To our knowledge, there has not been a systematic study to synthesize a closely related series of polyesters differing in their hydrophobicity/hydrophilicity to control water absorption, and thus its degradation. Such hydrophobically graded polymers may find important applications, especially in controlled drug delivery, since we can control the life time of these polymers by appropriate design of their structure. Here we wish to present the synthesis of such hydrophobically graded closely related polyesters, their characterization and use as controlled drug delivery devices in the form of microspheres.

Correspondence to: S. Tammishetti (shekharam@iict.res.in).

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MATERIALS AND METHODS

Succinc acid, Polyethyleneglycol (200) 1,4-Butanediol, benzoyl peroxide, and solvents were bought from

S. D. Fine Chem, Mumbai, India. Acryloyl chloride, benzophenone/hydroxycyclohexyl acetophenone, were purchased from Aldrich Chemical Company, Milwaukee, WI, USA. Benzoyl peroxide was recrystallized from chloroform before using and rest of the chemicals were used as received.

Synthesis of polyester polyols

Polyesterpolyols of the current study were synthesized using a procedure similar to that reported in our earlier work.[6–8] Polyesterpolyols of succinic acid and polyethylene glycol-200 (PEG-200) or a mixture of PEG-200 and 1,4-butanediol, as shown in Table I, were prepared as follows. Required amounts of acid and alcohols were taken in a three-necked round bottom flask fitted with an overhead mechanical stirrer, nitrogen bubbler, and a distillation condenser. The contents were slowly heated to 180°C and kept there for 4 h. Water formed in the reaction was collected in a round-bottomed flask. The products were characterized by ^{1}H NMR, IR, hydroxyl, and acid numbers.

^{1}H NMR: All NMRs were run in $CDCl_3$ and peaks are expressed as δ ppm from reference TMS. SAP Polyol: 2.5, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.2, m, O=C—O—CH_2. SAP25BD polyol: 1.7, m, O—$CH_2CH_2CH_2CH_2$—O—; 2.6, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.1, t, O=C—O—CH_2 (due to butanediol) and 4.25, t, O=C—O—CH_2 (due to PEG). SAP50BD polyol: 1.65, m, O—$CH_2CH_2CH_2CH_2$—O—; 2.6, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.1, t, O=C—O—CH_2 (due to butanediol) and 4.25, t, O=C—O—CH_2 (due to PEG). SAP75BD polyol: 1.65, m, O—$CH_2CH_2CH_2CH_2$—O—; 2.55, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.05, t, O=C—O—CH_2 (due to butanediol) and 4.25, t, O=C—O—CH_2 (due to PEG). IR spectra: All polyols had a 1740 cm^{-1} peak for carbonyl stretch.

Synthesis of polyesterpolyol acrylates

Polyester polyols made as above were acrylated using acryloyl chloride. Polyol (1 equiv) was dissolved in dry dichloromethane at 10% solids concentration in a two-necked RB flask protected from moisture using a $CaCl_2$ guard tube. Triethyl amine (2 equiv) was added and the contents cooled to 0°C followed by drop wise addition of acryloyl chloride (1.5 equiv). Stirred cold for 2 h and allowed to attain room temperature and stirred for 24 h. The reaction mixture was filtered to remove triethyl amine hydrochloride and then washed with dilute HCl and brine solution. Dried over Na_2SO_4 and concentrated at reduced pressure using a rotary evaporator.

^{1}H NMR: SAP.acrylate: 2.6, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.2, m, O=C—O—CH_2, 5.8 to 6.5, m, —CH=CH_2. SAP25BD acrylate: 1.7, m, O—$CH_2CH_2CH_2CH_2$—O—; 2.6, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.0 to 4.3, m, O=C—O—CH_2, 5.8 to 6.6, m, —CH=CH_2. SAP50BD acrylate: 1.75, m, O—$CH_2CH_2CH_2CH_2$—O—; 2.6, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.1 to 4.4, m, O=C—O—CH_2, 5.8 to 6.6, m, —CH=CH_2. SAP75BD acrylate: 1.7, m, O—$CH_2CH_2CH_2CH_2$—O—; 2.6, m, O=C—CH_2; 3.6, m, O—CH_2CH_2—O from PEG; 4.0 to 4.3, m, O=C—O—CH_2, 5.7 to 6.5, m, —CH=CH_2. IR Spectra: All acrylates had a 1740 cm^{-1} peak for carbonyl stretch and 1630 cm^{-1} peak for C=C stretch in their IR spectra.

UV polymerization for preparation of films for determination of water contact angles

Typically 1 g of above prepared macromer was mixed with 20 μL of photo initiator *viz*., benzophenone/hydroxycyclohexyl acetophenone. This solution was taken in a glass mold of ~0.6 mm thickness and photopolymerized by exposing to long wave length UV light for about 15 s using a medium pressure mercury vapor lamp (2.66 W/cm^2) in a lab cure unit (Wallace Knight, UK).

Contact angle measurement

Discs of 10 mm diameter were punched out of films obtained as above and equilibrium water contact angles determined. Typically 10 μL of water was put on the disc as a drop. Allowed to equilibrate for 3 min and contact angle was determined using Contact Angle Measuring Instrument G10 of Kruss,

TABLE I
Macromers Synthesized in This Study

Code	Acid (mol equiv)	PEG 200 (mol equiv)	Butane diol (mol equiv)	Acid no. initial	Acid no. final	Hydroxy no.	Contact angle (SD)
SAP200	Succinic acid (1.0)	1.10	0.00	348	50	145	44 (0.8)
SAP25BD	Succinic acid (1.0)	0.83	0.28	359	46	190	48 (0.9)
SAP50BD	Succinic acid (1.0)	0.55	0.55	408	40	211	53 (0.7)
SAP75BD	Succinic acid (1.0)	0.28	0.83	466	36	223	57 (0.1)

Germany. Five specimens were used for each polymer and average value and standard deviation reported.

Preparation of microspheres

Blank microspheres were prepared for studying the water equilibrium swell and *in vitro* degradation and microspheres loaded with sulfamethoxazole were prepared for studying the release characteristics *in vitro*. Typical procedure is as follows. Polyacrylate (10 g) and benzoyl peroxide (2.5% by weight of acrylate) [sulfamethoxazole, 5% by weight of acrylate in case of drug-loaded microspheres] were dissolved in dichloromethane (10 mL). This solution was added to silicone oil (70 mL) taken in a reaction kettle equipped with a over head mechanical stirrer and nitrogen bubbling and stirred at 200 rpm at ambient temperature for 1 h to allow dichloromethane to evaporate. The resulting suspension is then gradually heated to 80°C and maintained for 2 more hours. The resulting microspheres were separated by filtration and washed with hexane to remove adsorbed oil, washed with acetone, and dried at room temperature. All the microspheres were stored over $CaCl_2$ in a dessicator since they were found to be hygroscopic. The size distribution and morphology of microspheres were determined from SEM studies.

Determination of equilibrium swelling in water

Blank microspheres (0.3 g) made with each macromer were suspended in 30 mL of distilled water and left at room temperature (about 30°C) for 24 h. The swollen microspheres were then filtered by suction, pressed gently between fiber free filter papers, and weighed (W_1). Then the same sample was dried in an air circulating oven at 120°C for 20 min and weighed again after cooling back to room temperature in a dessicator (W_2). Equilibrium swells were calculated as $[(W_1 - W_2)/W_2] \times 100$. Five samples were studied for each and average and standard deviation are reported.

Determination of *in vitro* degradation times for microspheres

Microspheres (0.5 g) of blank samples were suspended in 50 mL of 0.01*N* sodium hydroxide taken in properly stoppered 100 mL polypropylene bottles. The bottles were then kept in a constant temperature (37°C) shaking water bath (100 rpm). Samples were observed frequently and total time taken for complete digestion of microspheres into soluble materials was determined. Five samples were run for each macromer and average and standard deviation are reported.

Microencapsulation efficiency of sulfamethoxazole

Microspheres (500 mg) were suspended in 100 mL of 0.1*N* sodium hydroxide in polypropylene bottles and kept in a constant temperature (37°C) shaking water bath. Upon complete digestion of the microspheres (2 days), aliquots from the extract were estimated for sulfamethoxazole concentration by comparing UV absorption at 264 nm with a standard. From this total amount of sulfamethoxazole present in the sample was calculated and encapsulation efficiency was calculated as $E = (Q_e/Q_t) \times 100$ where E is the percentage encapsulation of drug in microspheres, Q_e is the quantity of drug present in microspheres, and Q_t is the quantity of drug taken for encapsulation.

Study of *in vitro* drug release

Drug-loaded microspheres (0.5 g) were suspended in 100 mL of pH 7.4 phosphate buffer and kept in a constant temperature shaking water bath at 37°C and 100 rpm. Aliquots were collected at different times and concentration of sulfamethoxazole in them was determined by comparing their UV absorption at 264 nm with a standard curve. Three samples were run for each formulation and average values are reported along with maximum percentage deviation observed for each as error bars.

RESULTS AND DISCUSSION

As part of our investigation for *in situ* polymerizable biocompatible and degradable materials for tissue contacting application, we have been interested in PEG containing polyester polyol acrylates as liquid prepolymers, which could be polymerized into gels on target site.[6–8] These prepolymers were prepared by melt poly condensation of succinic acid and PEGs of different molecular weights. To control the polymers water absorption, degradation, and ability to properly wet different tissues, we needed a methodology for synthesis of closely related polymers with a graded hydrophobicity. This can be achieved by judicious selection of either diacid or diol components that go into the synthesis of these polymers. In the present work, we have investigated in detail the effect of choice of diol component on the polymer properties.

Synthesis and characterization of prepolymers

By replacing highly hydrophilic PEG200 with increasing amounts of hydrophobic 1,4-butanediol (Table I), we have prepared a series of prepolymers according to scheme shown in Figure 1. The polyester polyol

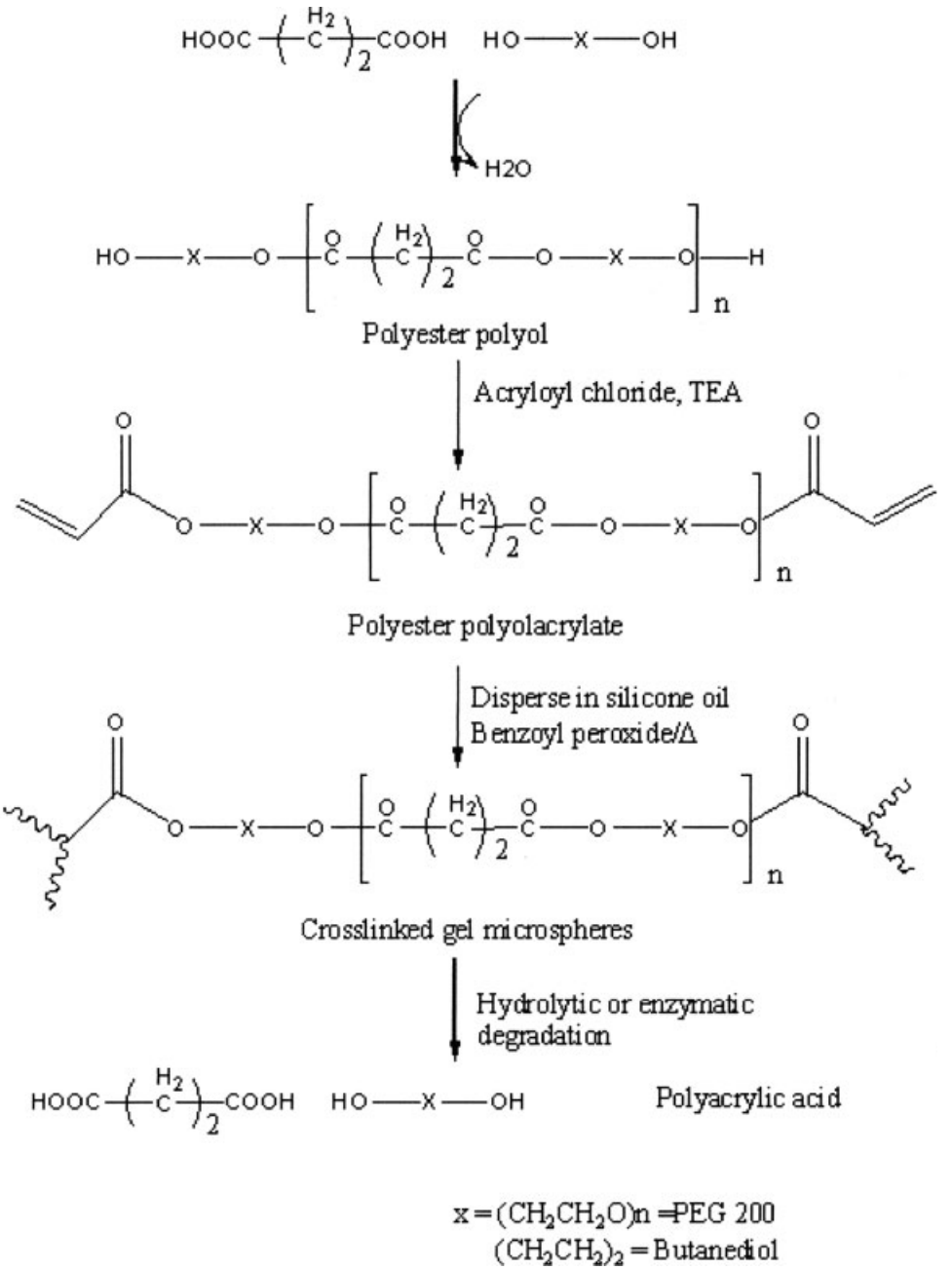

Figure 1 Scheme used for synthesis of the macromers of the study.

prepolymers were prepared by melt polycondensation at 180°C for about 4 h. As can be seen from Table I, the initial high acid numbers get reduced and became stable by the end of about two and a half hours. Further heating or heating at a higher temperature were not advantageous because they decomposed the prepolymer. The polyols were characterized mainly by ^{1}H NMR (Fig. 2).

In the polyol ^{1}H NMR, near 1:1 integrity for acid methylenes at 2.6 δ and ester methylenes between 4.0 and 4.3 δ suggest that no free acid is present in these polymers. In case of polyols made using both PEG200 and 1,4-butanediol, the incorporation ratio needed to be ascertained. The ester methylene peaks due to PEG 200 and 1,4-butanediol in proton NMR of polyols resonated slightly apart from each other and allowed to quantify their composition in their respective polymers (Fig. 3). As can be seen the integration of methylenes represent exactly the feed composition ratio. Thus, it can be concluded that under experimental conditions used, no butanediol escaped from the reaction. The terminal hydroxy groups of thus prepared polyols were then acrylated using acryloyl chloride. Acrylation was ascertained

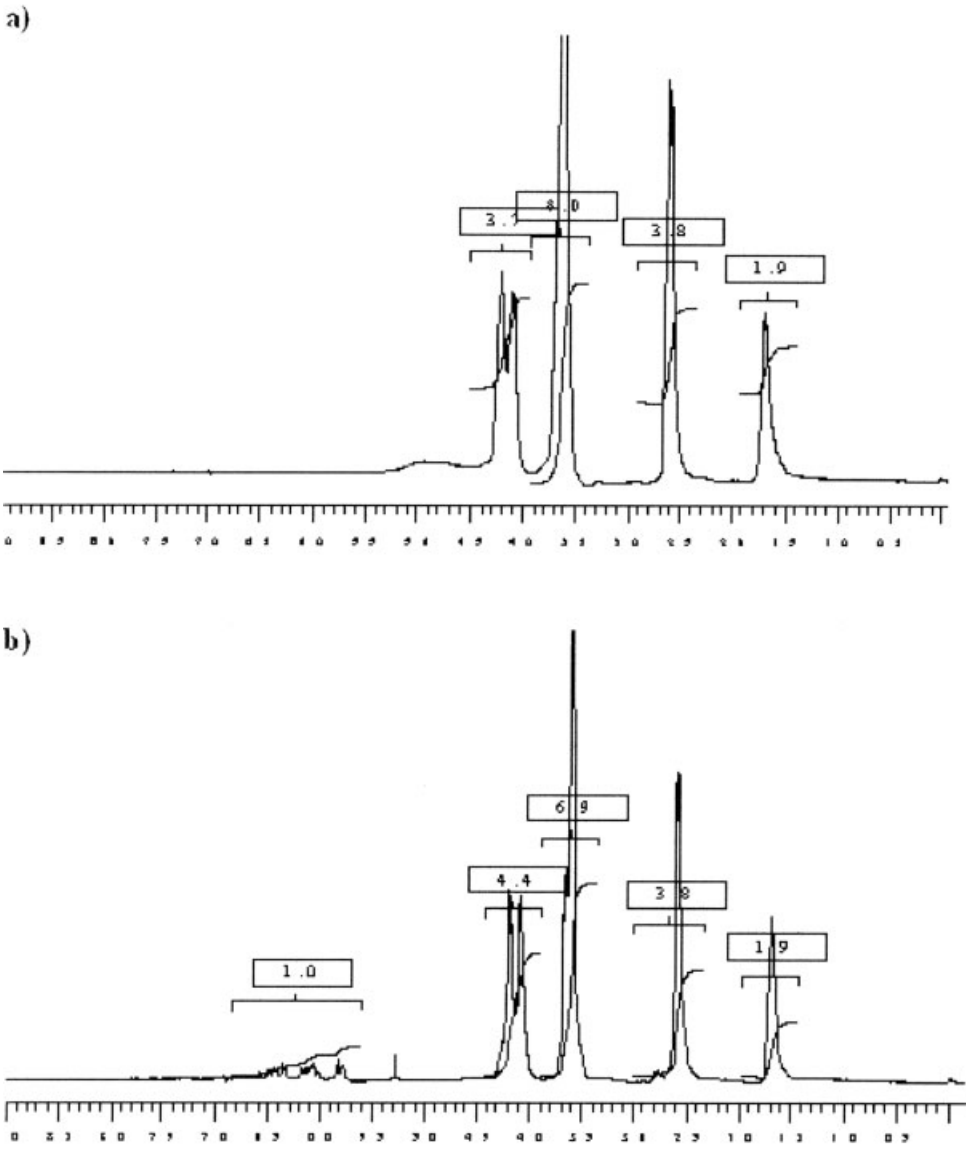

Figure 2 ^{1}H NMR of SAP 50 BD (a) Polyol and (b) Acrylate.

by appearance of —CH=CH_2 protons in the NMR spectra at 5.8 to 6.6 δ. In the infra red spectra of all the acrylates a new peak at 1630 cm^{-1} appeared corresponding to C=C stretch.

Having synthesized the required polyol acrylates with decreasing ratios of PEG to butanediol, we investigated the hydrophobicity of these polymers. For this, films of all polymers were prepared by photo crosslinking. Equilibrium contact angles were determined for all these films with water. The results are presented in Table I. As can be seen, as the ratio of PEG to butanediol decreased in the polymers, the water contact angle increased systematically. This suggests that by increasing the amount of butanediol in these polymers, it was possible to increase the hydrophobicity in a controlled fashion. Effect of this modification on hydrolytic and other properties of these polymers was studied next on microspheres made from these polymers.

Preparation of microspheres

There are a number of methods for preparation of microspheres from preformed polymers. For preparation of microspheres from free radically polymerizable prepolymers such as those used in this study, the best method is dispersion of the prepolymer in a suitable solvent, thermally polymerizing the prepolymer after achieving required particle size. Because the macromers of the study were amphiphilic in nature, usual solvents were found to be unsuitable for dispersion because the macromer was either soluble in them or formed an emulsion giving a too low particle size. Silicone oil was found to be a good

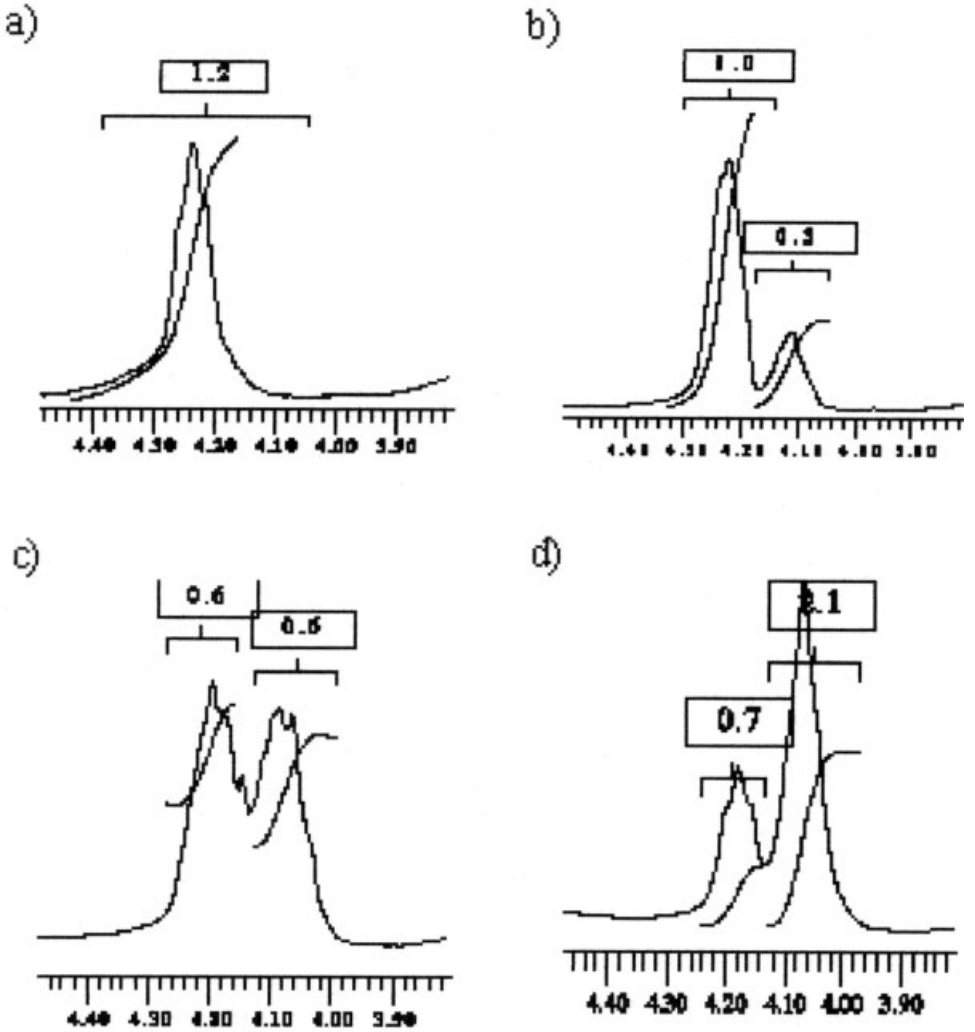

Figure 3 Ester methylene peaks of (a) SAP200, (b) SAP25BD, and (c) SAP50BD and SAP75BD polyols.

medium to disperse the prepolymer acrylates and thus all microsphere preparations were done in this medium only. First blank microspheres were prepared to study water absorption and degradation characteristics of these polymers. The morphology and particle size distribution of these microspheres was studied using scanning electron microscopy. As can be seen from Figure 4, all microspheres were found to be very spherical, entirely free of any defects, nonporous, and most of them were in the range of 10–20 μ in diameter.

Water equilibrium swelling and degradation times

The effect of above described subtle modification in structure of these polymers on their physical properties such as water equilibrium swell and time taken for degradation in aqueous environment were next investigated. Equilibrium swelling of microspheres made with different macromers decreased significantly with decreasing ratio of PEG to buatanediol (Table II). This reflects the trend expected from their hydrophobicity. Increasing amounts of butanediol in the polymer increased the hydrophobicity of the matrix, thus decreasing their capacity to absorb water. Hydrolytic degradation of these polymer microspheres was first attempted in phosphate buffer. But because of the long times required for degradation (more than 3 months for SAP75BD) and also unclear transitions, it was difficult to assign an end point and determine time for total degradation. Hence an expedited degradation study was resorted to in 0.01*N* NaOH. The times taken for total degradation of microspheres of various polymers of the study are presented in Table II.

As can be seen time taken for degradation of these microspheres increased with decrease in the ratio of

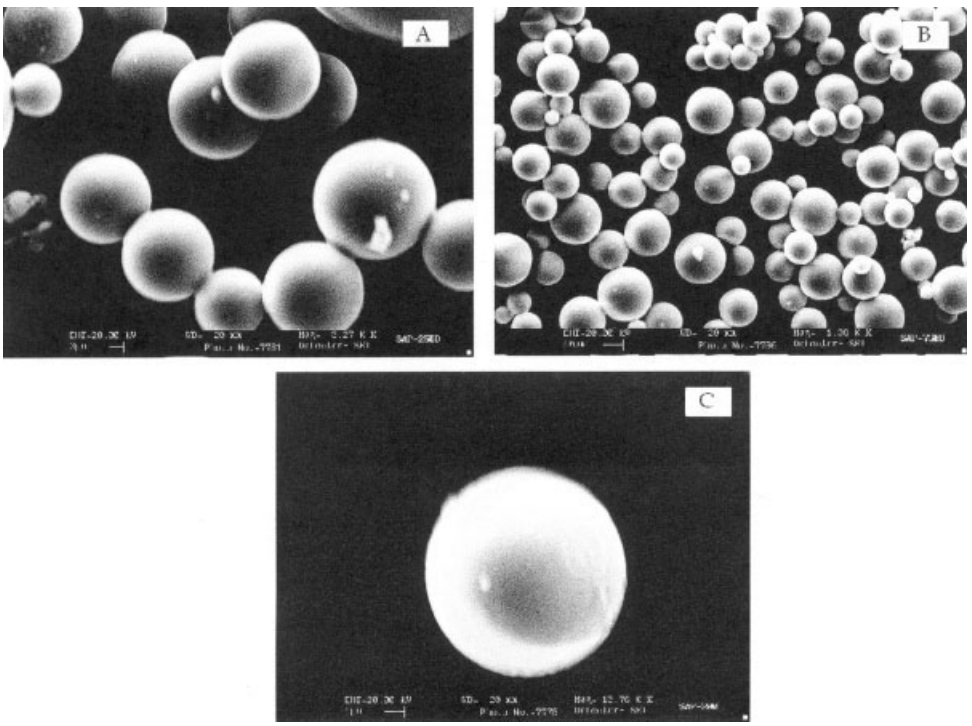

Figure 4 SEM pictures of sulfamethoxazole-loaded microspheres (a) SAP25BD, 3.27 K X; (b) SAP75BD, 1.38 K X; and (c) SAP200, 13.78 K X.

PEG/butanediol and reflects the trend seen in water contact angle and equilibrium swell depending on their hydrophobicity. The trends seen above encouraged us to investigate these polymers for controlled delivery of drugs.

Controlled release of sulfamethoxazole

Using sulfamethoxazole as a model drug, microspheres were prepared from all the macromers of the study. The microcapsule yield and drug entrapment efficiency of these polymers is given in Table II. Both yield and microencapsulation efficiency of these microspheres was moderate to good and no attempt was made to optimize these. The drug-loaded microspheres were subjected to *in vitro* release test and delivery data is presented in Figure 5. Since factors like drug concentration, dilution, temperature, and particle size of the microspheres are same, the differences seen in the delivery of sulfamethoxazole from different microspheres of the present study are essentially a manifestation of the difference in the polyester's structure. As can be seen from Figure 5, all the formulations have a burst release up to about two days probably due to release of sulfamethoxazole near the periphery of the microspheres. This burst release appears to be more for the most hydrophilic SAP and least for the least hydrophilic SAP75BD, suggesting that the difference is basically due the difference in their affinity to water and capability to swell in water. After the initial burst, the remaining drug is seen to be released in a controlled fashion by all the different polymers of the study. At the end of the study, it can be seen that the cumulative amount of drug released by these polymers also follows a trend that can be expected from their composition.

TABLE II
Degradation and Microencapsulation Efficiency of Different Microspheres

Code	% Water equiv. swell (SD)	Degradation time (SD) (h)	Microcapsule yield	Microencapsulation efficiency, %(SD)
SAP 200	109 (0.7)	106 (13)	79	83 (0.2)
SAP25BD	77 (0.5)	182 (13)	78	85 (0.4)
SAP50BD	53 (0.6)	283 (11)	79	89 (0.4)
SAP75BD	36 (0.7)	456 (17)	79	91 (0.1)

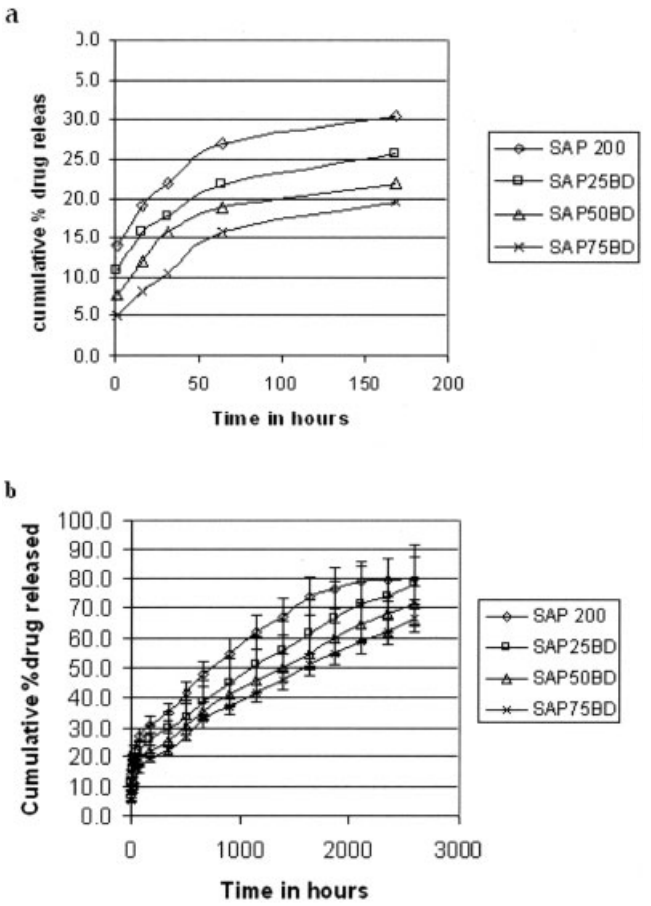

Figure 5 *In vitro* drug release from different microspheres of the present study (a) for the initial one week and (b) whole duration of the study.

CONCLUSIONS

Hydrophobically graded polyester polyols were prepared from succinic acid and different ratios of PEG200 and 1,4-butanediol. Polymers prepared from their acrylates showed a graded behavior in water contact angles, water equilibrium swells, and time taken for hydrolytic degradation and followed a trend representing their bulk hydrophobicity. Sulfamethoxazole-loaded microspheres were also prepared using these polymers and *in vitro* release studied. The release behavior coincided with a trend that can be expected from the polymers' hydrophobicity.

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