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Preparation and characterization of copolymers of new monomers from bile acid derivatives with methacrylic monomers and selective hydrolysis of the homopolymers*

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Polymers have been prepared by solution-free radical polymerization from new monomers made from bile acid derivatives. Two approaches were used to improve the hydrophilicity of the polymers: first, the monomers were copolymerized with methacrylic monomers such as methacrylic acid and 2-hydroxyethyl methacrylate; second, the methyl ester groups on cholic acid of the homopolymers were also removed selectively by hydrolysis. The copolymers and the hydrolysed polymers were characterized by solid-state n.m.r. spectroscopy and differential scanning calorimetry. The copolymers were found to be random and the chemical composition in the final products was close to the original monomer composition prior to polymerization. It was also found that the methyl ester groups can be removed selectively by controlling the time of hydrolysis in a basic media while keeping the methacrylate ester bond mostly intact. The methacrylamide bond in the polymers was more resistant to hydrolysis than the methacrylate bond.

(Keywords: bile acids; polymethacrylates; copolymerization)

INTRODUCTION

Biocompatibility is a necessity for polymers used in biomedical and pharmaceutical fields. Bile acids are natural compounds existing in the body which can emulsify hydrophobic substances by the formation of micellar aggregates and thus help in the digestion of fat and lipids[1]. Recently, many research interests have been drawn to the development of materials containing bile acids. For example, aliphatic chains can be attached to bile acids to obtain organized structures[2]; bile acids can also be used as building components in the preparation of macrocycles in molecular recognition studies[3]; and they can be attached to polymers for slow release in pharmaceutical applications[4]. When such natural biocompounds are introduced or used in the preparation of the polymer materials, they should be better tolerated biologically because of their amphiphilic properties, which allow the polymer to interact favourably with the biological environment. Even in case of a possible breakdown of the polymers, it is less likely to cause toxic effects, especially when used in the gastro-intestinal tract. Ahlheim *et al.* pioneered the synthesis of some polymers and copolymers from methacrylates of bile acids[2,5,6]. In these polymers, the bile acid residues existed as esters, which rendered the polymers rather hydrophobic as a whole. Recently, we have prepared new homopolymers from the methacrylamide monomers of cholic acid, one of the most commonly occurring bile acids, and found that the stereochemistry of the monomers determines the ease of polymerization as well as the solubility of the resulting polymers[7]. Because of the excellent amphiphilic properties of bile acids, it is interesting to explore the synthesis of some more hydrophilic forms of the polymers, which can be useful in the preparation of new hydrogels. The solubility of bile acids in water depends on the pH of the media ($pK_a = 5$–6): ranging from insoluble or slightly soluble at low pH (with the exception of certain tauro conjugates) to highly soluble at elevated pHs in their salt form. To date, there have been no other reports on the synthesis of polymers containing bile acids and their derivatives with free carboxylic acid groups.

Cholic acid, or 3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid, is one of the most commonly occurring bile acids and consists of a steroid skeleton, one carboxyl and three hydroxyl groups (*Figure 1*). The monomers from the 3-hydroxy and 3-amino derivatives can be prepared with relative ease[5,7] because of the higher reactivity of the OH group on the C-3 position relative to that of the other OH groups in positions C-7 and C-12[8,9]. The formation of an ester of the carboxylic acid group of bile acids proved to be quite useful in the synthesis of the new monomers as well as in the preparation of polymers, since it improves the solubility of bile acids in organic solvents. The transformation from 3-hydroxy to 3-amino cholic acid would improve the hydrophilicity of the

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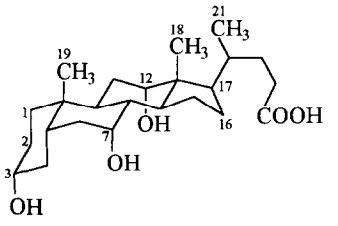

Figure 1 The chemical structure of 3α-cholic acid, one of the most commonly occurring bile acids

monomer (by the formation of an amide instead of an ester), but hydrophobicity still dominates the polymers[7]. There are two obvious ways to improve the hydrophilicity of the polymers: the selective removal of the ester groups to liberate the carboxylic acid groups of bile acids, and the copolymerization of these new monomers with monomers of higher hydrophilicity such as methacrylic acid and 2-hydroxyethyl methacrylate, which are often used in the preparation of polymeric hydrogels. In addition, the comonomers are expected to improve the flexibility of the polymer chains since they are smaller in size. Copolymerization with our newly prepared monomers should be possible since they all possess a common methacrylic structure. We report here the preparation and some characteristics of such copolymers and the selective hydrolysis of the methyl ester protecting groups.

EXPERIMENTAL

Materials and instruments

Cholic acid (CA) and its methyl ester (CAME) were purchased from Sigma and used as received. Methacrylic acid and 2-hydroxyethyl methacrylate, both purchased from Aldrich, were distilled before polymerization. Methacryloyl chloride was always freshly prepared from methacrylic acid and benzoyl chloride (also from Aldrich)[10]. 2,2′-Azo-bis(isobutyronitrile) (AIBN) was obtained from Eastman Kodak and recrystallized in chloroform–methanol prior to use.

N.m.r. spectra were recorded on a Bruker AMX-300 operating at 300 MHz for ^{1}H and 75.6 MHz for ^{13}C, in deuterated chloroform, which also served as a reference. Solid-state ^{13}C n.m.r. was performed at ambient temperature on a Varian VXR-300 instrument equipped with a Doty CP/MAS probe with spinning rate of 4–5 kHz. The contact time was optimized to be 1.5 ms and an interpulse delay of 5 s was used. All chemical shifts are relative to tetramethylsilane (TMS) at 0 ppm.

The molecular weights of the polymers soluble in tetrahydrofuran (THF) were determined by size exclusion chromatography (s.e.c.) at 33°C using a 5% w/v solution in THF on a Waters 410 system (Millipore) using polystyrene as the standard. The glass transition temperatures of the homo- and copolymer were determined on a differential scanning calorimeter (DSC 2910) from TA Instruments with a heating rate of 20°C per minute in the range of −50 to −320°C.

Preparation of monomers and polymers

The methacrylate and methacrylamide monomers can be synthesized by reacting freshly prepared methacryloyl chloride with the 3-hydroxy or 3-amino derivatives of cholic acid, due to the higher reactivity of the hydroxy or the amino group at position C-3[8,9]. Details of the preparation of the 3α- and 3β-methacrylamide monomers (MACAME) and their corresponding polymers were described in a previous report[7]. The 3α-methacrylate ester monomer (MECAME) was also prepared similarly[5,7] and had a melting temperature of 181°C as determined by d.s.c.. ^{1}H and ^{13}C n.m.r. spectroscopy was used to confirm the products and ^{13}C n.m.r. spectral assignments were made with the help of distortionless enhancement by polarization transfer (DEPT) and two-dimensional ^{13}C–^{1}H correlation experiments.

The polymers were prepared by free radical polymerization in toluene with AIBN as the initiator, as reported previously[7]. The procedure for the preparation of the copolymers was quite similar. Only the 3α-methacrylyl (ester and amide) monomers were used in the preparation of the copolymers. The comonomers were mixed in appropriate molar ratios and dissolved in freshly distilled toluene in an ampoule equipped with a magnetic stirrer. After purging with an inert gas (N_2), AIBN (ca. 5 mol%) was added before sealing the ampoule and placing it in an oil bath. The ampoule was sealed only to prevent the loss of solvent. The temperature was gradually raised to ca. 100°C over a period of over 24 h and maintained for another 4 h. The polymer itself was not very soluble in toluene and can be precipitated from methanol and filtered, washed and dried. The formation of the polymers was confirmed by solution and solid-state n.m.r. which showed the disappearance of the double bonds and also broadening of the n.m.r. signals.

Hydrolysis of the methyl ester of cholic acid on the polymers was accomplished by refluxing in an aqueous sodium hydroxide solution (2 N) mixed with some organic solvents such as THF. Water and THF at a ration of 1 : 3 were not completely miscible, but the hydrolyses were very close to completion upon refluxing for ca. 3 h. After separating the two phases, the organic phase was then washed with water and the aqueous phase was adjusted to slightly acidic pH with 10% HCl. It was then extracted with ether and the polymer was obtained by evaporating the solvents and drying the sample under reduced pressure.

RESULTS AND DISCUSSION

Characterization of the copolymers

The average molecular weights of the polymers and copolymers determined by s.e.c. (shown in *Table 1*) are reasonably high and the polydispersity indices (the ratio of weight-average to number-average molecular weights, M_w/M_n) are also within the range of those polymers obtained by free radical polymerization. The results of the MACAME polymers were published in a previous communication[7]. These measurements provide only an estimate of the molecular weights of the polymers since the standard used was polystyrene. Even so, the results unequivocally indicate the formation of polymers, not oligomers, which is further confirmed by the glass transition temperatures of the homo- and copolymers obtained from the d.s.c. analysis. In all cases, the making of the polymers from the methacrylic monomers

Table 1 Molecular weights of the polymers soluble in THF as measured by s.e.c.

Polymer[a]	M_n	M_w	M_w/M_n
MECAME homopolymer	212,300	505,100	2.37
MECAME: MAA 7:93	233,400	378,100	1.61
MECAME: MAA 37:63	206,000	366,000	1.77
MECAME: MAA 45:55	496,900	763,800	1.62
MECAME: HEMA 41:59	97,400	118,900	1.22

[a] The numbers in this column indicate the molar ratio of the monomers in the copolymers obtained

with bulky cholic acid derivatives proved to be relatively easy, confirming the previous reports on bile acid-containing polymers[5,7].

The chemical composition of the copolymers was determined by solid-state ^{13}C n.m.r. The spectra of the series of homo- and copolymers consisting of MECAME and HEMA are shown in *Figure 2*. The peaks in the ^{13}C n.m.r. spectra of the polymers can be divided into several groups, the carbonyl groups at very low field (175–190 ppm), the methyl groups at high field (10–30 ppm), and the CHOH groups (60–80 ppm) and the other carbons in the middle. As shown in *Figure 2*, the intensities of the peaks at 60–80 ppm (the CHOH groups) and of those at 10–45 ppm (the envelope of the other carbons), which belong to the monomer MECAME, increased steadily as the comonomer content increased in the copolymers.

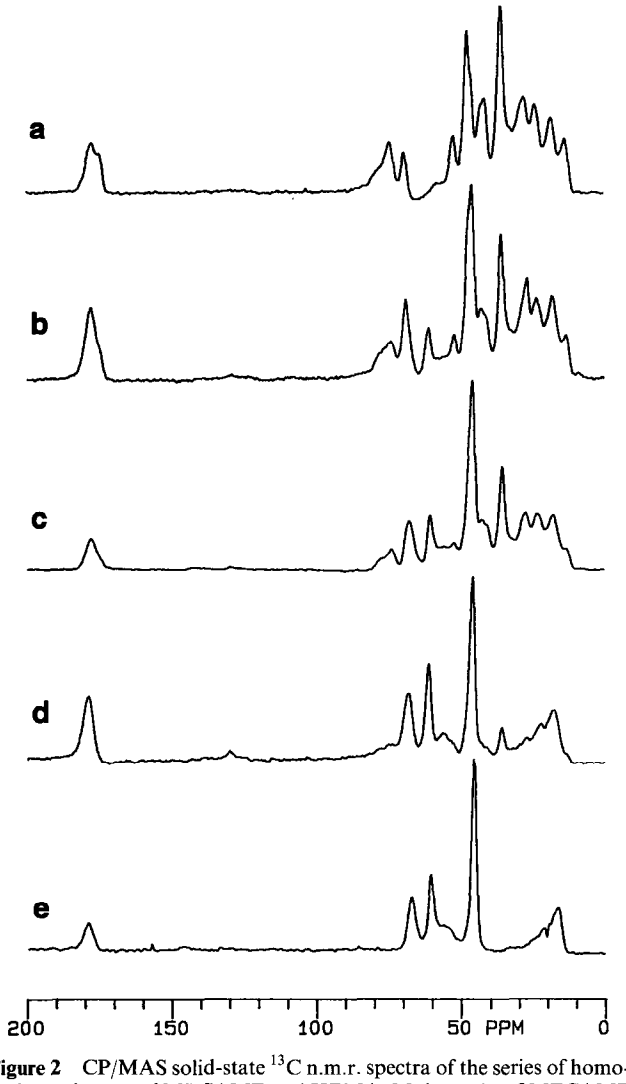

Figure 2 CP/MAS solid-state ^{13}C n.m.r. spectra of the series of homo- and copolymers of MECAME and HEMA. Molar ratio of MECAME to HEMA: (a) 100:0 (MECAME homopolymer); (b) 41:59; (c) 35:65; (d) 7:93; (e) 0:100 (HEMA homopolymer)

After optimization of the n.m.r. experimental conditions such as the contact time and relaxation delays, the relative molar composition of the copolymers can be estimated by comparison of the ratio of the intensities or the integrations of the characteristic peaks. The well-defined peak with a maximum at 42 ppm belongs to the quaternary carbon on the methacrylic backbone (MA-C) and existed for all the homo- and copolymers. Another sharp peak at ca. 34.5 ppm, certainly belonging to the cholic acid residue, can be attributed to the carbon at position 10. Therefore, the MA-C peak and its ratio to the peak at 34.5 ppm were used as the standard in calculation of the molar ratio of the two comonomers. It is of note that it is the ratios, not the absolute intensities or areas of the individual n.m.r. signals, that are important for the estimation. For the copolymers with methacrylic acid, the two methylene peaks from the 2-hydroxyethyl group between 55 and 70 ppm do not exist. In this case, additional information can be obtained by taking the ratio of the integration of the clearly defined peaks for the carbons at positions 3, 7 and 12 (65–85 ppm) to that of the standard MA-C peak at 42 ppm. The results obtained from the two calculations are comparable, confirming the molar ratios estimated for the final copolymers (*Table 2*). The estimated error for the values in *Table 2* should be within ±10%.

The conversion rates from monomers to polymers were 70–90%. At a lower conversion rate (e.g., 40%), the copolymer did not show any significant change in chemical composition within experimental error as determined by solid-state n.m.r. As shown in *Table 2*, the chemical compositions of the resulting copolymers are very close to the original monomer compositions added to the solution prior to polymerization, indicating similar reactivity of the comonomers. The similarity in structure of all the comonomers used is obvious. They all possess the same polymerizable group, i.e., the methacrylic group. Although the Price Alfrey reactivity parameters of copolymerization (the Q and e values)

Table 2 The chemical composition (molar ratio) of the copolymers as determined by solid-state ^{13}C n.m.r.

Copolymer	Monomer ratio before polymerization	Monomer ratio in the obtained polymer
MECAME–MAA	10:90	7:93
	30:70	37:63
	50:50	45:55
MECAME–HEMA	10:90	7:93
	30:70	35:65
	50:50	41:59
MACAME–MAA	10:90	11:89
	12:88	13:87
	17:83	23:77
	25:75	25:75
	50:50	42:58

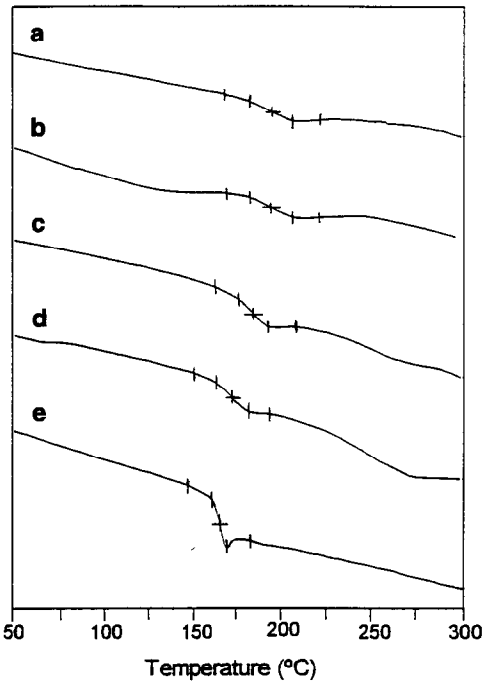

Figure 3 D.s.c. thermograms of the series of homo- and copolymers of MECAME and MAA. Molar ratio of MECAME to MAA: (a) 100:0 (MECAME homopolymer); (b) 45:55; (c) 37:63; (d) 7:93; (e) 0:100 (MAA homopolymer)

are not available for the new monomers, it can be expected that their reactivities should be close to those of the comonomers, which facilitates a random copolymerization.

The glass transition temperatures (T_g) of all the three series of copolymers were determined by d.s.c. and only the thermograms of the series of polymers consisting of MECAME and MAA, which are typical of all the polymers, are shown in *Figure 3*. These thermograms have been enlarged to show the relatively weak transitions (very small changes of heat capacity), ranging from only 0.05 to 0.10 W per gram of sample. Several features of the copolymers have been observed: all the copolymers are characterized by only one glass transition temperature and this temperature is always located between the T_gs of the two corresponding homopolymers. This is characteristic of random copolymers. The glass transitions of the copolymers as shown on the d.s.c. thermograms are broad, probably due to the relatively large polydispersity of the polymers. Even the homopolymer of MECAME has also a broad glass transition. In addition, different conversion rates and small changes of molecular weight did not show any significant effect on the glass transition of the copolymers. The d.s.c. analysis also showed that the polymers and copolymers were stable up to about 330–350°C, at which temperature some polymers started to decompose.

The T_gs of all the three series of copolymers determined by d.s.c. are shown in *Figure 4* as a function of copolymer composition. Variation of the T_gs of copolymers as a function of composition in the ideal case is linear, but the dependence on the weight fraction ω of the monomers is expected to follow equation (1)[11]

$$\frac{1}{T_g} = \frac{1}{1 + (K-1)\omega_2}\left[\frac{1-\omega_2}{T_{g1}} + \frac{K\omega_2}{T_{g2}}\right] \qquad (1)$$

where the subscripts 1 and 2 denote the two corresponding homopolymers and K is a weighting factor and is taken as a constant, which has a value of 1 in the ideal case. Note also that $\omega_1 + \omega_2 = 1$. The K values deduced from curve fitting for the copolymers are in the range 0.76–1.18, which are quite close to unity and thus are not far from an ideal case. These values provide another indication of the east of obtaining the random copolymers. It is important to note, however, that the molecular weight of the copolymers and their polydispersity in the series to be compared are not identical, which may have contributed to the scattering of the data points in *Figure 4*.

Hydrolysis of the ester protecting groups

The copolymers with MAA and HEMA have shown increased hydrophilicity. With high MAA content, the copolymers are readily soluble in water, even with the presence of the methyl ester group on the cholic acid residue. The copolymers with HEMA are less hydrophilic than their MAA counterparts. Hydrolysis of the methyl ester groups can liberate the carboxylic acid groups of cholic acid and should further increase the hydrophilicity of the polymers.

Since the ester or amide bonds between the cholic acid derivatives and the methacrylic group are also prone to hydrolysis, the hydrolysis has to be performed with care. During the selective hydrolysis, either incomplete or over-hydrolyses were often encountered. Therefore, the experimental condition of the hydrolysis was most critical. In order to find the most appropriate conditions of hydrolysis, we have carried out some systematic tests by varying the concentration of the bases, the temperature and the reaction time and characterized the hydrolysed polymers by ^{1}H n.m.r. in solution (by monitoring the methyl ester group OCH_3 at ca. 3.7 ppm) as well as solid-state ^{13}C n.m.r. (OCH_3 at ca. 52 ppm).

When the reaction was carried out in 2 N NaOH solution mixed with THF at a ratio of 1:3, at reflux temperature, we found that at 2 h the methyl ester group started to be hydrolysed and that, at 5 h or longer, some of the methacrylate bonds at position 3 were also broken

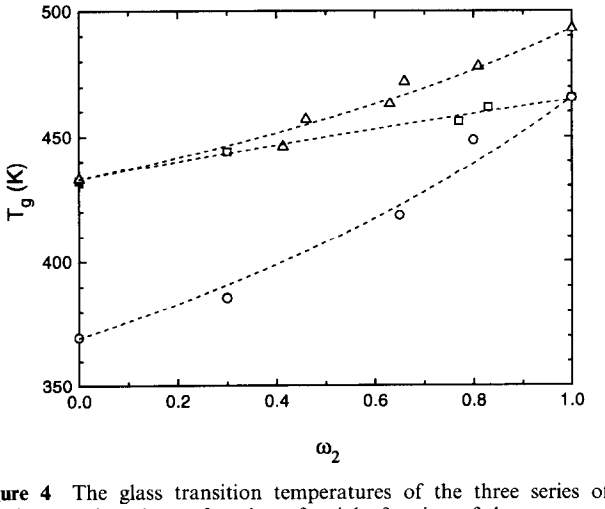

Figure 4 The glass transition temperatures of the three series of copolymers plotted as a function of weight fraction of the monomer units MECAME (methacrylate) or MACAME (methacrylamide): squares, MECAME-MAA; circles, MECAME-HEMA; triangles, MACAME-MAA. Dashed lines represent fitted curves according to equation (1)

since the ^{1}H n.m.r. spectrum showed a diminished peak at 4.6 ppm for the 3-CH ester group and a simultaneous appearance of a new peak at 3.5 ppm for the free hydroxy group. It was found that a reaction time of 3 h can hydrolyse most of the methyl ester groups while keeping the methacrylate bonds mostly intact. *Figure 5* shows the solid state ^{13}C n.m.r. spectra of the MECAME polymer before and after hydrolysis. Attention should be paid to the peak of the methyl ester group appearing at ca. 44–47 ppm in *Figure 5a*. After a 3 h hydrolysis, the methyl ester carbon signal has already disappeared, indicating removal of the ester group. Therefore, hydrolysis of the methacrylic ester and amide groups proved more difficult, most likely due to the steric hindrance created by the polymer backbone and by the methyl group on the methacrylic residue which helps to stabilize the ester or amide bonds.

D.s.c. analyses were also done on the hydrolysed samples. The d.s.c. analyses showed that upon hydrolysis of 3 h, the homopolymer of MECAME, now with the methyl ester groups removed, had a T_g of 252.2°C, a value very close to that of the homopolymer of the methacrylate of free cholic acid, 256.5°C[12], but much higher than that of the homopolymer of MECAME (192.7°C). Using the relationship in equation (1) by assuming an ideal case ($K = 1$), we can estimate that ca. 94% of the methyl ester groups were already hydrolysed at 3 h. The variance of K values between 1.18 and 0.76 does not change this estimate by any significant amount (between 93 and 95 wt%). Of course, given the closeness of molecular weights of cholic acid and its methyl ester, the molar fraction of the hydrolysed form should be approximately identical to the corresponding weight fraction.

Further prolongation of the hydrolysis beyond 3 h is not necessary and may cause excessive hydrolysis which also breaks the methacrylic ester or amide bonds. Increased reaction time may lead to a copolymer of methacrylic acid and methacrylate of cholic acid, which occurred at about 5 h. Hydrolysis of the methyl ester group on the MACAME polymers requires less restraint of the conditions because of the higher stability of the methacrylamide bond than the methacrylate ester.

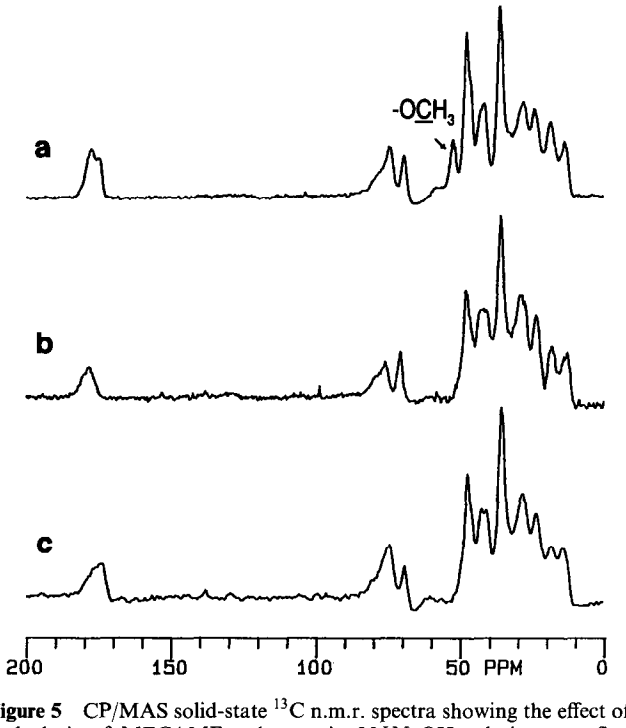

Figure 5 CP/MAS solid-state ^{13}C n.m.r. spectra showing the effect of hydrolysis of MECAME polymers in 2 N NaOH solution at reflux temperature. Reaction time of hydrolysis: (a) 0 h (MECAME polymer); (b) 3 h; (c) polymer of methacrylate of cholic acid[12]

It is important to point out that, after hydrolysis, the solubility of the polymer in water may increase significantly but depends on the degree of hydrolysis, ranging from insoluble at a lower degree of hydrolysis to quite soluble when completely hydrolysed, always in a basic solution. The hydrolysed form of the homopolymer was very soluble in the basic media. When the media was adjusted with dilute HCl solution to acidic pH, the transparent solution became milky and precipitation began. Therefore, the change of solubility was evident. Difficulties were encountered when redissolving the hydrolysed polymer in water after precipitation and drying. However, the polymer in its salt form is soluble in water and has very different solubilities in basic and acidic media.

CONCLUDING REMARKS

Polymers have been made by free radical copolymerization in solution of new monomers synthesized from bile acid derivatives and other methacrylic monomers such as methacrylic acid and 2-hydroxyethyl methacrylate. Characterization of the copolymers by d.s.c. and solid-state ^{13}C n.m.r. proved that the copolymers were random and that the chemical composition in the product was close to the original monomer ratio mixed prior to the polymerization, even at a relatively low conversion rate. Also as an effort to increase the hydrophilicity of the polymer, the homopolymers of methyl esters of the bile acid derivatives were selectively hydrolysed in bases. In a 2 N NaOH medium at reflux temperature, a reaction time of about 3 h was appropriate to remove the methyl ester groups while keeping the methacrylate ester bond mostly intact. It was also found that the methacrylamide bond is more resistant to hydrolysis than the methacrylate bond. With the increased water solubility after copolymerization or hydrolysis, it should be interesting to prepare hydrogels from the bile acid-containing monomers with a certain degree of cross-linking and to study their properties and potential applications.

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