BILE ACIDS AS BUILDING BLOCKS OF AMPHIPHILIC POLYMERS. APPLICATIONS AND COMPARISON WITH OTHER SYSTEMS

Alain DURAND

Laboratoire de Chimie Physique Macromoléculaire, UMR 7568 CNRS-Nancy-University, ENSIC, BP 20451, 1 rue Grandville, 54001 Nancy cedex, France; e-mail: alain.durand@ensic.inpl-nancy.fr

Received October 17, 2007
Accepted November 19, 2007

This review deals with the use of bile acids as building blocks of amphiphilic polymers. These natural polyfunctional organic molecules have been employed in the synthesis of macromolecules combining hydrophilic and hydrophobic sequences. The two main synthetic strategies are radical (co)polymerization after attachment of a vinyl group onto the bile acid and molecule grafting of bile acid onto a hydrophilic polymer. The physicochemical properties of the resulting polymers both as bulk materials and in aqueous solution are reviewed and compared with polymers of other structures. Whenever possible, semiquantitative correlations are established and discussed.

Keywords: Bile acids; Amphiphilic polymers; Polysaccharides; Macromolecules; Steroids; Copolymerizations; Hydrophilic materials.

Collect. Czech. Chem. Commun. 2007, Vol. 72, No. 11, pp. 1553–1578

doi:10.1135/cccc20071553

1. INTRODUCTION

Amphiphilic polymers have been widely studied during the last thirty years. These polymers were first obtained by polymerizing surface-active monomers (which led to the well-known polysoaps)[1]. It was intended to combine macromolecule characteristics to surface-active behaviour of individual units. The polymers obtained in this way were shown to form intramolecular aggregates called "necklaces" even at very low concentrations and exhibited very low water solubility. Permanent links between surface-active units favoured their aggregation on a thermodynamic basis since it strongly reduced the entropic loss accompanying aggregation of individual units. Later, other amphiphilic polymers were synthesized combining a large majority of hydrophilic units and a few hydrophobic ones[2]. Contrary to polysoaps, this kind of macromolecules has an "overall amphiphilic character" caused by the presence of hydrophilic and hydrophobic sequences within the chains (instead of being chains of individual amphiphilic units). Such polymers are generally water-soluble (depending on the amounts of the various units) and form intermolecular associations in sufficiently high concentrations[3]. Indeed, in addition to overlaps between macromolecules, the thermodynamic tendency of hydrophobic groups to associate so as to minimize contacts with the surrounding aqueous medium (hydrophobic effect) creates supplementary interactions between macromolecules. This leads to the formation of a physically crosslinked network and thus to aqueous solutions with high viscosities (associative polymers). A great deal of work has been devoted to the rheological behaviour of semidilute and concentrated aqueous polymer solutions. In particular, it has been shown that the viscosity level can be considerably modified by varying the nature of the hydrophobic units as well as their distribution within polymer chains. At interfaces between aqueous phase and hydrophobic surface, these macromolecules adsorb and lead to the formation of a thick polymeric layer[4,5]. In the case of liquid–liquid interfaces, this polymeric layer leads to specific mechanical response to surface deformation. This is the source of emulsifying properties of amphiphilic macromolecules. For solid–liquid interfaces, the solid surface is coated with a polymeric layer which controls interactions of the particles with the surrounding medium. Generally the polymeric layer is thick enough to prevent aggregation driven by van der Waals attractions. Another important characteristic of adsorption of macromolecules at interfaces is its "kinetic irreversibility". Indeed, the desorption of an individual macromolecule needs that all hydrophobic units desorb simultaneously from the interface, the

event that has a very low probability. Thus polymer-stabilized dispersions can generally be diluted without destabilization, which can be valuable in some cases[4].

The synthesis of amphiphilic polymers has been carried out in mainly two ways. The first one relies on the copolymerization of at least two monomers, one hydrophilic and one hydrophobic. The distribution of the various units in the chains is largely controlled by the chemical structure of the monomers (reactivity ratios) and by the reaction medium (homogeneous, heterogeneous, micellar, ...). Indeed, it has been shown that the blockiness of amphiphilic copolymers can be controlled by the amount of surfactant in micellar copolymerization[3].

The other procedure involves chemical modification of a pre-existing hydrophilic polymer through attachment of hydrophobic groups. In that case, the distribution of hydrophobic units along the hydrophilic backbone is mainly controlled by the reaction medium[6,7]. Using these two synthetic strategies and because of the abundance of chemical structures, it has been possible to obtain amphiphilic polymers exhibiting sensitivity to other physicochemical variables like temperature, ionic strength, UV-visible radiation and other. This leads to colloidal systems with original behaviours: inverse viscosity variation with temperature, switching the emulsion type by UV radiation or ionic strength, anti polyelectrolyte behaviour and other. More recently, other requirements appeared which were related to environmental concern. There was a growing interest for amphiphilic polymers obtained from renewable resources and exhibiting biodegradability and biocompatibility. Similar requirements were driven by a new type of applications related to the biomedical field such as materials for tissue repair and drug delivery devices. This led to the use of new materials for the preparation of amphiphilic polymers like polysaccharides. The hydrophilic polymers exhibit interesting properties like biodegradability and biocompatibility and can be chemically modified so as to obtain amphiphilic polymers.

Bile acids are a class of biological molecules with a common general structure (Chart 1)[8]. The main members are shown in Table I. These compounds exhibit limited solubility in water in the acid form and exhibit surface-active properties in the sodium salt form (Table II)[9–16]. The hydrophobicity of bile acids makes them attractive materials for preparation of amphiphilic polymers in which they are incorporated as hydrophobic segments. This has been performed following mainly three ways: grafting of bile acids onto a hydrophilic polymer (polysaccharide in most cases), attachment of bile acid molecules at the ends of hydrophilic macromolecules

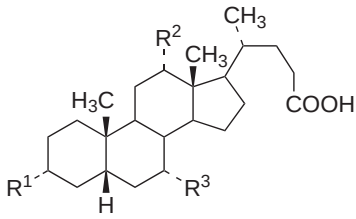

CHART 1
Chemical structure of bile acids

TABLE I
Chemical structure of some bile acids used as building blocks of amphiphilic polymers (for R^1, R^2 and R^3, see Chart 1)

Bile acid	R^1	R^2	R^3	M_w
Cholic acid	OH	OH	OH	408
Deoxycholic acid	OH	OH	H	392
Chenodeoxycholic acid	OH	H	OH	392
Lithocholic acid	OH	H	H	376
Ursodeoxycholic acid	OH	H	β-OH	392
5β-Cholanic acid	H	H	H	360

TABLE II
Physicochemical properties of bile acids

Bile acid	Acid		Sodium salt			
	Solubility[a] mol/l	log P[b]	CMC[c] mol/l	CMC[a] mol/l	CMC[d] mol/l	CMC[e] mol/l
Cholic	0.273×10^{-3}	0.72	18.4×10^{-3}	13×10^{-3}	10×10^{-3}	11×10^{-3}
Deoxycholic	0.028×10^{-3}	2.56	5.3×10^{-3}	10×10^{-3}	2×10^{-3}	4×10^{-3}
Ursodeoxycholic	0.0009×10^{-3}	1.05	–	19×10^{-3}	–	10×10^{-3}
Chenodeoxycholic	0.027×10^{-3}	2.20	7×10^{-3}	9×10^{-3}	–	4.6×10^{-3}
Lithocholic	0.05×10^{-3}	–	–	0.9×10^{-3}	0.25×10^{-3}	–
5β-Cholanic	–	–	–	–	0.03×10^{-3}	–

[a] Data in water at 25 °C from[16] except for the CMC of lithocholic acid sodium salt measured at 75 °C. [b] Octanol-water partition coefficient, data from[10]. [c] Data in water at 30 °C and pH 7.9 from[14]. [d] Data in water at 20 °C and pH 9 from[15]. [e] Data in water at 25 °C from[12].

and polymerization or copolymerization of bile acid-carrying vinylic monomers. In addition to their hydrophobicity, bile acids exhibit other specific properties in aqueous media which can be valuable in many biological applications such as complex formation with host molecules and micelle formation[17–19].

This review focuses on the use of bile acids as building blocks for the preparation of amphiphilic polymers. The various synthetic methods employed are examined with a special emphasis on the relation between the synthesis conditions and the chemical structure of amphiphilic polymers. In the second part, physicochemical properties of bile acid-based amphiphilic polymers are considered in aqueous solution and at air–water interfaces. We will try to relate the observed properties to the chemical structure of the polymers as well as to that of the bile acid employed. Whenever possible, we will try to compare the observed properties with those obtained with other amphiphilic polymers.

2. BILE ACIDS AS BUILDING BLOCKS FOR AMPHIPHILIC POLYMERS

The synthesis of macromolecules containing bile acid blocks has been carried out following the four strategies: attachment of a double bond to bile acid molecules followed by a chain polymerization, step-polymerization of bile acids, random grafting of bile acid molecules onto pre-existing macromolecules and end-functionalization of polymers by bile acid molecules. As far as amphiphilic polymers are concerned, the step-polymerization strategy will not be treated in detail since the polymers obtained in this way are not generally considered as amphiphilic macromolecules[20–26].

2.1. Chain Polymerization of Bile Acid-Carrying Monomers

This synthesis strategy involves at least two steps: (i) the preparation of a vinylic monomer by the introduction of a vinyl group into the bile acid structure and (ii) the (co)polymerization of the previous monomer often by the radical mechanism. This overall synthesis procedure can be directly compared with that leading to glycopolymers which is formally identical except that a mono- or disaccharide is used instead of a bile acid[27,28]. Glycopolymers are hydrophilic macromolecules with saccharide side groups while the polymers mentioned here are essentially hydrophobic ones with bile acids as pendant groups.

2.1.1. Synthesis of Monomers

This step is essential for final properties of the polymer and its structural originality.

Double bonds have been attached to bile acid molecules mainly through the formation of methacrylate and methacrylamide derivatives[29,30]. One hydroxyl group of the bile acid is involved in the formation of the ester or amide function. The difference in chemical reactivity of various hydroxyl groups contained in some bile acids provides regioselective fixation of the polymerizable function[31]. The acid group which remains is protected as methyl ester and eventually regenerated by hydrolysis. Instead of direct fixation of the double bond on the bile acid molecule, oligo(ethylene glycol) or C_{10} carbon spacers have been introduced[32–34]. These flexible spacers can import interesting properties to the final polymer but can also be essential for the polymerization step when acyclic diene metathesis was used in the polymerization step[35].

Another family of monomers derived from bile acids has been prepared by the fixation of acrylamide or methacrylamide groups via the carboxylic acid function. In that case a 2-carbon spacer separated the (meth)acrylamide function from the bile acid rigid core[36].

Finally, a monomer has been prepared by the use of lipase catalysis and contained an oligo(cholic acid) chain with one 11-(methacrylamido)-undecanoate ester group at one end[37]. This is the only example of a vinyl derivative of a bile acid in which there are on average two or three bile acid moieties per double bond.

2.1.2. Polymerization

Up to now, a large majority of polymerizations of monomers derived from bile acids have been radical polymerizations in organic solvents. Experiments were performed in which bile acid-based monomers were polymerized at the air–water interface[38]. Atom transfer radical polymerization (ATRP) was first reported recently with that kind of monomer[39]. This polymerization method provides a much better structural control (especially with regard to the length of macromolecules). Alternatively, well-defined polymers have been prepared and bile acids then grafted onto these macromolecules[40]. Recently, metathesis polymerization has been reported for the preparation of oligomers[35]. To our knowledge, no polymerization in disperse medium has been attempted despite its potential interest for the preparation of nanoparticles for drug delivery applications.

When compiling the available results of radical homopolymerization of bile acid monomers, several trends can be observed (Table III). The results concern mainly cholic acid (CA) and, for a minor part, lithocholic acid (LCA). Complex temperature cycles with progressive temperature rise seem to produce broad molecular weight distributions (see I_p values), which can be explained by differential variations of the initiator and monomer concentrations leading to a wider range of polymer chain lengths. The highest molecular weights are reported for polymerizations carried out in toluene instead of tetrahydrofuran or chloroform. These solvents are known to be efficient chain transfer agents in radical polymerization[41]. We should also notice that many polymerizations have been carried out at 60 °C. At that temperature, the half-life of the initiator (2,2′-azobisisobutyronitrile, AIBN) is about 23 h [41]. The chemical structure of the monomers seems to have an influence on the molecular weights obtained. Indeed, the axial or equatorial position of the methacrylate or methacrylamide functions seems to significantly change the molecular weights of the polymers under the same conditions of polymerization[29]. It must be noted that many values available for the molecular weights have been obtained by size exclusion chromatography using polystyrene standards. It is clear that differences in chemical structure between the investigated polymers and the standards make these values questionable. The very strong difference in molecular weights obtained by polymerizing 3α-MACAME and 3β-MACAME reported by Denike et al.[29] should be examined further considering possible artefacts related to conformational differences in THF since the two polymers exhibit significantly different solubilities. Recently, Gautrot el al.[35] showed that the use of polystyrene standards in size exclusion chromatography analysis of lithocholic acid oligomers leads to molecular weights overestimated by a factor 2. It is not possible to extrapolate these quantitative conclusions to the polymers mentioned in Table III since their chemical structure is different from that of the oligomers of the previously mentioned work.

Copolymers combining bile acid-derived monomers and other hydrophilic or hydrophobic monomers have been prepared by radical polymerization: styrene, methyl methacrylate, acrylamide and *N*-isopropylacrylamide are the main examples[30,34,36,42–46]. In the case of the copolymerization of 3α-MECAME and maleic anhydride (MAN), the reactivity ratios have been determined experimentally to be 11.6 for 3α-MECAME and 0.01 for MAN [46]. With these values of reactivity ratios, the preparation of random copolymers is not possible in a batch process. For the copolymerization of 3α-MECAME and styrene, the reactivity ratios 0.8–0.9 have been

TABLE III
Free-radical polymerization of bile acid-based acrylic monomers: reaction conditions and characteristics of the recovered polymers

Monomer	$[M]_0$ mol/l	Solvent	AIBN mole %[a]	Time of reaction %	T °C	$\overline{M}_n$ [b]	I_p	Yield %	Reference
oligo(CA)-COO-$(CH_2)_{10}$-MA	0.22	THF	8.4	24	60	33000	–	52	37
3α-MECAME	0.067	$CHCl_3$	3.0	12	60–70	29700	1.59	88	85
3β-MECAME	0.067	$CHCl_3$	3.0	12	60–70	112200	1.48	92	85
3α-MACAME	0.067	$CHCl_3$	3.0	72	60–70	20400	1.76	75.5	85
3β-MACAME	0.067	$CHCl_3$	3.0	72	60–70	32000	1.61	78	85
3α-$(CH_2)_{10}$-COO-CAME	0.06–0.08	THF	3.0	48	60	7100[c]	1.23	70–90	33
3α-$(CH_2)_{10}$-COO-LCAME	0.06–0.08	THF	3.0	48	60	8900[c]	1.29	70–90	33
3α-MELCAME	0.36	toluene	5.0	16.5	60	87000[d]	–	58	42
3α-MECAME	0.19	toluene/THF (8/1)	5.0	21.5	60	296000[d]	–	14	42
3α-MECAME	not given	toluene	5.0	28	[e]	212300	2.38	70–90	45
3α-MACAME	0.0613	toluene	4.0	ca. 40	[f]	26800	3.55	not given	29
3β-MACAME	0.0613	*tert*-butanol	4.0	ca. 40	[f]	550500	1.54	not given	29

[a] Relative to monomer. [b] Polystyrene standards. [c] The experimental method of determination is not detailed. [d] Membrane osmometry in toluene at 310 K. [e] Gradual rise in temperature up to 100 °C over 24 h. [f] Gradual rise in temperature up to 75 °C over 2 h, then maintained at 75 °C for 24 h, then heated at 100 °C and kept unchanged during 15 h.

reported for both monomers but the method of determination is not described in detail[42].

The recent use of atom transfer radical polymerization[39] opens the way to the synthesis of block copolymers which would lead to new properties compared with the random copolymers.

2.2. Grafting of Bile Acids onto Hydrophilic Polymers

This synthetic strategy for the preparation of amphiphilic polymers involves generally the reaction between a previously synthesized hydrophilic polymer and a bile acid. Other synthetic approaches were described in some specific cases[40,47].

Bile acids are often attached through their carboxylic acid group via the formation of an ester (agarose[48], dextran[49], 2-(hydroxyethyl)cellulose[50]) or an amide (heparin[51], chitosan[52] and glycol chitosan[53]) (Table IV). Sometimes, a flexible spacer is inserted between the bile acid groups and the polymer backbone which can be an aliphatic hydrocarbon chain or a sequence of several ethylene oxide units[34,49,50].

According to the reaction conditions, the amount of bile acid groups attached to the polymer backbone can be varied over a wide range. Most works use polysaccharides as starting polymer (dextran, chitosan and heparin) but a protein (bovine serum albumin, BSA) as well as a random copoly-

TABLE IV
Structural characteristics of bile acid-modified polymers

Polymer	$\overline{M}_w$	Bile acid	Content in the polymer, wt. %	Reference
Dextran	30 200	CA	<42[a]	49
Dextran	210 300	DCA	<42[a]	49
Agarose[b]		CA	–	48
Hydroxyethylcellulose[c]		3-Ac-LCA	53	50
Chitosan	70 000	DCA	6–11	96
Glycol chitosan	250 000	DCA	6–35	98
Heparin	12 400	DCA	7–24	103
BSA	69 000	DCA	<34	54

[a] The modified dextran samples are reported to be water soluble only for wt.% lower than 13. [b] Crosslinked polymer. [c] Not available.

mer of 1-acryloylbenzotriazole and 4-acryloylmorpholine have also been modified in that way[34,54].

The influence of the reaction solvent has been particularly detailed for dextran and glycol chitosan modification. It was demonstrated that the amount of bile acid attached to dextran changed significantly with the nature of the solvent; a ternary solvent mixture was shown to be optimal for dextran esterification with cholic acid as well as deoxycholic acid[49,55]. A more complex behaviour was reported for the modification of glycol chitosan by 5β-cholanic acid carried out in water–methanol mixtures. The optimal mixture composition varied with the targeted degree of substitution[56].

2.3. End-Functionalized Polymers with Bile Acids

The synthesis of hydrophilic polymers end-capped with bile acids was carried out essentially in the presence of poly(ethylene oxide) (PEO)[57–60] chains even if other examples can be found: PNIPA[61] and poly(anhydride)s[62,63] (Table V).

In many cases, bile acids are bound via their carboxylic group by the formation of amide bonds. Amino-terminated poly(ethylene glycol) was used together with an activator of the carboxylic acid function: *N*-hydroxysuccinimide or dicyclohexylcarbodiimide[57,60]. The formation of ester bonds

TABLE V
Structural characteristics of polymers end-functionalized with bile acids

Polymer	$\overline{M}_n$	Bile acid	wt. %[a]	Reference
Poly(ethylene oxide)	1100	DCA[b]	28	60
Poly(ethylene oxide)	5100	DCA[b]	8	60
Poly(ethylene oxide)	2000	DCA[b]	35	59
Poly(ethylene oxide)	2000	CA[b]	15	57
Poly(ethylene oxide)	194	UDA	67	58
Poly(ethylene oxide)	1000	UDA	28	58
Poly(*N*-isopropylacrylamide)	7880	CA[b]	5	61
Poly(sebacic anhydride)	3800	LCA[b]	20	63

[a] Weight fraction of bile acid in the end-capped polymer, calculated from the chemical formula. [b] One bile acid molecule attached at one end of the macromolecules. [c] Three bile acid molecules attached at one end of the macromolecules.

has been also reported with hydroxyl-terminated PEO, following a similar reaction scheme as mentioned above[58]. For the synthesis of PNIPA end-capped with cholic acid, amino-terminated PNIPA was first prepared by radical telomerisation in the presence of 2-aminoethane-1-thiol hydrochloride as the telogen[61]. In the case of polyanhydrides, bile acids were added to the polymerization mixture and acted as mono-functional monomers, which is equivalent, for step-polymerization, to chain terminators.

Mainly polymers end-capped at one end of polymer chain have been synthesized. One bile acid moiety was attached at one end of each macromolecule, with the exception where three bile acid molecules were linked at one end of a PEO molecule via the use of tris(hydroxymethyl)aminomethane. In the case of bile acid-terminated polyanhydrides, bile acid groups could be attached at both ends. Apart from the use of very short macromolecules (with a degree of polymerization, DP = 4), the weight fraction of bile acids in the final polymers is generally less than 35% (Table V).

3. SELF-ORGANIZATION IN AN AQUEOUS MEDIUM

3.1. Micelle-Like Self-Organization

The micellization behaviours of amphiphilic polymers consisting of hydrophilic macromolecules end-capped with bile acids have been reported. Generally, the critical aggregate concentration (CAC) was detected by static fluorescence using molecular probes like pyrene (Table VI). Due to a rather low number of experimental data, it is difficult to find a relation with the chemical structure similar to what has been done for PEO end-capped with aliphatic hydrocarbon groups[64–69].

TABLE VI
Critical micelle concentration of polymers end-functionalized with bile acids as determined by fluorescence techniques at 25 °C

Polymer	CMC mg/l	CMC mol/l	CMC of bile acid mol/l	Reference
PEO1000-DCA	43	3.07×10^{-5}	10×10^{-3}	60
PEO5000-DCA	36	7.06×10^{-6}	10×10^{-3}	60
PEO2400-3DCA	1.5	1.32×10^{-6}	10×10^{-3}	59
PEO2000-CA	50	1.84×10^{-5}	13×10^{-3}	57
PNIPA7880-CA	89	1.07×10^{-5}	13×10^{-3}	61

The critical aggregate concentrations of random copolymers containing limited amounts of bile acid repeating units have also been determined by static fluorescence measurements. The values as low as 1.5×10^{-3} wt.% were reported for random copolymers with *N*-isopropylacrylamide (NIPA) units[36,70,71]. This value is much lower than that of PNIPA or other acrylamide copolymers. Contrary to polymers end-capped with bile acids, these CAC of random copolymers may correspond to the formation of hydrophobic microdomains because of coiling of independent single macromolecules.

3.2. Formation of Physical Networks: The Associative Behaviour

Polysaccharides carrying bile acid moieties randomly attached to the backbone may cause intramolecular and intermolecular interactions in aqueous solutions depending on polymer concentration. Provided that the degree of substitution is sufficiently low, polymers remain water-soluble up to high concentrations (50 g/l). Static fluorescence allowed the determination of the critical concentration of formation of hydrophobic microdomains. This was shown to occur much below the limit of the dilute domain. Moreover, this concentration appeared to strongly depend on the length of polysaccharide chains[64]. Increasing the length of the hydrophilic backbone led to much lower critical concentrations for the formation of microdomains. The same effect is obtained by exchanging the attached bile acid for a more hydrophobic one, at a given degree of substitution (for example when CA is replaced by DCA).

Viscometric measurements give information about hydrodynamic behaviour of amphiphilic macromolecules: intrinsic viscosity ([η], specific volume of dissolved species, individual macromolecules or aggregates), Huggins coefficient (k_H, interaction constant between dissolved species) and association concentration (C_{assoc}, above which intermolecular interactions become predominant and lead to the formation of large aggregates in the solution).

For sufficiently low polymer concentrations, i.e. in the dilute region, the variation of solution viscosity follows the Huggins relation

$$\eta_{red} = \frac{\eta - \eta_s}{\eta_s C} = [\eta] + k_H [\eta]^2 C \,. \qquad (1)$$

In Eq. (*1*), η and η_s are the viscosities of the polymer solution and of the pure solvent (in Pa s), respectively, η_{red} is the reduced viscosity of the solution (in l/g) and C is the polymer concentration (in g/l)[72].

The semidilute domain corresponds to sufficiently high polymer concentrations to allow the formation of a physical network in which intermolecular interactions have a predominant influence, leading to a significant deviation of the solution viscosity from that indicated by Eq. (*1*). This viscosity increase is typical of the so-called "associative polymers".

With bile acid-modified dextrans, intrinsic viscosity decreases when the degree of substitution is increased, as compared with the intrinsic viscosity of native dextran (Fig. 1). This effect is common to other dextran derivatives and results from the formation of compact aggregates involving one or several macromolecules in dilute solution[73]. The decrease in intrinsic viscosity for a given degree of substitution is more pronounced when the dextran molecular weight is higher, a trend which is also observed with other hydrophobic substituents as well as for other amphiphilic polymers (Fig. 1). The Huggins coefficient varies in the reverse way of the intrinsic viscosity, which is a common (but not general) observation. The associate concentration depends on both the polymer molecular weight and the sticking groups (nature and number). A quite general trend is observed when plot-

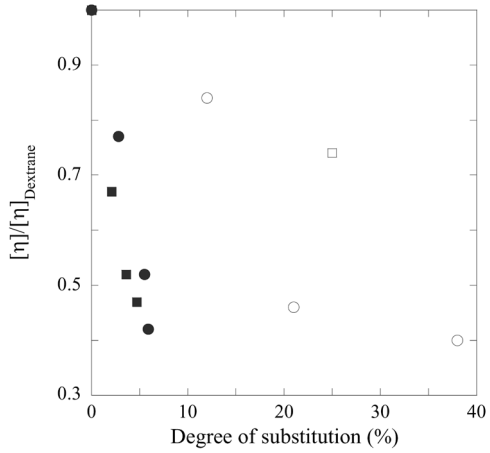

FIG. 1

Ratio of intrinsic viscosity of bile acid-modified dextrans to that of the corresponding native dextran as a function of the degree of substitution. The weight-average molecular weight of dextran is 30 000 (●), 210 000 (■), 40 000 (○) and 10 000 (□). The substituent groups are cholic acid (●, ■) (data from[49]), $-(CH_2)_5CH_3$ (data from[73])

ting the product $C_{assoc}[\eta]$ versus k_H for hydrophobically modified dextrans (Fig. 2). The values corresponding to dextran modified with various amounts of a bile acid are consistent with other results. In previous work, we found a semiempirical curve which gives the calculated course rather close to the experimental points (Fig. 2)[74–76].

By static and dynamic light scattering experiments, it was demonstrated that CA- and DCA-modified dextrans form compact aggregates in water at concentrations above 6 g/l with average hydrodynamic diameters of ca. 10 nm [77]. These results are consistent with the reported behaviour of other hydrophobically modified dextrans[73]. At much lower concentrations (below 0.2 g/l), Nichifor et al.[72] showed that CA- and DCA-modified dextrans form large and loose aggregates[77].

Other CA-modified dextrans have been prepared by the attachment of CA to one hydroxyl group. Consequently, an ionisable carboxylic function remained at the end of the hydrocarbon chain[78]. Although polyelectrolyte behaviour could be predicted in dilute aqueous solutions, it was not observed down to about 3 g/l. The authors attributed this observation to the formation of dense hydrophobic aggregates probably leading to a condensation of ion pairs and suppression of the polyelectrolyte behaviour.

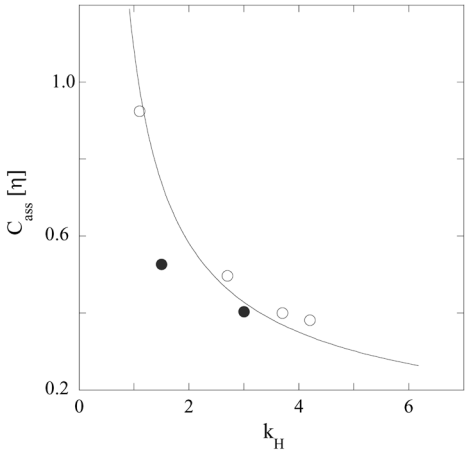

FIG. 2
Variation of the product of intrinsic viscosity to the critical association concentration with the Huggins coefficient for hydrophobically modified dextrans in aqueous solutions at 25 °C: ○ results from[73], ● results from[49]. The line represents a semiempirical prediction proposed in[74]

The addition of molecular surfactants to an aqueous solution of amphiphilic macromolecules has a great effect on solution viscosity, depending on their relative amounts. For sufficiently high amounts of a surfactant (above its critical micelle concentration (CMC)), amphiphilic macromolecules are fully dissociated from the neighbouring ones by the inclusion of their hydrophobic groups within surfactant micelles. This leads to a solution viscosity much lower than that obtained with the polymer alone. On the contrary, for intermediate surfactant amounts, mixed hydrophobic aggregates are formed involving hydrophobic segments of macromolecules and surfactant molecules. This mixed association efficiently contributes to the formation of a physical network in solution and much higher viscosities are obtained as compared with solutions of the polymer alone[79–81]. The former mechanism ignores a parameter that can become significant: the compatibility of the hydrophobic groups attached to macromolecules and the hydrocarbon tails of molecular surfactants. If the compatibility is limited, so is the variation of viscosity. This is what has been observed with DCA-modified dextran in the presence of various molecular surfactants[82]. Only limited viscosity enhancements are measured except for DCA (sodium salt) where a physical gel is formed but after several days (Fig. 3). Freshly

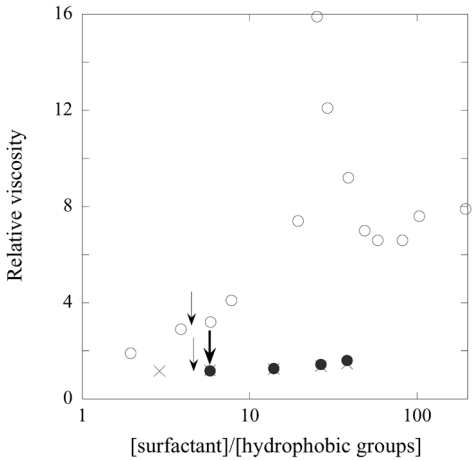

FIG. 3

Relative viscosities of mixtures of amphiphilic polymers and surfactants as a function of the molar concentration of surfactant normalized by the amount of hydrophobic groups of the polymer. ○ Cholesterol-modified pullulan (20 g/l) in the presence of SDS [80], ● deoxycholic acid-modified dextran (10 g/l) in the presence of SDS and × deoxycholic acid-modified dextran (10 g/l) in the presence of sodium deoxycholate[82]. Arrows indicate the position of the CMC of the surfactant, the bold one is for sodium deoxycholate

prepared mixtures exhibit viscosities similar to that of a solution containing the polymer alone. A transparent gel forms after a minimum of 10 days only in the presence of free DCA (sodium salt). DCA-modified dextrans seem to form compact hydrophobic aggregates which can include only free DCA molecules with rather slow kinetics. The CMC of sodium cholate was shown to decrease significantly in the presence of bile acid dimers[83]. Nevertheless, in the case of bile acid-modified polymers, other conformational restrictions may limit the interactions.

Apart from their associative behaviour, DCA-modified dextrans found applications in aqueous two-phase systems. Specific extractions were carried out with these polymers based on their interactions with solutes[84].

3.3. Thermal Properties of Bile Acid-Containing (Co)polymers

Three thermal properties of bile acid-containing polymers have been essentially studied: glass transition temperature of bulk materials, lower critical solution temperature of aqueous solutions and thermal stability. In what follows, we will detail the first two properties.

3.3.1. Glass Transition Temperatures of Bulk Materials

The introduction of bulky side chains like bile acids in macromolecules generally leads to materials with glass transition temperatures (T_g) close to 200 °C (Table VII). Configuration effects have been evidenced in the case of methacrylate and methacrylamide monomers carrying bile acid side groups. The β-position of the methacrylate (or methacrylamide) group led to polymers with T_g ca. 30 °C higher than the polymers with the α-position[85]. In order to decrease T_g two strategies were followed: (i) insertion of flexible spacers between the bile acid group and the main chain of the polymers, the spacers were chains of methylene or ethylene oxide units (up to 10) and (ii) copolymerization of bile acid-containing monomers with less sterically hindered monomers.

In the case of insertion of ethylene oxide (EO) units between main chain and bile acid side groups, experimental results are available[32]. Increasing the number of EO units from 1 to 6 lowers the T_g from more than 200 °C down to about 40 °C. Nevertheless, as stressed by the authors, the studied polymer samples differing both the repeating units and the molecular weight so that the variations of T_g cannot be attributed to a single effect of chemical structure.

On the basis of available experimental results, a semiquantitative analysis can be attempted of the link between T_g and the chemical structure of the macromolecules following the approach of the group contribution method and the formalism of van Krevelen[86,87]. Thus, the glass transition temperature is expressed as

$$T_g = \frac{\sum_i Y_{gi}}{M} . \qquad (2)$$

In Eq. (*2*) M is the molar mass of the repeating unit (in g/mol) and Y_{gi} (in K g/mol) is the contribution of the structural element n_i^0 to T_g (in K). In addition to the chemical structure of the repeating unit, it has been shown that T_g varies with the molecular weight of the polymer following the equation of the form[88]

$$T_g = T_g^{\infty} - \frac{A}{\overline{M}_n} . \qquad (3)$$

TABLE VII
Glass transition temperatures of some bile acid-containing homopolymers

Polymer	$\overline{M}_n$ [a]	T_g, °C	Reference
Poly(3α-MECAME)	212 300	192	30
Poly(3β-MECAME)	112 200	236	30
Poly(3α-MACAME)	26 800	219	30
Poly(3β-MACAME)	32 000	241	30
Poly(3α-$(CH_2)_{10}$-CAME)	7 100	≈50	33
Poly(3α-$(CH_2)_{10}$-LCAME)	8 900	≈25	33
Poly(LCA)	not given	85	20
Poly(3α-MECAME)	53 500	206	32
Poly(3β-ME-$(EO)_1$-CAME)	38 600	146	32
Poly(3β-ME-$(EO)_2$-CAME)	26 900	123	32
Poly(3β-ME-$(EO)_4$-CAME)	22 000	62	32
Poly(3β-ME-$(EO)_6$-CAME)	15 700	32	32
Poly(MATCA)	16 000	105	39

[a] Determined by size exclusion chromatography with polystyrene standards.

In Eq. (*3*), T_g^∞ is the glass transition temperature of the polymer with very high molecular weight and A is a constant (in g/mol) depending on the repeating unit.

Combining Eqs (*2*) and (*3*), we get

$$\overline{M}_n T_g = \overline{DP}_n \left(\sum_i Y_{gi} \right) - A \,. \qquad (4)$$

In Eq. (*4*), $\overline{DP}_n$ is the number-average degree of polymerization of the macromolecules. We will assume that the use of $\overline{M}_n$ values obtained with polystyrene standards allows a semiquantitative fitting of the effect of molar mass on T_g. The Y_{gi} terms will be estimated using the group contributions available as well as the experimental value of T_g for poly(3β-MECAME)[30,86]. In that way we obtain Y_g = 212 K kg/mol for the cholic acid group (Table VIII). Then by plotting the product $\overline{M}_n T_g$ as a function of $\overline{DP}_n$ ($Y_{gMeth} + Y_{gCA} + nY_{gEO}$) we obtain a linear variation with a slope equal to unity (with a good approximation) which is consistent with Eq. (*4*) (Fig. 4). We also obtain a value for A of about 2×10^6 g/mol but this should not be considered as more than a rough order of magnitude since the uncertainties are high. These simple calculations illustrate that the group contribution method conveniently accounts for the variation of T_g with chemical structure for bile acid-containing polymers provided that the effect of molecular weight variation is included.

A great deal of experimental results were available for random copolymers containing bile acid side groups. For all the reported results, a single T_g is detected which is an evidence of perfect compatibility between the different repeating units. The Gordon–Taylor equation[89] leads to a correct fitting of the experimental values over the whole composition range. Nevertheless, the parameter (K) contained in the Gordon–Taylor equation, although theoretically justified, is still empirical[90].

TABLE VIII
Group contributions for structure elements of CA-derived methacrylates, CH_2=C(CH_3)–COO–(CH_2–CH_2–O)$_n$–CA–CH_3

Structural element	Y_{gi} (K g/mol)[a]
CH_2=C(CH_3)–COO…–CH_3	37 800
–CH_2–CH_2–O–	18 000

[a] Values taken from[86].

3.3.2. Lower Critical Solution Temperature (LCST) of Aqueous Solutions

N-alkyl(meth)acrylamide monomers lead to polymers that exhibit very different solubility behaviours in aqueous solution depending on the nature of the alkyl group[91]. Long linear aliphatic alkyl groups lead to polymers with very low water solubilities. Hydrocarbon groups with less carbon atoms (isopropyl, propyl, ethyl, ...) produce polymers with inverse temperature sensitivity. Their solubility in water is high at low temperatures and strongly decreases above the critical value called the cloud point (CP) above which a macroscopic demixing is observed[92]. At the CP, phase separation proceeds between a polymer-rich and a solvent-rich phase. The minimum value of the CP as a function of polymer concentration is called the LCST. The value of LCST can be varied to large extent in two ways: (i) copolymerization of the "thermosensitive monomer" with hydrophilic or hydrophobic monomers and (ii) the introduction of additives (salts or surfactants are common examples) into the aqueous solution.

Bile acid-based monomers have been copolymerized with *N*-isopropylacrylamide (NIPA) and terpolymerized with *N*-ethylacrylamide (EA) and *N*,*N*-diethylacrylamide (DEA). Increasing the amount of bile acid monomer in the polymer up to 5 mole % decreases the CP of 7 to 20 °C depending on the copolymer[36,70,71].

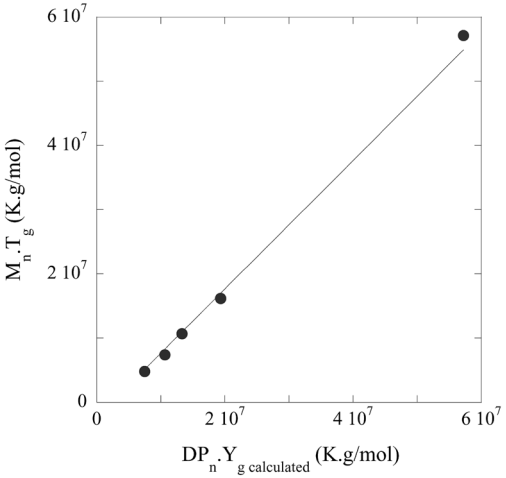

FIG. 4
Plot of experimental data according to Eq. (*4*). Points are calculated from data in[30,32]. The line is the curve corresponding to Eq. (*4*)

The effects of co-solutes like salt or surfactants on the LCST were also investigated. In the case of terpolymers, a nearly exponential decrease of the CP with salt concentration was observed. Nevertheless, for copolymers containing mainly NIPA, within the range of concentrations explored, linear variations of CP were observed with the concentration of added co-solute[70]. It is worth comparing the corresponding slope for a random copolymer of NIPA and bile acid-grafted methacrylate (ME-$(EO)_4$-CAME) with that observed for PNIPA homopolymer (Table IX). The salting-out effect of sodium chloride is sharper with the copolymer, which can be attributed to the presence of hydrophobic bile acid units. Similarly, the increase in the CP with SDS concentration is more pronounced in the case of the copolymer. When sodium cholate is used instead of SDS, the observed effects are much more limited and the presence of bile acid units does not change the trends. This effect of surfactants on the CP is explained by increased hydrophilicity of polymer chains caused by the adsorption of surfactant molecules[93]. This adsorption is favoured by the presence of more hydrophobic side chains such as bile acid moieties.

3.4. Nanoparticle Formation: The Segregation Behaviour

Hydrophobically modified polymers form submicronic aggregates of several macromolecules in aqueous medium and over a wide range of polymer concentrations. No physical network formation is observed since intramolecular associations predominate and favour the segregation of macromolecules into hydrophobic aggregates combining less than 10 macromolecules. The critical aggregate concentrations corresponding to nanoparticles forma-

TABLE IX
Slopes of cloud point dependences on solute for NIPA polymers with various solutes (the polymer concentration is 50 g/l except for PNIPA with NaCl for which it is 10 g/l)

Solute	Slope of cloud point variation (°C l/mol)	
	NIPA[a]	NIPA copolymer[b]
Sodium chloride	–10.3[c]	–14.8[d]
Sodium dodecyl sulfate	+572.5[d]	+798.0[d]
Sodium cholate	+61.3[d]	+121.0[d]

[a] Homopolymer. [b] Bile acid random copolymer. [c] Value from[104]. [d] Value from[70].

tion were reported to be lower than 0.2 g/l, depending on the hydrophilic/hydrophobic balance of the polymers[94].

Nanoparticles have been prepared varying the nature of polysaccharide, either anionic (CA-modified heparin)[51] or cationic (DCA- or CA-modified chitosan or glycol chitosan)[52,53,56,94–98] or neutral (dextran)[99]. A protein like BSA has also been modified to that goal with bile acid molecules[54]. Generally the obtained nanoparticles exhibit hydrodynamic diameters between 200 and 800 nm. This size can be controlled by molecular parameters of the macromolecules like the degree of hydrophobic substitution or by external variables like the ionic strength or pH [94]. Another important property of the formed nanoparticles is their colloidal stability in physiological medium. To improve colloidal stability, chitosan was replaced by glycol chitosan for gene delivery applications[56]. Hydrophobically modified chitosan nanoparticles were shown to readily complex AND [95] macromolecules and encapsulate hydrophobic molecules like adriamycin[52]. The kinetics of release was shown to be very slow, a characteristic which was attributed to the inner structure of hydrophobic aggregates formed by DCA moieties.

4. ADSORPTION AT AIR–WATER INTERFACES

To the best of our knowledge, a few studies have been devoted to the interfacial properties of amphiphilic polymers containing bile acids as hydrophobic groups. Dextran molecules randomly modified with bile acid groups have been considered as surface active polymers[49]. The surface tension of their aqueous solutions was measured as a function of polymer concentration. The obtained results can be compared with other polymeric surfactants derived from dextran with similar molecular weights (Fig. 5)[100–102]. Considering the kinetic effects observed in aqueous solution and especially when the polymers were mixed with surfactants[82], a kinetic study of their adsorption at interfaces (liquid–liquid or air–liquid) would be valuable.

At the present time no detailed study is available about the adsorption of such polymers at liquid–liquid interfaces. The preparation of emulsions stabilized by such polymeric surfactants is not yet available in literature despite their potential interest especially for drug delivery applications. This is certainly a scientific field to be investigated soon.

The surface active properties of bile acid-based monomers have been investigated by surface pressure measurements[38]. These monomers were synthesized by the attachment of a C_{11} cinnamic ester to cholic acid molecules. The effect of photopolymerization on the surface properties of the polymers was investigated.

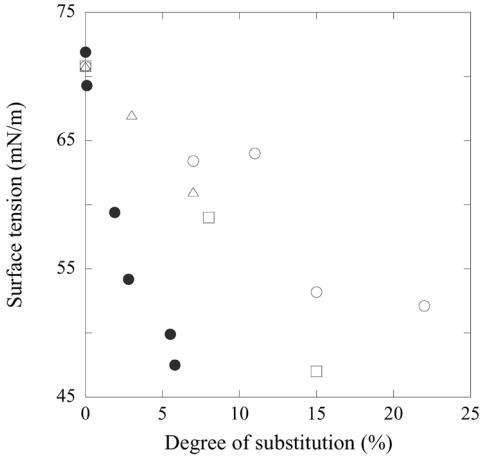

FIG. 5
Surface tension of 1 g/l aqueous solutions of hydrophobically modified dextrans as a function of the degree of substitution. ● Deoxycholic acid-modified dextran[49], ○ phenoxy-modified dextran, □ C6-modified dextran and △ C10-modified dextran[100]

5. CONCLUSION

Because of their multiple reactivities, functional groups and configurational properties, bile acids are real building blocks for the design of amphiphilic macromolecules. They have been used for preparation of a wide range of chemical architectures with a relatively good control of structure. Their self-organization in an aqueous medium was largely used for preparation of colloidal nanoparticles. The recent application of ATRP to bile acid-derived monomers will allow the synthesis of block copolymers and other well-defined structures. The synthesis of nanoparticles made of bile acid polymers by polymerization in disperse medium could be a new topic in the field. The application of bile acid-based amphiphilic polymers to stabilization of liquid–liquid dispersion could be another future development.

6. LIST OF ABBREVIATIONS

3-Ac-LCA	3-acetyllithocholic acid
AIBN	2,2′-azobisisobutyronitrile
CA	cholic acid
CAC	critical aggregate concentration
CMC	critical micellar concentration
CP	cloud point

3α-$(CH_2)_{10}$-CAME	methyl 3α-[11-(methacryloyloxy)undecanoloxy]-7α,12α-dihydroxy-5β-cholan-24-oate
3α-$(CH_2)_{10}$-LCAME	methyl 3α-[11-(methacryloyloxy)undecanoloxy]-5β-cholan-24-oate
DEA	*N*,*N*-diethylacrylamide
DCA	deoxycholic acid
LCA	lithocholic acid
LCST	lower critical solution temperature
3α-MACAME	methyl 3α-methacrylamido-7α,12α-dihydroxy-5β-cholan-24-oate
3β-MACAME	methyl 3β-methacrylamido-7α,12α-dihydroxy-5β-cholan-24-oate
MAN	maleic anhydride
MATCA	methacryloyl tri(ethylene glycol) cholanoate
3β-ME-$(EO)_n$-CAME	methyl 3β-$(oxymethoxy)_n$-methacryloyl-7α,12α-dihydroxy-5β-cholan-24-oate
3α-MECAME	methyl 3α-methacryloyl-7α,12α-dihydroxy-5β-cholan-24-oate
3β-MECAME	methyl 3β-methacryloyl-7α,12α-dihydroxy-5β-cholan-24-oate
3α-MELCAME	methyl 3α-methacryloyl-7α,12α-dihydroxy-5β-lithocholan-24-oate
NIPA	*N*-isopropylacrylamide
PEO	poly(ethylene oxide)
UDA	ursodeoxycholic acid

7. REFERENCES

1. Strauss U. P., Jackson E. G.: *J. Polym. Sci.* **1951**, *6*, 649.
2. Sau A. C., Landoll L. M.: *Adv. Chem. Ser.* **1989**, *223*, 343.
3. Candau F., Selb J.: *Adv. Colloid Interface Sci.* **1999**, *79*, 149.
4. Cohen Stuart M. A.: *Surf. Sci. Ser.* **2003**, *110*, 1.
5. Cosgrove T.: *Colloid Sci.* **2005**, 113.
6. Magny B., Lafuma F., Iliopoulos I.: *Polymer* **1992**, *33*, 3151.
7. Hirrien M., Desbrieres J., Rinaudo M.: *Carbohydr. Polym.* **1996**, *31*, 243.
8. Enhsen A., Kramer W., Wess G.: *DDT* **2003**, *3*, 409.
9. Fini A., Roda A., Fugazza R., Grigolo B.: *J. Solution Chem.* **1985**, *14*, 595.
10. Roda A., Fini A., Grigolo B., Simoni P., Rusticali G. A., Natalini B.: *Ann. Chim.* **1988**, *78*, 15.
11. Roda A., Hofmann A. F., Mysels K. J.: *J. Biol. Chem.* **1983**, *258*, 6362.
12. Nakashima T., Anno T., Kanda H., Sato Y., Kuroi T., Fuji H., Nagadome S., Sugihara G.: *Colloid Surf., B* **2002**, *24*, 103.
13. Coello A., Meijide F., Rodriguez Nunez E., Vazquez Tato J.: *J. Pharm. Sci.* **1996**, *85*, 9.
14. Simonovic B. R., Momirovic M.: *Mikrochim. Acta* **1997**, *127*, 101.
15. Vochten R., Joos P.: *J. Chim. Phys. Physico-Chim. Biol.* **1970**, *67*, 1372.
16. Hofmann A. F., Roda A.: *J. Lipid Res.* **1984**, *25*, 1477.
17. Tamminen J., Kolehmainen E.: *Molecules* **2001**, *6*, 21.
18. Zhu X. X., Nichifor M.: *Acc. Chem. Res.* **2002**, *35*, 539.
19. Gautrot J. E., Zhu X. X.: *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 1123.
20. Gouin S., Zhu X. X., Lehnert S.: *Macromolecules* **2000**, *33*, 5379.
21. Zuluaga F., Valderruten N. E., Wagener K. B.: *Polym. Bull.* **1999**, *42*, 41.
22. Ahlheim M., Hallensleben M. L.: *Makromol. Chem., Rapid Commun.* **1988**, *9*, 299.
23. Krasko M. Y., Ezra A., Domb A. J.: *Polym. Adv. Technol.* **2003**, *14*, 832.

24. Vijayalakshmi N., Maitra U.: *J. Org. Chem.* **2006**, *71*, 768.
25. Ropponen J., Tamminen J., Lahtinen M., Linnanto J., Rissanen K., Kolehmainen E.: *Eur. J. Org. Chem.* **2005**, *73*.
26. Shaikh V. A. E., Ubale V. P., Maldar N. N., Lonikar S. V., Rajan C. R., Ponrathnam S.: *J. Appl. Polym. Sci.* **2006**, *100*, 73.
27. Ladmiral V., Melia E., Haddleton D. M.: *Eur. Polym. J.* **2004**, *40*, 431.
28. Spain S. G., Gibson M. I., Cameron N. R.: *J. Polym. Sci., Part A* **2007**, *45*, 2059.
29. Denike J. K., Zhu X. X.: *Macromol. Rapid Commun.* **1994**, *15*, 459.
30. Zhang Y. H., Akram M., Liu H. Y., Zhu X. X.: *Macromol. Chem. Phys.* **1998**, *199*, 1399.
31. Denike J. K., Moskova M., Zhu X. X.: *Chem. Phys. Lipids* **1995**, *77*, 261.
32. Benrebouh A., Zhang Y. H., Zhu X. X.: *Macromol. Rapid Commun.* **2000**, *21*, 685.
33. Zhang J. H., Bazuin C. G., Freiberg S., Brisse F., Zhu X. X.: *Polymer* **2005**, *46*, 7266.
34. Ghedini N., Ferruti P., Andrisano V., Scapini G.: *Synth. Commun.* **1983**, *13*, 707.
35. Gautrot J. E., Zhu X. X.: *Anal. Chim. Acta* **2007**, *581*, 281.
36. Liu H., Avoce D., Song Z., Zhu X. X.: *Macromol. Rapid Commun.* **2001**, *22*, 675.
37. Noll O., Ritter H.: *Macromol. Rapid Commun.* **1996**, *17*, 553.
38. Ahlheim M., Hallensleben M. L.: *Makromol. Chem.* **1992**, *193*, 779.
39. Hao J., Li H., Zhu X. X.: *Biomacromolecules* **2006**, *7*, 995.
40. Melia E., Haddleton D. M.: *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **2005**, *46*, 166.
41. Odian G.: *Principles of Polymerization*, 3rd ed. John Wiley & Sons, Inc., New York, 1991.
42. Ahlheim M., Hallensleben M. L., Wurm H.: *Polym. Bull.* **1986**, *15*, 497.
43. Avoce D., Liu H. Y., Zhu X. X.: *Polymer* **2003**, *44*, 1081.
44. Aoai T., Sato K., Kodama K., Kawabe Y., Nakao H., Yagihara M.: *J. Photopolym. Technol.* **1999**, *12*, 477.
45. Zhu X. X., Moskova M., Denike J. K.: *Polymer* **1996**, *37*, 493.
46. Song Z., Li H., Zhu X. X.: *Chin. J. Polym. Sci.* **2003**, *21*, 521.
47. Chen W. H., Shao X. B., Regen S. L.: *J. Am. Chem. Soc.* **2005**, *127*, 12727.
48. Pattinson N., Collins D., Campbell B.: *J. Chromatogr.* **1980**, *187*, 409.
49. Nichifor M., Carpov A.: *Eur. Polym. J.* **1999**, *35*, 2125.
50. Shaikh V. A. E., Maldar N. N., Lonikar S. V., Rajan C. R., Ponrathnam S.: *J. Appl. Polym. Sci.* **2006**, *100*, 1995.
51. Diancourt F., Braud C., Vert M.: *J. Bioactiv. Compat. Polym.* **1996**, *11*, 203.
52. Lee K. Y., Kim J. H., Kwon I. C., Jeong S. Y.: *Colloid Polym. Sci.* **2000**, *278*, 1216.
53. Yoo H. S., Lee J. E., Chung H., Kwon I. C., Jeong S. Y.: *J. Controled Release* **2005**, *103*, 235.
54. Orienti I., Gianasi E., Aiedeh K., Zecchi V.: *J. Pharm. Belg.* **1996**, *51*, 125.
55. Bamford C. H., Middleton I. P., Al-Lamee K. G.: *Polymer* **1986**, *27*, 1981.
56. Kwon S., Park J. H., Chung H., Kwon I. C., Jeong S. Y.: *Langmuir* **2003**, *19*, 10188.
57. Kim I. S., Kim S. H., Cho C. S.: *Macromol. Rapid Commun.* **2000**, *21*, 1272.
58. Ghedini N., Ferruti P., Andrisano V., Cesaroni M. R., Scapini G.: *Synth. Commun.* **1983**, *13*, 701.
59. Huh K. M., Lee K. Y., Kwon I. C., Kim Y. H., Jeong S. Y.: *Langmuir* **2000**, *16*, 10566.
60. Kim C., Lee S. C., Kang S. W.: *Langmuir* **2000**, *16*, 4792.
61. Kim I. S., Jeong S. Y., Kim S. H.: *Arch. Pharm. Res.* **2000**, *23*, 87.
62. Teomim D., Domb A. J.: *J. Polym. Sci. A* **1999**, *37*, 3337.
63. Krasko M. Y., Shikanov A., Kumar N., Domb A. J.: *Polym. Adv. Technol.* **2002**, *13*, 960.

64. Vieira N. A. B., Tiera V. A. O., Tiera M. J., Moscardini M. S.: *Carbohydr. Polym.* **2003**, *53*, 137.
65. Grassl B., Billon L., Borisov O., François J.: *Polym. Int.* **2006**, *55*, 1169.
66. Kim M., Choi Y.-W., Sim J.-H., Choo J., Sohn D.: *J. Phys. Chem. B* **2004**, *108*, 8269.
67. Beaudoin E., Hiorns R. C., Borisov O., François J.: *Langmuir* **2003**, *19*, 2058.
68. Kim M., Sim J.-H., Sohn D.: *Macromolecules* **2003**, *36*, 9986.
69. Gourier C., Beaudoin E., Duval M., Sarazin D., Maître S., François J.: *J. Colloid Interface Sci.* **2000**, *230*, 41.
70. Benrebouh A., Avoce D., Zhu X. X.: *Polymer* **2001**, *42*, 4031.
71. Avoce D., Baille W. E., Zhu X. X.: *e-Polymers* **2004**, *7*.
72. Huggins M. L.: *J. Am. Chem. Soc.* **1942**, *64*, 2716.
73. Rotureau E., Chassenieux C., Dellacherie E., Durand A.: *Macromol. Chem. Phys.* **2005**, *206*, 2038.
74. Durand A.: *Eur. Polym. J.* **2007**, *43*, 1744.
75. Rotureau E., Dellacherie E., Durand A.: *Eur. Polym. J.* **2006**, *42*, 1086.
76. Durand A.: *Polym. Eng. Sci.* **2007**, *47*, 481.
77. Nichifor M., Lopes A., Carpov A., Melo E.: *Macromolecules* **1999**, *32*, 7078.
78. Nichifor M., Stanciu M., Zhu X. X.: *React. Funct. Polym.* **2004**, *59*, 141.
79. Sarrazin-Cartalas A., Iliopoulos I., Audebert R., Olsson U.: *Langmuir* **1994**, *10*, 1421.
80. Deguchi S., Kuroda K., Akiyoshi K., Lindman B., Sunamoto J.: *Colloids Surf., A* **1999**, *147*, 203.
81. Magny B., Iliopoulos I., Audbert R., Piculell L., Lindman B.: *Progr. Coll. Polym. Sci.* **1992**, *89*, 118.
82. Lopes A., Lindman B.: *Prog. Coll. Polym. Sci.* **2000**, *116*, 42.
83. Gouin S., Zhu X. X.: *Langmuir* **1998**, *14*, 4025.
84. Johansson G., Olde B., Joelsson M.: *J. Chromatogr., B: Biomed. Appl.* **1994**, *652*, 137.
85. Zhang Y. H., Zhu X. X.: *Macromol. Chem. Phys.* **1996**, *197*, 3473.
86. Van Krevelen D. W.: *Properties of Polymers*. Elsevier, Amsterdam, London, New York, Tokyo 1990.
87. Camacho-Zuniga C., Ruiz-Trevino F. A.: *Ind. Eng. Chem. Res.* **2003**, *42*, 1530.
88. Fox T. G., Flory P. J.: *J. Appl. Phys.* **1950**, *21*, 581.
89. Gordon M., Taylor S. J.: *J. Appl. Chem.* **1952**, *2*, 493.
90. Penzel E., Rieger J., Schneider H. A.: *Polymer* **1997**, *38*, 325.
91. Liu H., Zhu X. X.: *Polymer* **1999**, *40*, 6985.
92. Schild H. G.: *Prog. Polym. Sci.* **1992**, *17*, 163.
93. Schild H. G., Tirrell D. A.: *Langmuir* **1991**, *7*, 665.
94. Lee K. Y., Jo W. H., Kwon I. C., Kim Y. H., Jeong S. Y.: *Langmuir* **1998**, *14*, 2329.
95. Lee K. Y., Kwon I. C., Jo W. H., Jeong S. Y.: *Polymer* **2005**, *46*, 8107.
96. Lee K. Y., Jo W. H., Kwon I. C., Kim Y. H., Jeong S. Y.: *Macromolecules* **1998**, *31*, 378.
97. Park J. H., Kwon S., Nam J. O., Park R. W., Chung H., Seo S. B., Kim I. S., Kwon I. C., Jeong S. Y.: *J. Controlled Release* **2004**, *95*, 579.
98. Kim K., Kwon S., Park J. H., Chung H., Jeong S. Y., Kwon I. C.: *Biomacromolecules* **2005**, *6*, 1154.
99. Yuan X.-B., Li H., Zhu X. X., Woo H.-G.: *J. Chem. Technol. Biotechnol.* **2006**, *81*, 746.
100. Rouzes C., Durand A., Léonard M., Dellacherie E.: *J. Colloid Interface Sci.* **2002**, *253*, 217.
101. Durand A., Dellacherie E.: *Colloid Polym. Sci.* **2006**, *284*, 536.

102. Rotureau E., Léonard M., Dellacherie E., Durand A.: *J. Colloid Interface Sci.* **2004**, *279*, 68.
103. Lee K. Y., Moon H. T., Byun Y.: *Thromb. Res.* **1998**, *92*, 149.
104. Zhang Y., Furyk S., Sagle L. B., Cho Y., Bergbreiter D. E., Cremer P. S.: *J. Phys. Chem. C* **2007**, *111*, 8916.