Design of malolactonic acid esters with a large spectrum of specified pendant groups in the engineering of biofunctional and hydrolyzable polyesters

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Summary

The development of multimeric functionalized macromolecules with the strict adjustment of their structure and their properties, aimed at biological applications, leads to complex architecture and puts on the diversification of hydrolyzable polymers. Poly(β-malic acid) derivatives are very good candidates in the preparation of smart molecules for a large spectrum of applications in the release of bioactive molecules, due to the presence of a lateral carboxylic acid function besides stereogenic centers in the repeating units and main chain cleavable bonds. The opportunity for accessing to these structures comes from mastery of the corresponding functionalized β-substituted β-lactones synthesis. Two different synthesis routes have been established and the functional pendant groups is attached at the step preceding the lactone formation. A third way consists in the synthesis of malolactonic acid which is reacted with a specific molecule in presence of a coupling reagent. It is therefore possible to dispose of an important wealth of monomers and to tailor-make polymeric materials having a well-defined composition. Multimeric structures have been elaborated aimed at degradable micelles from block copolymers, nanoparticles starting from hydrophobic polyesters, biomimetic architecture for interacting with fibroblast growth factors and amphiphilic associating polymers for hydrogel networks. Biodegradable graft copolymers have been elaborated for bioactive molecules encapsulation and bioartificial membranes, including cholesterol and diacylglycerol, have been tailor-made.

Introduction

The need for polymers aimed at temporary therapeutic applications is increasing due to the different solutions which are now possible for drug administration. For each drug

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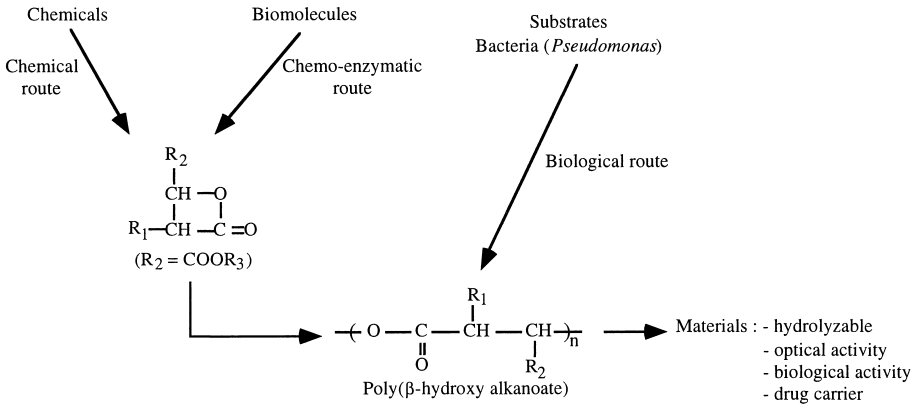
delivery system, it is necessary to fit material properties with well-defined prerequisites ; therefore, the tailor-making of polymers with different but complementary chemical structures and reproducible characteristics is necessary. The building of such polyvalent polymers can be achieved by copolymerization, cross-linking and chemical modification starting from a parent compound. These polymeric materials must be biocompatible, hydrolyzable and the presence of stereogenic centers in the repeating unit is a major structural factor for modifying physical and mechanical properties. Polyesters contain a degradable backbone and their configurational structure can be modulated by the presence of enantiomeric or diastereomeric repeating units. These polyesters, which are of the poly(3-hydroxyalkanoate) types, can be obtained by different routes : chemical, chemoenzymatic or biological (Scheme 1).

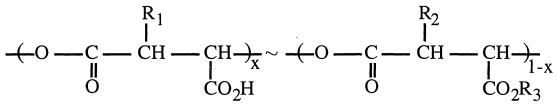


Scheme 1 : Access to poly(β-hydroxyalkanoates).

Besides functionalized bacterial polyesters which have been obtained from *Pseudomonas* strains and by using different substrates (1), polymers derivated from malic acid or 3-alkylmalic acid constitute a very large family of polymeric materials aimed at pharmaceutical applications.

High molecular weight polymers are prepared by anionic ring opening polymerization of β-substituted β-lactones obtained from bromosuccinic anhydride (2), (3-alkyl)aspartic acid

(3,4) or (3-alkyl)malic acid (5) enantiomers as chiral precursors. A wide spectrum of optically active or racemic, functional or reactive poly(β-(3-alkyl)malic acid) derivatives have been prepared by changing the chemical structures of the pendant ester groups for obtaining suitable properties such as hydrophobic/hydrophilic balance, degradation rate, bioactive or targeting molecules attachment, associating degradable hydrogels (Scheme 2).



R_1 and R_2 : H, methyl, ethyl, isopropyl
R_3 : alkyl, bioactive or targeting molecule, hydrophobic groups

Scheme 2 : Poly(β-(3-alkyl)malic acid) derivatives structures.

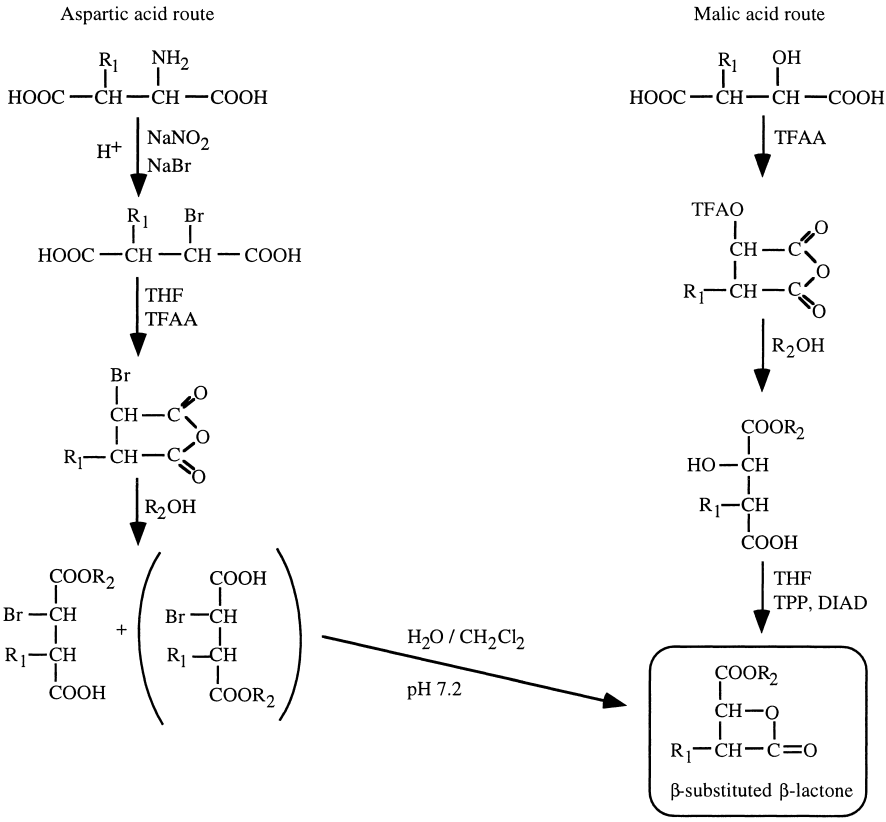
The limitation for obtaining such functional macromolecules lies in the possibility of synthesizing the corresponding monomers, i.e. β-substituted β-lactones.

In this paper, we wish to present different forms of malic acid polymers aimed at temporary therapeutic applications.

Discussion

As shown by Scheme 3, the large family of malolactonic and alkylmalolactonic acid esters (β-substituted β-lactones) can be synthesized starting from either (3-alkyl)aspartic acid (3,4) or (3-alkyl)malic acid (5). Yields of these multisteps reactions are quite similar (about 20%).

In the aspartic acid synthesis route, the chiral center undergoes two inversions of configuration during the first step thus leading to a retention of configuration (6). For both synthesis routes, an inversion of configuration occurs during the lactonization reaction (5,6). Worth to noting is that no racemization has been observed. Moreover, the strict control of chiral center configuration leads to a very high enantiomeric excess from 95% to 98% (5,6).

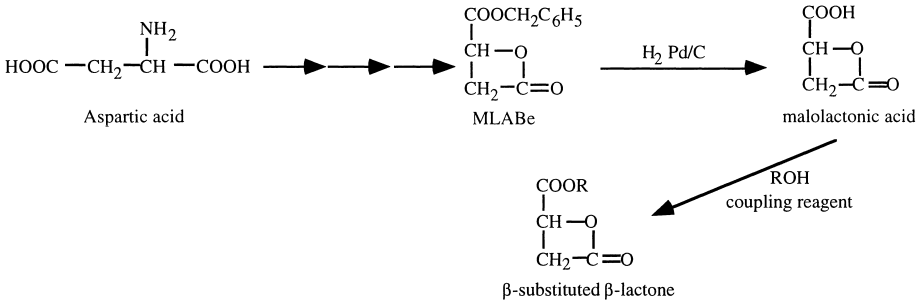


Scheme 3 : β-substituted β-lactones synthesis starting from aspartic acid or malic acid (TFAA : Trifluoroacetic anhydride, TPP : Triphenylphosphine, DIAD : Diisopropyl azodicarboxylate).

For both syntheses, the crucial step concerns the purification stage of the β-substituted β-lactone by column chromatography and distillations under vacuum (in case of liquid monomer). Indeed, we have shown that successful chemical modifications of the monomer and preparation of high molecular weight polymers are dependent on the purity degree of the monomer (5).

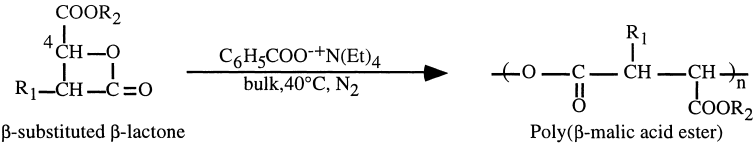
Despite the high efficiency of both synthesis routes in the obtaining of a large family of β-lactones, some monomers were not accessible using either aspartic acid route or malic acid

route. Therefore, in order to enlarge the family of malolactonic acid esters, we have successfully prepared malolactonic acid by catalytic hydrogenolysis of malolactonic acid benzyl ester (MLABe) as shown by Scheme 4 (7). Despite the presence of free carboxylic acid functions, this new functional monomer shows a good stability. Coupling reactions of alcohols were carried out in the presence of dicyclohexylcarbodiimide (DCC) leading to new β-substituted β-lactones (Scheme 4) (7).



Scheme 4 : Synthesis of β-substituted β-lactones from malolactonic acid.

In all cases, poly(β-malic acid esters) derivatives have been obtained by anionic ring-opening polymerization of the corresponding β-substituted β-lactones in presence of tetraethylammonium benzoate as initiator (Scheme 5).

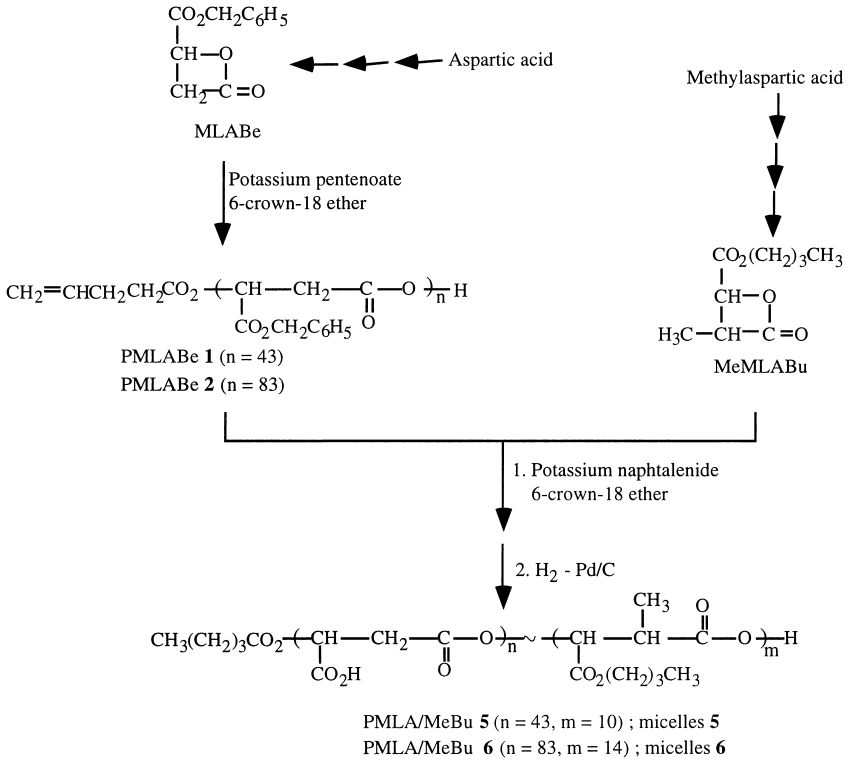


Scheme 5 : Anionic ring-opening polymerization of β-substituted β-lactones.

The anionic ring-opening polymerization proceeds by an attack on the carbon atom (C4) in β position of the carbonyl atom with an inversion of configuration of the C4 carbon atom without any racemization (6). Theoritical, molecular weight of the resulting polymeric material is deduced from the ratio monomer/initiator. However, we have demonstrated that transfer reactions took place during the polymerization, thus limiting molecular weights (8).

1 . Degradable macromolecular micelles (9)

In the field of particulate drug carriers, macromolecular micelles have attracted a lot of interest (10,11). However, it is essential to take into account interactions of the material with living species. This preoccupation has led to the development of degradable materials. In this context, we have designed amphiphilic block copolymers, with poly(β-malic acid) as hydrophilic block and poly(β-malic acid alkyl esters) as hydrophobic segments, which can be degraded into non-toxic or biocompatible low molecular weight molecules by simple hydrolysis.



Scheme 6 : Synthesis of amphiphilic degradable block-copolymers leading to degradable macromolecular micelles.

Preparation of such amphiphilic degradable copolyesters is a three steps synthesis starting from β-substituted β-lactones as shown by Scheme 6 (9).

We have first synthesized poly(β-malic acid benzyl ester) prepolymers by anionic ring-opening polymerization of MLABe using potassium pentenoate, 6-crown-18 ether complex as initiator. Molecular weight values measured by size exclusion chromatography (SEC in THF, polystyrene standard) and by ^{1}H NMR (relative intensity of peaks) were in good agreement with the theoretical ones controlled by the ratio monomer/initiator (Table 1) (9). Then, the carboxylic acid end-groups of these prepolymers were used as initiator for the anionic ring-opening polymerization of 3-methylmalolactonic acid butyl ester (MeMLABu) (Scheme 6) leading to the expected block copolymers (Table 1) (9).

Table 1 : Characteristics of amphiphilic degradable block-copolymers.

Polymer	M_{PMLABe} theo[(a)]	M_{NMR} PMLABe[(b)]	M_{NMR} PMLA[(b)]	n[(b)]	$M_{PMeMLABu}$ theo[(a)]	M_{NMR} PMeMLABu[(b)]	m[(b)]
PMLABe **1**	10 000	9 000	--	43	--	--	--
PMLABe **2**	20 000	17 000	--	83	--	--	--
PMLABe/MeBu **3**	10 000	9 000	--	43	5 000	2 000	10
PMLABe/MeBu **4**	20 000	17 000	--	83	10 000	3 000	14
PMLA/MeBu **5**	--	--	5 000	43	5 000	2 000	10
PMLA/MeBu **6**	--	--	9 600	83	10 000	3 000	14

(a). Determined by the ratio monomer/initiator ; (b). Measured by ^{1}H NMR in CD_3COCD_3.

Micellization of block polyelectrolytes has been studied and several methods to prepare well-defined micelles have been proposed (12-15). Degradable micelles were prepared by dissolving both type of poly(β-malic acid-co-β-methylmalic acid butyl ester) block copolymers in 10 mM phosphate buffered solution (PBS) at pH 7.4 containing 0.15M NaCl. Resulting micelles were characterized by dynamic light scattering (DLS), which gives access to the micelles diameter, and by fluorescence using pyrene as fluorescence probe, which allows to determine the critical micellar concentration (cmc).

As shown by Table 2, PMLA/MeBu micelles **5** and PMLA/MeBu micelles **6** have a similar diameter but very different cmc values. Indeed, micelles made up by short chain length block copolymers (micelles **5**) have a cmc of 32 mg/L and are therefore less stable than micelles made up by long chain length block copolymers (micelles **6**) with a cmc of 8 mg/L (9).

Table 2 : Characteristics of degradable macromolecular micelles.

Micelles	M_{NMR} PMLA	M_{NMR} PMLABu	Average diameter (nm) [a]	Polydispersity index [a]	cmc (mg/L) [b]
PMLA/MeBu micelles **5**	5 000	2 000	36	0.22	32
PMLA/MeBu micelles **6**	9 600	3 000	35	0.27	8

(a) . Average diameter and polydispersity indices were obtained by cumulant analysis of DLS in 10mM PBS, pH 7.4 + 0.15M NaCl at 25°C, 1mg/L. (b) . Determined by fluorescence using pyrene as fluorescence probe in 10mM PBS, pH 7.4 + 0.15M NaCl .

The higher thermodynamic stability of micelles **6** can be explained by the fact that the PMLA chain was longer than the PMLA chain of block copolymer **5** (molecular weight of 9 600 instead of 5 000). Since the hydrophilic chain was less extended, a better stability of the resulting micelles **6** was observed. Moreover, the hydrophobic block was also longer for micelles **6** than for micelles **5**, thus improving their stability by stronger hydrophobic interactions. Consequently, the micelles **6** have a similar diameter than the micelles **5** but a lower cmc and therefore a higher thermodynamic stability.

Moreover, we have shown that formation and stability of these micelles were depending on pH as a result of the polyelectrolyte nature of the outer-shell constituted by poly(β-malic acid) segment. In PBS containing 0.15M NaCl, no degradable micelles formation can be observed at low and high pH while at neutral pH (7.4) these micelles can be formed and are stable (9). This observation is one of the important conclusion of this study. Indeed, in addition to the degradability properties, these new macromolecular micelles can be useful as

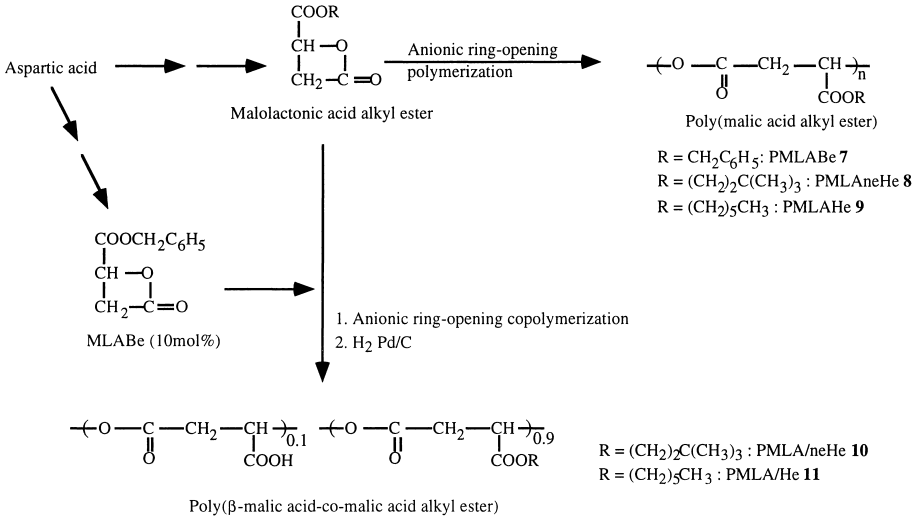
pH-sensitive drug carrier. Further studies on drug loading capacity, drug release and micelles degradation are under investigation.

2 . Degradable nanoparticles

Nanoparticles are solid colloidal particles made of artificial or natural polymers with a diameter ranging from 10 to 1 000 nm and in which the biologically active molecules can be entrapped, dissolved or encapsulated (16). The size and surface characteristics (charge, hydrophobicity, hydrophilicity) of the nanoparticles will influence their body distribution. Moreover, release and degradation properties of the nanoparticles are dependent on the nature, molecular weight and crystallinity of the constitutive polymers (16). Few years ago, Stolnik et al. have described the preparation of nanoparticles based on poly(β-malic acid-co-β-malic acid benzyl ester) copolymers (17). Size and properties of these nanoparticles have been found to be depending on copolymer composition (17).

Since these nanoparticles are designed to be used *in vivo* in contact with living species, an essential condition is that they have to be constituted by biocompatible polymers which can be degraded into non-toxic low molecular weight compounds in a well-defined rate adjusted to the duration of the treatment. Therefore, poly(β-malic acid esters) seem to be good candidates for such applications due to their hydrolyzable main chain ester bonds and the possibility of modifying their physico-chemical properties by varying the lateral ester groups nature.

We have synthesized three hydrophobic homopolymers bearing different kind of lateral ester group (benzyl, neohexyl and hexyl) as well as two copolyesters containing 10 mol% of hydrophilic units (malic acid units) in order to study influence of the presence of these hydrophilic units on nanoparticles preparation and degradation. As shown by Scheme 7, homopolymers and copolymers were obtained via anionic ring-opening polymerization or copolymerization of the corresponding β-substituted β-lactones prepared from aspartic acid.



Scheme 7 : Synthesis of hydrophobic polyesters and copolyesters used for nanoparticles preparation.

Poly(malic acid alkyl ester) and poly(β-malic acid-co-malic acid alkyl ester) have been characterized by ^{1}H NMR (structure), differential scanning calorimetry (DSC, glass transition temperature, Tg, and melting temperature, Tm) and by size exclusion chromatography (SEC, molecular weights and polydispersity) (Table 3). Worth to noting is that composition of both block copolymers were the same as the initial monomers feed.

Table 3 : Characteristics of poly(malic acid alkyl esters) and poly(β-malic acid-co-malic acid alkyl esters).

Polymer	Tg (°C)[(a)]	Tm (°C)[(a)]	Mw[(b)]	Ip[(b)]
PMLABe **7**	37	--	51 000	1.1
PMLAneHe **8**	22	86	17 000	1.2
PMLAHe **9**	-10	--	18 000	1.2
PMLA/neHe **10**	21	--	52 000	1.1
PMLA/He **11**	-11	--	55 000	1.1

(a). Determined by DSC ; (b). Measured by SEC in THF with polystyrene standards.

Nanoparticles were prepared by a nanoprecipitation method which consists in adding an acetone solution of the corresponding polyesters or copolyesters to water under vigorous stirring. Worth to noting is that we observed formation of well-defined nanoparticles without surfactant and that nanoparticles can be obtained from copolymers containing few percent of hydrophilic units. The resulting nanoparticles were characterized by light scattering, allowing to determine their size, and by zeta potential measurement, giving access to surface characteristics (Table 4).

Table 4 : Characteristics of degradable nanoparticles.

Polymer	Diameter (nm)[(a)]	Zeta Potential (mV)[(b)]
PMLABe **7** [(c)]	96	-51
PMLAneHe **8** [(c)]	90	-33
PMLAHe **9** [(c)]	171	-37
PMLA/neHe **10** [(d)]	104	-62
PMLA/He **11** [(d)]	109	-62

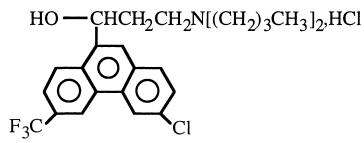
(a). Measured by light scattering with a Nanosizer (Coulter®N4 Plus Submicron Particles Sizer, detection angle 90°) at 20°C ; (b). Measured using a Zetasizer 4 (Malvern Instrument) ; (c). Nanoparticles prepared by adding 5ml of acetone solution containing 25mg of polyester to 10ml of water ; (d). Nanoparticles prepared by adding 5ml of acetone solution containing 100mg of copolyester to 10ml of water.

As shown by Table 4, the size of these new degradable nanoparticles varied from 96 to 170 nm, depending on the nature of the polyester used for nanoparticles preparation. Worth to noting is that this size is comparable to the one of nanoparticles obtained using the same procedure starting from different polyesters such as poly(lactic acid) and poly(ε-caprolactone) (18,19).

All the nanoparticles showed a negative surface charge from -30 to -60 mV (Table 4). Such negative surface charge can be related to the terminal carboxylic acid functions, created by the anionic ring-opening polymerization of the lactones, which are located at the surface of the nanoparticles. This phenomenon was also observed by Stolnick et al. for nanoparticles prepared from a copolymer constituted by malic acid benzyl ester and malic acid units (17). It is worth to noting that all morphological characteristics of the nanoparticles we have prepared were stable for at least three months.

Because of the ester bond in the polyester backbones, the corresponding nanoparticles should be degradable by hydrolysis of such bounds. However, the mechanisms of this degradation are probably complex because the formation of malic acid and/or malic acid ester derivatives and/or oligomers more or less esterified can be observed. Moreover, degradation rate is depending on the polyester molecular weight, on its morphology and on its hydrophobicity. Such evaluation is now under investigation. Comparison between degradation rate of the nanoparticles made up by hydrophobic homopolyesters and degradation rate of nanoparticles constituted by copolyesters containing few percent of hydrophilic units will be done. The presence of hydrophilic units should accelerate the degradation rate of the corresponding nanoparticles because water penetration and thus hydrolysis rate are favored by such hydrophilic units presence. Degradation will be studied by light scattering and size exclusion chromatography in various medium, phosphate buffered (PBS) at pH7.4, gastric and intestinal medium, which are in relation with an oral administration of these new drug carriers.

Drug loading capacity of such degradable nanoparticles was evaluated using poly(malic acid neohexyl ester) as hydrophobic polyester and halofantrin as a model drug. This molecule has a low water solubility (1.3 mg/L) (20) and is used in the treatment of malaria (Scheme 8).



Scheme 8 : Structure of halofantrin.

Encapsulation in nanoparticles can be foreseen in order to improve the bioavailability of such molecule when administrated by the oral route. The drug loadings were in the range of 5wt% when the nanoprecipitation method was used (dissolution of both hydrophobic homopolymer and halofantrin in acetone and addition of this solution in water) and no significant change in the nanoparticles diameter has been observed. However, we have shown that an inversion of the nanoparticles zeta potential occurred when the amount of halofantrin

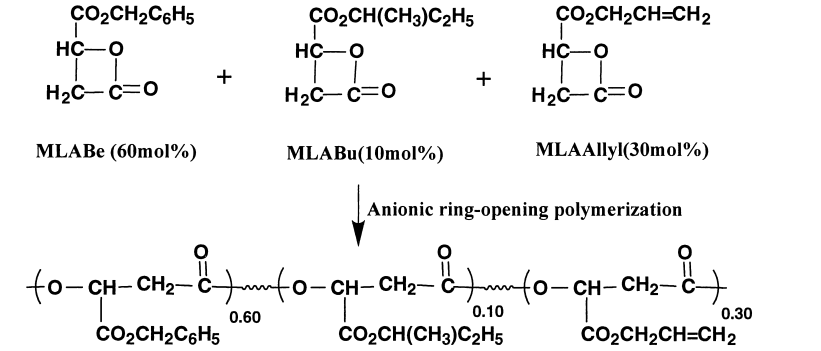
was increased in the nanoprecipitation medium, demonstrating that a part of halofantrin was adsorbed at the surface of the nanoparticles (21). Drug release will be studied in various medium (PBS at pH7.4, gastric and intestinal medium) and correlated to nanoparticles degradation rate and profile.

All these results demonstrate that polyesters of the poly(malic acid alkyl ester) family can be used for developing new particulate drug delivery systems.

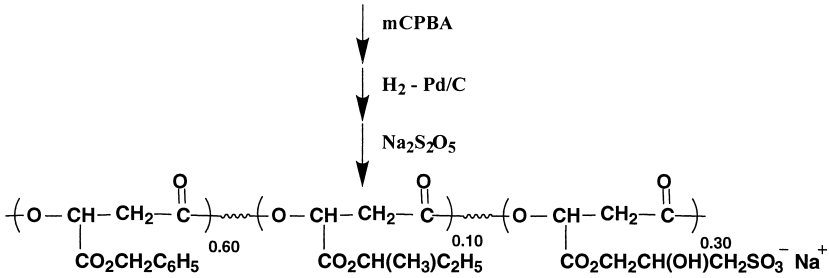
3 . Biologically active polymers (22)

Heparin sulfates (HS) play a key role in regulating Heparin Binding Growth Factors (HBGF) bioavailability. Fibroblast Growth Factors (FGFs) represent the paradigm of HBGF and numerous studies have described HS as the natural site for the storage and the protection of FGFs in the cellular environment. *In vitro*, HS can indeed protect FGFs against proteolytic degradation, potentiate FGFs binding to its high affinity receptor and its ability to stimulate cell proliferation. Similar functions are attributed to HS which are believed to play a key role in the control of HBGF bioavailability. Barritault and al. have recently developed functionalized dextran based biopolymers which could mimic HS functions towards HBGF, and showed that these compounds (named RGTA for regenerating agents) could stimulate tissue repair in various *in vivo* model (23). The development of artificial biopolymers with wound healing properties presents therefore therapeutic interests.

We have consequently prepared degradable polyesters with functional pendant groups selected in the goal to modulate the nature and the proportion of the potential interacting sites. Three β-substituted β-lactones, malolactonic acid benzyl ester (MLABe), malolactonic acid allyl ester (MLAAllyl) and malolactonic acid butyl ester (MLABu), have been synthesized starting from aspartic acid. These lactones have been copolymerized, via anioning ring-opening polymerization, in well-defined proportions (Scheme 9).



Terpolymer **12** (Mw = 6000, Ip = 1.4, SEC in THF with polystyrene standards; Tg = 17 °C)



Terpolymer **13** (Mw = 12500, Ip = 2.01, SEC in H_2O with PEG standards)

Scheme 9 : Synthesis of biologically active polyesters.

The terpolymer **12** has been characterized by ^1H NMR, SEC and DSC. ^1H NMR spectrum has shown that the composition of the terpolymer **12** was the same as the initial monomers feed. Lateral groups have been then chemically modified to introduced three interacting sites present in natural HS related molecules (24,25). Worth to noting is that the chemical modifications were realized without significant chain degradation and with quite good modification yield (22).

The power of wound healing of the final terpolymer **13** was tested in a skull defect model in rats (22). Defects of each experimental group were filled with a collagen plaster soaked at 4°C in a solution of final terpolymer **13**, of carboxymethyl benzylamide sulfonate dextrans (CMDBS) polymer which was the positive control and in a saline buffer solution

which was the negative control. The skulls were removed after a healing period of 35 days and submitted to radiomorphometric analysis. Results are summarized by Figure 1.

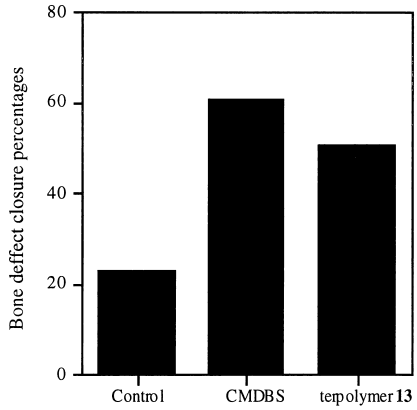


Figure 1 : Radiomorphometric results of bone defect closure percentages at 35 days for the three different experimental groups. Statistical analysis performed using Anova test.

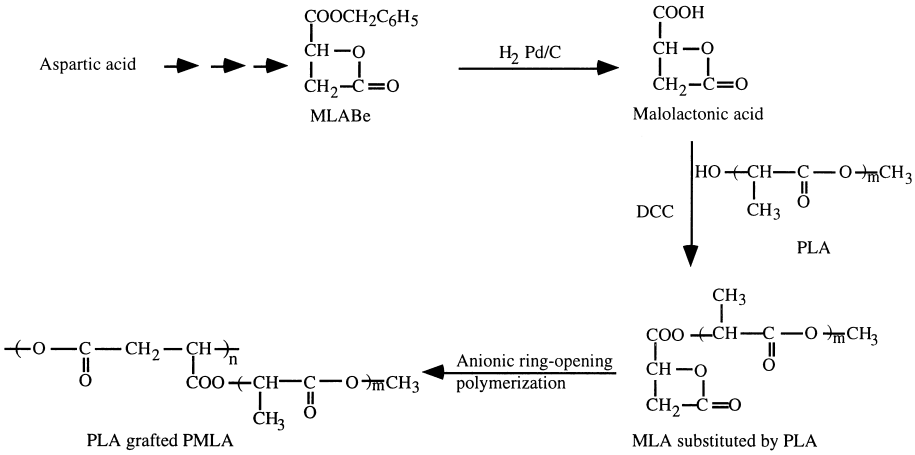
Results have shown that wound healing capacity of final terpolymer **13** was very close to the wound healing capacity of CMDBS, demonstrating that degradable polymers from poly(malic acid) family bearing appropriate pendant groups can be efficiently used as biologically active polymers for bone defect treatment. Further studies are now under investigation concerning wound healing properties of terpolymer **13** on muscle.

4 . Biodegradable graft polyesters

Biodegradable graft polyesters have been elaborated recently with several goals. First, such polymeric materials can be useful for bioactive molecules encapsulation. Because of the material structure, the bioactive molecule can be released as result of the slow polymeric matrix degradation. Poly(lactic acid), PLA, grafted poly(malic acid), PMLA, has been prepared in order to study effect of PLA presence on degradation rate of the resulting material.

Indeed, PLA is known to favour water penetration and therefore to accelerate polymeric materials degradation (26). Consequently, we assume that we will be able to modulate degradation rate of the materials in function of PLA amount in PMLA derivatives.

As shown by Scheme 10, we have successfully synthesized malolactonic acid substituted by P(D,L)-LA by a coupling reaction between PLA and malolactonic acid in presence of dicyclohexylcarbodiimide (DCC).



Scheme 10 : Synthesis of PLA grafted PMLA.

This new lactone has been characterized by Infra-Red (IR) spectroscopy, showing the presence of lactonic band at 1850 cm^{-1} ($n_{C=O}$), and by ^1H NMR, demonstrating the absence of side-products and that the coupling reaction took place without any degradation of the lactone and PLA chain.

We have then studied possibilities to polymerize malolactonic acid substituted by PLA. Homopolymerization of this bulky lactone has been possible by anionic ring-opening polymerization in presence of tetraethylammonium benzoate as initiator (Scheme 10). After purifications, the resulting material has been characterized by ^1H NMR, SEC in THF and DSC. First, ^1H NMR spectrum allowed us to show that the polymeric material effectively contained both kind of units : malic acid units and lactic acid units. Second, SEC chromatogram and DSC thermogram have demonstrated the obtaining of an homopolymer because of the presence of only one peak on SEC chromatogram and one Tg value on DSC thermogram

(Table 5). Moreover, PLA grafted PMLA showed a melting temperature at 37°C with a quite low ΔH value as a result of an organization due to the bulky lateral group.

Table 5 : Characteristics of PLA grafted PMLA.

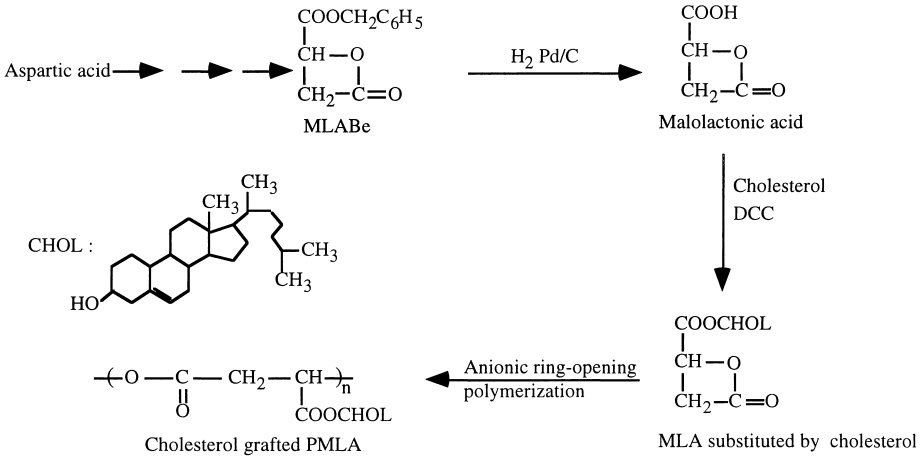
	Mw[(a)]	Ip[(a)]	Tg (°C)[(b)]	Tm (°C)[(b)]	ΔH (cal.g^{-1})[(b)]
PLA grafted PMLA	21 500	1.8	-17	37	-3

(a). Measured by SEC in THF with polystyrene standards ; (b). Measured by DSC.

Such new material is of interest due to the association of specific properties of both PMLA and PLA polymers. Degradation rate and profile of this new material is now under investigation. Moreover, we will study possibilities to copolymerize malolactonic acid substituted by PLA with other lactones in order to obtain polymeric materials having controlled degradation rate and well-defined properties for specific temporary biomedical applications.

The second goal for biodegradable graft polyesters elaboration is to obtain artificial membranes. Therefore, we have studied possibilities to prepare poly(malic acid) substituted by cholesterol. As shown by scheme 11, malolactonic acid substituted by cholesterol has been prepared by binding this group to malolactonic acid using DCC as coupling reagent.

The obtaining of expected monomer has been demonstrated by IR spectroscopy (presence of the lactonic band at 1850 cm^{-1}) and by ^{1}H NMR (structure and purity). Anionic ring-opening polymerization of malolactonic acid substituted by cholesterol has been successful and we were able to obtain cholesterol grafted poly(malic acid) (Scheme 11).



Scheme 11 : Synthesis of cholesterol grafted PMLA.

Table 6 summarizes characteristics of this new polymeric material which has quite high molecular weights with a narrow polydispersity. Cholesterol grafted PMLA showed a very interesting DSC thermogram. Indeed, besides the presence of a glass transition at -8°C, we have observed an exothermic peak at 220°C corresponding to crystallization followed by an endothermic peak at 290°C corresponding to the polymer melting temperature.

Table 6 : Characteristics of cholesterol grafted PMLA.

	Mw[(a)]	Ip[(a)]	Tg (°C)[(b)]	Exothermic peak (°C)[(b)]	ΔH (cal. g^{-1})[(b)]	Tm (°C)[(b)]	ΔH (cal. g^{-1})[(b)]
Cholesterol grafted PMLA	28 200	1.9	-8	220	35	290	-24

(a). Measured by SEC in chloroform with polystyrene standards ; (b). Measured by DSC.

This specific behavior can be related to the presence of cholesterol group which is known to confer liquid crystal properties to polymeric materials. Further studies are now under investigation to determine which kind of liquid crystal behavior this cholesterol grafted PMLA shows.

On the other hand, we have prepared malolactonic acid substituted by dicaprin (a diacylglycerol) using the method described for malolactonic acid substituted by cholesterol. After the study of dicaprin grafted PMLA properties, we will investigate possibilities to copolymerize malolactonic acid substituted by cholesterol, malolactonic acid substituted by dicaprin and malolactonic acid benzyl ester (to introduce hydrophilic units). Then, membrane properties of the resulting materials will be studied.

Conclusion

Mastery of β-substituted β-lactones synthesis and of their anionic ring-opening polymerization or copolymerization leads to the obtaining of polymeric materials having well-defined properties adjusted to the considered temporary biomedical application .

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