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Drug Carriers

Poly(β-Malic Acid) : A New Polymeric Drug-Carrier
Evidence for Degradation in vitro*

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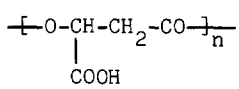
Summary

Poly(β-malic acid) is a new synthetic functional polyester of the poly(β-hydroxy-acid)-type whose properties are investigated in regard to possible uses as bioresorbable polyvalent drug-carrier. Degradation of polymer chains in 0.15 N phosphate buffer at pH=7.5 is monitored by aqueous GPC on SEPHADEX gels and by enzymatic titration of ultimate degradation products. It is shown that the rate of degradation obeys first order kinetics at the begining and that poly(β-malic acid) degrades to malic acid at last.

Introduction

Increasing interest is devoted to polymeric drugs and drug delivery systems by polymer chemists and pharmacologists (RINGSDORF 1975; GEBELEIN 1978). Water-solubility is generally a requisite for macromolecular prodrugs (polymer-drug conjugates) or for macromolecular drugs (KOPECEK 1977). It has been recognized that ionic groups born by polyelectrolyte chains provide means for both binding of substituents and solubilization of polymer-drug conjugates in water (KRAMER 1982). In this respect, polycarboxylic acids are extensively studied and various kinds of biological activities have been detected (OTTENBRITE 1980). However, only little attention has been payed, so far, to the need for systems to be biodegradable, in particular, when macromolecules are retained in the body because of chemical interactions or physical retention (high molecular weights).



PMLA 100

A few years ago, poly(β-malic acid) (PMLA 100) was selected as a potentially suitable candidate for drug-transportation and design of macromolecular prodrugs , and was synthesized for the first time (VERT and LENZ 1979&1981).

Since, efforts have been centered on the characterization of PMLA 100 and of some of its derivatives in order to bear out its suitability to fulfil the performance list of biodegradable polymeric drug-carriers. We have already shown that PMLA 100 is a relatively strong

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poly(carboxylic acid) soluble in water whatever the pH (BRAUD et al. 1981). PMLA 100 is non-toxic (LD_{50}=3.3g/kg intraperitoneally) and no immune response has been detected, so far (BRAUD et al. 1982). Furthermore, toxicity and physico- chemical properties of benzyl ester copolymer derivatives resulting from the modification of some of the COOH groups by hydrophobic benzyl substituents strongly depend on the distribution (block or random) of substituents on polymer chains (BRAUD and VERT 1983; BRAUD et al. 1983). Recently, it has been shown that compounds with activated hydrogen atoms such as alcohols and amines can be readily attached to PMLA 100 by using classical coupling reagents, and the feasability of the tailor-making of complex systems has been exemplified (BUNEL and VERT 1983).

In this paper, we wish to report data showing that sodium salt (PMLA 100, Na) does degrade in vitro at physiological pH and ionic strength, and that polymer chains turn to malic acid at last. For the sake of using aqueous GPC on SEPHADEX gels under reasonable flow rates, a sterilized low molecular weight PMLA 100,Na sample was allowed to age at various constant temperatures.

Experimental

Materials : A poly(β-malic acid benzyl ester) sample (MW ≃ 4,000 (Mw/Mn = 1.2) in THF by reference to polystyrene standards) was prepared from bromosuccinic acid using the initial route (VERT and LENZ 1979 & 1981) adjusted by using NaOH instead of $AgNO_3$ to close the β-lactone ring of the mono-benzyl ester monomer (JOHNS 1983). After hydrogenolytic cleavage of benzyl ester protecting groups of pendent COOH and evaporation of organic solvents, PMLA 100 was dissolved in water and turned to its sodium salt form by adding suitable amount of NaOH 1M. The resulting solution was dialized against pure water, concentrated and finally freeze-dried to yield solid PMLA 100,Na.

Polymer degradation : Aliquots of PMLA 100,Na (5 $mg.cm^{-3}$) in aqueous phosphate buffer (0.15 N, pH=7.5) were thermostated at 25°, 37° and 50°C respectively after sterilization by filtration on 0.22 μm MILLIPORE filters. Degradation of polymer chains was monitored by aqueous GPC using G-100 + G-50 + G-15 SEPHADEX gel columns (20 cm x 1 cm^2 each). This combination allowed MW determination with good resolution in the range 16,000 - 1,600 M_{GPC} units. Calibration was done by using polystyrene sulfonate standards. Eluent was 0.15 M NaCl with a flow rate of 12 $cm^3.h^{-1}$. Chromatograms were recorded using a UV detection at 214 nm.

Appearance of L-malic acid : L-malic acid produced during degradation of PMLA 100,Na was titrated using a diagnostic kit (BOEHRINGER) based on oxidation of L-malate by NAD^+. Resulting NADH, which is proportional to L-malic acid present in the medium, was assessed from absorbance at 340 nm.

Results and discussion

Decrease of molecular weight as monitored by GPC

Figure 1 shows typical patterns of UV-detected chromatograms versus time for a solution of PMLA 100,Na in 0.15 N phosphate buffer at pH=7.5 and at 50°C. The solution was sterilized in order to avoid fast growing of bacteries. At t=0, a small GPC peak is observed at $M_{GPC} \simeq$ 8,000 . As time

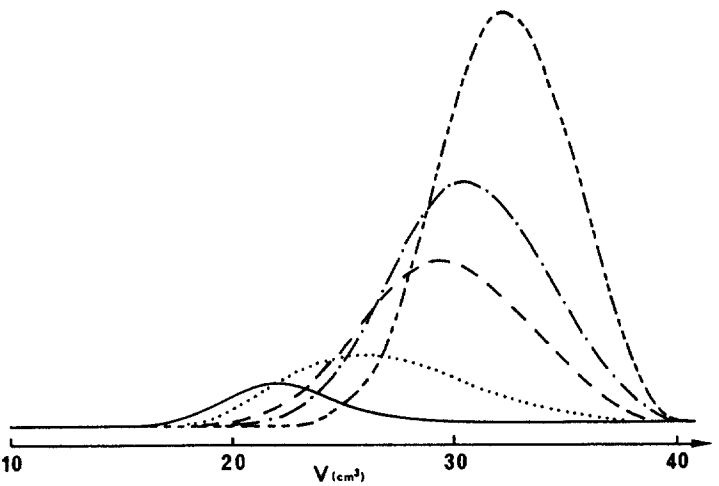
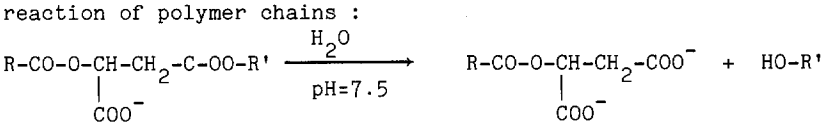


Figure 1 Typical UV-detected chromatograms of PMLA 100,Na in regard to aging time at 50°C in 0.15 N phosphate buffer at pH=7.5 (t=0 ——————— ;t=24h ····· ;t=70h — — — — — ; t=120h · —— · —— · —— ; t=330 - - — - - — - - —)

increases, this peak not only shifts to larger elution volumes (lower molecular weights) but its area enlarges because of great changes in UV-absorption at the wavelength used for detection. Both shift and magnification of the peak have been assigned to the following degradation reaction of polymer chains :



Accordingly, chain length decreases and cleaved main chain ester chromophores are replaced by hydroxyl and carboxylate ones as end groups. The former do not contribute to absorption above 200 nm. In contrast, the latter are known to absorb in the range of 210-220 nm as ester chromophores but with larger ε ($\varepsilon_{COO^-}/\varepsilon_{COOR} \simeq 20$).

Similar data have been obtained at 37° and 25°C. However, degradation depends very much on temperature. The lower the temperature, the slower the degradation.

Figure 2 shows the decrease of MW (maximum of GPC peak) versus time for the three temperatures. At 50°C, the curve levels off after c.a. 250 hours while c.a. 600 hours are necessary at 37°C and more than 1000 hours at 25°C.

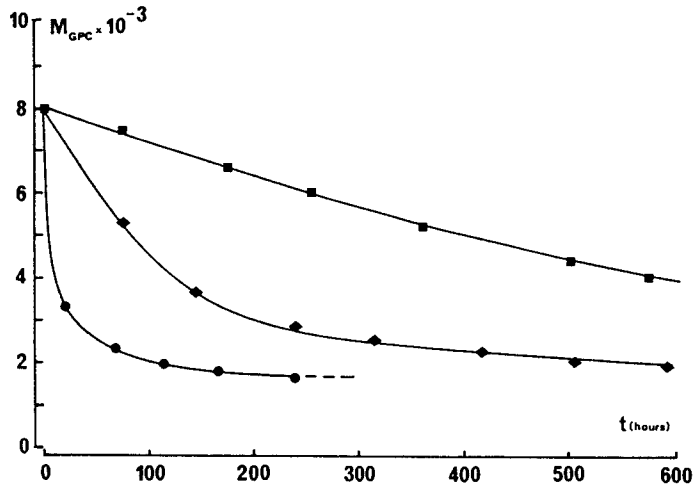


Figure 2 Decrease of M_{GPC} molecular weight in regard to aging time and temperature in 0.15 N phosphate buffer (■ 25°C ; ◆37°C ; ● 50°C)

Kinetics of chain degradation

The fact that degradation causes replacement of ester chromophores by carboxylate ones and that carboxylate chromophores absorb more that ester ones provided us with a means to determine kinetics parameters of the degradation reaction.

If residual absorption coefficients ε_{COO^-} and ε_{COOR} corresponding to main chain end COO^- chromophores and to main chain central COOR chromophores respectively, can be considered as independent of molecular weight, areas of the GPC peaks (A_t at time t) can be related to concentrations of COO^- and COOR chromophores, and, thus, to corresponding repeating units, by using eq (1) :

$$A_t = k.[\varepsilon_{COO^-}.\overline{DP}_n^o/\overline{DP}_n^t + \varepsilon_{COOR}.\overline{DP}_n^o/\overline{DP}_n^t.(\overline{DP}_n^t-1)] \qquad (1)$$

where $\overline{DP}_n^o$ is the initial degree of polymerization , $\overline{DP}_n^t$ the degree of polymerization at time t and k the calibration constant of the detector.

Considering eq. (1) at t=0 and t= ∞, one can write:

$$(1 - 1/\overline{DP}_n^o)/(1 - 1/\overline{DP}_n^t) = (A_\infty - A_o)/(A_\infty - A_t) \qquad (2)$$

Assuming that the cleavage of an ester bond in polymer main chains obeys eq. (3) corresponding to first order kinetics law :

$$d[ester]/dt = K.[ester] \qquad (3)$$

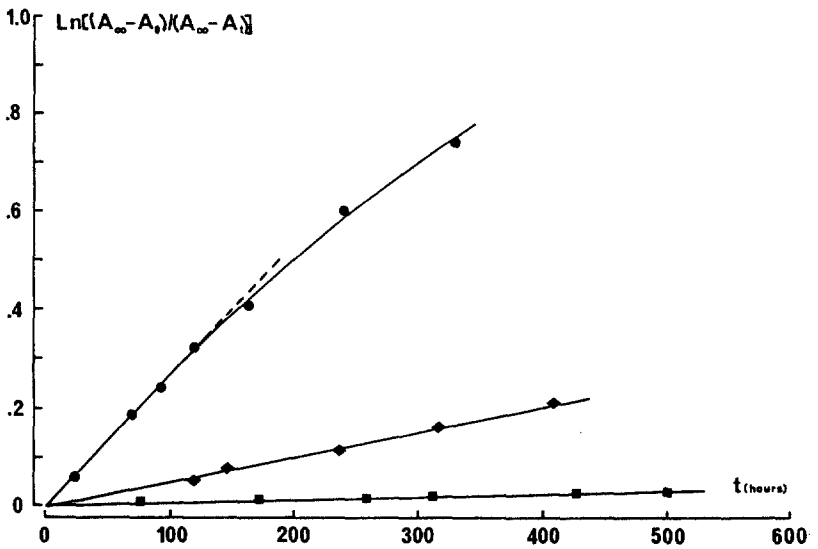


Figure 3 Plots of $Ln[(A_\infty - A_o)/(A_\infty - A_t)]$ vs t for determination of first order kinetics constants K at 25°(■), 37°(◆) and 50°C(●) using eq. (5)

$\overline{DP}_n^t$ and time can be related through eq. (4) after integration of eq. (3).

$$- Ln(1-1/\overline{DP}_n^t) = K.t - Ln(1-1/\overline{DP}_n^o) \quad (4)$$

Combination of eq. (2) and eq. (4) gives :

$$K = 1/t \; . \; Ln[(A_\infty - A_o)/(A_\infty - A_t)] \quad (5)$$

Figure 3 shows that degradation of PMLA 100,Na obeys eq. (5) at the three selected temperatures. Values of K (2.66×10^{-3} h^{-1} at 50°C, 0.52×10^{-3} h^{-1} at 37°C and 0.08×10^{-3} h^{-1} at 25°C) obtained by using eq. (5) fit Arrhenius law, $K = A.e^{-E/RT}$, with an activation energy E = 114.5 $kJ.mol^{-1}$. Furthermore, eq (5) shows that constant K should be independant of $\overline{DP}_n^o$ and, thus, of initial molecular weight, a feature which is still to be confirmed experimentally. These findings are in favor of equireactivity for all the central ester bonds. However, it is of value to note that eq. (5) and, thus, first order kinetics law does not hold up to total degradation as it is exemplified by the bending of the curve at 50°C for degradation times larger than 120 hours, i.e. when degradation is well advanced. It is likely that ε_{COO^-} and ε_{COOR} are no longer independent of the degree of polymerization when $\overline{DP}_n^t \to 1$ because of mutual perturbations of vicinal chromophores.

Degradation product

The ultimate step of the degradation of racemic PMLA 100,Na chains should be 50% sodium L-malate and 50% sodium D-malate. Search for formation of L-malate was carried out enzymatically and total malic acid was assessed as twice the formed L-malate assuming that cleavage of ester

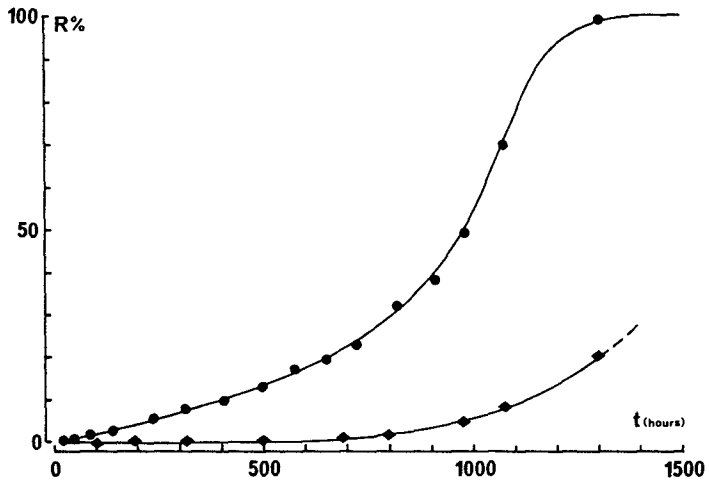


Figure 4 Yield in malic acid produced during the degradation of PMLA 100,Na at 37°(◆) and 50°C(●) in regard to malic acid initially present in polymer chains.

bonds occured at random. Figure 4 shows the yield in malic acid produced during the degradation reaction in regard to total malic acid initially present in the sample of polymer. Total degradation is observed after 1300 hours at 50°C. In contrast, 20% and 6% yields only are reached at 37° and 25° respectively for the same aging time.

Theories have been proposed several years ago to correlate degree of degradation, decrease of molecular weights and reaction time during degradation of polymer chains (MONNERIE and NEEL 1965a,b). Several of these theories led to similar general and exact relationships. However, practical calculations can be performed for given molecular weight distributions only and are necessarily limited to low degree of degradation (below 10%). Because of these limitations, experimental and theoretical appearance of malic acid could not be reasonably compared, so far.

Work is under way to investigate degradation of PMLA 100,Na in regard to structural parameters (DP, configuration,...) and experimental conditions (pH, concentration, temperature,...).

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