



obtained by heterogeneous catalysis and not for copolymers with the same gross composition but obtained by chemical coupling of benzyl alcohol to PMLA<sup>50</sup>H<sub>100</sub>. It was then concluded that the partial hydrogenolysis using the heterogeneous Pd-charcoal catalytic system should be a block-forming reaction.

Similar findings were also reported for benzyl ester derivatives of poly(acrylic acid) and poly(methacrylic acid)<sup>6</sup>. However, detailed investigations of the Pd-charcoal catalytic hydrogenolysis of these pendant benzyl ester-containing polymers has shown that access to any member of the copolymer family is not always feasible and depends on many factors<sup>6</sup>.

Among the techniques available to demonstrate the presence of blocks of similar repeat units in a copolymer, n.m.r. spectroscopy can be a powerful tool if atoms sensitive to the nearest neighbours are present<sup>7</sup>. However, selective degradation of one block is certainly one of the most convincing ways when one block can be degraded completely and exclusively, leaving the other block intact for subsequent analysis<sup>7</sup>. It is now well known that the water-soluble hydrophilic PMLA<sup>50</sup>H<sub>100</sub> polymer chains can be easily cleaved by hydrolysis in aqueous media. The rate of degradation increases when the temperature increases and when the pH decreases from neutral to acid<sup>8,9</sup>. On the other hand, a recent study has shown that PMLA<sup>50</sup>Be<sub>100</sub> does not degrade rapidly when in contact with aqueous media even under *in vivo* conditions<sup>10</sup>. Therefore, we have decided to take advantage of the hydrolytic sensitivity of PMLA<sup>50</sup>H<sub>100</sub> to investigate the sequence distribution in acid/ester copolymers obtained by Pd/C-catalysed hydrogenolysis. For this, two commercially available Pd-charcoal catalysts from different sources were used to cleave partially or totally the benzyl ester groups of five PMLA<sup>50</sup>Be<sub>100</sub> homopolymers with different initial molecular weights. The resulting compounds were analysed to show whether they were mixtures of homopolyacid and homopolyester forms and/or copolymer form with variable amounts of benzyl ester moieties. Sequence-selective degradation was carried out on one of the resulting compounds, which was identified as a water-soluble acid-rich copolymer, namely h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub>. The degradation of polymer chains was monitored by s.e.c. and the structure of the oligomeric end-products was investigated by FTi.r. and <sup>1</sup>H n.m.r.

## EXPERIMENTAL

### Materials

PMLA<sup>50</sup>Be<sub>100</sub> was synthesized by bulk ring-opening polymerization of benzyl malolactonate in the presence of triethylamine as the initiator and under a nitrogen atmosphere, as previously described<sup>1</sup>.

PMLA<sup>50</sup>H<sub>100</sub> and h-PMLA<sup>50</sup>Be<sub>(100-γ)</sub>H<sub>γ</sub> benzyl ester-containing compounds were obtained by hydrogenolysis of PMLA<sup>50</sup>Be<sub>100</sub>. Typically, a round-bottomed 500 cm<sup>3</sup> flask, equipped with a 80 cm<sup>3</sup> lateral reservoir, was loaded with 150 cm<sup>3</sup> of a mixture of ethyl acetate-ethanol (3:2 v/v) and with 400 mg of 5% (w/w) Pd-charcoal catalyst (Johnson 37 from Johnson Matthey), or with 200 mg of 10% (w/w) catalyst (from Janssen). One gram of PMLA<sup>50</sup>Be<sub>100</sub> was dissolved in 50 cm<sup>3</sup> of the same solvent mixture and placed in the lateral reservoir. The flask was then connected to a

graduated hydrogenation apparatus maintained at constant pressure and temperature. The flask and the hydrogenation apparatus were filled with hydrogen and the catalyst was allowed to saturate with the gas. The polymer solution located in the lateral reservoir was then poured into the main compartment. The mixture was stirred (2500 rpm) at room temperature until consumption of the theoretical volume of hydrogen corresponding to the desired Y value. The catalyst was then removed by filtration and the resulting copolymer was recovered as a solid powder after evaporation of the solvent system.

Ageing experiments were performed at 37°C using solutions of h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> (initial  $T_{\text{COOH}} = 5.6 \times 10^{-2} \text{ mol l}^{-1}$ ) either in a pH 7.4 buffer mixture of 1 M NaCl + 0.15 M sodium phosphate (named 'buffer' in the following) or in pure water.

### Techniques

*Aqueous s.e.c. analysis.* A Pharmacia FPLC equipment connected with a CM-Sepharose CL-6B gel column was used to monitor ageing experiments. Aliquots of 0.5 cm<sup>3</sup> of the ageing solution were diluted by adding 2.5 cm<sup>3</sup> of the buffer. Some 200 μl of this solution was injected and the chromatogram was recorded using both monochromatic u.v. detection at 214 nm (Pharmacia) and multi-wavelength u.v. spectral detection (Hewlett-Packard HP1040A). Results were expressed with respect to sodium polystyrene sulphonate standards.

*Organic s.e.c. analysis.* Relative molecular weights as referred to polystyrene standards and polydispersity were evaluated by using a Waters SEC apparatus equipped with μ-Styragel columns and refractive index (r.i.) detection.

*Turbidity monitoring.* The optical density (OD) at 500 nm (path length = 1 cm) of aliquots taken from the ageing stock solutions was recorded by using a Perkin-Elmer Lambda 15 at room temperature. Turbid solutions were defined as OD = 0.05–0.1 and milky ones when OD > 0.5.

*FTi.r. analysis.* Spectra recorded on a Perkin-Elmer 1760 spectrometer were obtained from films cast on an Irtran 2 window by evaporation of dioxane solutions of the polymers. Computed subtraction of Irtran 2 absorbance and normalization were done.

*<sup>1</sup>H n.m.r. spectra.* Spectra were obtained by using either a Varian T60A or a Bruker AM400 spectrometer and deuterated solvents (acetone or dioxane).

## RESULTS AND DISCUSSION

Partial and total catalytic cleavage of the pendant ester bonds of five PMLA<sup>50</sup>Be<sub>100</sub> homopolymers with different molecular weights whose characteristics are given in Table 1 were hydrogenolysed according to a procedure previously described<sup>1</sup> by using two Pd-charcoal catalysts. Data are presented in Table 1.

It is now known that hydrogenolysis depends on various factors, such as solvent, nature of catalyst and molecular weight of the poly(benzyl ester) as revealed by a systematic study of poly(benzyl acrylate) samples<sup>6</sup>, and does not yield copolymers systematically. As an example, the hydrogenolysis of PMLA<sup>50</sup>Be<sub>100</sub> ( $M_w = 33\,000$ ,

**Table 1** Characteristics of h-PMLA<sup>50</sup>Be<sub>(100-y)</sub>H<sub>y</sub> and h-PMLA<sup>50</sup>Be<sub>(100-y)</sub>Na<sub>y</sub> obtained by hydrogenolysis of PMLA<sup>50</sup>Be<sub>100</sub> in an ethyl acetate-ethanol (3:2 v/v) mixture

PMLA <sup>50</sup> Be <sub>100</sub>		Catalyst <sup>b</sup>	Y <sup>c</sup>	Aspect in water <sup>d</sup>	Aspect in buffer <sup>d</sup>
$\bar{M}_w^a$	I <sup>a</sup>				
20 000	1.5	C <sub>0</sub>	50	milky	milky
19 000	1.5	C <sub>0</sub>	50	milky	milky
10 000	1.2	C <sub>1</sub>	50	milky	milky
20 000	1.5	C <sub>0</sub>	78 <sup>e</sup>	turbid	clear
15 500	1.8	C <sub>0</sub>	78 <sup>e</sup>	turbid	clear
33 000	1.4	C <sub>1</sub>	80	milky	milky
19 000	1.5	C <sub>1</sub>	80 <sup>e</sup>	turbid	turbid
10 000	1.2	C <sub>0</sub>	80 <sup>e</sup>	clear	clear
20 000	1.5	C <sub>0</sub>	85	clear	clear
10 000	1.2	C <sub>0</sub>	90 <sup>e</sup>	clear	clear
33 000 <sup>f</sup>	1.4	C <sub>1</sub>	100 <sup>e</sup>	clear	clear
20 000	1.5	C <sub>0</sub>	100 <sup>e</sup>	clear	clear
10 000	1.2	C <sub>0</sub>	100 <sup>e</sup>	clear	clear

<sup>a</sup> Weight-average molecular weight and polydispersity as referred to polystyrene standards in dioxane<sup>b</sup> C<sub>0</sub> = Johnson 37 catalyst, C<sub>1</sub> = Janssen catalyst<sup>c</sup> Evaluated from hydrogen consumption<sup>d</sup> Solid samples ( $\approx 10$  mg) were poured into 1 ml of pure water or 1 ml of a mixture of 1 M NaCl + 0.15 M Na phosphate at pH 7.4<sup>e</sup> Controlled by <sup>1</sup>H n.m.r. analysis<sup>f</sup> Hydrogenolysis performed in dioxane

$I = 1.4$ ) in an ethyl acetate-ethanol (3:2 v/v) mixture consumed only 80% of the theoretical volume of hydrogen for total cleavage (Table 1). When dissolved in water or in the buffer after evaporation of the organic solvents, the recovered polymeric material gave a milky solution, which could be separated into a clear solution and a precipitate by filtration. Analysis of the two parts showed that the solution is pure PMLA<sup>50</sup>H<sub>100</sub> and that the precipitate is pure PMLA<sup>50</sup>Be<sub>100</sub> with a higher molecular weight ( $\bar{M}_w = 50\,000$ ,  $I = 1.1$ ) than the starting poly(benzyl malolactonate). This experiment led to the conclusion that hydrogenolysis performed in ethyl acetate-ethanol (3:2 v/v) is molecular-weight-dependent. In contrast, the total hydrogenolysis of high-molecular-weight PMLA<sup>50</sup>Be<sub>100</sub> ( $\bar{M}_w = 33\,000$ ) was achievable when dioxane was used as the solvent medium.

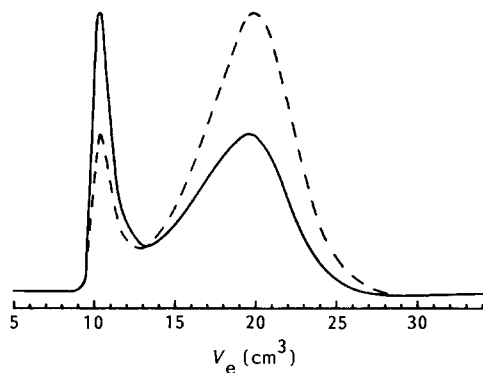
For all the other PMLA<sup>50</sup>Be<sub>100</sub> samples, which have lower molecular weights, hydrogenolysis was feasible in a mixture of ethyl acetate-ethanol (3:2 v/v), and led to corresponding PMLA<sup>50</sup>H<sub>100</sub> samples (Table 1).

Partially hydrogenolysed compounds were obtained by stopping the magnetic stirring before completion of the reaction (see 'Experimental'). When mixed with pure water or with the buffer that was used as eluant in aqueous s.e.c. measurements, the recovered solid materials yielded non-homogeneous solutions. The two media were considered because the carboxyl groups of the resulting copolymer solutions (or suspensions) were in their -COOH and -COO<sup>-</sup> forms respectively. All the crude h-PMLA<sup>50</sup>Be<sub>50</sub>H<sub>50</sub> hydrogenolysates gave milky aqueous solutions regardless of the ionization state of -COOH groups (Table 1). By filtration on 0.45  $\mu$ m Millipore membranes, clear filtrates were obtained. After evaporation and drying, analysis by <sup>1</sup>H n.m.r. of these water-soluble copolymers showed a content of about 90% of -COOH moieties. White solid materials recovered on the filters were identified as pure PMLA<sup>50</sup>Be<sub>100</sub> with higher molecular weights and lower polydispersity than the starting homopolymers. Similar features were observed for h-PMLA<sup>50</sup>Be<sub>20</sub>H<sub>80</sub> ( $\bar{M}_w = 19\,000$ ). Filtration of the buffered solution gave a clear solution containing macromolecules with about 90%

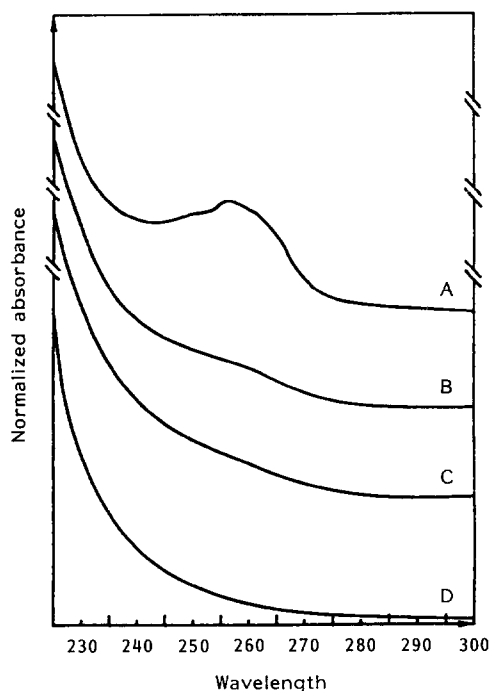
of -COOH moieties, i.e. with a composition close to that found in the case of h-PMLA<sup>50</sup>Be<sub>50</sub>H<sub>50</sub> hydrogenolysates.

These results suggest that hydrogenolysis of PMLA<sup>50</sup>Be<sub>100</sub> polymers proceeds as in the cases of poly(benzyl acrylates) and poly(benzyl methacrylates) to yield mixtures of macromolecules with a wide variety of composition and molecular weight<sup>6</sup>. Therefore, in the PMLA<sup>50</sup>Be<sub>100</sub> series, turbidity of aqueous solutions can also be assigned to the presence of residual non-modified poly(benzyl malolactonate) macromolecules or of water-insoluble h-PMLA<sup>50</sup>Be<sub>(100-y)</sub>H<sub>y</sub> copolymers with low Y values.

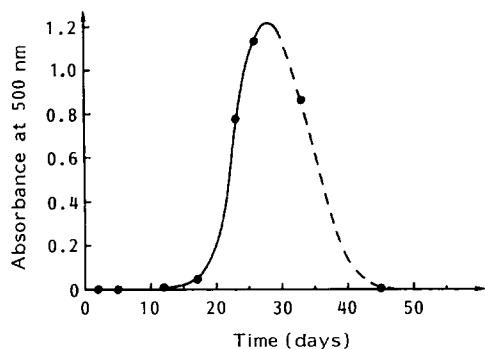
For the structural analysis of sequence distribution through selective degradation of polyester chains, only those hydrolysates with  $Y \geq 78$ , which gave clear solutions with no trace of insoluble homopolyester chains in the neutral buffer, were considered. Indeed, they were actual copolymers. Turbidity was found for some of these copolymers in water when they were turned to their acidic form. This turbidity reflected the presence of large aggregates. The clear solutions given by the salt forms were not true solutions. Indeed, bimodal chromatograms were found in aqueous s.e.c. analysis, the peak located at the exclusion volume being assignable to the presence of small aggregates<sup>5</sup>. In addition, polydispersity in chemical compositions was found by combining u.v. absorption and s.e.c. analyses. Figure 1 shows u.v.-detected s.e.c. chromatograms of h-PMLA<sup>50</sup>Be<sub>20</sub>Na<sub>80</sub> ( $\bar{M}_w = 10\,000$ ,  $I = 1.2$ ) recorded at 235 and 260 nm. The second wavelength corresponded predominantly to the <sup>1</sup>L<sub>B</sub> electronic transition of benzyl aromatic chromophores<sup>2</sup> whereas at 235 nm all the chromophores contributed. The relative magnitudes of the peaks centred at  $\approx 10.5$  cm<sup>3</sup> (exclusion volume) and at 19.5 cm<sup>3</sup> were reversed for detections performed at 235 and 260 nm. It was concluded that excluded aggregates had a higher content in benzyl ester moieties than the permeated macromolecules. This finding was confirmed by u.v. spectra recorded at different elution volumes  $V_e$  (Figure 2). As  $V_e$  increased, the u.v. contribution of the <sup>1</sup>L<sub>B</sub> absorption band due to the benzyl ester chromophores



**Figure 1** U.v.-detected chromatograms of h-PMLA<sup>50</sup>Be<sub>20</sub>H<sub>80</sub> ( $\bar{M}_w = 10\,000$ ) in aqueous medium recorded at  $\lambda = 235$  nm (broken curve) and  $\lambda = 260$  nm (full curve) recorded by using multi-wavelength u.v. detection



**Figure 2** Normalized u.v. spectra recorded during experiment reported in *Figure 1* at different elution volumes: A, 10.3 cm<sup>3</sup>; B, 17.7 cm<sup>3</sup>; C, 21.7 cm<sup>3</sup>; D, 23.8 cm<sup>3</sup>



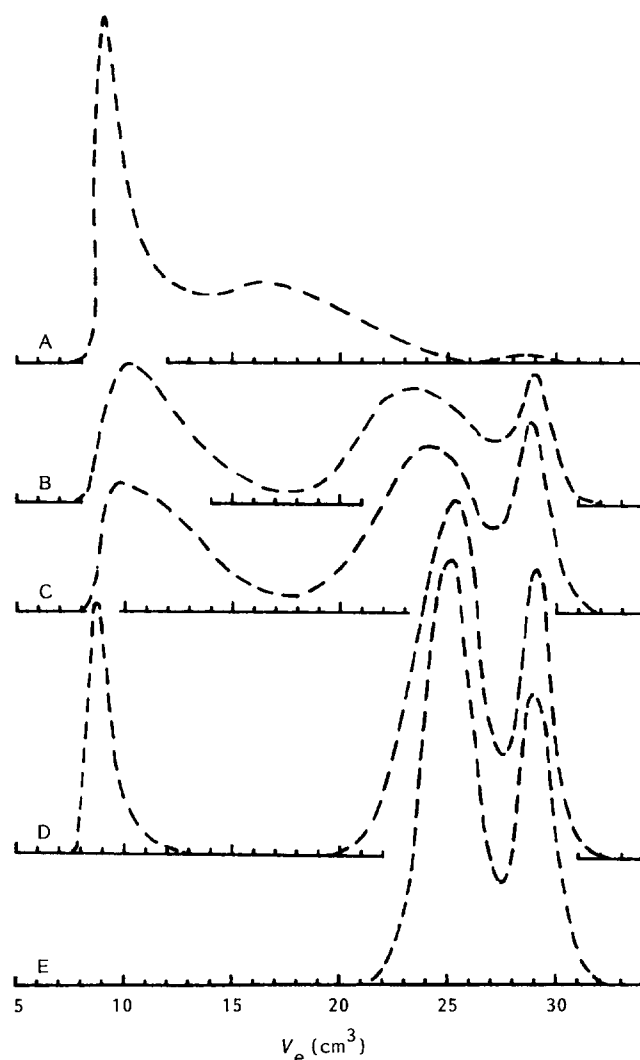
**Figure 3** Variation of turbidity as a function of time during ageing of h-PMLA<sup>50</sup>Be<sub>22</sub>Na<sub>78</sub> in the presence of buffer

decreased. The shape of the chromatogram also depended on the characteristics of the parent PMLA<sup>50</sup>Be<sub>100</sub>. For instance, h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> and h-PMLA<sup>50</sup>Be<sub>20</sub>H<sub>80</sub> copolymers, which had similar gross compositions but parent PMLA<sup>50</sup>Be<sub>100</sub> with different molecular weights (15 500 vs. 10 000), led to different u.v.-detected

chromatograms. h-PMLA<sup>50</sup>Be<sub>22</sub>Na<sub>78</sub> (*Figure 4*) showed a larger population of excluded species than h-PMLA<sup>50</sup>Be<sub>20</sub>H<sub>80</sub> (*Figure 1*). The h-PMLA<sup>50</sup>Be<sub>22</sub>Na<sub>78</sub> copolymer was used for the investigation of the sequence-selective degradation.

#### Ageing of buffered solutions of h-PMLA<sup>50</sup>Be<sub>22</sub>Na<sub>78</sub>

An aqueous solution of h-PMLA<sup>50</sup>Be<sub>22</sub>Na<sub>78</sub> buffered at pH 7.4 was allowed to age at 37°C. The ageing of the copolymer solution was first monitored by evaluating the absorbance at 500 nm for different times (*Figure 3*). Initially, the buffered solution was clear. After 12 days, the solution became slightly turbid. By the 17th day, the turbidity had increased significantly and the solution turned milky progressively. However, after about 30 days, turbidity started decreasing whereas a macroscopic precipitate was observed and separated by filtration. After 45 days, a clear solution could be easily separated from the precipitated particles, which could be recovered in amounts allowing characterization. Ageing was also monitored by aqueous s.e.c. Chromatograms were recorded for different ageing times on aliquots that were filtered before injection in order to remove the precipitated materials (*Figure 4*). The bimodal chromatogram found at  $t=0$  reflected the presence of two



**Figure 4** U.v.-detected chromatograms of h-PMLA<sup>50</sup>Be<sub>22</sub>Na<sub>78</sub> (at  $\lambda = 214$  nm) during ageing experiment in the presence of buffer: A,  $t = 0$ ; B,  $t = 2$  days; C,  $t = 5$  days; D,  $t = 17$  days; E,  $t = 45$  days

populations of macromolecules in the medium. The first population 'A' was composed of aggregated macromolecules eluted at  $V_e = 9 \text{ cm}^3$  and the second one 'NA' was composed of non-aggregated macromolecules giving a broad peak centred at  $V_e = 17 \text{ cm}^3$  ( $M_{\text{gpc}} = 30\,000$ ) as referred to sodium polystyrene sulphonate standards. After two days, the chromatogram changed drastically. 'A' and 'NA' peaks moved to  $V_e \approx 10 \text{ cm}^3$  ( $M_{\text{gpc}} = 300\,000$ ) and to  $V_e > 23 \text{ cm}^3$  ( $M_{\text{gpc}} < 5000$ ), respectively, in a zone corresponding to the permeation volume of the column. At the same time, a third peak appeared at  $V_e = 29 \text{ cm}^3$  far beyond the permeation volume. Between 2 and 5 days, only changes in relative magnitudes of the three peaks were observed. After 17 days, the first broadened peak turned back to a thin peak at the excluded volume. This is regarded as in good agreement with the appearance of tiny solid particles, which finally gathered to form a macroscopic precipitate visible after 30 days. On day 45, no more aggregates were present in the clear supernatant, which gave a chromatogram composed of only the 'NA' and the  $29 \text{ cm}^3$  peaks. The increase in magnitude of the 'NA' peak was due to the cleavage of ester main-chain groups, which formed degradation products with higher molecular absorption coefficients<sup>8</sup>. The peak centred at  $V_e = 29 \text{ cm}^3$  reached a maximum value after 17 days, i.e. when the solution started to become turbid. Spectral multi-wavelength u.v. detection showed that the compound eluting at  $29 \text{ cm}^3$  absorbed strongly at  $260 \text{ nm}$ . Because, in aqueous media, affinity phenomena can occur between the gel and the hydrophobic molecules<sup>11</sup>, the possibility of the presence of benzyl alcohol due to the cleavage of side-chain ester groups was suspected. This was confirmed by the injection of aqueous benzyl alcohol, which showed a peak at  $V_e = 29 \text{ cm}^3$ . A calibration curve established by injection of known amounts of benzyl alcohol showed that about 10% of the benzyl ester groups present in the starting PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> sample were cleaved during the experiment.

#### Ageing of self-ionized h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> in water

In pure water, h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> gave a turbid solution whose pH was acidic and depended on the self-ionization of the carboxylic groups present on the polymer chains. The chromatograms obtained for ageing the copolymer solution were similar to those found for the ageing in the buffered medium. However, the timescale of the degradation was contracted since macroscopic precipitation of solid material was observed after 19 days instead of 45 days at neutral pH. This finding is in good agreement with the fact that faster rates of degradation of PMLA<sup>50</sup>H<sub>100</sub> were found in acidic media with respect to neutral<sup>9</sup>. When the experiment was carried out in the absence of salt, it was possible to monitor the degradation by s.e.c. in dioxane upon recovery and freeze-drying of aliquots of the ageing polymer solution. Results are presented in Figure 5. It must be pointed out that no aggregation could occur in dioxane, as this solvent was a good solvent for both the acidic and the ester repeat units. The initial sample (Figure 5A;  $M_{\text{gpc}} = 18\,000$  as referred to polystyrene standards in dioxane) gave a typical high-molecular-weight polymer profile. After 2 days, the s.e.c. changed dramatically. The high-molecular-weight peak appeared as a shoulder on a larger peak located at larger elution volumes. The trend was confirmed after 5 days with the

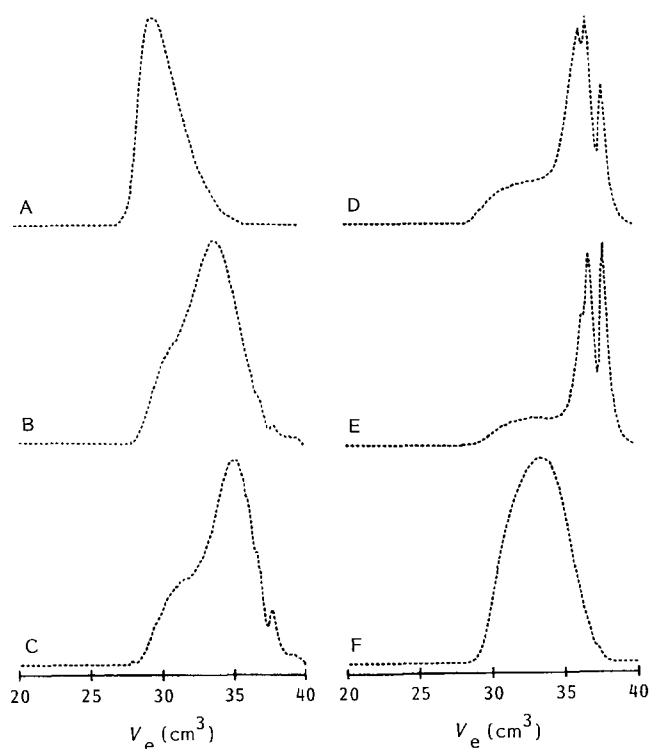
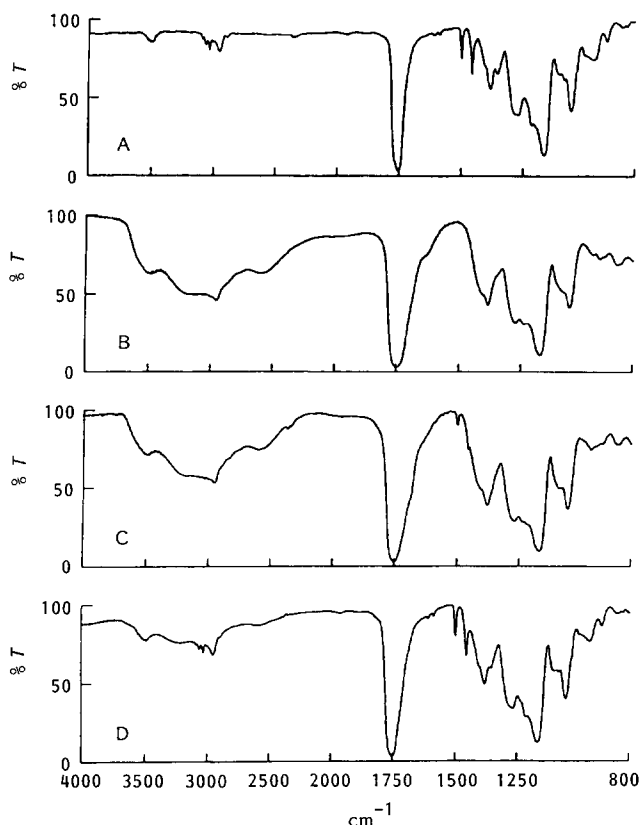


Figure 5 R.i.-detected chromatograms of recovered samples of h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> dissolved in dioxane after ageing in pure water: A,  $t = 0$ ; B,  $t = 2$  days; C,  $t = 5$  days; D,  $t = 12$  days; E,  $t = 19$  days; F, precipitate recovered at  $t = 19$  days

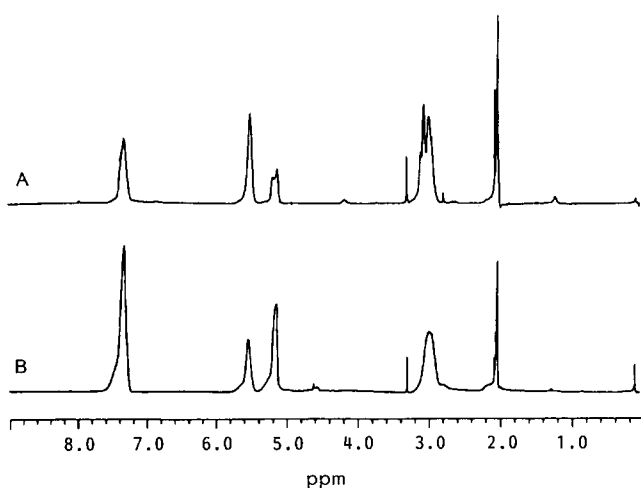
low-molecular-weight peak at  $35 \text{ cm}^3$  and the appearance of a thin peak in the range where small molecules eluted. After 12 days, the peak of high-molecular-weight species was small whereas three well defined peaks appeared close to the permeation volume. After 19 days, the same pattern was observed with a relative increase of the peak corresponding to the lower-molecular-weight species. It is likely that these well defined low-molecular-weight peaks corresponded to oligomers with low degree of polymerization.

#### Analysis of recovered precipitates

The solid material that precipitated at  $t = 19$  days after degradation in water was recovered by centrifugation after washing with water. The collected solid weighed 20% of the original hydrogenolysate. The chromatogram of this precipitate (Figure 5F) showed a peak at  $\bar{M}_w = 5500$  with rather large polydispersity  $I = 1.9$ . The FTi.r. spectrum of the precipitate was compared with the spectra of PMLA<sup>50</sup>Be<sub>100</sub>, PMLA<sup>50</sup>H<sub>100</sub> and h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> (Figure 6). The i.r. spectrum of PMLA<sup>50</sup>Be<sub>100</sub> is mainly characterized by the presence of two thin bands located at  $1455 \text{ cm}^{-1}$  and  $1499 \text{ cm}^{-1}$ , which are assigned to aromatic C–C stretching, and a relatively thin band at  $1750 \text{ cm}^{-1}$  characteristic of the stretching vibration of ester groups (main chain + benzyl ester side-chain). In contrast, the i.r. spectrum of PMLA<sup>50</sup>H<sub>100</sub> is characterized by a broader  $1750 \text{ cm}^{-1}$  band because of the overlapping of the C=O bands corresponding to side-chain COOH and main-chain COOR carbonyl groups. The second characteristic of the i.r. spectrum of PMLA<sup>50</sup>H<sub>100</sub> is the rather strong absorption visible in the  $2500\text{--}3300 \text{ cm}^{-1}$  region due to the O–H vibration of the OH bond in –COOH groups. The i.r. spectrum of h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> exhibited the



**Figure 6** I.r. spectra of: A, PMLA<sup>50</sup>Be<sub>100</sub>; B, PMLA<sup>50</sup>H<sub>100</sub>; C, h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> before degradation; D, precipitate recovered at  $t = 19$  days after ageing in pure water



**Figure 7** <sup>1</sup>H n.m.r. spectra of: A, h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> before degradation; B, precipitate recovered at  $t = 19$  days after ageing in pure water

characteristics of the acid and benzyl ester repeat units as they are in PMLA<sup>50</sup>Be<sub>100</sub> and in PMLA<sup>50</sup>H<sub>100</sub> but with a larger proportion of the units present in PMLA<sup>50</sup>H<sub>100</sub> in agreement with the large  $Y$  value. In contrast, the precipitate showed an i.r. spectrum that reflected the predominance of the units present in PMLA<sup>50</sup>Be<sub>100</sub>. The <sup>1</sup>H n.m.r. spectrum of the precipitate (Figure 7B) was recorded in deuterio-acetone. The comparison with the spectrum of the starting copolymer bears out the enrichment in benzyl units. A composition of 20% acid units and 80% benzyl ester units was deduced

from the integration of the signals observed at 7.32 and 5.18 ppm, which correspond to aromatic and methylene protons of side-chain ester groups, and of the signals at 5.58 and 3.00 ppm, respectively, assigned to the methine and methylene main-chain protons. Similar features were found for the precipitate recovered 45 days after degradation of h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> in the buffer and washed with diluted HCl in order to turn the polymer acidic for comparison with the precipitate recovered from the non-buffered ageing medium. The only difference seemed to be related to the amount of recovered material (11% instead of 20%), a feature that might be due to the cleavage of side-chain ester detected by s.e.c. Anyhow, at the ultimate step of the hydrolytic degradation of h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub>, i.e. when benzyl ester-enriched oligomeric compounds precipitated, one ended up with rather large amounts of degraded macromolecules in which the average length of benzyl ester sequences corresponded to tetrads (one acid for four benzyl ester repeat units). These amounts (20% or 11% of the initial copolymer) are much larger by far than the probability for finding tetrads in a PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> random copolymer ( $p_{\text{tetrad}} = 0.22^4 \approx 0.2\%$ ). Therefore, one can conclude that the starting h-PMLA<sup>50</sup>Be<sub>22</sub>H<sub>78</sub> did have a blocky structure. One can extend this conclusion to the partially hydrogenolysed benzyl esters of poly(acrylic acid) and poly(methacrylic acid), which were also suspected to be blocky copolymers.

## CONCLUSIONS

In conclusion, we have borne out that hydrogenolysis of benzyl ester-protected poly(carboxylic acids) proceeds through a complex pattern as exemplified by the various compositions of the compounds isolated during the hydrogenolysis of PMLA<sup>50</sup>Be<sub>100</sub> samples. Furthermore, we have shown that h-PMLA<sup>50</sup>Be<sub>(100- $\gamma$ )</sub>H <sub>$\gamma$</sub>  copolymers soluble in water are blocky copolymers, as strongly suspected before from the investigation of physico-chemical properties.

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## REFERENCES

- 1 Vert, M. and Lenz, R. W. *ACS Polym. Prep.* 1979, **20**, 608
- 2 Braud, C., Vert, M. and Lenz, R. W. 'Proc. 27th IUPAC Int. Symp. Macromolecules' (Strasbourg), 1981, p. 1086
- 3 Braud, C. and Vert, M. in 'Polymers as Biomaterials' (Eds S. W. Shalaby, A. S. Hoffman, B. D. Ratner and T. A. Horbett), Plenum Press, New York, 1984, p. 1
- 4 Selb, J. and Gallot, Y. *Makromol. Chem.* 1980, **181**, 809
- 5 Braud, C., Bunel, C., Garreau, H. and Vert, M. *Polym. Bull.* 1983, **9**, 198
- 6 Caron, A. Thesis, Rouen, 1988
- 7 Noshay, A. and McGrath, J. E. 'Block Copolymers. Overview and Critical Survey', Academic Press, New York, 1977
- 8 Braud, C., Bunel, C. and Vert, M. *Polym. Bull.* 1985, **13**, 293
- 9 Braud, C., Caron, A., Francillette, J., Guérin, Ph. and Vert, M. *ACS Polym. Prep.* 1988, **29**, 600
- 10 Christel, P., Vert, M., De Roquencourt, A. and Brocherion, P. INSERM Grant No. 8480024, Final Report, 1987
- 11 Barth, H. G. *Adv. Chem. Sci.* 1986, **213**, 31