Safety Assessment of Polymeric Additives for Food Packaging: Hydrolysis of Polymeric Plasticizers by Digestive Fluids

M. HAMDANI,[1] L. THIL,[2] G. GANS,[2] A. E. FEIGENBAUM[1]

[1] Institut National de la Recherche Agronomique, SQuAlE-UPBP, Centre de Physicochimie et Biotechnologies Agro-industrielles, F 51687 Reims, Cedex, France

[2] BASF AG, Industrial Chemicals, D 67056 Ludwigshafen, Germany

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ABSTRACT: The *in vitro* hydrolysis of poly(1,2-propylene adipate) by digestive fluid liquids was studied to assess the safety of polymeric plasticizers that could migrate into food. A high extent of hydrolysis was obtained with freshly prepared intestinal fluid solutions. High performance size exclusion chromatography analysis indicated that the bulk plasticizer completely disappeared and that low molecular weight oligomers were formed within 4 h. Hydrolysis did not result in a significant conversion to the free monomers, like adipic acid, as was shown by gas-phase chromatography. Measurements by NMR indicated that the cleavage selectively occurred at primary ester linkages. Fractionation of the hydrolysis products on silica gel gave six oligomeric fractions, which were recovered and analyzed. The absolute molecular weight of the plasticizers and the average molecular weight of the hydrolysis products were evaluated using proton NMR. Hydrolysis did not take place (<2%) under simulated gastric and saliva conditions. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 956–966, 2002

Key words: hydrolysis; polymeric plasticizer; digestive fluid simulants; oligomers; pancreatin; food packaging; regulation

INTRODUCTION

Plastic packaging materials release some of their low molecular weight constituents into foodstuffs; substances like residual monomers, oligomers, additives, or their degradation products are potential migrants. In Europe the substances used to manufacture food contact plastic materials must therefore be evaluated by the Scientific Committee for Food (SCF) before being accepted on the European Union positive list.

The evaluation made by the SCF uses a risk assessment approach. The potential danger represented by consumer ingestion of these food contaminants is considered, and the amount of toxicity data that is required depends on the level of exposure. The following criteria are taken into account by the SCF[1]:

1. If the migrant has a molecular weight higher than 1000 g/mol, SCF considers that there is little absorption in the gastrointestinal tract; thus, in principle, no toxicological data are required for the substance itself. Therefore, for polymeric additives and oligomers, only the fraction with a molecular weight below 1000 g/mol is relevant for safety evaluation.

Correspondence to: A. E. Feigenbaum (feigenba@lille.inra.fr).

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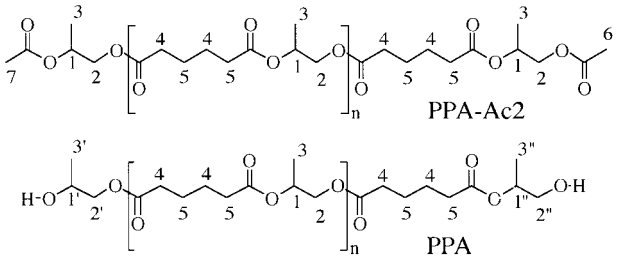


Scheme 1 The structures of the polymeric plasticizers in the study. The atom numbering is used in Figures 1, 2, 4, and 5.

2. If migration is shown to be lower than 5 mg/kg of food in worst case situations and if the substance is not mutagenic, a reduced compendium of toxicological information is required.
3. If the substance hydrolyzes in digestive fluids, the toxicological information available on hydrolysis products may be taken into account. In favorable situations, when hydrolysis is complete and hydrolysis products are already authorized, no other information is needed.

These criteria are essential for the evaluation of polymeric additives. Polymeric plasticizers are increasingly used in poly(vinyl chloride) films and tubing. They often give rise to high migration levels.[2] The hydrolysis of poly(propylene adipate) (PPA) and poly(butylene adipate) (PBA; Scheme 1) in digestive fluids *in vitro* was studied.[3] Based on the very low yield in adipic acid (one of the comonomers) formed, the authors concluded that PPA and PBA did not hydrolyze significantly in digestive fluid simulants.[3]

However, it can be envisioned that hydrolysis does occur but without yielding adipic acid. We therefore investigated the reactivity of PPA terminated with acetyl groups (PPA-Ac2) and PPA (Scheme 1) by focusing on the possible formation of hydrolysis products other than adipic acid. Reaction mixtures were fractionated on the basis of their acidic behavior and polarity and then analyzed.

EXPERIMENTAL

Methods

High Performance Size Exclusion Chromatography Analysis

High performance size exclusion chromatography (HP-SEC) was carried out using two coupled PL Gel columns [300 × 7.5 mm, 10 μm poly(styrene-divinylbenzene)] of nominal pore sizes of 100 and 500 Å. The mobile phase was dichloromethane/cyclohexane (1:1 v/v, 1.0 mL/min). UV detection at 230 nm was used.

Gas Chromatography Analysis

Gas chromatography (GC) analysis for the trimethylsilyl derivative of adipic acid was performed on a Fisons instrument using a DB5MS® (15 m × 0.25 mm i.d., 0.12 μm film thickness) and a flame ionization detector (at 280°C). Helium was used as the carrier gas (30-kPa inlet pressure). Samples were injected at 220°C with a 15:1 split. The column temperature was maintained for 1 min at 60°C, raised to 160°C (20°C/min) and held for 2 min, and raised to 200°C (49.9°C/min) and maintained for 2 min.

NMR Analysis

Proton and carbon-13 NMR (^{1}H-NMR and ^{13}C-NMR) spectra were recorded in $CDCl_3$ on a Bruker AC 250 spectrometer.

Monitoring the hydrolysis by ^{1}H-NMR was based on the peak integration of terminal diol units (CH-1″ or CH-2″) versus the protons of units within the chain (CH-1). The detection limit was determined by systematically diluting PPA (as a 25 mg/mL solution in $CDCl_3$) in PPA-Ac2 (as a 25 mg/mL solution in $CDCl_3$) until the relevant peak of proton CH-1″ could hardly be distinguished in the baseline. It was thus possible to detect a signal of CH-1″ in a mixture of PPA and PPA-Ac2 with a ratio of 1/5. In this case, the ratio of CH-1″/CH-1 was 1/66, which corresponded to a 1.5% concentration of end-chain units. This suggested that the detection limit was 1.5%.

Digestive Fluid Simulants

The European Commission recently defined three simulants to be used for digestive fluids,[1] which are saliva, gastric, and intestinal fluid simulants.

Saliva fluid simulant[1] was $NaHCO_3$ (4.2 g), NaCl (0.5 g), and K_2CO_3 (0.2 g) dissolved in distilled water with the volume adjusted to 1 L. The pH of the solution was 8.9 ± 0.1.

The gastric fluid simulant[1] was a 0.07*M* hydrochloric acid solution (pH 1.2 ± 0.1) prepared from a 0.1*M* standard solution.

The intestinal fluid simulant[1] consisted of a solution of pancreatin. First KH_2PO_4 (6.8 g) was dissolved in distilled water (250 mL) in a 1-L volumetric flask. Then 0.2*M* NaOH (190 mL), so-

dium taurocholate (0.5 g), and distilled water (around 400 mL) were added. A solution of pancreatin (10 g, 8 USP) was prepared separately by gradually adding the enzyme into distilled water (150 mL) to avoid the formation of lumps, and it was then transferred into the volumetric flask with gentle shaking. The pH was adjusted to 7.5 ± 0.1 with NaOH (0.2*M*), and the resulting solution was diluted to 1 L with distilled water.

Procedures

Hydrolysis Monitored by HP-SEC and NMR Analysis

Gastric Hydrolysis. The plasticizer (5 mg as 500 μL of a 10 mg/mL solution in acetonitrile) was added to a 250-mL round-bottom flask, and the solvent was removed under a vacuum. The simulant (100 mL) was then added and the sample stirred at 37 ± 0.5°C for 4 h. Dichloromethane (20 mL) was added and the mixture transferred to a separating funnel. The aqueous phase was separated and reextracted with dichloromethane (20 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in either $CDCl_3$ (0.4 mL) for NMR analysis or 100 μL of solvent (dichloromethane-cyclohexane 1:1, v/v) for HP-SEC analysis.
Blank control experiments included the analysis of reaction media without plasticizer.

Intestinal Hydrolysis. The plasticizer (5 mg added as 500 μL of a 10 mg/mL solution in acetonitrile) was added to a 250-mL round-bottom flask, and the solvent was removed under a vacuum. The simulant (amount adjusted according to the concentration in the table below) was then added and the sample stirred at 37 ± 0.5°C for 4 h. The sample was centrifugated and separated into two fractions: neutral and acidic. In the neutral fraction the mixture was extracted twice by dichloromethane (2 × 10 mL) in a separating funnel. The organic phase was dried over $MgSO_4$, evaporated to dryness, and analyzed as in the gastric hydrolysis. In the acidic fraction the aqueous phase was recovered, acidified to pH 2 with HCl (2*M*), and extracted with diethyl ether (2 × 10 mL). The organic phase was dried over $MgSO_4$, evaporated to dryness, and analyzed as in the gastric hydrolysis.

Experiment	Concentration (mg/l) of plasticizer in the intestinal fluid simulant	Detection of adipic acid by GC	Detection of oligomeric hydrolysis products (NMR, HP-SEC)
25, 250	Separation of hydrolysis products + NMR and HP-SEC	2500	

Hydrolysis Monitored by GC Analysis: Investigation for Possible Adipic Acid Formation

Gastric Hydrolysis. Plasticizer (1 mg as 100 μL of a 10 mg/mL solution in acetonitrile) was added to a 50-mL round-bottom flask, and the solvent was removed under a vacuum. Pimelic acid as an internal standard (200 μg as 100 μL of a 2 mg/mL solution in water) and a prewarmed solution of the simulant (40 mL) were then added. The reaction medium was stirred at 37 ± 0.5°C for 4 h. After cooling, the reaction mixture was extracted twice with ether (2 × 5 mL). The ether extracts were joined, dried over $MgSO_4$, and evaporated under a vacuum. Acetonitrile (100 μL) was then added and the sample was transferred to a microvial. The derivatization reagent bissilyltrifluoroacetamide (100 μL, Aldrich) was added, and the sample was heated for 60 min at 70°C and analyzed as described in the Experimental section.

Intestinal Hydrolysis. Plasticizer (1 mg as 100 μL of a 10 mg/mL solution in acetonitrile) was added to a 50-mL round-bottom flask, and the solvent was removed under a vacuum. Pimelic acid as an internal standard (200 μg as 100 μL of a 2 mg/mL solution in water) and a prewarmed solution of the simulant (40 mL) were then added, and the sample was stirred at 37 ± 0.5°C for 4 h. The sample was acidified to pH 2 with HCl (2*M*) and extracted with diethyl ether (2 × 5 mL). The ether extract was treated and derivatized as above.

Control experiments included the analysis of reaction blanks without plasticizer but with pimelic acid.

RESULTS AND DISCUSSION

Structure of PPA and PPA-Ac2 Polymeric Plasticizers

Understanding the structure of the hydrolysis products requires a good knowledge of the starting plasticizers and their spectroscopic properties and chromatographic behavior.

The HP-SEC profiles of the two plasticizers showed no significant difference. They looked very similar to those shown by Castle et al.[2] In each case, in addition to the bulk plasticizer, there were peaks on the lower molecular weight

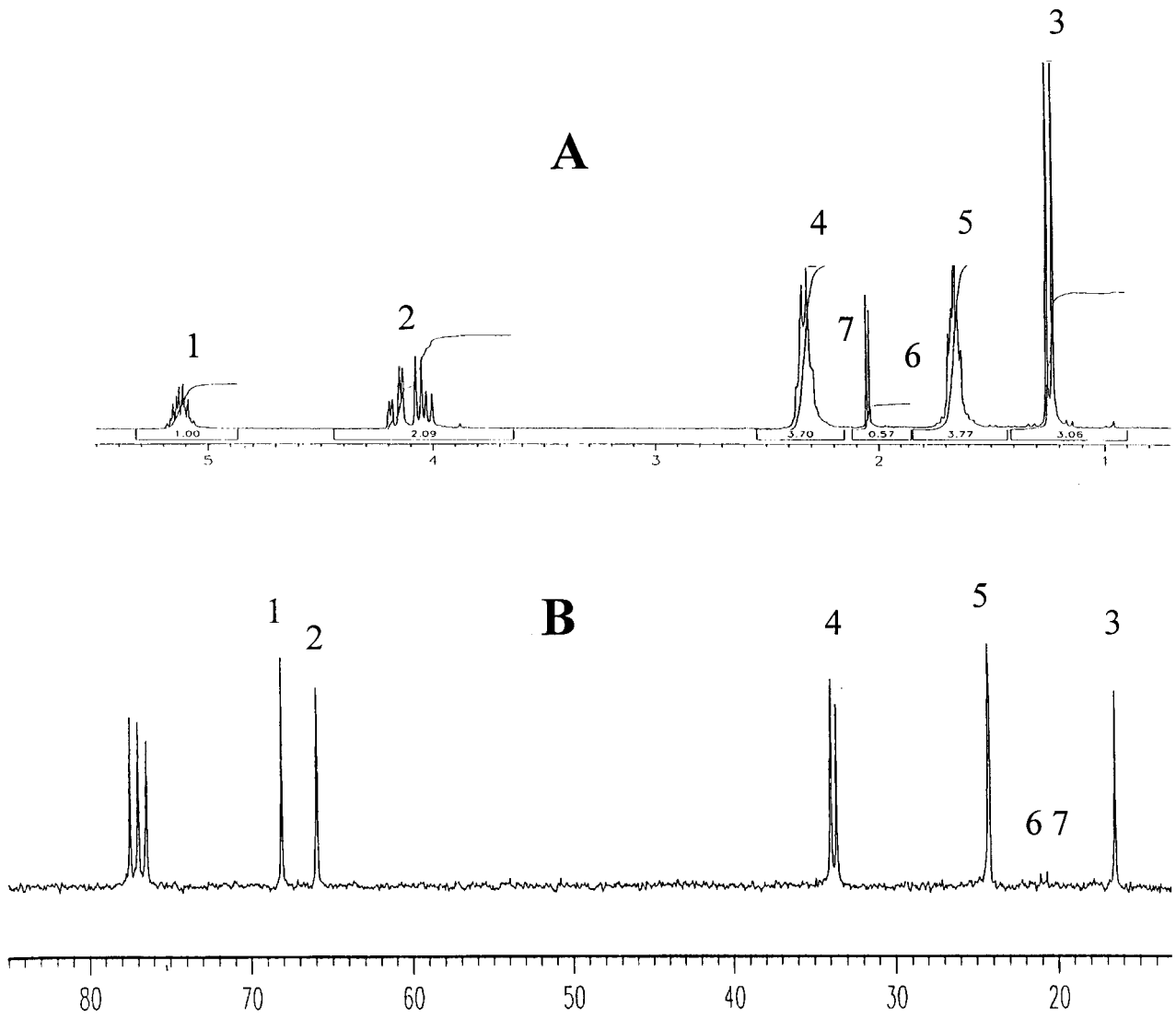


Figure 1 The (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of PPA-Ac2.

side. The corresponding peak area was lower than 15% of the total.

If hydrolysis of the polymeric plasticizers had occurred, ester groups would be cleaved into alcoholic and carboxylic functional groups. Such functional groups were expected to induce different chemical shifts of the nearest atoms in the NMR. Therefore, we carefully analyzed the ^{1}H- and ^{13}C-NMR spectra of PPA-Ac2 (Fig. 1) and PPA (Fig. 2).

Figure 1 shows the ^{1}H- and ^{13}C-NMR spectra of PPA-Ac2 (acetylated). These well-resolved ^{1}H- and ^{13}C-NMR spectra enabled an unambiguous identification of the signals to be carried out.

The ^{1}H-NMR spectrum of PPA-Ac2 displayed very large peaks that could be easily allocated to protons in the chain (CH_2-5, CH_2-4, CH_3-3, CH_2-2, and CH-1; see Table I). It was interesting to observe two different acetyl groups (CH_3-6 and CH_3-7), corresponding to the two possible chain ends (—CH_2OAc or —CHOAc). Integration indicated that CH_3-7 was slightly more abundant (CH_3-7/CH_3-6 = 55/45).

The allocation of the carbon atoms on the ^{13}C-NMR spectra was straightforward (Table I). The acetyl chain ends were easily distinguished.

Because it was possible to recognize chain-end protons, the average chain length could be deduced from the ratios of integration of chain-end protons (acetyl) over in-chain protons. For example, when the intensity of CH_2-4 was taken for in-chain protons,

$$CH_2\text{-}4/(CH_3\text{-}6 + CH_3\text{-}7) = 7.0$$

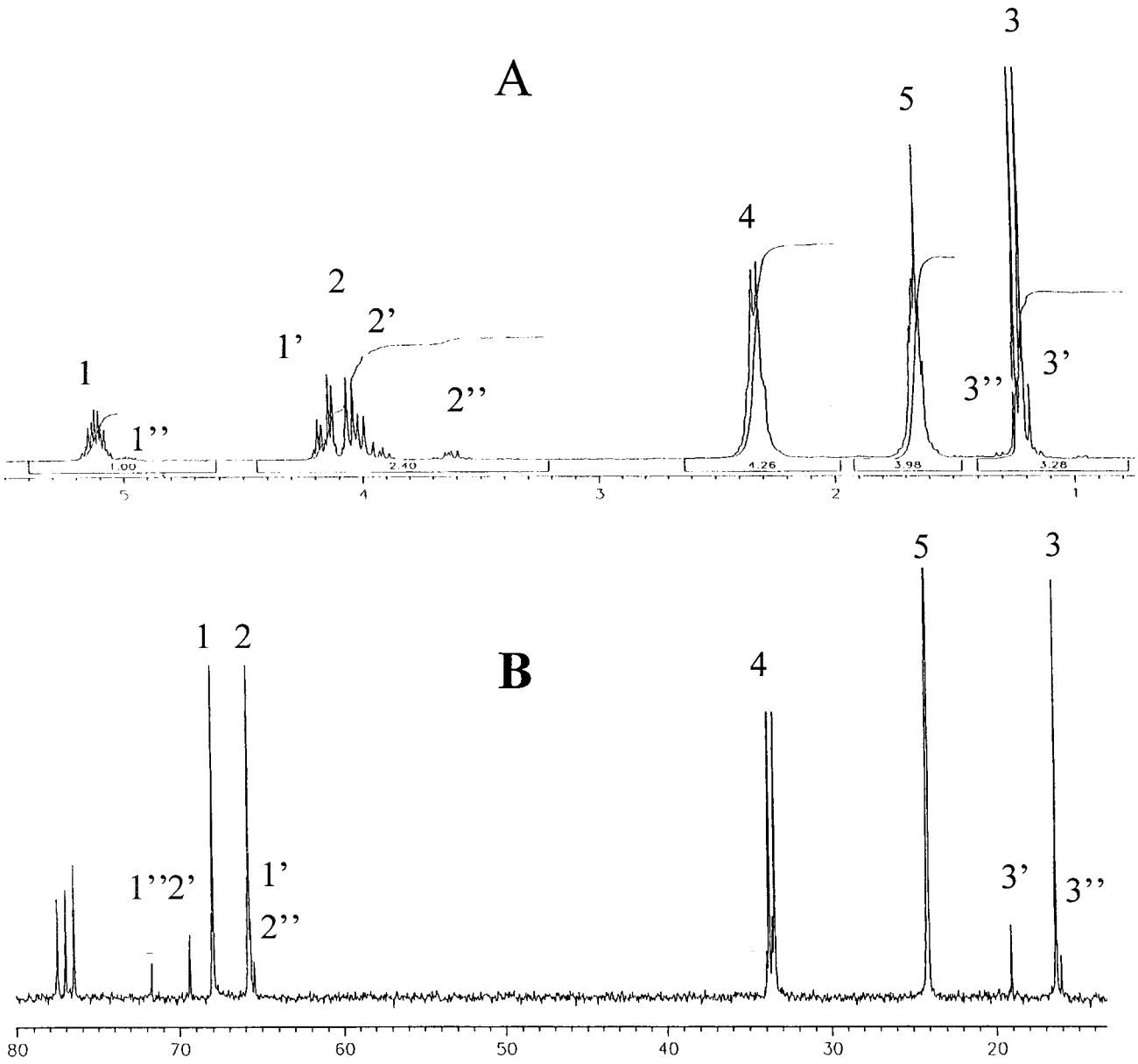


Figure 2 The (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of PPA.

This ratio was related to the number of repeating adipate units in the chain (Scheme 1).

$$CH_2\text{-}4/(CH_3\text{-}6 + CH_3\text{-}7) = 4n/6$$

which gave $n = 10.50$.

When the intensity of CH-1 was taken for in-chain protons, the following ratio was found:

$$CH\text{-}1/(CH_3\text{-}6 + CH_3\text{-}7) = (n + 1)/6 = 1.92$$

This was related to the number $(n + 1)$ of 1,2-propanediol units within the chain and gave $n = 10.57$. This led to the raw formula $A_{10.5\pm0.1}P_{11.5\pm0.1}(Ac)_2$ for PPA-Ac2.

Using any other in-chain proton gave the same result. This good consistency between the integration ratios of all peaks suggested that the substance was very pure. This approach was very simple and could be applied to the study of other similar polyesters.

NMR provided other information on the structure of the plasticizers. Figure 2 shows the ^{1}H- and ^{13}C-NMR spectra of PPA (not acetylated). Obviously, the ^{1}H-NMR spectrum of PPA revealed some similarities to that of PPA-Ac2, as far

Table I ^{1}H- and ^{13}C-NMR Chemical Shifts (ppm) and Coupling Constants (Hz) of PPA-Ac2

NMR Signal	Carbon Number						
	1	2	3	4[a]	5[a]	6	7
^{1}H	5.12 d quin, 3.8, 6.5 Hz	4.16, 4.08 ABX; 11.8, 6.5, 3.8 Hz	1.23 d, 6.5 Hz	2.36–2.04 m	1.68–1.62 m	2.04 s	2.06 s
^{13}C	67.95	65.79	16.32	33.92, 33.61	24.15, 24.09	21.11	20.71

[a] In ^{13}C-NMR, two signals correspond to this atom. It is likely that they correspond to the two possible neighbor ester groups (primary and secondary).

as the in-chain protons were concerned. The acetyl group of PPA-Ac2 was not present in PPA and was replaced by hydroxyl groups. It was possible to distinguish the protons of the two possible end chains 2′ (secondary) or 1″ (tertiary) linked to adipic acid and 2″ (secondary) or 1′ (tertiary) bearing the free hydroxyl group (Table II).

The fact that CH-1″ and CH-2″ appeared without an overlap in the ^{1}H-NMR suggested that the average chain length of PPA could be determined from the integration ratios. For example, using either of the two ratios

$$CH_2\text{-}4/CH\text{-}1'' = 4n = 42.36$$

$$CH\text{-}1/CH\text{-}1'' = n = 10.62$$

led to the same raw formula of PPA: $A_{10.5\pm0.1}P_{11.5\pm0.1}(OH)_2$.

Because this approach could be used to monitor the presence of possible hydrolysis products of PPA-Ac2, it was therefore essential to confirm the ^{13}C-NMR assignments. This was achieved by a complementary study of monoacetylated 1,2-propanediol. We synthesized propanediol acetates **I** and **II** (Table III). These were obtained as a 70:30 mixture by the reaction of 1,2-propanediol with acetic anhydride.

As can be seen from Tables I and III, the chemical shifts of the acetyl groups of **I** and **II** were close to those of PPA-Ac2. A COSY correlation confirmed the spectroscopic allocations of the peaks as indicated in Tables I and II.

Hydrolysis in Simulated Intestinal Fluid

Influence of Initial Concentration of Substrates

The EC guidelines for simulated digestive hydrolysis suggests a test compound concentration that is approximately equivalent to that resulting from its ingestion under normal circumstances. Taking into account the migration data of polymeric plasticizers into different foodstuffs, Castle et al.[2] found that the *in vivo* concentration of plasticizer would be 1–3 mg/L gastric juice and considered that the range of 5–8 mg/L was appropriate for *in vitro* hydrolysis experiments.

Castle et al.[2] also refer to analytical difficulties due to substances coextracted from the enzymatic reagent, which strongly interfered with HP-SEC analysis of the polymeric plasticizers. Consequently, these authors could not carry out this experiment.

In order to facilitate the analytical work, we carried out the hydrolysis of PPA-Ac2 at 10, 25, 250, and 2500 mg/L concentrations. To the extent possible, we ascertained that the results were qualitatively the same at all of these concentrations.

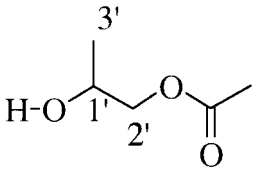
However, it must be noted that if hydrolysis was observed at high concentrations, it would also occur at the lower concentrations.

Table II ^{1}H- and ^{13}C-NMR Chemical Shifts (ppm) and Coupling Constants (Hz) of PPA

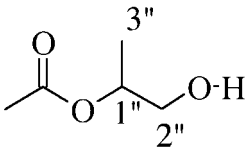
NMR Signal	Carbon Number					
	1′	2′	3′	1″	2″	3″
^{1}H	4.09–3.88 m	4.09–3.88 m	1.19 d, 6.1 Hz	4.98 d quin; 3.4, 6.5 Hz	3.66, 3.58 ABX; 12.0, 6.5, 3.4 Hz	1.21 d, 6.8 Hz
^{13}C	65.69	69.42	19.05	71.67	65.47	16.01

Table III ^{1}H- and ^{13}C-NMR Chemical Shifts (ppm) and Coupling Constants (Hz) of 1,2-Propanediol Monoacetates I and II

		CH	CH_2	CH_3	CH_3 (Ac)
I	^{1}H	4.05 d quin; 3, 6.5 Hz	4.11, 3.93 ABX; 10, 3, 6.5 Hz	1.21 d, 6.5 Hz	2.10 s
	^{13}C	65.76	69.42	19.23	20.64
II	^{1}H	4.98 d quin; 3.8, 6.5 Hz	3.61, 3.55 ABX; 9, 3, 6.5 Hz	1.24 d; 6.5 Hz	2.08 s
	^{13}C	71.80	65.38	16.01	21.05



I



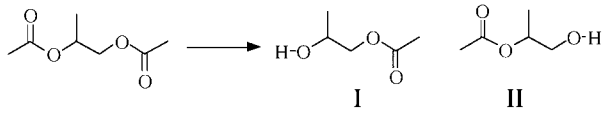
II

Pancreatin, the catalyst added to the intestinal simulant, contains enzymes and possibly some other impurities that are likely to interfere with any analytical method. A blank was therefore extracted with ether and analyzed prior to any further experiment. It showed the presence of substances in the region of the HP-SEC chromatogram where oligomeric hydrolysis products were expected. Similarly, in ^{1}H-NMR the blank exhibited many signals [5.39 (t), 2.35 (t), 2.03 (m), 1.65 (m), 1.27 (m), 0.90 (t), and 0.92 ppm (t)] that could hide those of hydrolysis products.

Influence of Stability of Intestinal Fluid Simulant

The efficiency of the enzymatic reagent was first optimized with 1,2-propanediol diacetate, whose reaction products could be easily analyzed by GC (Scheme 2). The digestive fluid was stored at 4°C as reported in the literature.[3] The hydrolysis of 1,2-propanediol diacetate was investigated at 37°C. The results summarized in Table IV show that the enzyme rapidly lost its activity during storage at 4°C.

It is interesting to note that the **I/II** ratio depended on the reaction conditions (Table IV). The primary ester groups were cleaved faster than the secondary groups. This could be related to the greater sterical hindrance of the secondary ester group.



Scheme 2 The intestinal hydrolysis of 1,2-propanediol diacetate.

To avoid artifacts during hydrolysis, all reactions described below were carried out with freshly prepared intestinal simulant solution.

Plasticizer Hydrolysis

The pH of the simulant was 7.5, so the acidic hydrolysis products were not extracted by dichloromethane. This allowed a fractionation of the hydrolysis products (Scheme 3). The direct extraction with dichloromethane provided a fraction of nonacidic hydrolysis products, which were called the neutral fraction; subsequent acidification (pH 2) followed by extraction with ether gave products with at least one carboxyl group. The extraction yields of typical compounds were not determined. However, the extraction steps were repeated several times to have a satisfactory mass balance. This fraction was called the acidic fraction.

Investigation for Adipic Acid by GC Analysis

When the plasticizer (10 and 25 mg/L) was incubated with the intestinal fluid for 4 h at 37°C, GC analysis of the acidic fraction showed that adipic acid ($t_r \approx 8.7$ min.) formation was very low (<2%) or there was none. The absence of adipic acid was not attributable to an inefficient extraction because pimelic acid, the internal standard ($t_r \approx 9.6$ min) was present in large amounts. The concentration of pimelic acid was such that if the hydrolysis was quantitative, the areas of the peaks of both acids would be close.

Table IV Influence of Aging (4 h at 37°C) of Enzymatic Reagent on Hydrolysis of 1,2-Propanediol Diacetate

	Freshly Prepared Enzyme Reagent	Reagent Prepared 3 Days before Use	Reagent Prepared 3 Weeks before Use
Hydrolysis (%)	39	31	3
Ratio **I/II**	28/72	35/65	55/45

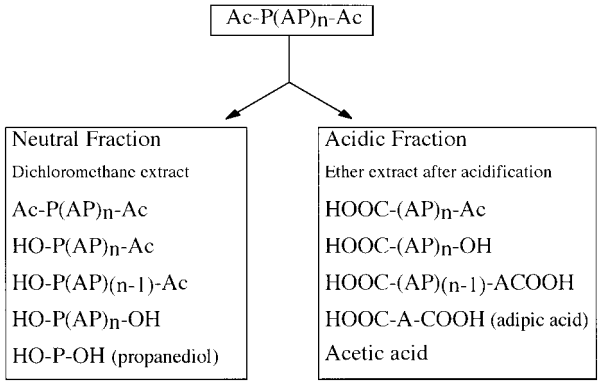
Analysis of Hydrolysis Products by HP-SEC and FTIR

The HP-SEC analysis of the plasticizers before and after treatment under intestinal hydrolysis conditions (250 mg/L concentration) showed that the bulk plasticizer had completely disappeared (Fig. 3). Unfortunately, the chromatogram region corresponding to low molecular weight compounds could not be analyzed because of strong interferences with the blank.

The FTIR analysis of the acidic fraction showed a broad OH carboxyl band (3000–2800 cm^{-1}). Weak OH hydroxyl bands were detected in the neutral fraction.

Structure of Hydrolysis Products by NMR Analysis

The two extracts (neutral and acidic) were analyzed separately by ^{1}H-NMR. At low plasticizer concentrations [10 and 25 mg/L; Fig. 4(A)] there were weak signals that emerged from the noise at around 4 ppm, a region where there was no absorption for the blank. This region was the one where C$\underline{H_2}$OH (2″) signals of hydrolysis products were expected. However, the signals of the blank in other regions of the spectrum were too intense, and no firm conclusion could be drawn.



Scheme 3 The separation of the possible hydrolysis products of PPA-Ac2: P, propylene glycol unit; A, adipoyl unit.

At higher concentrations [250 mg/L; Fig. 4(B)] there were important CHOH (1″) and C$\underline{H_2}$OH (2″) signals that could be seen in the ^{1}H-NMR spectra of both fractions, confirming a large extent of hydrolysis that could be evaluated from the ratios

$$\text{CH-1/CH-1}'' = 7/6.5 = 1.08$$

in the neutral fraction and

$$\text{CH-1/CH-1}'' = 32/8 = 4.00$$

in the acidic fraction.

It was interesting to note that the intensities of the remaining acetyl groups were very different in both fractions: the signal of CH_3-6 (acetyl group linked to primary ester) was much less intense than the signal of CH_3-7 (acetyl group linked to secondary ester), indicating that hydrolysis at the chain ends had cleaved the primary ester group with a high extent of selectivity. This corresponded to the results obtained with 1,2-propanediol diacetate (Fig. 3).

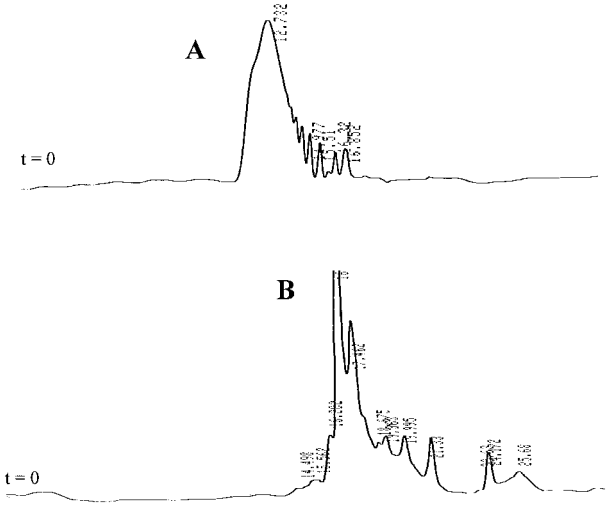


Figure 3 The HP-SEC analysis of (A) PPA-Ac2 and (B) its hydrolysis products in intestinal fluid simulant.

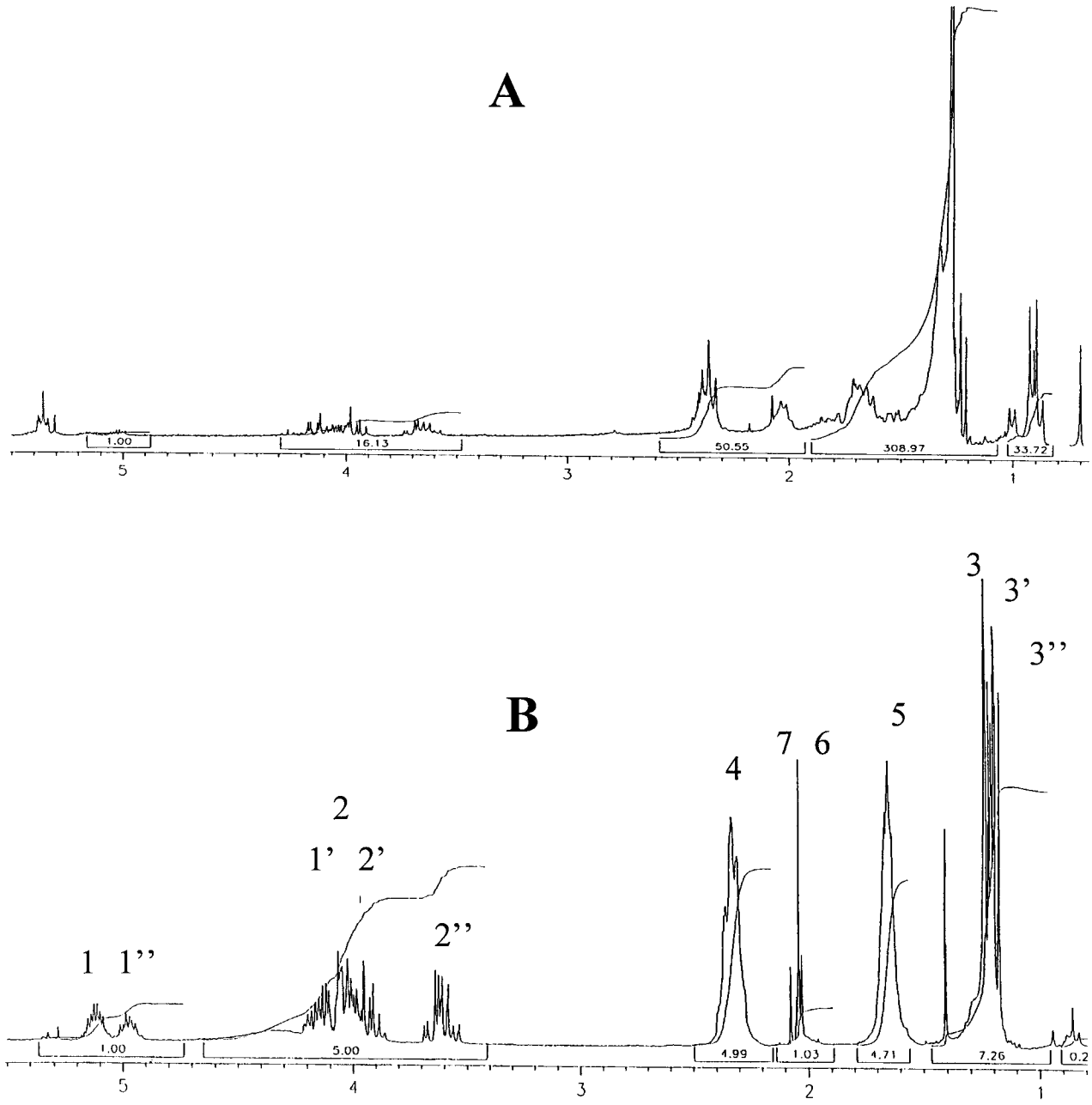


Figure 4 ^{1}H-NMR spectra of the dichloromethane extract of PPA-Ac2 hydrolyzate with (A) 25 and (B) 250 mg/L intestinal simulant.

The GC, HP-SEC, and ^{1}H-NMR results showed that under intestinal conditions the hydrolysis of polymeric plasticizer took place and led to low molecular weight oligomers. The GC showed that hydrolysis was not accompanied by a significant formation of adipic acid.

Separation and Identification of Hydrolysis Products

Isolation of these products was attempted in order to identify the hydrolysis products. To have enough material available, hydrolysis was carried out on a 2500 mg/L scale. The products were separated as above (Scheme 3) into neutral and acidic fractions. The ^{1}H-NMR spectra of these crude fractions exhibited the same signals as the experiment with 250 mg/L with the following intensity ratios of CH-1/CH-1″:

$$\text{CH-1/CH-1}'' = 1.14$$

in the neutral fraction and

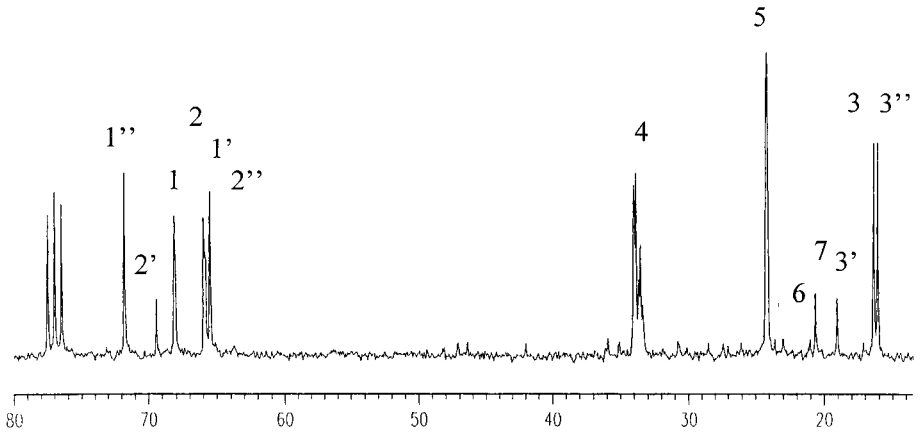


Figure 5 The ^{13}C-NMR spectrum of the hydrolysis product of PPA-Ac2 (2500 mg/L intestinal simulant).

$$\text{CH-1/CH-1}'' = 3.63$$

in the acidic fraction.

These values were close to those obtained with a 250 mg/L concentration. This suggested that the use of a high concentration of plasticizer led to similar hydrolysis products.

The ^{13}C-NMR analysis gave the same result, because the spectra of both fractions exhibited high CH-1″, CH-1′, CH_2-2″, and CH_2-2′ signals (Fig. 5), confirming a large extent of hydrolysis. It was also possible to recognize CH_3-3″ and CH_3-3′ signals. The intensity of CH_3-3″ was greater than that of CH_3-3′, indicating that hydrolysis at the chain ends had selectively cleaved the acetyl group CH_3-6.

Each fraction was then further separated by flash chromatography on silica gel, based on the order of polarity. The neutral fraction was thus split into three fractions (N1, N2, and N3 in increasing order of elution). Similarly, three fractions of acidic compounds (A1, A2, and A3) were obtained.

The separation was not complete and these fractions were still mixtures as determined by their HP-SEC chromatograms. Each fraction was analyzed by ^{1}H- and ^{13}C-NMR to establish the composition and average empirical formula based on the peak area ratio of end-chain protons (CH-1″ or CH_3-6 + CH_3-7) versus in-chain protons (CH-1). Table V gives these ratios for the N1, N2, N3, A1, A2, and A3 fractions. This made it possible to evaluate the average number of diol units within the oligomer chain, which varied from 2 to 7 units.

If PPA-Ac2 was recovered, it could only be present in the less polar fraction N1. Its absence was confirmed by HP-SEC and thin layer chromatography: N1 was much more polar than the PPA-Ac2 starting material.

Table V Peak Height Ratio and Compositional Analysis (from ^{1}H-NMR Data) of Hydrolysis Products of PPA-Ac2 (2500 mg/L) in Intestinal Fluid

	1	1″	2″	6	7	1/1″ [a] or 1/(6 + 7)[a]
Neutral						
N1	10	3.5	7	13.5	2.5	2.85
N2	10	ND	ND	3.9	3.9	7.69
N3	10	2.5	5	1	2	4.00
Acidic						
A1	10	0.7	1.4	10.7	21.4	1.86
A2	10	5	10	ND	ND	2.00
A3	10	5	10	1.6	ND	2.00

ND, not detected.
[a] This ratio was related to the number of in-chain diol units.

The N2 contained a large proportion of monoacylated compounds with the two type of chain ends (primary and secondary acetates).

In all other fractions the signal of acetyl CH_3-6 was much lower than that of CH_3-6, confirming once more that enzymatic hydrolysis at the chain ends had selectively cleaved the primary acetate group.

The recovery yield of the separations was around 50% for the acidic and neutral fractions.

The hydrolysis of the polymeric plasticizers mainly led to low molecular weight oligomers. The signal intensity of free alcohol units was always well above the detection limit in all experiments.

Hydrolysis under Gastric and Saliva Conditions

The plasticizer was allowed to react under exposure to simulated gastric juice. The analysis of the HP-SEC chromatograms showed that at low concentration the bulk plasticizer was recovered intact while the low molecular weight constituents were hidden by impurities from the blank. At high concentration there was no significant difference between the chromatographic profiles of PPA-Ac2 before and after hydrolysis. The same results were observed with the addition of pepsin and mucin as catalysts.[3] These results were confirmed by ^{1}H-NMR, because no free hydroxyl signal of the end-chain groups could be detected, even after prolonged exposure (3 days). The GC analysis showed that there was no detectable formation of free adipic acid.

Hydrolysis under saliva conditions gave identical results; the polymeric plasticizer was recovered unchanged.

It could therefore be deduced that under gastric and saliva conditions the hydrolysis of the polymeric plasticizer PPA-Ac2 was insignificant.

CONCLUSION

The hydrolysis of polymeric plasticizers under *in vitro* digestive fluids was carried out. There was no detectable hydrolysis under saliva and gastric conditions. However, PPA-Ac2 was hydrolyzed to a large extent when exposed to simulated intestinal fluid. Enzymes selectively catalyzed the hydrolysis of the primary alcohol ester linkage and led to low molecular weight oligomers. Hydrolysis to free adipic acid and to 1,2-propanediol did not occur during the 4-h digestion phase. The formation of adipic acid, which was considered as a probe for hydrolysis,[2] would result from a nonselective cleavage of both primary and secondary ester bonds. Obviously the secondary ester groups exhibited a greater resistance to the enzymatic reagents.

Other polymeric plasticizers (esters of adipic acid and ethanediol or butanediol) are likely to undergo the same kind of hydrolysis and are expected to result in similar mixtures of hydrolysis products. These hydrolysis products have not been evaluated individually. However, the toxicological evaluation of PPA-Ac2 could be representative of its hydrolysis products.

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