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Polymer 48 (2007) 1517–1525
polymer
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Lipase-catalyzed synthesis and characterization of copolymers from ethyl acrylate as the only monomer starting material

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Received 19 September 2006; received in revised form 17 January 2007; accepted 19 January 2007
Available online 25 January 2007

Abstract

Several acrylic copolymers containing, at random, sequences of poly(ethyl acrylate) and poly(*N*-(2-hydroxyethyl)acrylamide) were obtained from ethyl acrylate as the only monomer starting material in a chain polymerization process, catalyzed by *Candida antarctica* lipase B. In the presence of ethanolamine, the enzyme not only catalyzes the chain polymerization of ethyl acrylate but also aminolysis the pendant ester groups. The products, characterized by FTIR, ^{1}H and ^{13}C NMR and UV-MALDI-TOF-MS, show low molecular weight and high monodispersity. The activity showed by *C. antarctica* lipase B in the polymerization reaction is a new example of enzyme promiscuity.

Keywords: Lipase-catalyzed; Chain polymerization; Ethyl acrylate

1. Introduction

Binary and ternary polymers of hydroxyalkylacrylamides, ethyl acrylate and acrylic acid, known as hydrophobically modified polyacrylamides, have attracted increased interest over the past decades due to their ability in controlling viscosity at various shear rates. Due to this property, they are used for enhanced oil recovery, aqueous solid–liquid separation and water modification [1]. They are useful as catalysts for Knoevenagel condensation [2] and show several biomedical applications [3].

This family of polymers is generally obtained by free-radical polymerization, in particular micellar radical polymerization [4]. The polydispersity ($\overline{M}_w/\overline{M}_n$) of the products synthesized by this method, determined by Gel Permeation Chromatography (GPC), is between 3.1 and 5.8 depending on time of reaction. The limiting factor in this methodology has been the ability to control free-radical polymerization and therefore the molecular weight distribution of the resulting polymer [5].

In the last years, reversible addition–fragmentation chain transfer (RAFT) polymerization was employed to solve this problem of increased polydispersity [6]. Following another approach, modified polyacrylamides have also been prepared by chemical modification of polyacrylamide in dioxane at 100 °C [7].

Considering that the application of the above mentioned methodologies could not decrease the polydispersity of these polymeric materials and the experience of our laboratory on application of enzymes in the synthesis of organic compounds, we decided to explore the possibilities of enzymes as catalysts in the synthesis of hydrophobically modified polyacrylamides.

Biotransformations represent an effective and sometimes preferable alternative to conventional chemical synthesis for the production of fine chemicals and optically active compounds [8]. Recently the use of enzymes in polymer science has been discussed within comprehensive reviews [9]. These enzyme-catalyzed polymerizations are examples of a new

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doi:10.1016/j.polymer.2007.01.040

methodology of polymer syntheses and, in many cases, enzymes enable the synthesis of polymers, which otherwise are difficult to prepare [10].

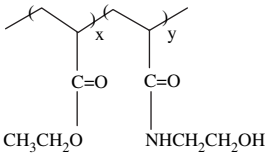
Isolated lipases are currently under study as catalysts for polymer synthesis. They are used in polyester synthesis as a good alternative to transition metals that are toxic and require careful and drastic reaction conditions such as high temperature and inert gas atmosphere. Lipase-catalyzed condensation polymerizations are metal free and can be performed at lower temperatures [11]. Biodegradable polyesters have been synthesized by incorporating lipase catalyst from various monomer combinations [12]. In our laboratory we have performed the polymerization of glycerol with ethyl adipate in dry dioxane using the lipase from *Candida antarctica* B as catalyst. Working at 30 °C, a low molecular weight poly(1,3-glyceryladipate) was formed in high regioselectivity and low polydispersity $\overline{M}_w/\overline{M}_n = 1.1$ [13].

Other enzymes, such as horseradish peroxidase, have been used to catalyze addition polymerizations of commodity vinyl monomers [14–16]. But at this moment, there are no reports of lipase-mediated vinyl monomer polymerization.

In a previous work we have synthesized hydroxyalkylacrylamides by reaction of ethyl acrylate and alkanolamines through an enzymatic approach using diisopropylether as solvent. The presence of a polymeric material as by product of the acrylamides prompted us to study the polymerization reaction [17]. So, in this paper we describe the polymerization of ethyl acrylate with variable amounts of ethanolamine, catalyzed by immobilized *C. antarctica* lipase B. The potential of using lipases from different sources was investigated. The effect of solvent, enzyme:substrate ratio and reaction temperature over ranges from 0.2 to 2 and from 15 to 55 °C were studied. The polymers formed were analyzed by FTIR, ^{1}H and ^{13}C NMR spectroscopies and matrix-assisted ultraviolet laser-desorption/ionization time-of-flight mass spectrometry (UV-MALDI-TOF-MS) to determine their composition, average molecular weight and polydispersity.

By variation of the ratio of ethanolamine:ethyl acrylate, we have obtained four low molecular weight polymers with different proportion of ester and substituted amide as pendant groups in the polyacrylate chain. According to ethanolamine:ethyl acrylate ratio the products were named **1–4** as follows: **1** (0.5), **2** (1), **3** (2.5) and **4** (5), where ethanolamine:ethyl acrylate ratio is indicated in brackets.

Scheme 1 shows the structure of the binary polymers prepared through the enzymatic approach.



Ethanolamine/ Ethyl acrylate	0.5	1	2.5	5
Product	**1**	**2**	**3**	**4**

Scheme 1. General formulae of the products **1–4** prepared through lipase-catalyzed polymerization of ethyl acrylate. The sequence of *x* and *y* is at random.

2. Experimental

2.1. Materials

Ethyl acrylate, ethanolamine, oxalic acid, methanol, tetrahydrofuran, dioxane and acetonitrile (Sigma–Aldrich), dimethylsulfoxide and trifluoroacetic acid (Merck) were used as purchased without further purification. Water of very low conductivity (Milli Q grade; 56–59 nS/cm with PURIC-S, ORUGANO Co., Ltd., Tokyo, Japan) was used. Lipase from *Candida rugosa* (CRL) (905 U/mg solid) and type II crude from porcine pancreas (PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *C. antarctica* lipase B (CAL B), Novozym® 435 (7400 PLU/g) and Lipozyme RM 1M (LIP) (7800 U/g) were generous gifts of Novozymes Latinoamerica Ltda and Novozymes A/S. All enzymes were used 'straight from the bottle'. Calibrating chemicals for UV-MALDI-TOF-MS analysis: Proteins: aprotinin (A1153, m.w. 6512), bovine insulin (I5500, m.w. 5733.5), angiotensin 1 (m.w. 1296.49) and angiotensin 2 (m.w. 1046.21) were obtained from Sigma. Carbohydrates (neutral cyclic oligosaccharides): α-cyclodextrin (cyclohexaamylose, m.w. 972.9), β-cyclodextrin (cycloheptaamylose, m.w. 1135) and γ-cyclodextrin (cyclooctaamylose, m.w. 1297) were purchased from Sigma–Aldrich. Matrices for UV-MALDI-TOF-MS: β-carboline (9*H*-pyrido[3,4-*b*]indole) nor-harmane (nor-Ho), 2,5-dihydroxybenzoic acid (DHB), gentisic acid (GA), *trans*-3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid (SA)) and 2-(4-hydroxyphenylazo)-benzoic acid (HABA) were obtained from Aldrich Chemical Co.

Precautions while handling ethyl acrylate: avoid contact with eyes, skin, and clothing. Use only with adequate ventilation. Keep away from heat, sparks and flame. Avoid breathing vapor or mist [18].

2.1.1. Synthesis of poly(ethyl acrylate)

Poly(ethyl acrylate) was prepared at 45 °C from ethyl acrylate using 2,2′-azobisisobutyronitrile (AIBN) (0.05%) as initiator. The polymerization mixture was diluted with dichloromethane and poured in water to wash the polymer. The organic layer was dried and the solvent evaporated. FTIR (film): 1720 cm^{-1}.

2.1.2. Synthesis of hydrophobically modified polyacrylamides

In a typical polymerization, to a 9 M solution of ethyl acrylate in acetonitrile, variable amounts of ethanolamine (ethanolamine/ethyl acrylate ratio: 0.5, 1, 2.5 and 5) and lipase (enzyme/substrate ratio: 0.5) were added. The suspension was stirred (200 rpm) at 25 °C and the progress of reaction was monitored by ^{1}H NMR. After 48 h, the enzyme was filtered off and the solution was washed four times with dioxane. The solvent of reaction was evaporated and the excess of ethanolamine was eliminated with oxalic acid and methanol.

Four different polymeric products were obtained depending on the ethanolamine/ethyl acrylate ratio (ratio: product): 0.5: **1**, 1: **2**, 2.5: **3** and 5: **4**).

2.2. Analysis of polymers

2.2.1. FTIR spectroscopy

FTIR measurements were performed on a Shimadzu FTIR-8300 spectrophotometer in film (poly(ethyl acrylate)) and in KBr pellets (products **1**–**4**).

2.2.2. NMR spectroscopy

^{1}H and ^{13}C NMR mono- and bidimensional spectra were recorded on a A Bruker AM-500 MHz NMR instrument operating at 500.14 and 125.76 MHz for ^{1}H and ^{13}C, D_2O was used as solvent in products **1**–**4** and $CDCl_3$ for poly(ethyl acrylate). ^{1}H–^{13}C heteronuclear chemical shift correlation spectrum (HSQC) was recorded in D_2O, using the standard pulse sequence. A total of 32 scans were accumulated with a relaxation delay of 2 s for each of the 512 t_1 experiments. The double quantum filter homonuclear shift correlated spectroscopy (DQF-COSY) experiment, with 32 scans being collected for each t_1 value, was carried out in D_2O. A total of 256 spectra, each containing the 1 K data points, were accumulated.

2.2.3. UV-MALDI-TOF mass spectrometry

2.2.3.1. Calibration of UV-MALDI-TOF-MS spectra. Spectra were calibrated using as external calibration reagents the proteins: aprotinin, bovine insulin, angiotensin 1 and angiotensin 2 (10 μM in MeCN:H_2O–TFA 0.1% (2:3, v:v) solution with SA as matrix (saturated solution in MeCN:H_2O–TFA 0.1% (2:3, v:v)) in positive-ion mode (sandwich sample preparation method, see details below) and the carbohydrates: α-, β- and γ-cyclodextrin (10 μM in MeOH:H_2O (1:1, v:v)) with nor-Ho (saturated solution in MeOH:H_2O (1:1, v:v)) as matrix in positive- and in negative-ion modes (mixture sample preparation method, see details below). The Kratos Kompact calibration program was used.

2.2.3.2. UV-MALDI-TOF-MS experiments. Measurements were performed using a (i) Shimadzu Kratos, Kompact MALDI III laser-desorption/ionization time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a pulsed nitrogen laser ($\lambda_{em} = 337$ nm; pulse width = 3 ns) and (ii) a Shimadzu Kratos, Kompact MALDI 4 (pulsed extraction) laser-desorption/ionization time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a pulsed nitrogen laser ($\lambda_{em} = 337$ nm; pulse width = 3 ns), tunable PDE, PSD (MS/MS device), and both with a secondary electron multiplier (SEM) detector. Experiments were performed using firstly the full range setting for laser firing position in order to select the optimal position for data collection, and secondly fixing the laser firing position in the sample sweet spots. The samples were irradiated just above the threshold laser power for obtaining molecular ions and with higher laser power for studying matrix cluster formation. Thus, the irradiation used for producing a mass spectrum was analyte-dependent with an acceleration voltage of 20 kV. Usually 50–100 spectra were accumulated. All samples were measured in the linear and the reflectron modes, in both positive- and negative-ion mode. The spectra obtained in the linear, positive-ion mode were the best, and only the peaks clearly distinguishable from the matrix baseline were taken into account. The stainless steel polished surface 20-sample slides were purchased from Shimadzu Co., Japan (P/N 670-19109-01). Polished surface slides were used in order to get better images for morphological analysis with a stereoscopic microscope (NIKON Optiphot, Tokyo, Japan; magnification ×400) and with a high-resolution digital microscope (Keyence VH-6300, Osaka, Japan; magnification ×800).

2.2.3.3. Sample preparation. Matrix stock solutions were prepared by dissolving 1 mg of the selected compound, GA and nor-harmane, in 0.5 ml of MeOH:H_2O (1:1, v:v) and HABA (1 mg) in 0.25 ml of THF. Analyte solutions were freshly prepared by dissolving the sample **1**–**4** (1 mg) in water (1 ml). To prepare the analyte-matrix deposits two methods were used [19]. *Method A (sandwich method)*: typically 0.5 μl of the matrix solution (M) was placed on the sample probe tip and the solvent was removed by blowing air at room temperature. Subsequently, 0.5 μl of the analyte solution (A) was placed on the same probe tip covering the matrix and partially dissolving it, and the solvent was removed and the tip was dried with a stream of forced room temperature air. Then, two additional portions (0.5 × 2 μl) of the matrix solution (M) were deposited on the same sample probe tip, producing a partial dissolution of the previously deposited thin-film matrix and analyte layers. The matrix to analyte ratio was 3:1 (v/v), and the matrix and analyte solutions loading sequence were: (i) matrix, (ii) analyte, (iii) matrix, (iv) matrix (M:A:M:M). *Method B (mixture method)*: the analyte stock solution was mixed with the matrix solution in 1:1–1:12 v/v ratio. A 0.5 μl aliquot of this analyte-matrix solution was deposited onto the stainless steel probe tip and dried with a stream of forced room temperature air. Then, an additional portion of 0.5 μl was applied to the dried solid layer on the probe, causing it to re-dissolve partially, and the solvent was removed by blowing air. Among the different UV-MALDI matrices tested (HABA, GA and nor-Ho) and the different experimental conditions used, best results were obtained with nor-Ho as matrix in positive-ion mode and sample preparation method B (analyte to matrix solution ratio 1:1 (v:v)). When GA was used as matrix, sample signals were obtained from the same sweet spot only by the first and second laser shots.

3. Results and discussion

3.1. Enzymatic reaction conditions

3.1.1. Enzyme selection

Several commercial lipases were evaluated in the polymerization reaction of ethyl acrylate with ethanolamine: *C. rugosa* lipase (CRL), *C. antarctica* lipase B (CAL B); lipozyme from the fungus *Rhizomucor miehei* (LIP) and porcine pancreatic

lipase (PPL). Reactions were carried out at 25 °C using an enzyme:substrate ratio of 0.5 and an ethanolamine:ethyl acrylate ratio of 1. The progress of the reaction in each case was determined from the ^{1}H NMR spectrum of the crude reaction mixture of each sample by taking the ratio of peak intensities due to polymer and monomer. For ethanolamine/ethyl acrylate-based polymers, the disappearance of signals of vinyl protons of ethyl acrylate at 6.30 (dd, 1H, C*H*$_2$=CH–), 6.15 (dd, 1H, CH$_2$=C*H*–) and 5.70 ppm (dd, 1H, C*H*$_2$=CH–) was used to determine the end of the reaction. This was also the criterion used to identify the lipase that is able to promote the polymerization.

After 48 h of reaction all enzymes showed the absence of vinyl signals in the ^{1}H NMR spectrum. The analysis of the product formed by catalysis of lipases from CRL, PPL and LIP or without biocatalyst showed that the Michael adduct **5** was obtained in variable yield (10–15%) (Scheme 2).

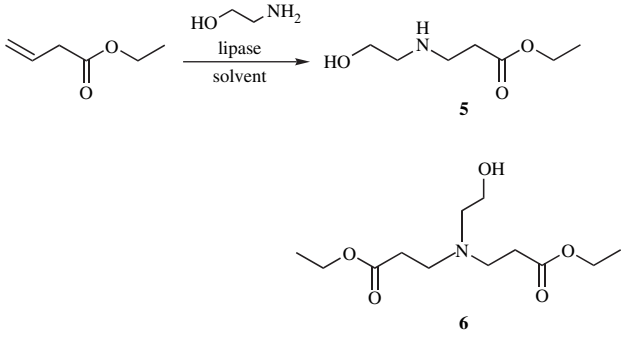
Differentially enough, in the case of the reaction catalyzed by CAL B using acetonitrile as solvent, spectroscopic data were consistent with a polymeric material, so CAL B was the only lipase giving satisfactory results.

3.1.2. Enzyme:substrate ratio

The optimum enzyme:substrate ratio was studied for CAL B in acetonitrile using an ethanolamine:ethyl acrylate ratio of 1:1. The enzyme:substrate ratio was studied between 0.2 and 2. As we have reported in a previous work, an enzyme: substrate ratio higher than 2 afforded hydroxyethylacrylamide as the main product [17]. The best results in terms of polymerization were achieved with E/S = 0.5. Control experiments with ethanolamine and ethyl acrylate (ethanolamine:ethyl acrylate ratio: 0.5–5) in acetonitrile in the absence of enzyme did not afford polymeric products.

3.1.3. Solvent effect

Attempts to carry out the reaction in a solvent free system were unsuccessful, recovering the starting materials. Using a non-polar solvent such as hexane afforded the di-Michael product **6** by a double Michael reaction between ethyl acrylate and ethanolamine to form initially **5** and a subsequent Michael addition of ethanolamine to **5** (Scheme 2) [20]. Ethers such as tetrahydrofuran, dioxane and diisopropylether gave the wanted polymeric material but in low yield. The best results in the polymerization corresponded to the process occurring in acetonitrile. This solvent completely dissolved the reactants and the products of reaction.



Scheme 2. Michael products of reaction of ethyl acrylate with ethanolamine.

3.1.4. Influence of the temperature

Temperature is a key factor in all biocatalytic processes since high temperatures usually allow high conversions, generally despite the loss in the selectivity. The polymerization was studied in the presence of CAL B over a range of temperatures between 15 and 55 °C. At low temperatures only the product of Michael addition **5** was obtained. Above room temperature, no increase in polymer production was observed, so 25 °C was chosen as the optimum reaction temperature.

3.2. Synthesis of the copolymers *1–4*

Considering the results of enzymatic screening, CAL B was the enzyme of choice for the polymerization of ethyl acrylate, using acetonitrile as solvent. Then we performed a series of CAL B-catalyzed polymerizations of ethyl acrylate by using variable amounts of ethanolamine. Depending on the amount of ethanolamine in the feed we obtained four different polymeric products **1–4**, according to the ethanolamine/ethyl acrylate ratios showed in Scheme 1.

The analysis of the compounds **1–4** showed that, besides ethyl carboxylate, the hydroxyethylamido moiety was present as pendant group in the products. These results indicated that, under these reaction conditions, CAL B carried out two catalytic actions: (i) polymerization of ethyl acrylate to form the polymeric chain and (ii) aminolysis of some ethyl ester groups using the amino group of ethanolamine as nucleophile (Scheme 3).

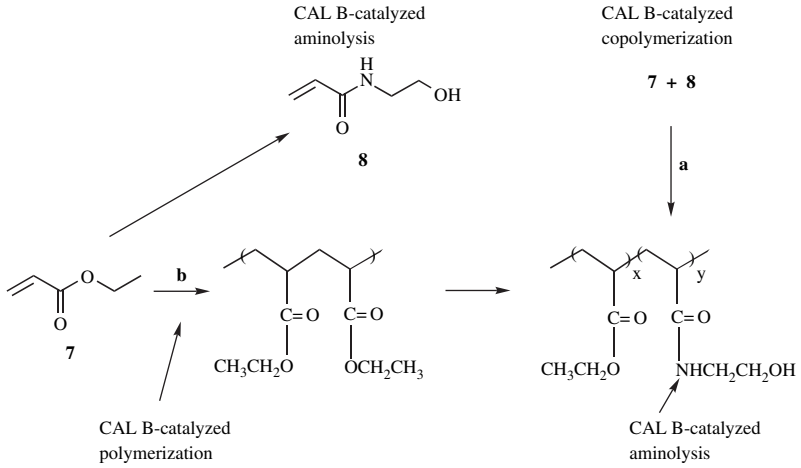
This double activity displayed by the lipase leads to the formation of copolymers containing two different pendant groups using ethyl acrylate as the only vinyl monomer starting material.

The results would suggest the possibility of two alternative ways to obtain the copolymers. At the moment, it is not possible to determine if the enzyme catalyzes the formation of poly(ethyl acrylate) chain before the aminolysis reaction (Scheme 3, b) or, on the contrary, initially the enzyme catalyzes the aminolysis of some molecules of the ethyl acrylate **7** to give hydroxyethylacrylamide **8** and later the copolymerization of the mixture of the two monomers: ethyl acrylate **7** and hydroxyethylacrylamide **8** (Scheme 1, a). Mechanistic studies of the reactions via computer simulations are in progress in our laboratory.

The behavior of the lipase constitutes a new example of enzymatic catalytic promiscuity defined by Bornscheuer and Kazlauskas as the ability of enzyme active sites to catalyze distinctly different chemical transformations [21]. The normal activity in a lipase is ester hydrolysis. In this paper we report that, in the synthesis of hydrophobically modified polyacrylamides **1–4**, the lipase is also catalyzing the addition reaction in the polymerization and the aminolysis of ester pendant group.

3.3. Analysis of products

Products **1–4** were analyzed by four independent analytical techniques: FTIR, ^{1}H, ^{13}C NMR and UV-MALDI-TOF-MS.



Scheme 3. Possible enzymatic ways in the formation of copolymers.

3.3.1. FTIR

FTIR analysis confirms the presence of ester and amide groups as pendant groups of the polymeric chain. The IR spectra of compounds **1**–**4** show strong bands at 3331 and 1524 cm^{-1} due to NH stretching and NH bending, respectively. In the carbonyl region, the typical amide bands I to C═O stretching and the amide II due to the CONHR group are observed at 1620 and 1524 cm^{-1}, respectively, and the corresponding to COOR at 1720 cm^{-1} in **1**–**4**.

Fig. 1 shows FTIR spectrum of compound **1**.

3.3.2. ^{1}H NMR

The ^{1}H NMR spectra of the products are quite complex. As an example, Fig. 2 shows the spectrum of **1** (ethanolamine: ethyl acrylate ratio: 0.5), recorded at 500 MHz using D_2O as solvent. The assignments of resonances to various types of protons are shown directly in the spectrum in Fig. 2.

The different regions in the complex ^{1}H NMR spectrum of **1** has been completely analyzed using 2D HSQC and COSY spectra. The protons of methylene adjacent to nitrogen of ethanolamine (f) and the belonging to methylene of the polymer chain (i) gave resonance signals between 2.41 and 2.98 ppm.

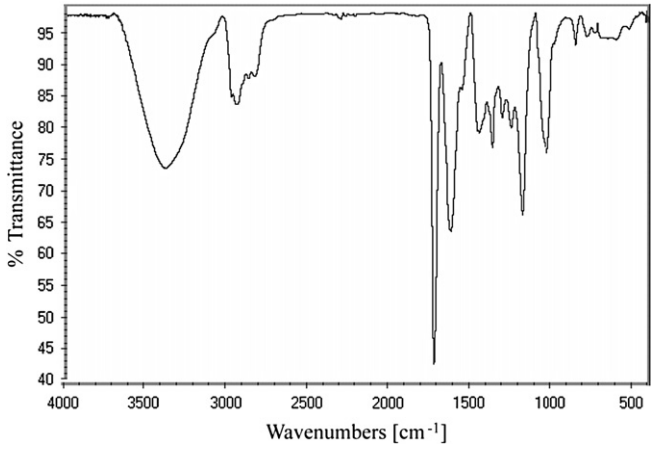


Fig. 1. FTIR of product **1**.

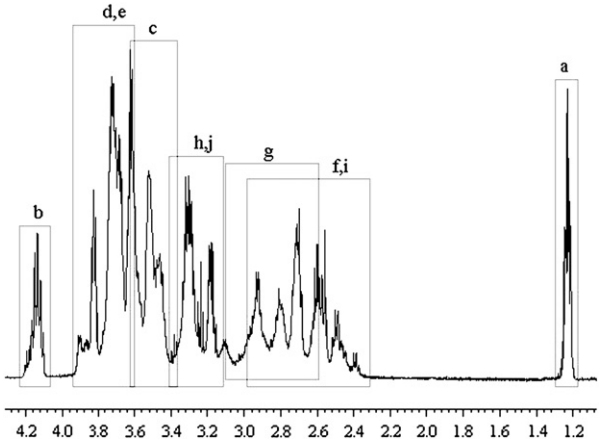


Fig. 2. ^{1}H NMR of product **1** at 500 MHz, solvent D_2O.

The protons of the methylene (g) of the first unit in the polymer chain gave resonance signals between 2.60 and 3.10 ppm. The protons of methyne (h and j) show signals in the range 3.16–3.47 ppm. A signal can also be observed at 1.25 and 4.19 ppm corresponding to methyl (a) and methylene (b), respectively, in the ethyl group of ethyl carboxylate of ester pendant group. The peaks in the range 3.35–3.65 ppm can be assigned to the methylene group (c) contiguous to the amino group from hydroxyethylamide pendant group and the peaks in the range 3.64–3.95 to the methylene group (d) contiguous to the hydroxyl from hydroxyethylamide pendant group and the methylene group attached to the amino group in ethanolamine chain-ending (e).

3.3.3. ^{13}C NMR

As an example, the proton decoupled ^{13}C NMR spectrum of **1** is shown in Fig. 3a.

The chemical shift assignments were made from the off-resonance decoupled spectra of the polymer and based on DQF-COSY and HSQC correlations. The ester carbonyl carbon resonance of the pendant carboxyethyl unit (k) appears at 173.8 ppm and the amide carbonyl carbons of the hydroxyethylamide unit (l) at 170.5 ppm (Formula 1). In the

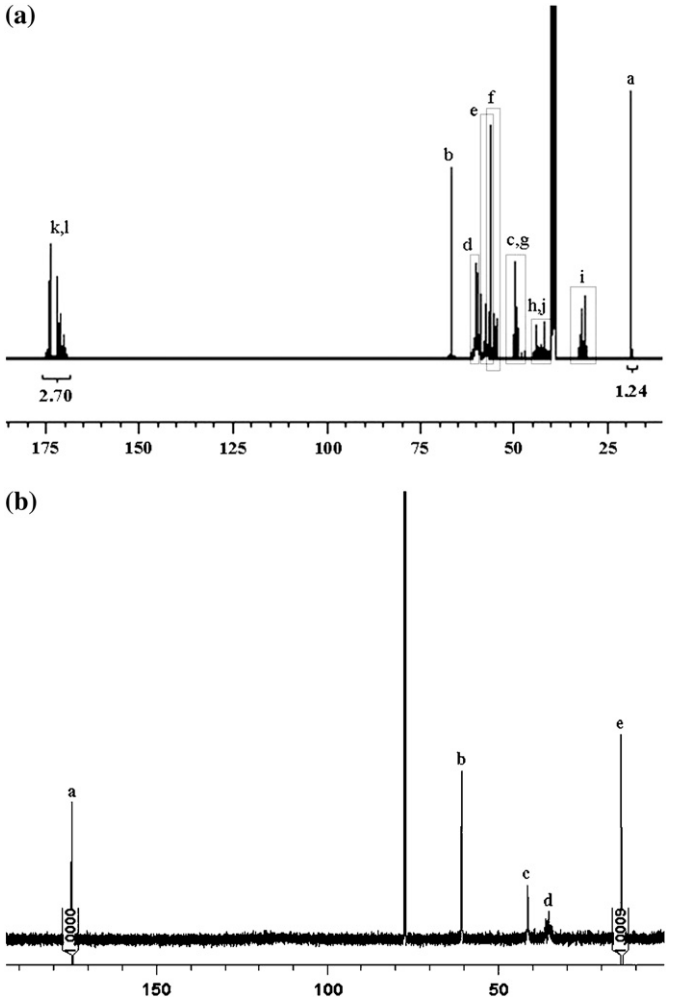
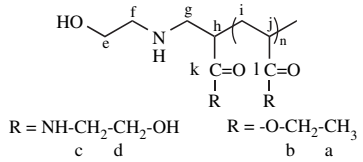


Fig. 3. ^{13}C NMR of (a) product **1** at 500 MHz (solvent D_2O) and (b) poly(ethyl acrylate) at 500 MHz (solvent $DCCl_3$).

carboxyethyl unit of pendant ester group the methyl group gave a resonance signal at 18.9 (a) and the methylene (b) at 67.7 ppm. The methylene group attached to −OH (d) gave resonance signals between 59.0 and 60.5 ppm and the methylene group attached to −NH in amide (c) between 49.2 and 50.5 ppm. Accordingly, the polymer backbone −CH− signal (h and j) appeared between 40.3 and 44.0 ppm and the $-CH_2-$ (i) between 29.4 and 34.8 ppm. The carbon of the methylene (g) of the first unit in the polymer chain gave resonance signals between 49.0 and 50.5 ppm. The carbon of methylene adjacent to nitrogen of ethanolamine (f) gave resonance signals between 53.9 and 58.4 ppm and that belonging to methylene group attached to the amino group in ethanolamine (e) between 56.2 and 60.4 ppm.



Formula 1.

^{13}C NMR was also useful for the determination of the ester/amide ratio of pendant groups in copolymer **1** considering the relative areas of signals of carbonyl groups and methyl carbon in the pendant carboxyethyl unit. The resonances of these signals are well separated from others and their relative resonance areas can be easily measured. The carbonyl carbon resonance is due to both pendant groups amide and ester, but the methyl group carbon resonance is due only to ethyl acrylate units, so the ratio of the relative areas of methyl (A_{Me}) to carbonyl ($A_{C=O}$) carbon resonance can be used to calculate the mole fraction of ethyl acrylate (F_{ester}) in the copolymer **1**. This is easily expressed in the Eq. (1).

$$F_{ester} = \frac{A_{Me}}{A_{C=O}} \tag{1}$$

According to the results by Kim and Hardwood [22] on the analysis of sequence distribution in methyl methacrylate–methyl acrylate copolymers, the copolymer compositions obtained by ^{13}C NMR spectroscopy are in good agreement with distributions calculated based on monomer feed compositions, conversion and reactivity ratios in copolymerization.

We applied this approach to our system by comparison between ratios of methyl to carbonyl areas in two different polymer systems: the copolymer product **1** and the corresponding homopolymer, poly(ethyl acrylate) (Formula 2).

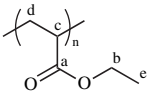
The analysis of the ^{13}C NMR spectrum of a sample of poly(ethyl acrylate) showed that the ratio of methyl to carbonyl (F_{ester}) in the poly(ethyl acrylate) was 1 (Fig. 3b).

This result shows the same amount of methyl and carbonyl groups in the pendant groups of the homopolymer.

The area of carbonyl groups (k, l) 2.70 and methyl group (a) 1.24 in the ^{13}C NMR spectrum of product **1** is indicated in Fig. 3a. By applying these data to the Eq. (1) and assuming that all carbonyl groups (ester and amide) are resonating with the same intensity, it could be determined that the product **1** contains 46% of ester as pendant group in the copolymer. This result was also corroborated by UV-MALDI-TOF mass spectrometry.

3.3.4. UV-MALDI-TOF-MS

UV-MALDI-TOF analysis was conducted in a comparative way by using HABA, GA and nor-harmane (nor-Ho) as matrices in positive- and negative-ion modes. Reproducible spectra were obtained only in the former. When GA was used as matrix, sample signals were only obtained after the first and second laser shots. After that, signals could not be obtained anymore by shooting the same sweet spot. Thermal decomposition of the pendant amide groups (B_y) (Scheme 4) assisted by GA as acid catalyst during the ablation process, changing the chemical structure of the sample surface, would be the reason



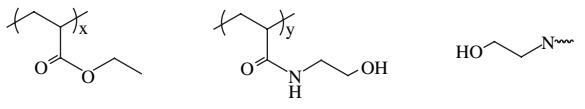
Formula 2.

of this behavior. Thus, only nor-Ho yielded reproducible spectra in positive-ion mode showing the molecules as $[M+H]^+$ and/or $[M+Na]^+$ species as it is detailed in Tables 1–4. As an example Fig. 4 shows the UV-MALDI-TOF mass spectrum of the product **1** and the assignment of the main peaks is observed in Table 1.

In Tables 1–4, the assignment of the main peaks appearing in the mass spectra of products **1**–**4** is attempted. Scheme 4 shows the schematic representation of the structural moieties A, B and T, present in the different products obtained. The stoichiometric numbers x and y (odd numbers) of each unit in **1**–**4** are also indicated in Scheme 4 and in Tables 1–4, where $n = x + y$ for compounds **1**–**4**.

3.4. $\overline{M}_w/\overline{M}_n$ and PD

Using FTIR, ^1H and ^{13}C NMR, as characterization techniques, we observed that the polymers **1**–**4** are a mixture of structures containing A and B functional moieties as pendant groups. The use of UV-MALDI-TOF-MS enabled us to



A_x B_y T

Scheme 4. Structural moieties (A, B and T) present in the products **1**–**4**.

Table 1
Assignment of peaks of UV-MALDI-TOF mass spectra for product **1**

m/z (exp.)	Species	*n*	*m/z*	
			Theor. + H^+	Theor. + Na^+
1261.8	TA_6B_5H	11		1260.5
1315.5	$TA_{10}B_2H$	12		1315.5
1375.8	TA_6B_6H	12		1375.6
1434.1	$TA_2B_{10}H$	12		1435.6
1492.9	TA_6B_7H	13		1491.1
1550.1	$TA_2B_{11}H$	13		1550.7
1608.8	$TA_{12}B_3H$	15	1608.9	
1665.8	$TA_2B_{12}H$	14		1665.9
1723.9	$TA_{12}B_4H$	16	1724.1	
1781.9	$TA_2B_{13}H$	15		1781.0
1839.4	$TA_5B_{12}H$	17	1839.2	
1897.7	$TA_2B_{14}H$	16		1896.1
1954.3	$TA_{12}B_6H$	18	1955.1	
2013.7	$TA_8B_{10}H$	18	2014.4	
2070.5	$TA_{12}B_7H$	19	2069.4	
2128.9	$TA_8B_{11}H$	19	2129.5	
2187.3	$TA_{21}H$	21		2186.6
2303.2	$TA_4B_{16}H$	20	2304.7	
2359.0	$TA_8B_{13}H$	21	2359.8	
2446.2	$TA_{19}B_4H$	23		2446.9
2537.2	$TA_{13}B_{10}H$	23		2536.9
2747.3	$TA_{22}B_4H$	26		2747.2
3182.3	$TA_{26}B_3H$	29		3182.7
3651.7	$TA_{14}B_{19}H$	33	3651.2	
3898.7	$TA_{22}B_{14}H$	36		3898.5

m/z, Da; theoretical values correspond to ionization with H^+ and Na^+; $n = x + y$ (Schemes 1 and 4).

Table 2
Assignment of peaks of UV-MALDI-TOF mass spectra for product **2**

m/z (exp.)	Species	*n*	*m/z*	
			Theor. + H^+	Theor. + Na^+
507.7	TAB_3H	4	507.6	
530.4	TAB_3H	4		529.6
622.9	TAB_4H	5	622.7	
645.3	TAB_4H	5		644.7
660.6	TB_5H	5		659.7
737.5	TAB_5H	6	737.9	
760.2	TAB_5H	6		759.8
776.2	TB_6H	6		774.8
860.2	TA_2B_5H	7		860.0
874.4	TAB_6H	7		875.0
891.0	TB_7H	7		890.0
990.5	TAB_7H	8		990.1
1089.9	TA_2B_7H	9		1090.2
1106.2	TA_2B_8H	9		1105.2
1120.6	TB_9H	9		1120.2
1179.7	$TA_{10}BH$	11	1178.4	
1204.2	TA_2B_8H	10	1205.4	
1222.0	TAB_9H	10	1220.4	
1336.0	$TAB_{10}H$	11	1335.5	
1405.0	TA_4B_8H	12	1405.9	
1566.0	$TAB_{12}H$	13	1565.8	
1680.5	$TAB_{13}H$	14	1680.9	
1779.7	$TA_2B_{13}H$	15	1781.0	

m/z, Da; theoretical values correspond to ionization with H^+ and Na^+; $n = x + y$ (Schemes 1 and 4).

confirm the presence of these functional groups and to compare the calculated theoretical *m/z* values for each polymer molecule with the experimental *m/z* values measured.

Thus, by analysis of the UV-MALDI-TOF mass spectra of the compounds **1**–**4** it was possible to determine the number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity (PD). Moreover it was possible to estimate the composition of each sample in terms of ratio of ester (A) and amide (B) molar fraction in each case. The results are summarized in Table 5.

The products are low molecular weight polymers. The molecular weight varies with the ethanolamine/ethyl acrylate ratio in the polymerization feed. The highest molecular weight was obtained for a ethanolamine/ethyl acrylate ratio of 0.5. The composition of the products was also dependent of this ratio. It was observed that a maximum content of hydroxyethylamide in the pendant groups occurs for a ethanolamine/ethyl acrylate ratio of 1. At this point we could suppose that the enzyme shows its maximum aminolytic activity. From the analysis of UV-MALDI-TOF mass spectra it can be observed that ethanolamine appears as the ending group in the polymer chain. At high amounts, it would seem that ethanolamine is used more as ending group than as nucleophile in the aminolysis reaction, giving products of lower molecular weight and with a lower proportion of hydroxyalkylacrylamide as pendant group.

It is remarkable to indicate the fact of high monodispersity exhibited by UV-MALDI-TOF spectra of lipase-catalyzed synthesized polymers (1.04–1.13), which is difficult to achieve by conventional polymerization procedures.

Table 3
Assignment of peaks of UV-MALDI-TOF mass spectra for product **3**

m/z (exp.)	Species	*n*	*m/z*	
			Theor. + H^+	Theor. + Na^+
177.2	TBH	1	177.2	
199.1	TBH	1		199.2
284.9	TA_2H	2		284.3
291.9	TB_2H	2	292.4	
314.2	TB_2H	2		314.1
363.7	TA_3H	3	362.5	
386.4	TA_3H	3		384.4
407.2	TB_3H	3	407.5	
430.2	TB_3H	3		429.5
479.7	TA_4H	4	477.6	
502.4	TA_3BH	4		499.6
522.6	TB_4H	4	522.6	
545.2	TB_4H	4		544.7
594.2	TA_3B_2H	5	592.8	
619.2	TAB_4H	5	622.7	
637.8	TB_5H	5	637.8	
660.0	TB_5H	5		659.8
706.7	TA_3B_3H	6	707.9	
	TA_3B_3H	6		730.8
752.9	TB_5H	6	752.9	
774.8	TB_6H	6		774.8
809.3	TA_4B_3H	7	808.0	
824.8	TA_3B_4H	7	823.0	
829.7	TA_4B_3H	7		830.0
858.1	TAB_6H	7		860.0
874.4	TB_7H	7		875.0
888.8	TA_3B_5H	8		890.0
939.3	TAB_7H	8	938.1	
967.0	TA_9H	9	968.1	
985.9	TA_9H	9		985.2

m/z, Da; theoretical values correspond to ionization with H^+ and Na^+; $n = x + y$ (Schemes 1 and 4).

Table 4
Assignment of peaks of UV-MALDI-TOF mass spectra for product **4**

m/z (exp.)	Species	*n*	*m/z*	
			Theor. + H^+	Theor. + Na^+
177.0	TB_1H	1	177.2	
198.9	TB_1H	1		199.2
284.8	TA_2H	2		284.3
292.1	TB_2H	2	292.4	
314.1	TB_2H	2		314.3
363.4	TA_3H	3		362.5
386.3	TA_3H	3		384.4
407.5	TB_3H	3	407.5	
429.8	TB_3H	3		429.5
458.0	TA_4H	4	462.6	
479.1	TA_3BH	4	477.6	
502.4	TA_3BH	4		499.6
522.6	TB_4H	4	522.6	
545.2	TB_4H	4		544.6
594.9	TA_3B_2H	5	592.8	
619.2	TAB_4H	5	622.7	
635.6	TB_5H	5	637.8	
666.1	TA_6H	6	662.8	
706.7	TA_3B_3H	6	707.8	
730.8	TA_3B_3H	6		729.8
753.3	TAB_5H	6		752.9
774.7	TB_6H	6		774.8
809.0	TA_4B_3H	7	808.0	
824.8	TA_3B_4H	7	823.0	
858.1	TA_2B_5H	7		860.0

m/z, Da; theoretical values correspond to ionization with H^+ and Na^+; $n = x + y$ (Schemes 1 and 4).

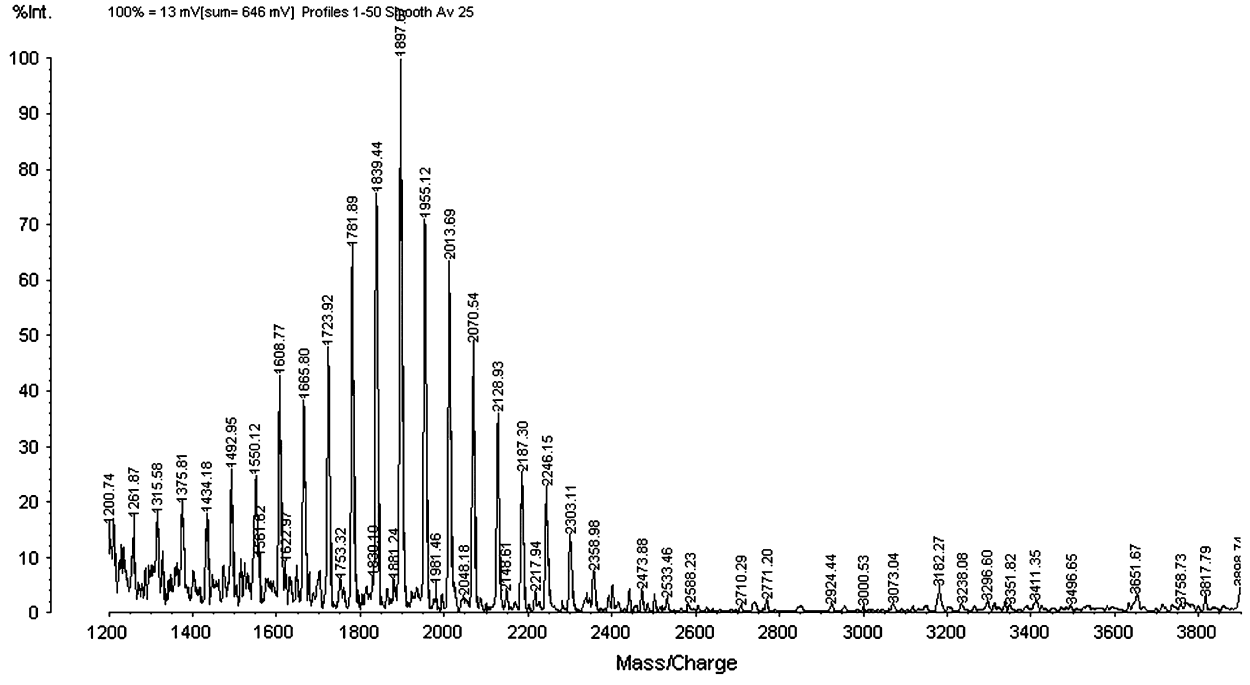


Fig. 4. UV-MALDI-TOF-MS of **1** (matrix: nor-Ho; positive-ion mode; sample preparation method: sandwich).

Table 5
Molecular weight, polydispersity and composition of products **1**–**4**

Product	Ethanolamine/ethyl acrylate	$\overline{M}_w$	$\overline{M}_n$	PD	Molar fraction	
					Amide	Ester
1	0.5	1956	1883	1.04	0.54	0.46
2	1	851	801	1.06	0.86	0.14
3	2.5	554	491	1.13	0.73	0.27
4	5	537	484	1.11	0.62	0.38

3.5. Solubility and pH

The products are soluble in polar solvents. According to their composition, the products **1**–**4** show functional groups of different polarities as pendant groups such as ethyl carboxylate (low polarity), and hydroxyethylamide (high polarity). Therefore, in this system there are a variety of hydrophilic/hydrophobic balances as well as many inter- and intramolecular interactions. For this reason and taking into account that the distribution of pendant groups in the products is at random, no relationship between the amount of ethanolamine in the feed and the solubility of products can be found. While **1** and **2** are soluble in MeOH and water, **3** and **4** are only soluble in H_2O.

The pH of aqueous solution of the products **1**–**4**, containing hydroxyethylamide and ethyl carboxylate as pendant groups, is 7.

4. Conclusion

This paper reports the synthesis and characterization of several acrylic copolymers obtained from ethyl acrylate as the only monomer starting material in a chain polymerization process catalyzed by *C. antarctica* lipase B. The lipase not only catalyzed the chain polymerization of ethyl acrylate forming the polyacrylate chain but also active in aminolysis of the pendant ester groups using ethanolamine as nucleophile. As a consequence of the double action developed by the lipase in the presence of ethyl acrylate and ethanolamine, and depending on the ethanolamine:ethyl acrylate ratio in the reaction feed, it was possible to obtain copolymers of poly(ethyl acrylate) and poly(*N*-(2-hydroxyethyl)acrylamide). The products, characterized by FTIR, ^{1}H and ^{13}C NMR and UV-MALDI-TOF-MS, showed low molecular weight and high monodispersity. The activity showed by *C. antarctica* lipase B in chain polymerization is reported for the first time and opens a novel pathway for the synthesis of acrylic copolymers.

The green and mild reaction conditions, medium reaction and the easy handling of the enzymatic catalyst are obvious advantages of the present method. Moreover, the work-up is reduced to a filtration and evaporation of the solvent. Finally the enzyme can be recovered by washing with solvent and used again at least three times with negligible loss in its activity.

Acknowledgements

We thank UBA (X089 and X022), ANPCyT (PICT 06-08293 and 06-12312) and CONICET (PIP 5443) for partial financial support. A.B. and R.E.B. are Research Members of CONICET. UV-MALDI-TOF-MS were performed as part of the Academic Agreement between Rosa Erra-Balsells (FCEyN-UBA, Argentina) and Hiroshi Nonami (CA-EU, Japan) with the facilities of the High-Resolution Liquid Chromatography-integrated Mass Spectrometer System of the United Graduated School of Agricultural Sciences (Ehime University, Japan).

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