Functionalization of Poly(2-Hydroxyethyl Methacrylate) with Chloroacetate Groups: Immobilization of Bioactive 1-Naphthylacetic Acid

Roman Jantas, Zbigniew Draczyński, Dawid Stawski

Department of Physical Chemistry of Polymers, Technical University of Łódź, 90-924 Łódź, Poland

In recent years, much attention has been directed to speciality polymers [1]. Poly(vinyl alcohol) or polysaccharides are good examples of macromolecular carriers for bioactive agents immobilization [2-4]. In most cases these polymers have been previously transformed into suitable reactive derivatives, in order to achieve the attachment of bioactive compound as well as to introduce a spacer between the carrier and the bioactive compounds. A gradual release of the bioactive agent can be achieved by hydrolytic or enzymatic cleavage of the linking bond. In this context, poly(2-hydroxyethyl methacrylate) (PHEMA) containing reactive hydroxyl groups, may be used for coupling of bioactive compounds.

In this paper, we report the applicability of pendant chloroacetate groups previously linked to PHEMA in coupling of bioactive carboxylic acid (1-naphthylacetic acid) by reaction with its potassium salt. A study of the hydrolysis of resulting adduct in the heterogeneous phase was also made in order to evaluate the release of the bioactive acid.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA) was dried and distilled under reduced pressure. N,N-dimethylacetamide (DMAc), dimethylsulfoxide (DMSO), lithium chloride (LiCl), chloroacetyl chloride, pyridine and potassium 1-naphthylacetate (KNA) were obtained from Aldrich. All reagents were used without purification. PHEMA was prepared by the procedure described in reference [5]. The number average molecular weight of PHEMA was M_n =23.600 g/mol, and M_w/M_n=1.93.

Esterification of PHEMA with chloroacetyl chloride

Esterification of PHEMA with chloroacetyl chloride was carried out in DMAc/5% LiCl as a solvent and in the presence of pyridine as catalyst at 30 ^oC [6].

Reaction of chloroacetylated PHEMA with the potassium 1-naphthylacetate

The chloroacetylated PHEMA was dissolved in DMSO at room temperature. The calculated amount of potassium salt of 1-naphtylacetic acid was added while stirring. All the reactions were performed at 30 ^oC. After 5 h the product was isolated by precipitation with distilled water. All samples were purified by reprecipitation, using DMSO as solvent and ethanol as precipitant and then dried under reduce pressure at 60 ^oC to constant weight.

Study of heterogeneous hydrolysis of PHEMA-1-naphthylacetic acid adduct

The approximately 0.1 g of PHEMA-1-naphthylacetic acid adduct powder-form samples were compressed at high pressure to form disks with diameter of 12 mm. The resulting disks were placed in conical flasks with 100 cm^3 of NaOH solution (pH = 12.7 ÷ 13.7). Flasks were put into water bath heated to the 25 ^oC. At fixed intervals, solution

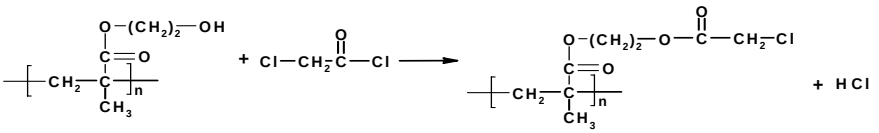
specimens were taken from the liquid above the padded of tables samples. The released bioactive agent in the homogeneous solution, was quantitatively determined by UV spectroscopy at the absorption wavelength of 1-naphthylacetic acid (NAA) at λ=281 nm using calibration curves (aqueous solution of sodium hydroxide as solvent). Tests were performed for different hydrophilic character of adducts and various pH values of reaction environment.

Measurements

Infrared spectra were recorded using Perkin-Elmer 2000 (FTIR) instrument. ^{1}H-NMR and ^{13}C-NMR spectra were obtained using Bruker DPX 250 MHz spectrometer. The UV-VIS spectra were obtained using Perkin Elmer UV/VIS Lambda 2 spectrometer. The degree of the esterification of the PHEMA was determined from the elemental analysis of chloride. The values of number average molecular weight (M_n), average molecular weight (M_w) and the polydispersity (M_w/M_n) of the PHEMA were determined by gel permeation chromatography.

RESULTS AND DISCUSSION

PHEMA modified with chloroacetate groups (mPHEMA) to different degrees of substitution were synthesized in a homogenous medium by using the method described for chloroacetylation of poly(vinyl alcohol) [7]. The following scheme shows the reaction:

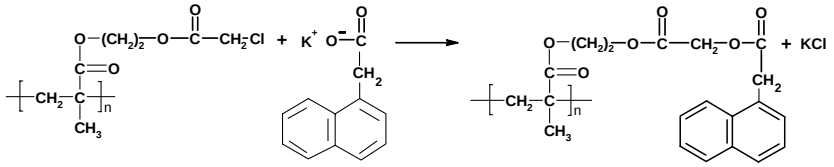


The effect of component ratio on the degree of substitution is summarized in Table 1. As follows from the data in Table 1, the extent of substitution increases with an increase in the ratio of chloroacetyl chloride to PHEMA. For example, the degree of substitution increases from 13.4 to 98.1 mol % as chloroacetyl chloride/hydroxy groups of PHEMA increases from 0.4 to 1.2.

Table 1. Effect of component ratio on the degree of substitution for the esterification of PHEMA with chloroacetyl chloride at 25^{o}C

Sample	$ClCH_2COCl$ /-OH	Cl in mPHEMA	Degree of substitution
	mole/mole	wt %	mol %
1	0.4	3.32	13.4
2	1.0	14.89	80.5
3	1.2	16.95	98.1

The coupling of bioactive carboxylic acid to PHEMA functionalized, was carried out by using the KNA according to the following scheme:



In this way mPHEMA-1-NAA adduct was obtained. The elemental analysis of the products obtained from mPHEMA and KNA showed the absence of chlorine, which allowed to assume that the substitution degree in the adduct was the same as for corresponding of mPHEMA.

The FTIR spectrum of mPHEMA (no shown) has a new absorption band at 1760 cm^{-1} of carbonyl groups in $–COO-CH_2-Cl$, which is superimposed on the spectrum of >C=O band of ester groups of PHEMA. There is also visible an absorption peak of $–CH_2Cl$ groups at 760 cm^{-1}. Moreover in spectrum of the adduct mPHEMA-1-NAA absorption band appears at 1560, 1513 and 790 cm^{-1}, which results from scissoring vibrations bands >C=C< and C-H in the naphthyl ring [8].

The ^{1}H-NMR spectrum of the same mPHEMA (Fig. 1a) shows a characteristic band of protons of chloroacetate groups at 4.2 ppm, which is superimposed on one of the signals of $–OCH_2CH_2O-$ groups. There are also visible bands of $–CH_2-$ in the main chain at 1.31 – 2.41 ppm, a signal of $\alpha–CH_3$ groups at 0.63 - 1.31 ppm. The spectrum of mPHEMA-1-NAA adduct (Fig. 1b) shows additional signals of naphthyl ring at 7.3 – 8.2 ppm.

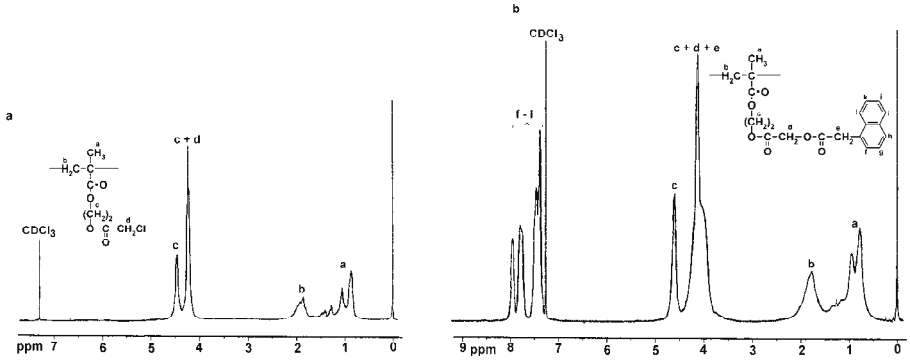


Fig. 1. Spectra ^{1}H-NMR of: a - chloroacetylated PHEMA (98.1 mol% of chloroacetate groups), b - adduct of PHEMA-1-naphthylacetic acid (98.1 mol% of 1-naphthylacetate groups)

The ^{13}C-NMR spectrum of mPHEMA (Fig. 2a) is characterized by chemical shifts at 41.6 and 167.7 ppm, which correspond to chloromethyl and carbonyl carbon atoms of chloroacetate groups, respectively.

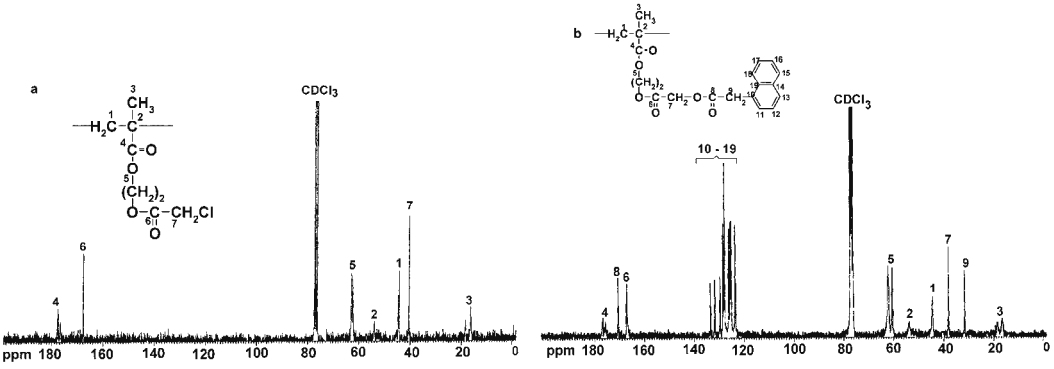


Fig. 2. Spectra ^{13}C-NMR of: a - chloroacetylated PHEMA (98.1 mol% of chloroacetate groups), b - adduct of PHEMA-1-naphthylacetic acid (98.1 mol% of 1-naphthylacetate groups)

The spectrum of the mPHEMA-1-NAA adduct (Fig. 2b) shows additional peaks between 121.5 and 135.0 ppm, which due to the resonance of carbon atoms in the naphthyl ring and the signals at 170.9 ppm can be assigned to the $C_{10}H_7$-CH_2-CO- groups.

All these spectroscopic results confirm the presence of chloroacetate and naphthylacetate groups in modified PHEMA.

Fig. 3 shows the release behavior of naphthylacetic acid (at 25 ^oC and pH=12.7) from three mPHEMA-1-NAA adducts, (containing naphthylacetate groups from 13.4 to 98.1 mol%). From the course of kinetic curves it follows that the release of the active compound is the quickest in the case of the adduct with the lowest content of naphthylacetate groups. This seems to be connected with interaction between the polymer and water. The decreased content of naphthylacetate groups makes the polymer more hydrophilic and consequently facilitates the penetration of hydroxyl ions to active sites in the tablet, effectively increasing the relative of hydrolysis.

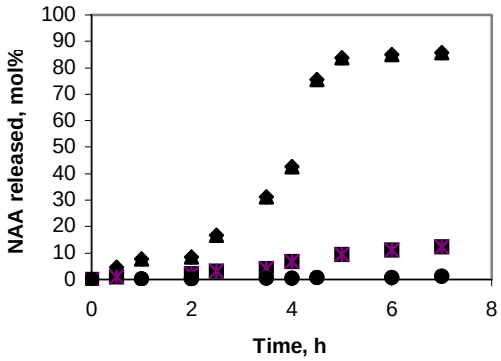


Fig. 3. The release of the bioactive compound (NAA) from mPHEMA-1-NAA adducts of 3 different composition (pH = 12.7 at 25 ^oC): (▲) 13.4 mol% 1-naphthylacetate groups; (■) 80.5 mol% 1-naphthylacetate groups; (●) 98.1 mol% 1-naphthylacetate groups

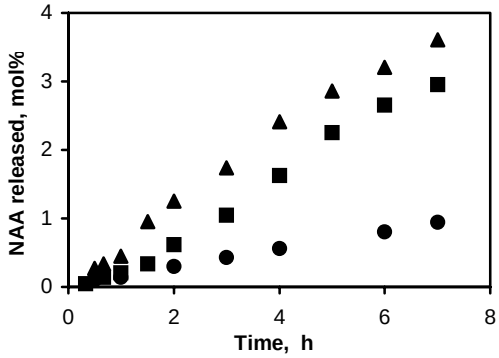


Fig. 4. The release of the bioactive compound (NAA) from mPHEMA-1-NAA adduct at 3 different pH value: (●) pH = 12.7, (■) pH = 13.0 and (▲) pH = 13.7 (98.1 mol% 1-naphthylacetate groups, at 25 ^oC)

Fig. 4 shows a typical course of the heterogeneous hydrolysis of mPHEMA-1-NAA adduct (containing 98.1 mol% of naphthylacetate groups) in alkaline medium at 25 ^oC from pH=12.7 to 13.7. The presented results clearly indicate the increase in the release of bioactive carboxylic acid with the increase in the alkalinity of reaction medium. The hydrolysis rate of adduct is the lowest at pH = 12.7. This is consistent with the results obtained by Arranz *et al.* [7] for the poly(vinyl alcohol)-1-naphtylacetic acid adduct.

Reference

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