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ORIGINAL PAPER

Preparation and characterization of the poly(2-hydroxyethyl methacrylate)–salicylic acid conjugate

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Abstract A new polymeric system containing hydrolysable ester bond linked to salicylic acid to be used for controlled drug release was synthesized. Poly(2-hydroxyethyl methacrylate) (PHEMA) functionalized with chloroacetate groups was obtained by the reaction between PHEMA and chloroacetyl chloride using the *N,N*-dimethylacetamide/5% lithium chloride system as a solvent and pyridine as a catalyst. The degree of substitution was calculated from the chlorine content and ranged from 32.2 to 98.1 mol.% depending on the ratio of chloroacetyl chloride to PHEMA. The coupling of salicylic acid to PHEMA functionalized with chloroacetate groups was carried out by the reaction between PHEMA and the sodium salt of salicylic acid. The structures of chloroacetylated PHEMA and PHEMA–salicylic acid conjugates were determined by means of FTIR, ^{1}H-NMR and ^{13}C-NMR spectra. The hydrolysis in the heterogeneous system of PHEMA–salicylic acid conjugates were performed in buffer solutions (pH 7.6 and 8.5) at 37 °C and showed that the release of the drug (sodium salicylate) from tablets was dependent on the hydrophilic character of conjugate as well as on the pH value of the medium.

Keywords Polymer–drug conjugate · Poly(2-hydroxyethyl methacrylate) · Chloroacetylation · Sodium salicylate · Controlled release

Introduction

In recent years, much attention has been directed to specialty polymers of the most useful materials for biomedical application. Polymer materials have been designed and proposed as matrices, membrane for biosensors, prosthesis, soft tissue substitutes, coatings or drug delivery systems. One particular approach towards

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the improved use of drugs is the design of polymer–drug conjugates or polymeric prodrugs [1–9]. The chemical attachment of low molecular weight drugs to synthetic or natural polymers has been frequently investigated as a means of improving the efficacy of drug control release devices through a constant but prolonged release of drugs with minimum side effects. The drugs may be linked to the polymeric carriers using a number of chemical reactions with the participation of functional groups such as –OH, –COOH, $–NH_2$ or –SH which are either originally present in the polymer chain or alternatively formed by functionalization. Another possibility is the use of functionalized monomers in the synthesis of a reactive polymeric precursor. In most cases, drugs bound directly to the polymer chain exhibit either a reduced or zero biological activity. For this reason, drugs should be separated from the polymeric backbone by means of a spacer. Once the drug conjugate reaches the target compartment, the drug can then be split off more readily in its active form. In order to facilitate the release of the drug it must be attached to the macromolecular chain by covalent bonds of limited stability in a biological environment [10].

The advantages of poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(ethylene oxide), poly(2-hydroxypropyl methacrylamide), copolymers of 2-hydroxyethyl methacrylate or polysaccharides as a macromolecular carrier for drug immobilization are well acknowledged, as is apparent from the literature data. In most cases, the polymers has been previously transformed into a suitable reactive derivative to achieve the attachment of drug molecules and to introduce a spacer between the carrier and the bioactive compounds [11–18]. The selection of the spacer arm is critical as it opens the possibility of controlling the site and the rate of release of the drug from the conjugates either by hydrolytic or by enzymatic cleavage of the linking bond [5, 18].

Some synthetic or natural polymers possess multiple primary and secondary hydroxyl groups and therefore can be easily conjugated with drug molecules with reactive groups either by direct conjugation or by incorporation of a spacer arm. In this article, PHEMA with reactive primary hydroxyl groups may be used as a polymeric carrier for coupling pharmaceutical compounds.

Recently, Babazadeh [9] has used only copolymers of 2-hydroxyethyl methacrylate with *N*-vinyl-2-pyrrolidone, methacrylamide and *n*-butyl methacrylate to preform covalent bond of diclofenac followed by testing the release of the drug from the obtained conjugates.

Salicylates are used in medicine as analgesic and antipyretic agents. They also act as non-steroidal anti-inflammatory drugs (NSAIDs) and induce apoptosis in cancer cells. Recent years have witnessed many studies on the synthesis and hydrolysis of polymer–drug conjugates of NASAIDs such as naproxen [16, 19] indomethacin [20, 21] ibuprofen [22, 23] ketoprofen [24] diclofenac [9, 25] and fenoprofen [26].

The aim of the present study was to synthesize and characterize PHEMA–salicylic acid conjugates in a two-stage procedure. During the first stage, PHEMA was chloroacetylated with chloroacetyl chloride, while in the second stage, chloroacetate groups were reacted with sodium salicylate. A study of the hydrolysis of the resulting conjugates in the heterogeneous system was also conducted in

aqueous buffer solutions (pH 7.6 and 8.5) and the quantity of the released drug was detected by UV spectroscopy. The influence of neighbouring groups on the release of the drug from the conjugates was also studied.

Experimental

Materials

2-Hydroxyethyl methacrylate (HEMA) was purified by distillation under reduced pressure, and the fraction of bp 87–89 °C/5 mmHg was collected. Poly(2-hydroxyethyl methacrylate) (PHEMA) was prepared by polymerization of a 10% solution of monomer in isopropanol. The concentration of AIBN was 20% by wt. in relation to HEMA. The reaction temperature was 75 °C, and the reaction time was 5.5 h. The polymer was precipitated with benzene–heptane mixture 1:1 by vol, washed with acetone, and dried under reduced pressure at 50 °C. The yield was 69%. The number average molecular weight of PHEMA was $\bar{M}_n = 23,600$ g/mol, $(DP_n = 181)$, and the polydispersity index was $\bar{M}_w/\bar{M}_n = 1.93$.

N,N-Dimethylacetamide (DMAc) (Aldrich) and dimethylsulfoxide (DMSO) (Aldrich) were purified by distillation and then stored over 4 Å molecular sieves. Lithium chloride (LiCl) (Aldrich) was dried under reduced pressure in the presence of phosphorus pentoxide. Chloroacetyl chloride (Aldrich) was purified before use by distillation under reduced pressure. Pyridine (POCh) was refluxed over CaH_2 under a nitrogen atmosphere and then distilled. Salicylic acid (Fluka, Buchs, Switzerland) was used without further purification. Sodium salicylate (SSA) was obtained by dissolving 8.05 g (0.05 mol) of the acid in 50 mL of chloroform, then neutralized with 2.0 g (0.05 mol) of NaOH dissolved in 50 mL of ethyl alcohol. The product was precipitated by pouring reaction mixture into 600 mL of dry acetone. After filtration, the salt was dried under reduced pressure at 50 °C to constant weight.

Esterification of PHEMA with chloroacetyl chloride

The typical procedure of esterification was as follows: 3.9 g (30.0 mmol, OH groups) of PHEMA was dissolved in 60 mL of the DMAc/LiCl solvent system. The solution was then charged into a three-necked flask equipped with a nitrogen inlet and outlet, dropping funnel, magnetic stirrer and thermometer. 2.6 mL (33.0 mmol) of pyridine was added to the flask as an acid acceptor. 10 mL of DMAc solution containing 2.4 mL (30.0 mmol) of chloroacetyl chloride was then added dropwise at about 0 °C with stirring. The reaction mixture was heated at 25 °C for 8 h and after that, the solution was poured into a large amount of cold 2 M HCl to precipitate the product. The precipitated product was filtered and washed several times with cold distilled water. It was purified by reprecipitation using THF as a solvent and cold distilled water as a precipitant, then dried under reduced pressure at 50 °C to constant weight. The yield was 84%.

Reaction of chloroacetylated PHEMA with sodium salicylate

The typical procedure of the reaction was as follows: 2.1 g (10 mmol of $ClCH_2CO$– groups) of chloroacetylated PHEMA was dissolved in 20 mL of DMSO at room temperature and then 1.92 g (12 mmol) of sodium salicylate was added while stirring. The reaction was performed at 30 °C under stirring for about 4 h. The obtained product was isolated by precipitation using distilled water as precipitant and then washed with ethanol to remove unreacted sodium salicylate. All samples were purified by reprecipitation, using DMSO as a solvent and ethanol as a precipitant and then dried under reduce pressure at 50 °C to constant weight. The yield was 71%.

Study of the heterogeneous hydrolysis of PHEMA–salicylic acid conjugate

About 0.1 g samples of the PHEMA–salicylic acid conjugate (containing from 32.2 to 98.1 mol.% of salicylate groups) in the form of powder were pressed in a steel cylindrical cell with a diameter of 12 mm in a hydraulic press under a pressure of about 12 MPa to make disks. The resulting tablet was placed in conical flasks with 100 mL of an aqueous buffer solution (pH 7.6 and 8.5). The flaskes were put into a water bath heated to 37 °C. 2 mL samples were taken at appropriate intervals from the liquid above of the tablets, and equal volume of same dissolution medium was added to maintain a constant volume. The solution contained the released drug, which was quantitatively determined by UV spectroscopy at the absorption wavelength of sodium salicylate ($\lambda = 295$ nm) using calibration curves obtained previously under the same conditions. Tests were performed for different degrees of substitution of the conjugates and various pH values of the reaction medium. We noticed that of PHEMA–salicylic acid conjugates remained insoluble in the reaction environment along the whole hydrolysis process investigated.

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 with $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal reference. The UV–VIS spectra were obtained using the Perkin Elmer UV/VIS Lambda 2 spectrometer. The values of number average molecular weight ($\bar{M}_n$), average molecular weight ($\bar{M}_w$) and the polydispersity index ($\bar{M}_w/\bar{M}_n$) of PHEMA were determined by gel permeation chromatography (GPC). The chromatogram in DMF at 35 °C was obtained in the Waters modular system using Ultrastyragel Linear Column and RI-detector Waters 410. The average molecular weight was calculated on the basis of a polystyrene calibration curve. Elemental analysis (Cl) was carried out on a Carlo Erba 1106 EA-instrument.

Results and discussion

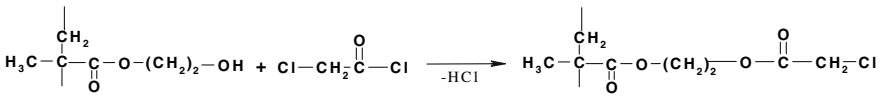
Synthesis and characterization of the PHEMA–salicylic acid conjugates

In order to provide a uniform distribution of chloromethyl groups along the polymer chain, the esterification was carried out in a homogeneous system, previously dissolving PHEMA in the DMAc/LiCl system. PHEMA modified with chloroacetate groups with different degrees of substitution was synthesized using the method described for chloroacetylation of poly(vinyl alcohol) [11] according to the reaction presented by Scheme 1.

The effect of reaction conditions on the degree of substitution is summarized in Table 1. The degree of the esterification of PHEMA was calculated from the content of chlorine determined by the elemental analysis. As follows from the date in Table 1, the extent of modification increases with the increase in the ratio of chloroacetyl chloride to PHEMA. For example, the degrees substitution increases from 32.2 to 98.1 mol.% chloroacetate groups as chloroacetyl chloride/hydroxyl groups of PHEMA increase from 0.5 to 1.2.

The coupling of bioactive carboxylic acid to PHEMA functionalized with chloroacetate groups was carried out using the sodium salicylate according to the following Scheme 2.

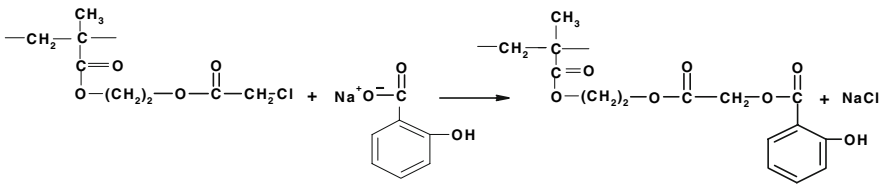
The elemental analysis of the products obtained from chloroacetylated PHEMA with various degrees of substitutions and sodium salicylate showed the absence of



Scheme 1 Reaction between PHEMA and chloroacetyl chloride

Table 1 Effect of reaction conditions on the degree of substitution for the esterification of PHEMA with chloroacetyl chloride at 25 °C

Sample	$ClCH_2COCl$/–OH (mole/mole)	Cl (%)	Degree of substitution (mol.%)
1	0.5	7.37	32.2
2	1.0	14.79	80.6
3	1.2	16.89	98.1



Scheme 2 Synthesis of PHEMA–salicylic acid conjugate

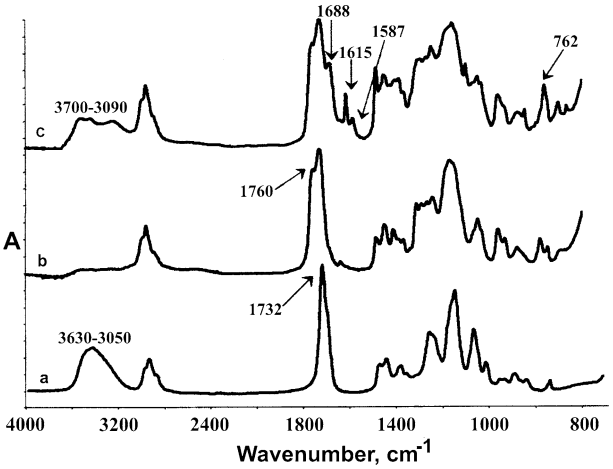


Fig. 1 FTIR spectra of **a** PHEMA, **b** chloroacetylated PHEMA (98.1 mol% of chloroacetate groups) and **c** conjugate of PHEMA–salicylic acid (98.1 mol% of salicylate groups)

chlorine, which allowed one to assume its total replacement with the substitution degree of the conjugate being the same as that for corresponding chloroacetylated derivatives of PHEMA.

Figure 1a–c shows exemplary the FTIR spectra of unmodified PHEMA, chloroacetylated PHEMA (98.1 mol.% of chloroacetate groups) and PHEMA–salicylic acid conjugate (98.1 mol.% of salicylate groups). As is seen, the spectrum of chloroacetylated PHEMA (Fig. 1b), unlike the spectrum of PHEMA (Fig. 1a), has a new absorption band at 1760 cm^{-1} of carbonyl group (in –COO–CH_2–Cl), which is superimposed on the spectrum of >C=O band of ester group of PHEMA. On the other hand, there disappears the band within the range 3630–3050 cm^{-1} derived from hydroxyl groups. Moreover, in the spectrum of the PHEMA–salicylic acid conjugate (Fig. 1c), the absorption bands appear at 1688, 1615, 1587 and 762 cm^{-1}, which results from scissoring vibrations bands of >C=C< and C–H in the benzene ring.

The ^{1}H-NMR spectrum of the same chloroacetylated PHEMA (Fig. 2a) shows a characteristic peak of protons of chloroacetate groups at 4.2 ppm, which is superimposed on one of the signals of –OCH_2CH_2O– group. There are also visible peaks at 1.47–2.31 ppm, which belong to protons of –CH_2– in the main chain, and a signal of protons of –CH_3 group at 0.43–1.39 ppm. The spectrum of PHEMA–salicylic acid conjugate (Fig. 2b) shows characteristic signals at 6.7–8.2 ppm and 10.2 ppm, which can be assigned to the protons of the benzene ring and OH groups, respectively.

The ^{13}C-NMR spectrum of chloroacetylated PHEMA (Fig. 3a) is characterized by signals at 40.7 and 167.2 ppm, which correspond to chloromethyl and carbonyl carbon atoms of chloroacetate groups, respectively. The spectrum of the PHEMA–salicylic acid conjugate (Fig. 3b) shows additional peaks between 112.4 and

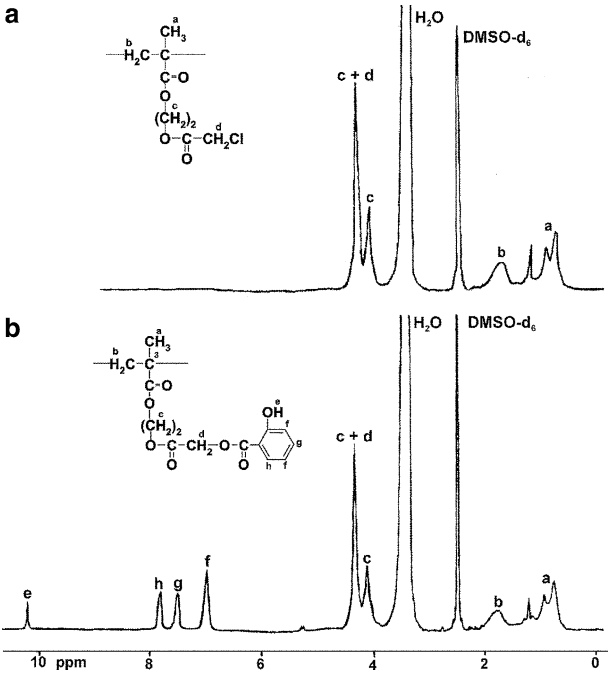


Fig. 2 ^{1}H-NMR spectra of **a** chloroacetylated PHEMA (98.1 mol% of chloroacetate groups) and **b** conjugate of PHEMA–salicylic acid (98.1 mol% of salicylate groups)

135.6 ppm, which is due to the resonance of carbon atoms in the benzene ring and the signals at 176.1 ppm can be assigned to C_6H_4–CO– groups. All these spectroscopic results confirm the presence of chloroacetate and salicylate groups in the modified PHEMAs.

Drug release by the alkaline hydrolysis of PHEMA–salicylic acid conjugates

From the literature reports it follows that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the polymer–drug bonds, the structure of polymer and medium conditions. The hydrolysis of the bond is also dependent on its distance from the main polymer chain. The length and hydrophilicity of the spacer between the drug and polymer backbone can also influence the drug release rate [27]. The in vitro hydrolysis behaviour of polymer–drug conjugate was studied in buffer solutions (pH 7.6 and 8.5) at 37 °C. Three hydrolyzable ester groups were present in PHEMA–salicylic acid conjugates. The investigation of the hydrolyzing solution by UV spectroscopy showed that the polymer–drug conjugates released the sodium salicylate gradually under mild conditions, by the hydrolysis of the ester bond between the drug and the side chain of the polymer during the reaction. The direct ester linkage to the main chain of the

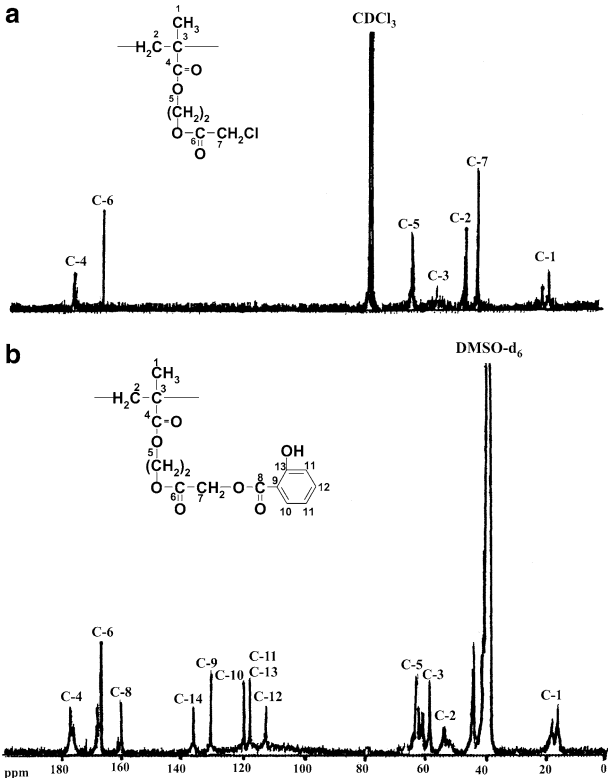


Fig. 3 ^{13}C-NMR spectra of **a** chloroacetylated PHEMA (98.1 mol% of chloroacetate groups) and **b** conjugate of PHEMA–salicylic acid (98.1 mol% of salicylate groups)

polymer was less susceptible towards hydrolysis. This may be connected with the steric hindrance of the polymeric chain that reduces the bond mobility [28].

Figure 4 shows the release behaviour of sodium salicylate at 37 °C and pH 8.5 from three PHEMA–salicylic acid conjugates with various compositions, containing from 32.2 to 98.1 mol.% of salicylate groups. From the kinetic curves it follows that the release of the active compound is the quickest in the case of the conjugate with the lowest content of salicylate groups. This seems to be connected with the interaction between the polymer and water. The decreased content of salicylate groups makes the polymer more hydrophilic and consequently facilitates the penetration of hydroxyl ions to ester groups in the conjugate, effectively increasing the relative hydrolysis rates. This is consistent with the results obtained by Babazadeh [9] for conjugates of copolymers 2-hydroxyethyl methacrylate.

Figure 5 shows a typical course of the heterogeneous hydrolysis of PHEMA–salicylic acid conjugate (containing 98.1 mol.% of salicylate groups) in slightly alkaline medium from pH 7.6 to 8.5 at 37 °C. The presented results clearly indicate the increase in the release of sodium salicylate with the increase in the alkalinity of

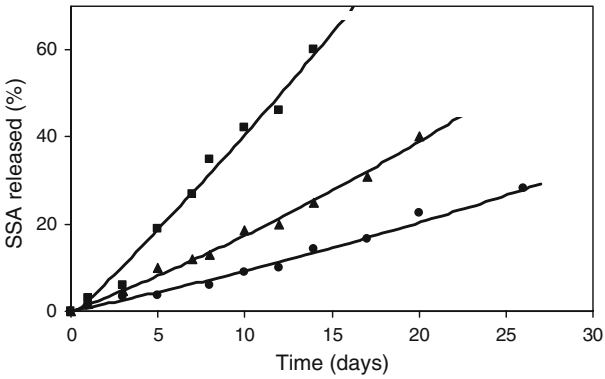


Fig. 4 The release of the sodium salicylate (SSA) from the PHEMA–salicylic acid conjugates depending on the conjugate compositions: (*filled square*) 32.2 mol% salicylate groups; (*filled triangle*) 80.6 mol% salicylate groups; (*filled circle*) 98.1 mol% salicylate groups (pH 8.5 at 37 °C)

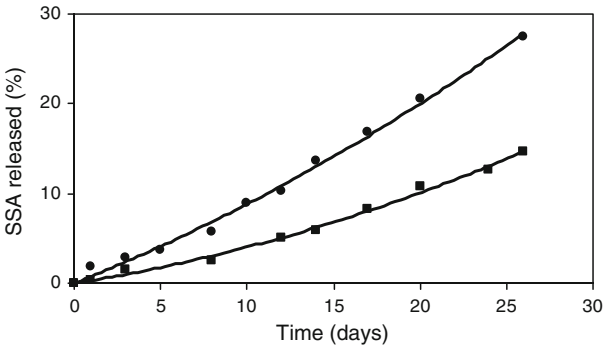


Fig. 5 The release of the sodium salicylate (SSA) from the PHEMA–salicylic acid conjugates depending on the pH value of reaction environment: (*filled square*) pH 7.6, (*filled circle*) pH 8.5 (98.1 mol% salicylate groups, at 37 °C)

the reaction medium. Sanchez-Chaves et al. [14] have shown the similar influence of pH in the case of 2-acetoxybenzoic-dextran conjugates.

Conclusions

As a result of the PHEMA esterification with chloroacetyl chloride, using pyridine as catalyst and DMAc/LiCl system as a solvent, PHEMA with chloroacetate groups was produced. The presence of chloroacetate groups was used to obtain a conjugate with salicylic acid by the reaction between its sodium salt and PHEMA. On the basis of the results of conjugate heterogeneous hydrolysis, it has been found that the rate of drug release depends on the pH of reaction medium and the composition of the PHEMA–salicylic acid conjugate.

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