ELSEVIER

International Journal of Pharmaceutics 241 (2002) 223–230

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Macromolecular prodrugs X. Kinetics of fenoprofen release from PHEA-fenoprofen conjugate

T. van der Merwe [a], B. Boneschans [b,*], B. Zorc [c], J. Breytenbach [a], M. Zovko [c]

[a] *Department of Pharmaceutical Chemistry, Potchefstroom University for CHE, P.B. X6001, Potchefstroom 2520, South Africa*
[b] *Centre for Quality Assurance of Medicines, P.B. X6001, Potchefstroom 2520, South Africa*
[c] *Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia*

Received 14 August 2001; received in revised form 25 March 2002; accepted 15 April 2002

Abstract

The kinetics of fenoprofen release from poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate (PHEA-Fen) in aqueous buffer solutions (pH 10 and 1.1), simulated gastric (SGF) and intestinal fluids (SIF) was studied. In borate buffer pH 10, the following rate constants were obtained: $k = 0.2659$ ($t = 60$ °C) and $k = 0.0177$ h^{-1} ($t = 37$ °C) and in glycine buffer solution pH 1.1 $k = 0.0036$ h^{-1}. In SGF and SIF fenoprofen release did not occur in significant extend within 12 h. The hydrolysis of the ester bond between the polymeric carrier and fenoprofen followed the pseudo first-order kinetics, with activation energy indicative for the breakage of a sigma bond ($E_a = 100.6$ kJ mol^{-1}). The concentration of the released fenoprofen was determined by high performance liquid chromatography (HPLC).

Keywords: Fenoprofen; Polymer–drug conjugate; Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]; Kinetic study; HPLC

1. Introduction

A promising approach to improve drug delivery and solubility, prolong drug release, reduce doses, dosing intervals and drug toxicity or to achieve targetability is to bind a low molecular mass drug to a polymeric carrier (Al-Shamkhani and Duncan, 1995; Giammona et al., 1995, 1998; Duncan et al., 1996, 1998; Vasey et al., 1999; Lovrek et al., 2000).

Our research is focused on polymer-fenoprofen conjugates, in which fenoprofen, a well-known non-steroidal anti-inflammatory drug, is covalently bound to hydrophilic polymers of polyaspartamide type. In previous papers the synthesis of poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-fenoprofen conjugate (PHEA-Fen) and several related conjugates have been described (Zorc and Butula, 1994; Zovko et al., 2001). In these conjugates fenoprofen is bound to hydroxyl or amino bearing polymeric carriers by cleavable ester or amide bonds. In addition, the synthesised conjugates differed in type and/or length of spacer and drug loading.

* Corresponding author. Tel.: +27-18-299-2265; fax: +27-18-299-2284
E-mail address: fmsbb@puknet.puk.ac.za (B. Boneschans).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00197-7

In this paper the physico-chemical properties of PHEA-Fen conjugate and kinetics study of fenoprofen release are reported. These tests are crucial for evaluation of PHEA-Fen as a potential macromolecular prodrug for peroral use.

2. Materials and methods

2.1. Materials

The high performance liquid chromatography (HPLC) system (Thermo Separations, USA) consisted of a spectraSYSTEM AS 3000 autosampler with a variable volume loop injector, a spectraSYSTEM P1000 isocratic pump, spectraSYSTEM SN 4000 signal converter and a spectraSYSTEM UV 1000 programmable variable wavelength detector set at 272 nm, with a 10 mm analytical flow cell. A Phenomenex Luna C_{18} and C_8 columns (Separations, South Africa) were used. The mobile phase was acetonitrile (60 ml), concentrated phosphoric acid to pH 2, water up to 100 ml. The mobile phase was flushed at a flow rate of 1.75 ml min^{-1} at room temperature (25 $\pm$ 2 °C) and the injection volume was 20 μl. Raw data were processed by means of Pentium MMX 166 MHz computer and TSP PC 1000 software package with an IBM OS/2 Warp version 3 operating system. IR spectra were recorded on a FT-IR Paragon 500 spectrometer (Perkin–Elmer, UK) and UV spectra on a Shimadzu UV 2100 spectrophotometer (Shimadzu, Japan). DSC thermogram was taken with a Shimadzu DSC-50 instrument. The measurement conditions were as follows: sample weight, approximately 2 mg; sample holder, aluminium crimp cell; gas flow, nitrogen at 45 ml min^{-1}; heating rate, 10 °C min^{-1}. X-ray powder diffractometry was performed at room temperature with a Bruker D8 advance diffractometer (Bruker, Germany). The measurements conditions were: target, Cu, K_β-filter, Ni; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; scanning speed, 2° per min (step size 0.025°, step time, 0.75 s). Approximately, 200 mg sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals. Dissolution tests were performed in a Vankel VK 7000 apparatus (Vankel Industries Inc., USA) utilising the paddle dissolution method of the USP (USP, 2000a). For thin layer chromatography, silica gel sheets Kieselgel 60 F_{254} (Merck, Germany) were used. Solvent systems were dichloromethane/methanol (9.5:0.5), cyclohexane/ethyl acetate (1:1) and methanol. For spot detection iodine vapour was used.

Fenoprofen was purchased from Eli Lilly Company (South Africa). The borate buffer consisted of 3.1 g boric acid, 3.7 g potassium chloride, 0.2 mol l^{-1} sodium hydroxide (to adjust pH to 10) and water to 1000 ml. The glycine acid buffer consisted of 6.0 g glycine, 4.6 g sodium chloride, 1.0 mol l^{-1} hydrochloric acid (to adjust pH to 1.1) and water to 1000 ml. Simulated gastric fluid (SGF) and intestinal fluids (SIF) were prepared according to USP (USP, 2000b). SGF contained 2 g sodium chloride, 3.2 g purified pepsin, 0.2 mol l^{-1} hydrochloric acid (to adjust pH to 1.2) and water to 1000 ml. SIF was prepared from 6.8 g monobasic potassium phosphate, 10 g pancreatin, 0.2 mol l^{-1} sodium hydroxide (to adjust pH to 6.8) and water to 1000 ml. Purified pepsin (with activity 800–2500 units mg^{-1} protein) and pancreatin from porcine pancreas were supplied by Merck (Merck, South Africa). Solvents used for syntheses were of analytical grade purity and dry while solvents used in HPLC were of HPLC grade quality.

2.2. Chemistry

2.2.1. Synthesis of PHEA-Fen conjugate

PHEA-Fen conjugate was prepared according to the procedure previously published (Zorc and Butula, 1994). A solution of 2.86 g PHEA (0.018 mol of PHEA repeat units), 1.72 g (0.005 mol) fenoprofen benzotriazolide and 4.04 g (0.04 mol) triethylamine in 90 ml DMF was left for free days at room temperature with occasional shaking. The solvent was evaporated in vacuum to a small volume. The PHEA-Fen conjugate was precipitated by adding acetone. The product was filtered off and washed several times with a small amount of acetone, until benzotriazole was completely washed off.

2.2.2. Determination of fenoprofen drug loading

2.2.2.1. HPLC method. The amount of 0.075 g PHEA-Fen conjugate was dissolved in 50 ml of borate buffer solution pH 10. The reaction mixture was maintained at 60 °C for 72 h after which it was neutralised with hydrochloric acid ($c = 2$ mol l^{-1}). The amount of fenoprofen released from the conjugate was determined by HPLC. The experimental procedure was conducted in triplicate and five replicates of the sample were injected. The drug loading in the conjugate was calculated to be 14%.

2.2.2.2. UV method. Two solutions were prepared: the first contained 43.4 μg ml^{-1} of fenoprofen and the second contained PHEA-Fen conjugate equivalent to 43.4 μg ml^{-1} of fenoprofen assuming that the drug loading was 14% (m/m) (0.00618 mg PHEA-Fen conjugate dissolved in 20 ml water). The absorbances of both solutions measured at 272 nm coincided.

2.2.3. Solubility test

Solubility of PHEA-Fen conjugate was determined by preparing saturated solutions of the conjugate in different solvents and allowing it to dissolve for 48 h at room temperature (22 °C). Excess PHEA-Fen was then filtered off and the amount of the conjugate remained in solution was determined by UV spectrophotometry.

2.2.4. Fenoprofen release from PHEA-Fen conjugate at pH 10

Two experiments with the same PHEA-Fen concentration (0.075 g of the conjugate dissolved in 50 ml borate buffer pH 10) at two different temperatures were performed. The first reaction mixture was maintained at 60 ± 0.1 °C for 72 h and the second one at 37 °C for 300 h. Samples taken at different time intervals were neutralised with hydrochloric acid ($c = 2$ mol l^{-1}) and analysed by HPLC. Experiments were conducted in triplicate and five replicates of the samples were injected. The fenoprofen concentration was determined utilising the regression equation for the standard curve.

To ensure that fenoprofen itself was stable under these circumstances, two fenoprofen calcium samples (equivalent to 52.1 μg ml^{-1} of free fenoprofen) were kept under the analogous conditions (same buffer, temperature, time span) and analysed.

2.2.5. Fenoprofen release from PHEA-Fen conjugate at pH 1.1

0.075 g of PHEA-Fen conjugate was dissolved in 50 ml glycine buffer solution pH 1.1 and 5 ml dimethylsulphoxide (DMSO) as co-solvent. The reaction mixture was maintained at 37 ± 0.1 °C for 800 h. Samples taken at different time intervals were neutralised with sodium hydroxide ($c =$ 2 mol l^{-1}) and analysed by HPLC. To avoid hydrolysis of the conjugate during the neutralisation process, a cold NaOH solution was added under agitation. Six replicates of the experimental procedure were conducted and five replicates of each sample were injected. DMSO contributed to UV interference resulting in a fenoprofen peak area reduction. To compensate this phenomenon a standard curve for fenoprofen in the presence of DMSO was utilised for the HPLC quantitative analysis.

A fenoprofen calcium sample (equivalent to 52.1 μg ml^{-1} of free fenoprofen) dissolved in glycine buffer was kept at the same temperature for the same time period to ensure that fenoprofen is stable under these conditions.

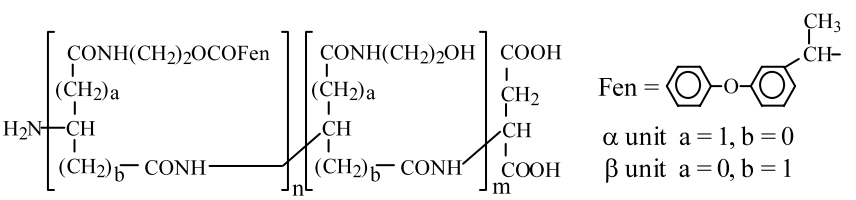

Fig. 1. Chemical structure of PHEA-Fen conjugate.

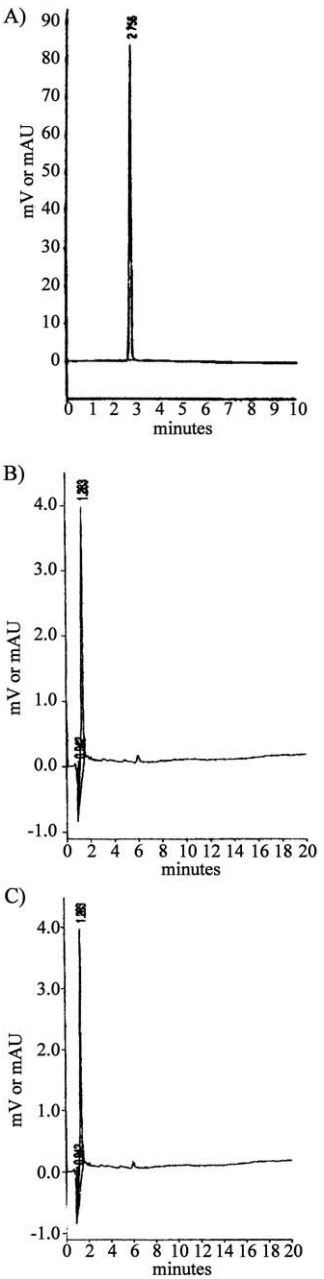

Fig. 2. Chromatogram of fenoprofen (A), PHEA-Fen (B) and PHEA (C).

2.2.6. PHEA-Fen conjugate powder dissolution tests in simulated biological fluids

Six samples of 0.100 g PHEA-Fen conjugate were vortexed in 10 ml of dissolution medium (SGF or SIF) for 2 min and then introduced into the dissolution vessel containing 900 ml dissolution medium. The solution in the vessels was stirred at 100 rpm. Samples taken at different time intervals (5, 10, 15, 30, 45, 90, 120, 200, 500, 700 min) were analysed by HPLC.

3. Results and discussion

PHEA-Fen conjugate (Fig. 1) was prepared from PHEA and fenoprofen benzotriazolide according to the procedure previously published, with a small change in the reactants ratio (Zorc and Butula, 1994). IR and UV spectra were identical with the previously described product, but the conjugates differed in fenoprofen content. The drug loading was estimated both by HPLC method and UV spectroscopy. The HPLC data were obtained by determining the concentration

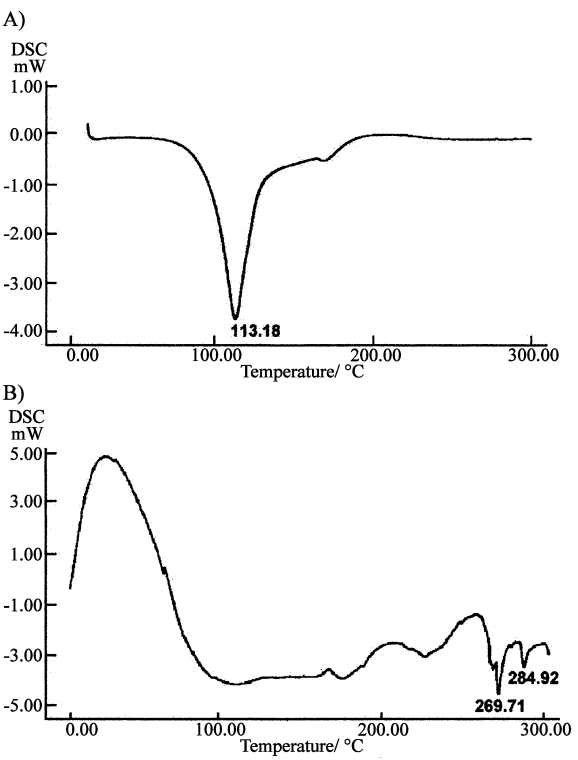

Fig. 3. DSC-thermogram of fenoprofen calcium (A) and PHEA-Fen (B).

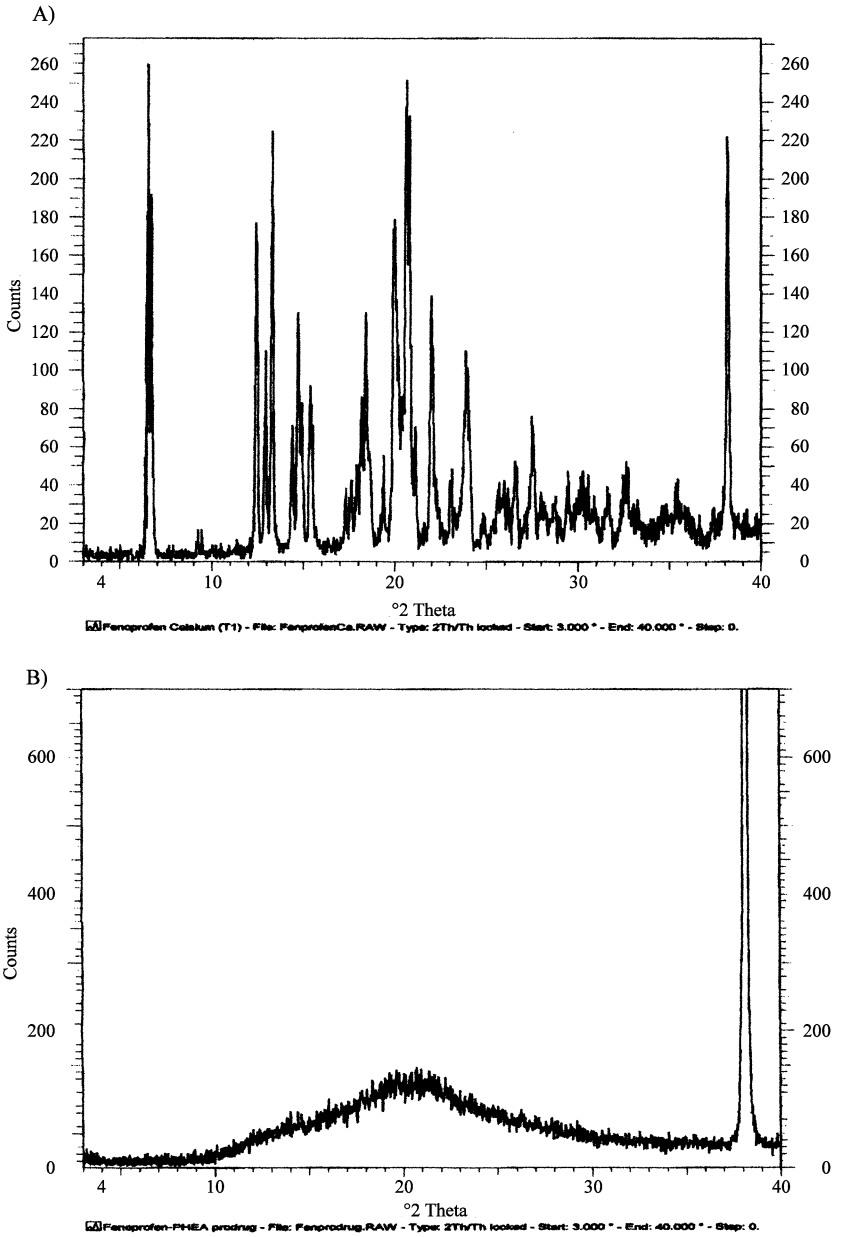

Fig. 4. X-ray powder diffractometric pattern of fenoprofen calcium (A) and PHEA-Fen (B).

of free fenoprofen after total hydrolysis of the polymer–drug linkages. The UV spectroscopy data were obtained by comparing the absorbance of fenoprofen and the conjugate at 272 nm. Both results were in good agreement and the fenoprofen loading was estimated as 14% (m/m). The HPLC chromatogram of the conjugate showed that all fenoprofen was chemically bound to the PHEA carrier and not merely dispersed or incorporated into polymer (Fig. 2). Additionally, the absence of the free drug in the conjugate was proved by thin layer chromatography and DSC

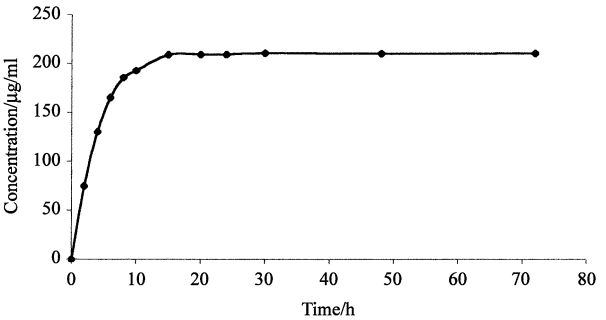

Fig. 5. Fenoprofen released versus time in borate buffer pH 10 (60 °C) (RSD 0.12–1.10%).

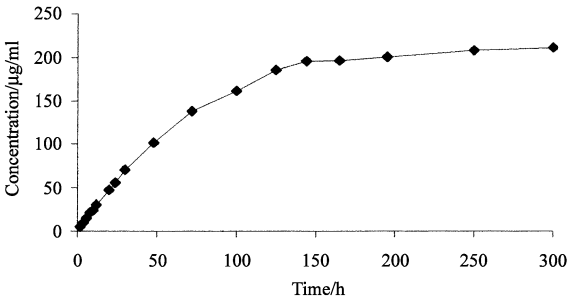

Fig. 6. Fenoprofen released versus time in borate buffer pH 10 (37 °C) (RSD 0.01–0.69%).

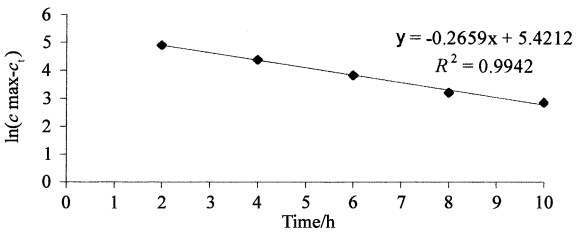

Fig. 7. Plot of $\ln(c_{max} - c_t)$ versus time for the hydrolysis at pH 10 (60 °C).

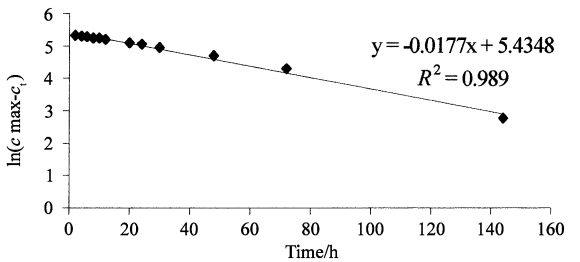

Fig. 8. Plot of $\ln(c_{max} - c_t)$ versus time for the hydrolysis at pH 10 (37 °C).

thermography. PHEA-Fen melted at 270 °C (Fig. 3), while fenoprofen calcium melting point was 118–123 °C. In the DSC thermogram of the conjugate no peak was visible near the fenoprofen melting point. The PHEA-Fen showed a totally new X-ray powder diffractometric pattern, in comparison to fenoprofen calcium (Fig. 4).

In order to fully characterise PHEA-Fen conjugate, solubility test was performed. The conjugate was totally insoluble in the following organic solvents: ethanol, methanol, acetonitrile, THF, cyclohexane, diethyl ether, chloroform. In water, DMSO and DMF it was soluble but turned into a gel at high concentrations, making it difficult to determine the saturation level (in water the gel transformation occurred at concentration 90 mg ml^{-1}).

In a previous paper the development and validation of a HPLC method for the determination of fenoprofen in PHEA conjugates was described (Boneschans et al., 2002). The same method was utilised to follow the kinetics of fenoprofen release from the PHEA-Fen conjugate. Kinetics studies were performed in aqueous buffer solutions and simulated biological fluids (SGF and SIF). Fenoprofen release from the conjugate in basic conditions was performed in borate buffer solution pH 10 at 60 and 37 °C, respectively (Figs. 5 and 6).

The chemical rate constants (k) for the reactions were determined from the slope of plot $\ln(c_{max} - c_t)$ versus time, where c_{max} was the average maximum concentration of fenoprofen released and c_t fenoprofen concentration in a particular time interval (Figs. 7 and 8). The values of c_{max} were obtained by calculating the average amount of fenoprofen present after 30, 50 and 70 h for hydrolysis at 60 °C ($c_{max} = 210.50$ µg ml^{-1}) and after 300 h for hydrolysis at 37 °C ($c_{max} =$ 211.47 µg ml^{-1}). The following chemical rate constants were obtained: $k_1 = 0.2659\ h^{-1}$ at $t_1 =$ 60 °C (338 K) and $k_2 = 0.0177\ h^{-1}$ at $t_2 = 37$ °C (315 K). From these data the activation energy (E_a) was calculated using the Arrhenius equation:

$$\ln \frac{k_2}{k_1} = \frac{E_a}{R} \frac{T_2 - T_1}{T_1 T_2}.$$

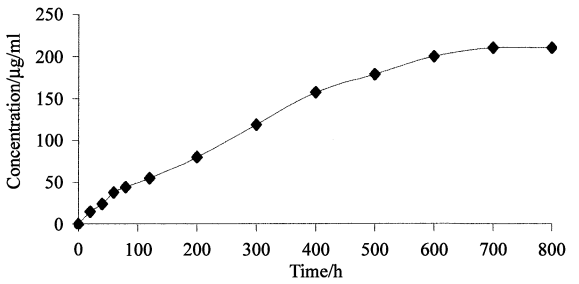

Fig. 9. Fenoprofen released versus time in glycine buffer pH 1.1 (37 °C) (RSD 0.05–1.98%).

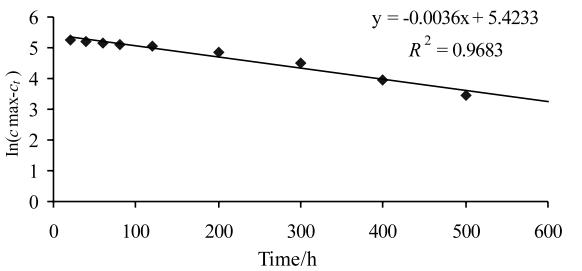

Fig. 10. Plot of $\ln(c_{max} - c_t)$ versus time for the hydrolysis at pH 1.1 (37 °C).

The value 100.6 kJ mol^{-1} was obtained, which was indicative for the breakage of a sigma bond, proving once again that fenoprofen was covalently bond to the polymeric backbone.

Hydrolysis in acidic medium was performed in glycine buffer solution pH 1.1 at 37 °C. The average concentration of fenoprofen was plotted as a function of time to show progress of the reaction (Fig. 9). The hydrolysis rate constant was determined as 0.0177 h^{-1} (Fig. 10), showing that fenoprofen release in an acidic medium was much slower than in a basic medium ($c_{max} = 209.73$ μg ml^{-1} was reached after 800 h). On the other hand, in SGF and SIF no significant hydrolysis was observed within 12 h.

4. Conclusion

In order to fully characterise PHEA-Fen conjugate the drug loading was estimated, solubility test was performed and DSC thermogram and X-ray powder diffractometric pattern were recorded. The hydrolysis of ester bonds between polymeric carrier and fenoprofen occurred in basic and acidic conditions showing that the conjugate was able to gradually release the active fenoprofen and therefore it could be a potentially useful macromolecular prodrug.

Acknowledgements

This work was supported by Grant 006243 of the Ministry of Science and Technology of the Republic of Croatia.

References

Al-Shamkhani, A., Duncan, R., 1995. Synthesis, controlled release properties and antitumor activity of alginate-*cis*-aconityl-daunomycin conjugates. Int. J. Pharm. 122, 107–119.

Boneschans, B., van der Merwe, T., Wessels, A., Zorc, B., 2002. Validation of the HPLC method for model determination of fenoprofen in conjugates with PHEA. Acta Pharm. 52, 37–43.

Duncan, R., Dimitrijević, S., Evagorou, E.G., 1996. The role of polymer conjugates in the diagnosis and treatment of cancer. S.T.P. Pharma Sci. 6, 237–263.

Duncan, R., Coatsworth, J.K., Burtles, S., 1998. Preclinical toxicology of a novel polymeric antitumor agent HMPA copolymer-doxorubicin (PK1). Hum. Exp. Toxicol. 17, 93–104.

Giammona, G., Puglisi, G., Cavallaro, G., Spadaro, A., Pitarresi, G., 1995. Chemical stability and bioavailability of acyclovir coupled to α,β-poly(*N*-hydroxyethyl)-DL-aspartamide. J. Controlled Release 33, 261–271.

Giammona, G., Cavallaro, G., Fontana, G., Pitarresi, G., Carlisi, B., 1998. Coupling of the antiviral agent zidovudine to polyaspartamide and in vitro drug release studies. J. Controlled Release 54, 321–331.

Lovrek, M., Zorc, B., Boneschans, B., Butula, I., 2000. Macromolecular prodrugs. VIII. Synthesis of polymer–gemfibrozil conjugates. Int. J. Pharm. 200, 59–66.

The United States Pharmacopeia 24, Supplement 1, 2000a. United States Pharmacopeial Convention, Inc., Rockville, p. 2697.

The United States Pharmacopeia 24, 2000b. United States Pharmacopeial Convention, Inc., Rockville, pp. 2235–2236.

Vasey, P.A., Kaye, S.B., Morrison, R., Twelves, C., Wilson, P., Duncan, R., Thomson, A.H., Murray, L.S., Hilditch, T.E., Murray, T., Burtles, S., Fraier, D., Frigerio, E., Cassidy, J., 1999. Phase I clinical and pharmacokinetic study of PK1 [*N*-(2-hydroxypropyl)methacrylamide co-

polymer doxorubicin]: first member of a new class of chemotherapeutic agents-drug-polymer conjugates, Cancer Research Campaign Phase I/II Committee. Clin. Cancer Res. 5, 83–94.

Zorc, B., Butula, I., 1994. Macromolecular prodrugs. III. Esters of fenoprofen and probenecid. Acta Pharm. 44, 103–108.

Zovko, M., Zorc, B., Lovrek, M., Boneschans, B., 2001. Macromolecular prodrugs. IX. Synthesis of polymer–fenoprofen conjugates. Int. J. Pharm. 228, 129–138.