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Synthesis and analysis of viscous poly (ortho-ester) analogs for controlled drug release

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Abstract

Viscous poly(ortho-ester)s (POE), synthesised by a transesterification reaction between a substituted orthoacetate and a triol have been shown to be bioerodible polymers and are currently under investigation as a drug carrier material. Main efforts of the polymer development so far have been focused on a polymer prepared of trimethyl orthoacetate and 1,2,6-hexanetriol. The present investigation describes the synthesis and characterisation of an analog polymer. By replacing the 1,2,6-hexanetriol with 1,2,10-decanetriol, a higher molecular weight aliphatic triol, the physio-chemical characteristics of the polymer can be changed. The new POE, prepared by a transesterification reaction of trimethyl orthoacetate and 1,2,10-decanetriol is less viscous and more hydrophobic. Due to the decreased viscosity the polymer is easier to use for injectable applications and due to the increased hydrophobicity, the release rate of the chosen compound, the water-soluble 5-fluorouracil (5-FU) is slowed down, compared to the POE described earlier. © 1997 Elsevier Science B.V.

Keywords: Poly(ortho-ester); Bioerodible polymer; Polymer synthesis; Polymer characterisation; Controlled release, 1,2,10-decanetriol

1. Introduction

Viscous poly(ortho-ester) (POE) prepared by a transesterification reaction between substituted orthoacetate and a triol are currently under investigation as injectable bioerodible polymers for controlled release of 5-fluorouracil (5-FU) and mitomycin C in glaucoma filtering surgery (Merkli et al., 1993, 1994, 1995; Bernatchez et al., 1994; Zignani et al., 1997a,b). The use of an aliphatic triol produces a polymer with viscous characteristics at room temperature due to flexible segments in the polymer chain. The semi-solid

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nature allows the incorporation of therapeutic agents by simply mixing without the need to use solvents or elevated temperatures.

The POE developed so far is synthesised by a transesterification reaction between trimethyl orthoacetate and 1,2,6-hexanetriol. The relatively short aliphatic chain length of 1,2,6-hexanetriol does not result in a very flexible structure and therefore the viscosity is rather high, especially for higher molecular weight POE (Merkli et al., 1993, 1994). Thus, the incorporation of drugs by simple mixing and direct injection at the target side becomes difficult. However, in vitro release studies have shown that only high molecular weight POE or POE stabilised with basic excipients are able to release the drugs over a time period of up to 2 weeks as desired for the controlled release of antiproliferative agents in glaucoma filtering surgery (Blandford et al., 1992). The stabilisation of POE with basic excipients, based on the pH-sensitive structure of POE, is possible but leads to drug release predominantly controlled by diffusion (Merkli et al., 1995).

The present investigation evaluated possible structural modification of the earlier described viscous POE_{C6} (C6 stands for 1,2,6-hexanetriol) in order to modulate the characteristics and the release behaviour of POE. Changes can be done by the substitution of the orthoacetate as well as by the variation the number of carbons of the triol. A triol with an increased number of carbons was used to prepare the polymer and was compared to the POE used earlier. The studies have been carried out with a polymer POE_{C10} (C10 stands for 1,2,10-decanediol) prepared from 1,2,10-decanetriol and trimethyl orthoacetate.

2. Materials and methods

The materials have been purchased as follow: 9-decene-1-ol from Alfa®, Johnson Matthey GmbH, Germany, osmium tetraoxide (OsO_4) from Johnson Matthey GmbH, Zurich, Switzerland. 1,2,6-hexanetriol and trimethyl orthoacetate from Aldrich®-Chemie, Steinheim, Germany, 5-fluororacil (5-FU) from Sigma® Chemie AG, Buchs, Switzerland and tetrahydrofuran (THF) from Romil Chemical, Leicester, England. All other chemicals and solvents have been purchased from Fluka® -Chemie AG, Buchs Switzerland and used as received. The reference polymer POE_{C6} has been synthesised in our laboratory and purified as described earlier (Merkli et al., 1993).

2.1. Synthesis

To synthesise the precursor 1,2,10-decanetriol, 25.0 g (160 mM) of 9-decene-1-ol was dissolved in a solution made up of t-butanol (100 ml), acetone (90 ml) and distilled water (10 ml). Then, 26.6 g (240 mM) of trimethylamine-*N*-oxide and 2 ml of a solution of OsO_4 in t-butanol (2% m/v) was added. The resulting solution was stirred for 20 h and 50 ml of an aqueous solution of sodium bisulfite (10%) was added. The mixture was concentrated, taken up in trichloromethane, and washed twice with water, dried with Na_2SO_4 and concentrated to give 1,2,10-decanetriol. Before drying under vacuum the solid triol was washed twice with diethylether.

The polymer POE_{C10} (III) was prepared and purified accordingly to POE_{C6} by an acid catalyzed transesterification reaction of 7.21 g (60 mmol) trimethyl orthoacetate (I) and 11.42 g (60 mmol) 1,2,10-decanetriol (II) (Merkli et al., 1993) (Fig. 1). The product was a slightly yellowish viscous material.

2.2. Characterisation

The precursor and polymer structures have been confirmed using Fourier transform infra-red spectroscopy (FT-IR) and nuclear magnetic resonance spectroscopy (NMR). The FT-IR spectra were obtained with a 1600 series FT-IR spectrometer (Perkin-Elmer AG, Küsnacht, Switzerland), whereas ^1H NMR and ^{13}C NMR spectra of the triol and POE were performed in Acetone-D6 and $CDCl_3$, respectively, at room temperature using a AC200S-Bruker® spectrometer (Spectrospin, Fällanden, Switzerland). Thermal analysis of the precursor has been performed using a Perkin-Elmer DSC-4 microcalorimeter (Küsnacht, Switzerland). The microcalorimeter was calibrated with indium. The samples were heated at 5°C/min

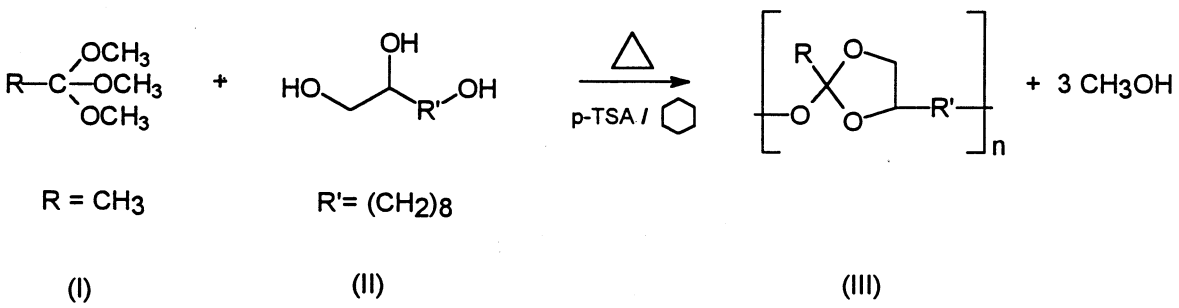


Fig. 1. Synthesis of POE_{C10}.

under a flow of nitrogen. The average molecular weights of the synthesised POE were determined by gel permeation chromatography (GPC) using a modified method reported earlier (Merkli et al., 1996). The new method was developed on a Waters® 600E instrument with a series of four Waters®-Styrogel HR® columns 1–4 (Waters®, Rupperswil, Switzerland). All determinations were carried out in THF at 30°C with a 1 ml/min flow rate. A refractometer Waters® 410 was used as a detector. To calibrate the system, monodispersed polystyrene standards of the following molecular weights were used: 500, 2630, 5970, 9100, 18100, 37900, and 96400 Da (Tosoh, Tokyo, Japan).

The viscosity determination was carried out as described earlier on a Bohlin® Controlled Stress Rheometer with a parallel plate PU 20 device (Bohlin® Rheology GmbH, Mühlacker, Germany) (Merkli et al., 1994).

The volatile compounds were analysed quantitatively by equilibrium headspace gas-chromatography with the technique of multiple head-space extraction (Guimbard et al., 1991; Kolb, 1982). The method was developed on a HP 7694 Headspace connected to a HP 5890 (Series 2) gas-chromatograph (Hewlett Packard®, Urdorf, Switzerland) equipped with either a Trio-2 VG Masslab Automated Mass spectrometer (Fisons, Instruments, Folsom, CA) for qualitative analysis or a flame ionisation detector (FID) for quantitative analysis. The column used for the analysis was a filled column bonded with polyethylene glycol (HP INNOWax, 30 m × 0.25 mm × 0.5 μm). Samples of 20.0 mg POE were analysed in airtight closed 10 ml vials by performing four extraction steps (injections) per vial. The concentration of residual solvents were calculated form calibration curves which were effectuated in benzylalcohol using a concentration range of 0.004–0.063% (w/w) for cyclohexane, of 0.1–1.75% (w/w) for THF and of 0.04–0.65% (w/w) for methanol.

Release studies on 5-FU were conducted in thermostated cells at 37°C containing 200 mg of drug-POE-mix (1%, w/w) (Merkli et al., 1994). Phosphate buffer at pH 7.4 (0.15 M) was circulated through the cell at a rate of 6.67 ml/h, and collected every 6 h. The amount of drug release was measured at 266 nm with a diode array 8452A spectrophotometer (Hewlett-Packard®, Urdorf, Switzerland).

3. Results and discussion

3.1. Synthesis and analysis

Since this higher molecular aliphatic triol is not commercially available, it had to be synthetisized in our laboratory. The triol has been prepared by a *cis*-hydroxylation of 9-decene-1-ol with OsO_4 (Felgner and Eppstein, 1986). The melting point of 62°C and the purity have been determined by thermal analysis. Fig. 2 shows the FT-IR spectrum and the characteristic functional group of the 1,2,10-decanetriol. The structure has been confirmed by NMR and MS-analysis.

Structural analysis by NMR and FT-IR have shown that the structure of the new POE_{C10} is similar to the earlier described POE_{C6} (Merkli et

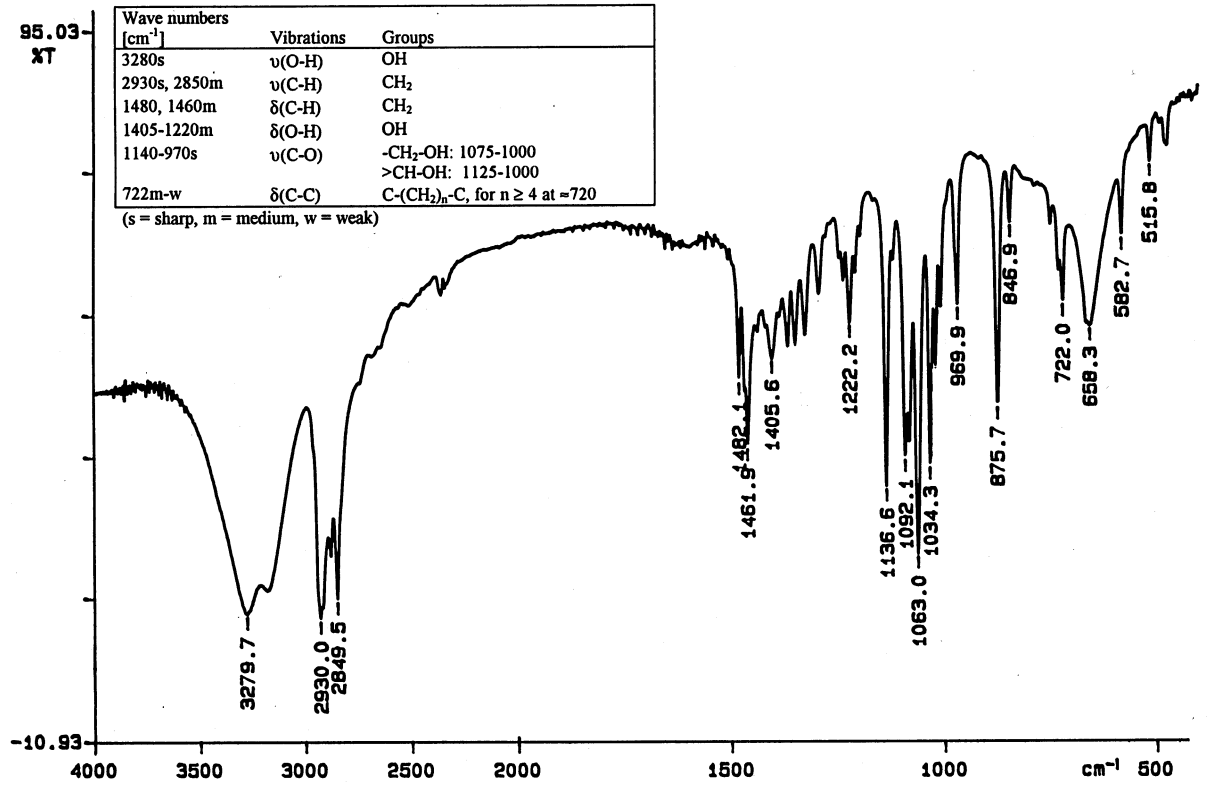


Fig. 2. Infrared absorption spectrum, characteristic vibrations and functional groups of the 1,2,10-decanetriol (KBR disc).

al., 1993). The characteristic functional groups of the IR-spectrum are listed in Table 1. The spectrum of POE_{C10} differs from the spectrum of POE_{C6} (Merkli et al., 1993) by the intensity of the vibrations for CH_2-groups at 1460 cm^{-1} as well as the shift of the peak from 740cm^{-1} for POE_{C6} to 724 cm^{-1} for POE_{C10}. Also the ^1H NMR and ^{13}C NMR spectra of the compared POE are in agreement. Table 2 compared the ^{13}C NMR spectra of POE_{C10} to the earlier published data of POE_{C6} (Merkli et al., 1993). The major difference between the two spectra are the additional four CH_2 groups for POE_{C10}. The additional CH_2 groups can also be observed in the ^1H NMR spectra of POE_{C10}, where they are expressed in the integral of the signal at the chemical shift δ of 1.46 to 1.62 ppm.

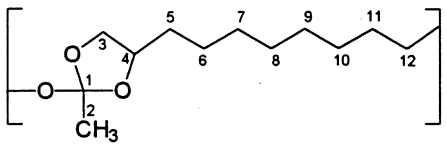
Table 1
Characteristic vibrations and functional groups of the POE_{C10}.

Wave numbers [cm^{-1}]	Vibrations	Groups
3530w	υ(O–H)	OH (terminal chain)
3000s, 2930s	υ(C–H)	CH_3, CH_2
2860s	υ(C–H)	OCH_3 (terminal chain)
1460m	δ(C–H)	CH_2
1380s	δ(C–H)	C–CH_3
1220s, 1160s	υ(C–O)	C–O–C (cycle)
1040s	υ(C–O)	C–OH (terminal chain)
724m-w	δ(C–C)	C–$(CH_2)_n$–C, for $n \geq 4$

s = sharp; m = medium; w = weak.

Gel permeation chromatography allowed the determination of weight average molecular weight M_w, number average molecular weight M_n and the polydispersity index (M_w/M_n). Polymers POE_{C10} with the following molecular weights have been synthesised: (a) M_w of 22 330 Da (M_n: 8 650 Da; M_w/M_n: 2.58), (b) M_w of 15 360 Da (M_n: 11 540 Da; M_w/M_n: 1.33), (c) M_w of 14 660 Da (M_n: 6600 Da; M_w/M_n: 2.22), and (d) M_w of 3110

Table 2
Comparison of the chemical shifts of 50 MHz carbon spectra of POE_{C6} [13] and POE_{C10}.



Carbon no.	Chemical shift δ POE_{C6} ^{13}C-NMR (ppm)	POE_{C10} ^{13}C-NMR (ppm)	Assignment	
1	121.31 and 121.67	121.21 and 121.56	C	(quarternary)
2	22.44 and 22.92	22.32 and 22.80	CH_3	
3	69.75 and 70.03	69.74 and 69.77	CH_2	(cycle)
4	76.31 and 76.84	76.92 and 77.62	CH	(cycle)
5	22.17 and 22.63	25.36 and 25.78	CH_2	
6	29.60 and 29.67	25.36 and 25.78	CH_2	
7	33.12 and 33.54	26.16 and 29.30	CH_2	
8	61.50 and 62.04	29.30 and 29.31	CH_2	POE_{C6}: terminal
9		29.40 and 29.50	CH_2	
10		29.68 and 29.78	CH_2	
11		33.35 and 33.75	CH_2	
12		61.92 and 62.42	CH_2	POE_{C10}: terminal

Da (M_n: 1 860 Da; M_w/M_n: 1.68), whereas POE_{C6} had the following molecular weights: (e) M_w of 33 300 Da (M_n: 7250 Da; M_w/M_n: 4.59) and (f) M_w of 11 930 Da (M_n: 8620 Da; M_w/M_n: 1.38).

The hypothesis has been confirmed that the additional CH_2-groups in the triol lead to a higher flexibility of the polymer structure, the viscosity has been measured. A 22.3 kDa POE_{C10} polymer shows a dynamic viscosity of 530 Pa·s at 37°C and of 1500 Pa·s at 25°C. For POE_{C6}, a logarithmic linear relationship of the average molecular weight (M_w) and the dynamic viscosity has been found (Merkli et al., 1994). The dynamic viscosity of a polymer depends on molecular weight and temperature. Based on this relationship the calculated dynamic viscosity for a 22.3 kDa polymer POE_{C6} at 37°C is 1161 Pa·s.

3.2. Residual solvents

For toxicity and quality control reasons, the analysis of residual solvents in the final product is necessary. The amount of residual solvents for both POE's is in the same range, for POE_{C10} 0.05% cyclohexane, 0.7% THF and 0.2% methanol, whereas for POE_{C6} 0.01% cyclohexane, 0.4% THF and 0.9% methanol has been found. The ICH guidelines (Igarashi, 1996; Wigman et al., 1996; Erni et al., 1996) defines residual solvents as impurities and classified the residual solvents into three groups based on their toxicity. According to the latest guidelines (Igarashi, 1996) cyclohexane and methanol belong to the class 2 solvents with an upper limit at 38.8 mg per day or 0.39% for cyclohexane and at 30.0 mg per day or 0.3% for methanol. THF belongs to the solvent of low toxicity with an upper limit at 50 mg per day or 0.5% without further justification. Since POE is applied as a dose of 100 mg, the requirements are not completely fulfilled; THF in POE_{C10} and methanol in POE_{C6} exceeds the limits of 0.5% for THF and 0.3% for methanol. However, the daily limits are not exceeded. Even subconjunctival injections have shown, that the amount of residual solvents found in POE does not affect the biocompatibility of the polymer (Zignani et al.,

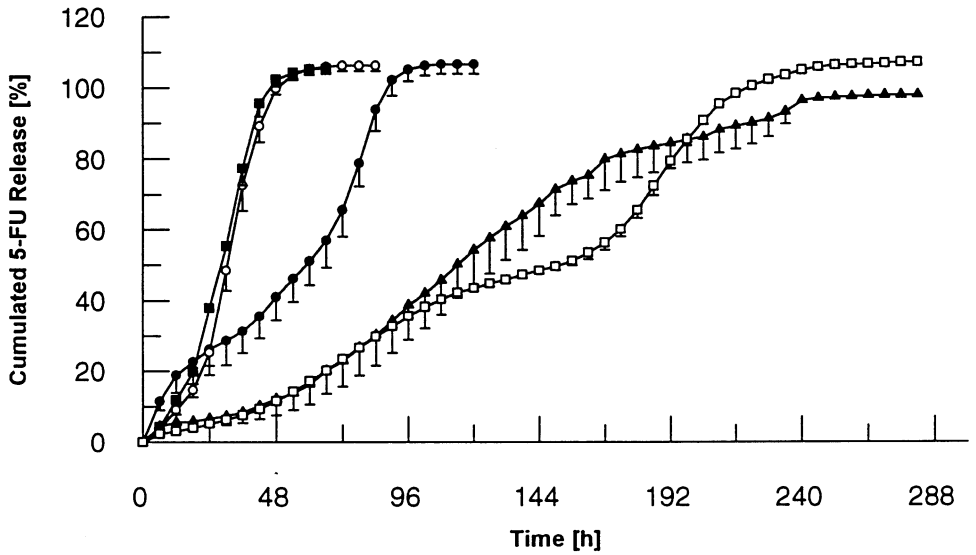


Fig. 3. Cumulative in vitro release of 5-FU (1% w/w) from different molecular weight POE_{C6} (open symbols) and from POE_{C10} (closed symbols) in phosphate buffer, pH 7.4 at 37°C. (○) 11.9 kDa; (□) 33.3 kDa; (■) 3.0 kDa; (●)15.3 kDa and (▲) 22.3 kDa. ($n = 3$, sdm).

1997b), further improvements should be made in order to further reduce the amount of residual solvents.

3.3. Release studies

A number of studies on POE_{C6} have shown that the release of 5-FU can be modulated by either the molecular weight of the carrier or by the incorporation of a small amount of acidic or basic excipients into the polymer (Merkli et al., 1994, 1995). The preliminary data of drug release studies from POE_{C10} are represented in Fig. 3. It is shown that the overall release profile is similar to the one observed with POE_{C6}, but the release rate is substantially decreased. The release of 5-FU follows over a wide range zero-order kinetics and depends on the molecular weight of the carrier. The retardation of the drug release rate from POE_{C10} is due to the higher hydrophobicity of the decanetriol compared to the hexanetriol. Therefore the release of 5-FU from a 3.1 kDa POE_{C10} is similar to the release from a 11.9 kDa C6-polymer. The 22.3 kDa POE_{C10} shows almost constant 5-FU release for more than a week and as it can be observed in Fig. 3 the release rate is almost similar to a 33.3 kDa POE_{C6}.

4. Conclusion

A structural variation of the earlier described viscous POE has been prepared. Due to the longer aliphatic chain length of the triol used, the characteristics of the polymer can be modulated. The new POE_{C10} is compared to POE_{C6} and is less viscous and more hydrophobic. Due to the reduced viscosity, the incorporation of drugs by simple mixing and the direct injection at the target side are easier. Due to the higher hydrophobicity of POE_{C10}, the release rate of 5-FU is slowed down.

This preliminary studies have shown, that it would be interesting to synthesise viscous POEs with other triols, such as 1,2,12-dodecanetriol or 1,2,14-tetradecanetriol in order to obtain a complete picture of the influence of the aliphatic chain length and their characteristics of viscous POE.

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