Synthesis and characterisation of polyethylene glycol conjugates of hydrocortisone as potential prodrugs for ocular steroid delivery

S.M. Foroutan, D.G. Watson *

Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G1 1XW, Scotland, UK

Abstract

A method for the synthesis of polyethyleneglycol esters of hydrocortisone 21-hemisuccinate (H-PEGs) as potential ocular prodrugs, was developed using dicylohexylcarbodiimide to promote esterification. The products were purified by semipreparative high performance liquid chromatography (HPLC). The steroid derivatives were, mainly, highly water soluble. The products were characterised by NMR, fast atom bombardment mass spectrometry and elemental analysis. The polyethylene glycol esters were studied with respect to their stability in aqueous solution and their partition coefficients. © 1997 Elsevier Science B.V.

Keywords: Polyethyleneglycol esters; Ocular drug delivery; Prodrugs; Hydrocortisone

1. Introduction

When a drug is applied to the eye only a small fraction of the dose is absorbed most of the dose is lost via the nasolachrymal duct, one approach to improved drug delivery to the eye is the synthesis of prodrugs (Lee and Li, 1989). Polyethylene glycols (PEGs) are water soluble lipophilic polymers which are widely used in pharmaceutical preparations because of their safety, biocompatibility and lack of antigenicity and toxicity (Pang, 1993). PEGs have been considered for use as promoieties for the synthesis of prodrugs in which the properties of the original drug are altered advantageously. These polymers when covalantly bound to drugs, enzymes or other bioactive molecules, alter their physicochemical and biological properties. They have been found to change the pharmacokinetic behaviour of drugs and improve the stability of protein drugs in vivo. In previous work drugs such as asparginase (MacEwen et al., 1987), 5-chloro-8-quinoline (Rashcov et al., 1993), 2-benzoxazolone-3-yl-

* Corresponding author. Tel./fax: +44 141 5484400; e-mail:D.G.Watson@strath.ac.uk

acetic acid (Mincheva et al., 1994), taxol (Green-wald et al., 1994), proteins (Fuke et al., 1994; Vestling et al., 1993; Weiner, 1993), phenytoin (Dal-Pozzo and Acquasaliente, 1992), penicillin V, aspirin, amphetamine, quinidine, atropine (Zalipsky et al., 1983) and indomethacin (Bonina et al., 1995), have been attached to PEGs to form prodrugs.

In previous studies involving synthesis of drug-PEG conjugates, complex extraction procedures have been used to isolate the products from the reaction mixtures and in some cases protective groups have been attached to one hydroxyl end group to facilitate purification of product and to ensure attachment of the drug at one end only (Dal-Pozzo et al., 1989). The present work aims to establish a simple procedure for the synthesis for corticosteroid-PEGs as potential prodrugs to improve ocular corticosteroid delivery.

2. Materials and methods

2.1. Chemicals

All the chemicals used were readily available from the usual sources, e.g. Sigma–Aldrich, Poole, Dorset.

2.2. Instrumentation

The hydrocortisone 21-hemisuccinate polyethylene glycol esters (H-PEGs) were characterised by a variety of analytical techniques. ^{1}H NMR spectra were recorded in CDCl$_3$ solution with TMS as a reference standard at 250 MHz by using of Bruker Spectrospin NMR spectrometer. Mass spectra were obtained using a JEOL JMS-AX505HA mass spectrometer with a FAB ion source using xenon fast atoms and dissolving the compound at ca 0.5 mg/ml in a m-nitrobenzyl alcohol matrix. The instrument was connected to a PC with JEOL complement software. Chemical analyses of samples were obtained using a Perkin Elmer 2400 CHN analyser. For analytical high-performance liquid chromatography (HPLC) a Thermo Separation Products model P100 pump was used and this was linked to a Knauer UV

detector. The system was fitted with a Rheodyne injection valve with a 20 μl loop. The detector was connected to a Hewlett Packard integrator model HP 3395. A column (25cm × 4.6 mm i.d.) packed with hypersil RP-18 with 5 μm particle size (Phenomenex, Macclesfield, UK) was used for analytical separation. A Gilson model 132 refractive index detector was used in some preliminary analyses to establish where polyethylene glycols eluted. Preparative purification of the products was achieved using a Gilson preparative HPLC system fitted with a model 118 UV/vis detector with a peparative scale flow cell. The system was fitted with a Rheodyne injection valve fitted with a 0.5 ml loop. A column (30 cm × 10 mm i.d.) packed with Econo-prep C18 with 5 μm particle size (Phenomenex) and fitted with a guard column (5 × 10 mm) was used for semipreparative separation of the product from reagents and starting materials.

2.3. Synthesis and purification of H-PEGs

Hydrocortisone-21-hemisuccinate (460 mg, 1 mmol) and 4-dimethylaminopyridine (60 mg, 0.5 mmol) were added to a solution of polyethylene glycol or (2 mmol of either PEG200, PEG400, PEG600, PEG900, PEG 2000) and dicyclohexyl-carbodiimide (DCC, 412 mg, 2 mmol) in tetrahydrofuran (15 ml). The mixture was stirred and after 15–30 min a white precipitate of dicyclohexyl urea (DCU) was formed. The reaction was monitored using HPLC and allowed to proceed until the hydrocortisone 21-hemisuccinate was completely consumed. After about 6–8 h the precipitate was filtered off and the filtrate was evaporated to dryness. The residue was redissolved in acetone, filtered to remove further DCU and then evaporated again and the residue was dissolved acetonitrile (8 ml) and injected in 0.5 ml aliquots onto the semi-preparative HPLC column. The column was eluted at ambient temperature with 40:60 acetonitrile and phosphate buffer (0.05 M, pH 3.5) or 65:35 methanol and phosphate buffer (0.05 M, pH 3.5) at a rate of 6 ml/min. The column effluent was monitored at 242 nm with the detector range setting at 20 absorbance units full scale (AUFS). Fractions were collected at the

Table 1
Analytical data of polyethylene glycol esters of hydrocortisone 21-succinate

Compound	Formula	Average MW	Synthetic yield (%)	Elemental analysis (%)	
				Calculated	Found
Hydrocortisone	$C_{21}H_{30}O_5$	362			
Hydrocortisone 21-hemisuccinate	$C_{25}H_{34}O_8$	462			
H-PEG200	$C_{33}H_{50}O_{12}$	638	80	C 61.56	61.69
				H 7.99	7.77
H-PEG400	$C_{43}H_{70}O_{17}$	858	67	C 60.12	59.85
				H 8.21	7.98
H-PEG600	$C_{51}H_{86}O_{21}$	1034	65	C 59.98	59.16
				H 8.37	8.70
H-PEG900	$C_{65}H_{114}O_{28}$	1342	60	C 57.98	57.98
				H 8.57	8.70
H-PEG2000	$C_{115}H_{40}O_{53}$	2442	56	C 56.55	56.52
				H 8.33	8.33

[a] Average values of n are given since commercially available PEGs are mixtures of oligomers.

outlet from the detector. The fractions containing the product were combined and then concentrated under vacuum using a rotary evaporator and the remaining buffer solution (200 ml) was extracted with dichloromethane (3 × 250 ml). The purified products were obtained after evaporating off the dichloromethane under vacuum were subjected to analysis by mass spectrometry, ^{1}H NMR and to elemental analysis. The analytical results confirmed the formation of pure polyethylene glycol esters of hydrocortisone 21-succinate. The polyethylene glycol 200, 400, 600, 900 and 2000 derivatives of hydrocortisone 21-succinate are referred to H-PEG200, H-PEG400, H-PEG600, H-PEG900 and H-PEG2000 respectively. The elemental analyses (C, H, O) of the H-PEGs were all within ± 0.23% of the theoretical values (Table 1).

All of the H-PEGs contained the following typical features in their ^{1}H NMR spectra: ca δ 5.65 singlet for C4-H in the hydrocortisone ring A; ca δ 4.95 for hydrocortisone 21-CH_2 which appears as a doublet of doublets due to the conformational restriction introduced by the bulky PEG side chain; ca δ 4.43 multiplet due to C11-H of hydrocortisone; ca δ 4.23 two overlapping multiplets due to ($-COO-\underline{CH_2}-CH_2-O-$) where the two protons are non-equivalent due to conformational restriction; ca δ 3.56–3.76 multiplet for ($-O-CH_2CH_2-O-$) of the polyether chain; ca δ 1.42 singlet for 19-CH_3; ca δ 0.90 Singlet 18-CH_3. The FAB mass spectra were all similar to that of H-PEG 400 which is shown in Fig. 1. The ions at m/z 727.4, 771.4, 815.4, 859.4, 903.5, 947.5, 991.5, 1035.5 are for H-PEGs with ($n = 6, 7, 8, 9, 10, 11, 12, 13$) oxyethylene units, FAB gives molecular ions as $[M + H]^+$. The series of molecular ions is parallelled by a series of ions in which H_2O has been lost from the molecular ion, e.g. the ion at m/z 841 in Fig. 1. The ion at m/z 489.3 is due to [hydrocortisone $-$ $COCH_2CH_2COO$ $-$ CH_2CH_2]$^+$ and the ion at m/z 471.3 is due to [CH_2CH_2COO-$(CH_2CH_2O)9H$]$^+$.

2.4. HPLC method for analysis of H-PEGs

The various PEG esters and the parent steroid were characterised by analytical reversed phase HPLC. The esters were dissolved in phosphate buffer (pH 3.5) and chromatographed with a solvent system consisting of methanol/water mixed in the ratio 60:40 v/v. A flow rate of 1 ml/min was used and the sensitivity was maintained at 0.32 AUFS. The column effluent was monitored at 242 nm.
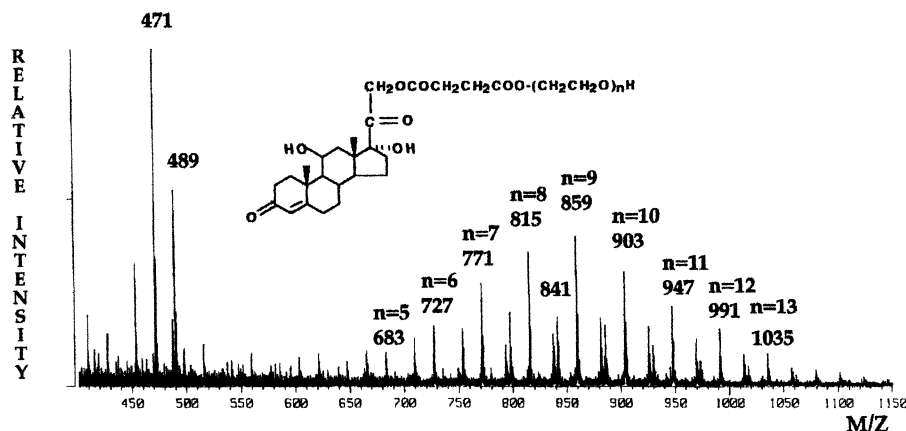
Fig. 1. FAB mass spectrum of H-PEG 400 obtained with a *m*-nitrobenzyl alcohol matrix and with xenon fast atoms.

2.5. Studies on the stability of H-PEGs

The chemical stability of the esters was studied in aqueous buffer solutions. The buffers used were a range McIlvaine buffered solutions with pH values of 2.2, 3.0, 4.0, 5.0, 6.0, 7.0 and 7.4. The buffers contained 0.1 M citric acid and 0.2 M disodium hydrogen phosphate solutions in various proportions. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride (Perrin and Boyd, 1974). Stock solutions of H-PEGs were prepared by dissolving the appropriate amount of prodrug in acetonitrile in order to obtain a concentration of 20 μmol/ml. All stock solutions were kept in screw-capped vials at 4°C. The degradation reactions were initiated by adding 50 μl of a stock solution of the H-PEG to 3 ml of pre-heated buffer solution containing 0.1% of Dowcil 200 in screw capped test tubes. The solutions were kept in a water bath at a constant temperature of 37°C and at appropriate intervals samples were taken and chromatographed immediately. Samples were analysed for remaining H-PEG as well as for free hydrocortisone using the HPLC assay described above, analyses at each pH were repeated three times. Quantification of the H-PEGs and the hydrocortisone formed upon hydrolysis was carried out by measuring peak areas in relation to those of standards of known concentration chromatographed under the same conditions.

2.6. Determination of partition coefficient of H-PEGs

The partition coefficients of the hydrocortisone PEG esters were determined between *n*-octanol and phosphate buffer buffer (pH 7.4) at 22°C. In order to prepare the buffer an isotonic buffer diluting solution was used with the following composition: KH_2PO_4, 1.9 g; $Na_2 HPO_4$, 8.1 g; NaCl, 4.11 g made up to 1000 ml with water. The octanol and buffer phases were mutually saturated before use. Aliquots (40 μl) of the hydrocortisone PEG ester stock solutions were added to 2 ml of aqueous buffer phase and were shaken with 0.5 ml octanol for 5 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase before and after distribution, could be measured readily using the HPLC method. At distribution equilibrium the two phases were separated by centrifugation for 2 min. The partition coefficients were calculated from following equation:

$$P = \frac{C_i - C_w}{C_w} \times \frac{V_w}{V_o}$$

where C_i and C_w represent the solute concentrations in the aqueous phase before and after distribution, respectively. V_w represents the volume of the aqueous and V_o the volume of the octanol phase.

3. Results and discussion

Esterification is a widely employed procedure in organic synthesis and many methods have been developed to accomplish it. A variety of reactions have been carried out using cationic ion exchange resins as catalysts for esterification (Charabarti and Sharma, 1993). Carboxylic acids and alcohols have been esterified using Amberlyst-15, a macro reticular ion exchange resin which contains strongly acidic sulfonic groups, as a catalyst (Rosini, 1988). However, when this approach was used in the current study it was found by analysis using FAB-MS that the amberlyst-15 catalyzed the dehydration of hydrocortisone, probably at the 17-position. A common method for esterification of carboxylic acids at room temperature is through the use of DCC as a coupling agent (Hassner and Alexanian, 1978; Neises and Steglich, 1978). DCC was found to be a good coupling agent for promoting the esterification of hydrocortisone 21-succinate with PEGs. Dimethyl amino pyridine, a strong base, (Zalipsky et al., 1983) was used as a catalyst for the reaction of the PEG with hydrocortisone 21-hemisuccinate. The degradation of a functionalised PEG has been reported to occur when pyridine was used as acid scavenger, when the stronger base triethylamine was used as acid scavenger instead of pyridine no degradation was observed (Huang, 1992). In the current study THF was found to be a good solvent for the reaction because of the limited solubility of the DCU byproduct in this solvent.

At room temparature H-PEG 2000 is solid and H-PEGs 200, 400, 600 and 900 are oils. All of the conjugates are soluble in water and in the organic solvents ethanol, chloroform, THF and dichloromethane. As is indicated in Table 1 with increasing chain length of the yield H-PEG from the reaction decreases and this is due to poorer extraction from the aqueous solution remaining after HPLC separation of the higher molecular weight PEGs. It may be better to recover the larger H-PEGs by freeze drying or continuous extraction.

For analysis of the H-PEGs a reversed phase chromatographic method was developed. The chromatograms obtained from chromatography of H-PEG 200 and H-PEG 400 on an ODS column under the same mobile phase conditions are shown in Fig. 2. It was found that a methanol/water mixtures allowed the satisfactory separation of ca 12 oligomers. The number of peaks obtained in the chromatogram was consistent with the molecular weight distribution of the polyethylene glycols observed in the mass spectra.

PEGs have two equivalent hydroxyl end groups and to avoid the formation of disubstituted products, a large excess of the PEG must be used in the reaction mixture. The final products may not be sufficiently different from the starting polyethylene glycols for isolation by extraction methods. Dal-Pozzo and co-workers reported generally applicable methods for the preparation of PEG monoderivatives of molecular weight between 200 and 1000 (Dal-Pozzo et al., 1989). They used a trityl group for protection of one hydroxyl end group. This type of protection proved to be successful because the trityl group was sufficiently large to allow separation of the product from the reagents and starting materials. Also a general method was described for separating a monosubstituted PEG from reaction mixtures especially in
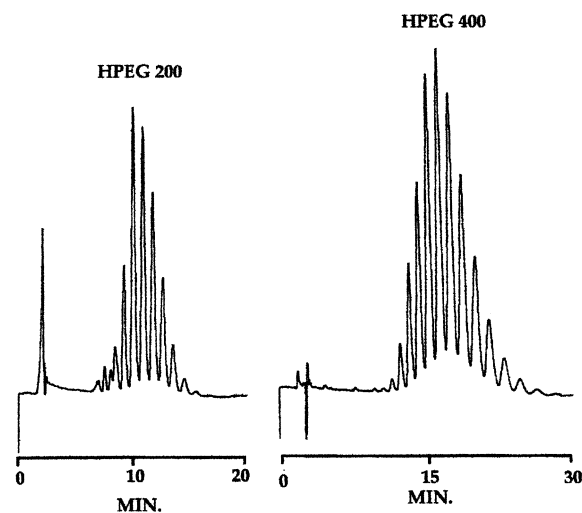
Fig. 2. HPLC chromatograms of H-PEG 200 and H-PEG 400. The analysis was carried out by reversed phase HPLC using an ODS column (4.6 × 25 cm). Conditions: Mobile phase: methanol/water (60:40 v/v), flow rate 1 ml/min; UV detector wavelength 242 nm.

the case of reaction with a high molecular weight PEG > 1000 (Huang, 1992). In the case of high molecular weight PEGs there is only a small difference in physical properties between the monosubstituted PEG and the unsubstituted PEGs and most studies have used an excess of the compound being derivatised over the PEG. The advantage of these methods is that at the end of reaction the amount of raw PEG in reaction mixture is low but the disadvantage is the risk of producing of by-products. For example in the case of preparation of esters of 2-benzoxazolon-3-yl-acetic acid (BOAA) an excess of BOAA was used and resulted in the formation of N-acylurea derivatives as a side products (Mincheva et al., 1994). In the present work, in order to obtain pure monosubstituted PEGs, excess PEG was used in the reaction with hydrocortisone 21-hemisuccinate and a chromatographic method was developed in which the separation of hydro-cortisone esters from the excess PEG in the reaction mixture could be readily achieved. The PEGs are essentially unretained by the reverse phase column under the conditions used. This enabled the preparation of predominantly the monosubstituted PEG derivative and decreased the likelihood of the formation of N-acylurea derivatives which are formed as intermediates by reaction between DCC and the acidic compound being conjugated to the PEG, the excess of PEG encourages the complete reaction of these intermediates. The HPLC method gave wide separation between PEG2000 and H-PEG2000 even though their water/organic solvent partitioning properties are very similar. As a general method the use of DCC in promoting esterification with and acid bearing a chromophore might prove useful in the development of analytical methods for PEGs and other non-ionic surfactants.

The degradation kinetics of the H-PEGs was followed by adjusting the HPLC mobile phase conditions so that the H-PEG oligomers eluted as a single peak. Fig. 3 shows the chromatograms obtained from a partly degraded solution of polyethylene glycol esters of hydrocortisone 21-succinate in boric acid at pH 4.7. Calibration curves were constructed for each compound by plotting peak area versus the concentration for a
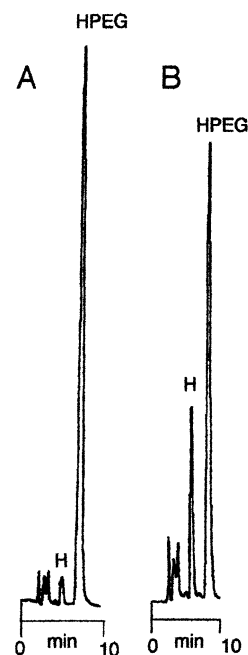

Fig. 3. HPLC chromatogram showing partial degradation of HPEG 200 after: (a) 6 and (b) 24 h in 2% boric acid solution (pH 4.7) at 60°C. H, hydrocortisone.

series of calibration standards. The instrument response were found to be linear (correlation co-efficients 0.999–1.000) over the range of concentrations (0.05–0.25 μmol/ml) used in our studies.

Most hydrolysis reactions are catalysed by hydrogen and hydroxide ions and their rates therefore depend upon the pH of the solution in which the compound is dissolved. A useful graphical display of this dependence consist of plots of kobs against pH or of log kobs against pH (Carstensen, 1990; Connors, 1993). At constant pH and temperature pseudo first order kinetics was observed for hydrolysis of the H-PEGs. For a reactant which is susceptible to either acid or base hydrolysis, if the rate constant is apparently first order the rate constant at a given pH can be determined using the following equation:

$$k_{obs} = k_H[H]^+ + k_0 + k_{OH}[OH]^- \qquad (1)$$

Here H^+ and OH^- refer to the hydrogen ion and hydroxide ion concentrations respectively, k_H and k_{OH} are second-order rate constants for specific

Table 2
Observed pseudo first-order rate constant (log k_{obs}) for the degradation of polyethylene glycol esters of hydrocortisone in aqueous solution at different pH values and at 37°C ($\mu = 0.5$)

pH	H-PEG200 (log $k_{obs} \pm$ S.D.)	H-PEG400 (log $k_{obs} \pm$ S.D.)	H-PEG600 (log $k_{obs} \pm$ S.D.)	H-PEG900 (log $k_{obs} \pm$ S.D.)	H-PEG2000 (log $k_{obs} \pm$ S.D.)
2.2	-3.401 ± 0.0737	-3.423 ± 0.0155	-3.456 ± 0.0405	-3.38 ± 0.0582	-3.492 ± 0.0355
3	-3.763 ± 0.058	-3.756 ± 0.0703	-3.773 ± 0.05	-3.716 ± 0.0451	-3.842 ± 0.0333
4	-4.087 ± 0.0681	-4.111 ± 0.0467	-4.143 ± 0.119	-4.205 ± 0.165	-4.148 ± 0.0418
5	-3.748 ± 0.0546	-3.812 ± 0.0315	-3.812 ± 0.018	-3.768 ± 0.0297	-3.795 ± 0.067
6	-2.991 ± 0.021	-3.06 ± 0.0156	-3.069 ± 0.018	-3.053 ± 0.0197	-3.065 ± 0.0286
7.4	-2.225 ± 0.00458	-2.252 ± 0.0046	-2.276 ± 0.003	-2.298 ± 0.0127	-2.304 ± 0.00416

acid and base catalysis, respectively and k_0 is a first-order constant for spontaneous degradation. It is possible to obtain k_0 from the minimum of the plot of log k_{obs} against pH and k_H and k_{OH} are obtained from the for the most alkaline and acidic conditions used by subtracting k_0. Table 2 shows the primary data used to calculate the k_H, k_{OH} and k_0 values for the HPEGs prepared in the current study which are shown in Table 3. The effect of pH on the rates of hydrolysis at 37°C is shown by the data points in Fig. 4 in which the logarithm of k_{obs} has been plotted against pH. The smooth curve ovelaying the data points was calculated with Eq. (1). Good correlation was observed between the calculated and experimental data. The pH at the minimum of curves can be easily derived from Eq. (1):

$$pH\ min = 1/2\ pk_w + 1/2\ \log k_H/k_{OH}$$

Here the pH of the minima which is the calculated pH of maximum stability is 4.18 ± 0.03 for H-PEG200, H-PEG400, H-PEG600, H-PEG900 and H-PEG2000. At all pH values there was no sig-

nificant difference between the hydrolysis kinetics of H-PEGs with different polymer chain length.

The log P values for hydrocortisone, hydrocortisone 21-hemisuccinate and the H-PEGs between n-octanol and buffer are shown in Table 4 as well as the log P values determined from HPLC capacity factors (Lein, 1993) and calculated by the fragment constant method (Dunn, 1986). During the entire procedure less than 2% hydrocortisone was formed as a result of ester hydrolysis as determined by HPLC. The following values have been reported for log P of hydrocortisone be-

Table 3
Rate constants obtained for the hydrolysis of H-PEGs in aqueous solution ($\mu = 0.5$, 37°C)

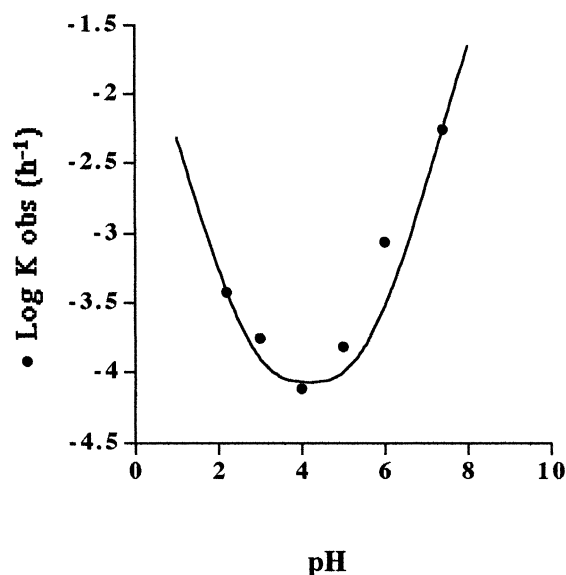| Compound	k_H ($M^{-1}\ h^{-1}$)	k_0 (h^{-1})	k_{OH} ($M^{-1}\ h^{-1}$)
H-PEG200	0.0500	0.0000818	23388
H-PEG400	0.0476	0.0000774	21976
H-PEG600	0.0441	0.0000719	20800
H-PEG900	0.0563	0.0000624	19796
H-PEG2000	0.0398	0.0000711	19486

Fig. 4. pH-rate profile for the degradation of H-PEG400 in aqueous solution at 37°C ($\mu = 0.5$). The points are experimental while the curve is calculated from Eq. (1). Each experimental point is the mean of three determinations.

Table 4
Partition coefficients of H-PEGs obtained by the three different methods

Compound	No. of units[a]	Rt[b]	Log K[c]	$P_{obs} \pm$ S.D.	Log P_{obs}	Log P_{cal}	Solubility (M)
Hydrocortisone	—	4.968	−0.184	40.5 ± 1.0	1.61	1.61	0.002
Hydrocortisone 21-hemisuccinate	—	3.348	−0.939	90.6 ± 1.5	1.96	1.74	
H-PEG 200	4	7.328	0.158	36.3 ± 0.6	1.55	2.16	0.102
H-PEG 400	9	7.775	0.200	11.8 ± 0.4	1.07	2.31	0.334
H-PEG 600	13	7.920	0.213	4.2 ± 0.7	0.622	2.43	1.012
H-PEG 900	20	8.152	0.237	0.72 ± 0.04	−0.139	2.64	2.273
H-PEG2000	45	8.770	0.283	—	—	3.39	High

[a] Number of oxyethylene units in H-PEGs.
[b] Rt, retention time of compounds obtained from HPLC. Mobile phase 40/60 acetonitrile/water.
[c] Log K obtained from the following equation: $K' = (t_r − t_0)$, where t_r is the retention time of the solute and t_0 is the elution time of the solvent ($t_0 = 3.005$ min).

tween *n*-octanol and water: 1.20 (Grass and Robinson, 1986), 1.35 (Lein et al., 1982), 1.50 (Fleisher et al., 1988), 1.58 (Dunn, 1986). The log P value of hydrocortisone determined in the current study was 1.61 agreeing with those reported by other authors. The log P value determined for hydrocortisone 21-hemisuccinate is suprising since it would be assumed that carboxylic acid would be highly ionised at pH = 7.4 thus reducing the partitioning of the molecule. For the H-PEGs the partition coefficients obtained using octanol-buffer decreased with increasing oxyethylene chain length. The greater the number of oxyethylene groups the stronger hydrogen bonding interactions with water and therefore the longer chain length gives higher water solubility and a lower partition coefficient.

The water solubilities of the H-PEGs were calculated from the partition coefficient data using the equations reported by Yalkowsky et al., 1983 that enables the estimation of the aqueous solubility of liquid organic non-electrolytes:

$$\text{Log } S_w = -1.072 \log P + 0.672$$

where S_w represents water solubility of the compound. The calculated solubilities of the H-PEGs are shown in Table 4.

Substituent or fragment constants were also used to estimate the partition coefficients of the H-PEGs. When a close congener with a measured log P value is available it is easier to use $\Sigma \pi$ method in calculating the log P of a structurally similar new derivatives. The results, Table 4, showed that from the theoretical point of view that the lipophilicity of H-PEGs increased with increasing chain length. A good correlation between calculated log P and the increase in capacity factor upon reverse phase HPLC with increasing chain length of the PEGs was observed. The interactions between the analytes and the HPLC stationary phase are more complex than simple partitioning between two immiscible phases. In the shake flask method presence of oxygen atoms close together in the polymer chains, gives greater hydrogen bonding between oxygen and water than is predicted by calculation. In the literature numerous examples have been presented to indicate that solute liquid chromatographic retention data can be use for measuring partition coefficient or solubility within a series of compounds, in the case of the H-PEGs it appears to have little validity.

Studies are currently being conducted on the hydrolysis of H-PEGs by corneal esterases and on their corneal penetration. The prodrugs are rapidly hydrolysed by corneal enzymes.

References

Bonina, F.P., Montenegro, L., Caprariis, D., Palagiano, F., Trapani, G., Liso, G., 1995. In vitro and in vivo evaluation of polyoxyethylene indomethacin esters as dermal prodrugs. J. Control Release 34, 223–232.

Carstensen, J.T., 1990. Solution kinetics. In: Drug Stability Principles and Practices. Marcel Dekker, New York, pp. 15–108.

Charabarti, A., Sharma, M.M., 1993. Cationic ion exchange resins as catalyst. Reactive Polym. 20, 1–45.

Connors, K.A., 1993. Hydrolysis of drugs. In: Swabrick, J., Boylan, J.C. (Eds.), Encylopedia of Pharmaceutical Technology. Marcel Decker, New York, 1993, pp. 1–29.

Dal-Pozzo, A., Acquasaliente, M., 1992. New highly water-soluble phenytoin prodrugs. Int. J. Pharm. 81, 263–265.

Dal-Pozzo, A., Vigo, A., Donzelli, G., 1989. New monofunctional derivatives of polyethylene glycols via monotrityl intermediates. Makromol. Chem. 190, 2457–2461.

Dunn, W.J., 1986. Partition Coefficient Determination and Estimation. Pergamon, Oxford, 1986.

Fleisher, D., Johnson, K.C., Stewart, B.H., Amidon, G.L., 1988. Oral absorption of 21-corticosteroid esters: A function of aqueous stability and intestinal enzyme activity and distribution. J. Pharm. Sci. 77, 3–14.

Fuke, I., Hayashi, T., Tabata, Y., Ikada, Y., 1994. Synthesis of polyethylene glycol derivatives with different branchings and their use for protein modification. J. Controlled Release 30, 27–34.

Grass, G.M., Robinson, J.R., 1988. Mechanisms of corneal drug penetration II: ultrastructural analysis of potential pathways for drug movement. J. Pharm. Sci. 77, 15–23.

Greenwald, R.B., Pendri, A., Bolikal, D., Gilbert, C.W., 1994. Highly water soluble taxol derivatives: 2′-polyethyleneglycol esters as potential prodrugs. Bioorg. Med. Chem. Lett. 4, 2465–2470.

Hassner, A., Alexanian, V., 1978. Direct room temperature esterification of carboxylic acids. Tetrahedron Lett. 46, 4475–4478.

Huang, J., 1992. Syntheses of polyethylene glycol (PEG) with different functional groups at each end I. Preparation of monotrityl PEG using polyvinyl alcohol (PVA) matrix. J. Appl. Polym. Sci. 46, 1663–1676.

Lein, E.J., 1993. Partition coefficients. In: Swabrick, J., Boylan, J.C. (Eds.), Encylopedia of Pharmaceutical Technology. Marcel Decker, New York, pp. 293–307.

Lein, E.J., Alhaider, A.A., Lee, V.H.L., 1982. Phase partition: Its use in prediction of membrane permeation and drug action in the eye. J. Parenter. Sci. Technol. 36, 86–93.

Lee, V.H.L, Li, V.H.K., 1988. Prodrugs for improved ocular drug delivery. Adv Drug Delivery Rev. 3, 1–38.

MacEwen, E., Rosenthal, R., Matus, R., 1987. A preliminary study on the evaluation of asparaginase. Cancer 59, 2011–2015.

Mincheva, Z., Stambolieva, N., Rashkov, I., 1994. Preparation and properties of di-, tri- and polyethyleneglycol esters of 2-benzoxazolon-3-yl-acetic acid. Eur. Polym. J. 30, 761–765.

Neises, B., Steglich, W., 1978. Simple method for esterification of carboxylic acids. Angew. Chem. Int. Ed. Engl. 17, 522–524.

Pang, S.N.J., 1993. Final report on the safety assessment of polyethylene glycols (PEGs) -6, -8, -32, -75, -150, -14M, -20M. J. Am. Coll. Toxicol. 12, 429–457.

Perrin, D.D., Boyd D., 1974. Buffers for pH and Metal Ion Control. Chapman and Hall, London, p. 153.

Rashcov, I., Angelova, N., Manolova, N., 1993. Preparation and properties of poly oxyethylenes with 5-chloro-8-quinolinoxyl end-groups.. Eur. Polym. J. 29, 1407–1417.

Rosini, G., 1988. Amberlyst 15: A practical, mild and selective catalyst for methyl esterification of carboxylic acids. Synth. Commun. 18, 847–853.

Vestling, M.M., Murphy, C.M., Keller, D.A., Fenselau, C., Dedinas, J., Ladd, L., Olsen, M.A., 1993. Strategy for characterization of polyethylene glycol-derivatized proteins. Drug Metabol. Dispos. 21, 911–917.

Weiner A.L., 1993. Lipids in pharmaceutical dosage forms. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology, vol. 8. Marcel Decker, New York, pp. 417–476.

Yalkowsky, S.H., Valvani, S.C., Roseman, T.J. 1983. Solubility and partitioning VI: Octanol solubility and octanol water partition coefficient. J. Pharm. Sci. 72, 866–870.

Zalipsky, S., Gilon, C., Zilkha, A., 1983. Attachment of drugs to polyethylene glycols. Eur. Polym. J. 19, 1177–1183.