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Short Communication

Determination of polyethylene glycols by high-performance liquid chromatography–thermospray mass spectrometry

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Introduction

Drug absorption and distribution are dependent on the rates of drug flux across biomembranes in the body. For most drugs passive diffusion is the most important mechanism of movement across the membranes. Drug permeability in the membranes is influenced both by the properties of the penetrant and membrane. Permeability is affected by the lipid–water partitioning, charge, hydrogen bonding capacity, and molecular size of the compound [1]. Since these drug properties usually change simultaneously upon structural changes, it is difficult to differentiate the effect of each parameter on permeability. In the membrane structure, for example the number of cellular layers and tightness of the intercellular spaces affect its permeability.

The dependence of membrane permeability on molecular size has been often studied using polyethylene glycols (PEGs) as permeability probes [2–5]. PEGs are polydisperse polymeric mixtures available in a wide range of molecular weights. The structure of this polyoxyethylene polymer is H-$(C_2H_4O)_n$-OH. The physicochemical properties, particularly the partition coefficient of PEGs, do not change drastically with increasing molecular size as happens within a series of alkyl homologues. This makes them suitable for studying molecular size dependence of permeability. Because PEGs are hydrophilic molecules their permeability gives information about the paracellular route of penetration. They are stable to metabolism [2, 5] and are widely used as emulsifiers, surfactants and cosmetics [6].

In the permeability studies PEGs have been analysed by HPLC or by using radioactively labelled compounds. Although HPLC is the technique of choice for separation of PEGs, subsequent detection has proved to be difficult as the molecules do not contain a chromophore. The refractive index detector has been reported to be suitable, when the sample contains 10–100 μg of PEG 400 per injection [3, 7]. PEG 600 has been detected after precolumn derivatization with benzoyl chloride using UV-detection at 230 nm. The detection limit of the method was 1 μg per injection. However, in this method only total PEG 600, not the individual oligomers, were determined [4]. The separation of PEGs by supercritical fluid chromatography and HPLC using high column temperatures and low-wavelength UV-detection has been studied by Escott and Mortimer [6].

PEGs have been used as calibration compounds in thermospray (TSP) ionization liquid chromatography–mass spectrometry (LC–MS). The TSP mass spectra of PEGs and polypropylene glycols (PPGs) show a series of

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ammoniated molecules as well as some protonated molecules [8]. PEGs have also been used as model compounds in TSP LC–MS tuning and optimization experiments. The TSP response for low molecular weight PEGs (M.W. below 700) is better than for larger PEGs. The sensitivity of the high molecular weight PEGs has been increased by using a needle tip repeller electrode in the ion source and a smaller vaporizer probe orifice [9]. TSP HPLC–MS has been used for identification of PEGs in waste water treatment plants [10].

In this study a TSP LC–MS method was developed for the simultaneous determination of a wide molecular weight range of PEGs (238–986). The method proved to be suitable for transmembrane permeability studies performed with an *in vitro* apparatus. The conjunctiva of the eye was used as a model membrane. The quantitation limit of the method for PEG 600 was 60 ng per injection.

Experimental

Materials

A female albino rabbit (New Zealand strain) weighing 4.0 kg was used as the animal model. Lighting was maintained on a 10 h dark/14 h light cycle, and the animal was fed a regular diet. PEG 300 (M_r 285–315), PEG 600 (M_r 570–630) and PEG 1000 (M_g 950–1050) were obtained from Fluka Ag (Buchs, SG, Switzerland). They all were of technical grade. Polypropylene glycol (PPG) 425, which was used as an internal standard, was obtained from Aldrich (Steinheim, Germany). The structure of PPG is $H\text{-}(C_3H_6O)_n\text{-}OH$. All other chemicals used were of analytical grade.

Preparation of drug solution

PEG 300 (final concentration 30 mg ml^{-1}), PEG 600 (60 mg ml^{-1}) and PEG 1000 (100 mg ml^{-1}) were weighed into the same beaker. Glutathione bicarbonated Ringer's solution (GBR) was prepared as described earlier [11]. The pH of the solution was adjusted to 7.65 at 37°C with O_2–CO_2 (95:5) bubbling and 15 ml of GBR solution was added to the PEG-mixture.

In vitro *permeability study*

The rabbit was killed by a marginal vein injection of a lethal dose of T-61 vet. (Hoechst, Munich, West Germany). The palpebral conjunctivas were cut from the lower eyelids. They were positioned between two rings and placed with 20 min of death in mounts between Ussing modified perfusion chambers as described by Schoenwald and Huang [11]. The exposed surface of the conjunctiva between the two sides was 0.283 cm^2. GBR solution (6.5 ml) was added to the receptor (serosal) side. Immediately thereafter, an equal volume of PEG-mixture was added to the donor (mucosal) side. Mixing on both sides and constant pH (7.65) was achieved by bubbling a O_2–CO_2 (95:5) mixture at a rate of three to five bubbles per second. The experiments were conducted at 37°C for a period of 4 h. At intervals, 1 ml samples of the solution were withdrawn from the receptor side and replaced with an equal amount of GBR buffer.

Analytical procedure

Samples (1 ml) of the receptor side solutions were collected and diluted (1:1) with the internal standard solution containing 25 μg ml^{-1} of PPG 425. The samples were analysed without any extraction procedure by LC–MS.

HPLC conditions. The HPLC pump used with the mass spectrometer was a model 2900-0374 solvent delivery system (Applied Biosystems, USA). The injector was a Rheodyne Model 7125 instrument with a 50 μl loop. The column was a PRP-1 (150 × 4.1 mm i.d., 10 μm particle size; Hamilton, Reno, NE, USA) polymeric styrene divinylbenzene reversed-phase column. The isocratic eluent consisted of ammonium acetate (0.1 M, pH 6.0)–acetonitrile (79:21, v/v). The flow rate was set to 1.0 ml min^{-1}. An eluent mixture containing less acetonitrile (85:15, v/v) was used to achieve better chromatographic separation of the low molecular weight PEG oligomers, when their mass spectra were recorded.

Mass spectrometry. The LC–MS system used was a VG thermospray probe coupled to a VG Trio-2 quadrupole mass spectrometer (VG Masslab, Manchester, UK). The ion source was modified by changing the original blunt tip repeller electrode to a needle tip electrode located 3 mm from the ion exit aperture [12]. The ion source temperature was 200°C, the vaporizer temperature was 175°C and the ion repeller potential was set to 320 V. Other source parameters were tuned daily with [PEG + NH_4]$^+$ ions at *m/z* 388, 608 and 916 by

injecting PEG via a loop. The mass spectra of the PEG oligomers were recorded after chromatographic separation by scanning the instrument from *m/z* 200 to 1200 in 2 s.

The oligomers in the PEG sample mixtures were quantitated using selected ion recording. SIR was based on the ammoniated molecules (MNH_4^+ ions): *m/z* 384 for internal standard, PPG, and *m/z* 256, 300, 344, 388, 432, 476, 520, 564, 608, 652, 696, 740, 784, 828, 872, 916, 960 and 1004 for PEGs.

Calibration and quality control of the assay. Quantitation of the 18 PEG oligomers (molecular weight range 238–986) was based on the internal standard method. The internal standard, PPG 425 (25 μg in 1 ml of water), was added to the samples and quantitation standards. The quantitation standards were made by diluting the donor side PEG solution with Ringer buffer. The dilutions were 1:100, 1:500, 1:2000, 1:10000 and 1:50000. These dilutions correspond to 1, 0.2, 0.05, 0.01 and 0.002% transmembrane permeation of the PEG, respectively. This method allows direct measurement of the exact permeation percentage for each PEG oligomer, even though the molecular weight distribution of the original PEG solution is not exactly known. Four point calibration graphs (triplicate injections) were collected for the range corresponding to 0.002–0.2% permeation by plotting the ratios of analyte and internal standard peak areas versus the amounts of analyte. Linear regression analysis was used to calculate the curve parameters. The 95% confidence limits for the slope and the intercept were also calculated. The precision of the LC–MS system was tested by analysing seven replicate samples.

Results and Discussion

As suggested by Delahunty and Hollander [7] the polymeric styrene divinylbenzene HPLC column can be successfully used for the analysis of PEGs. The chromatographic peaks show some tailing, but this might also result from the memory effect, caused by contamination of the ion source with PEG [13]. The use of 21% acetonitrile–0.1 M ammonium acetate buffer solution allows the separation of the low molecular weight PEGs from the solvent peak. All the high molecular weight PEGs and the internal standard (PPG, MNH_4^+ at *m/z* 384) are eluted in 9 min (Fig. 1), which means that six samples may be analysed in one hour. The chromatographic separation of the low mol-

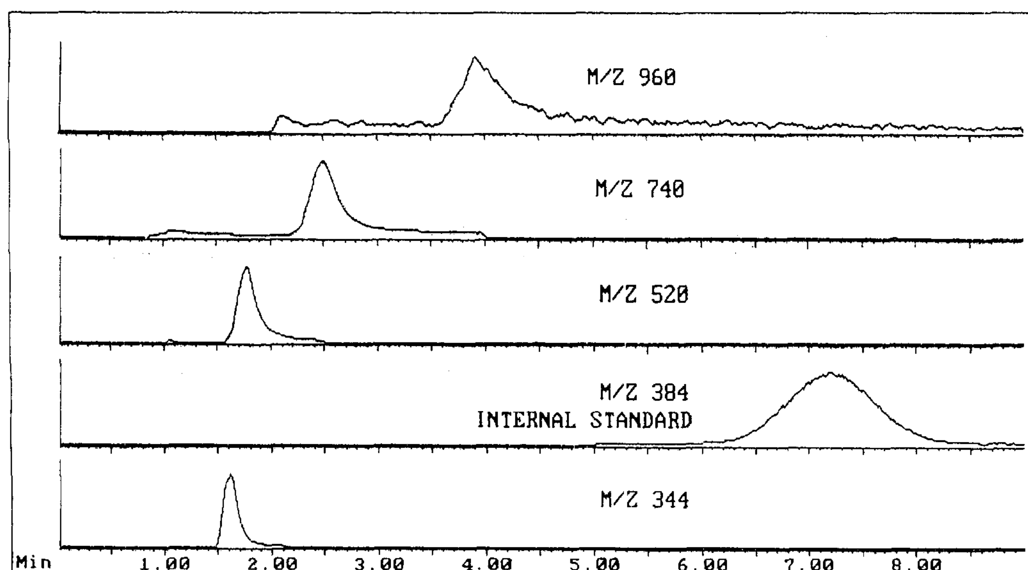

Figure 1
Selected ion chromatograms of a polyethylene glycol sample. The intensities of the chromatograms were independently normalized. The ions recorded at *m/z* 960, 740, 520 and 344 represent MNH_4^+ ions of PEGs (total of 18 different molecular weight PEGs were measured simultaneously). The sample containing a mixture of PEG 300, PEG 600 and PEG 1000 was taken from the acceptor side of the *in vitro* apparatus at 45 min. The peak monitored at *m/z* 384 originated from the internal standard, polypropylene glycol. Conditions: ammonium acetate (0.1 M, pH 6.0)–acetonitrile (79:21, v/v); flow rate 1 ml min^{-1}; PRP-1 styrene divinylbenzene column (150 × 4.1 mm i.d.); ion source temperature 200°C; vaporizer temperature 175°C; ion repeller potential 320 V.

ecular weight PEG oligomers was improved by using less organic modifier in the eluent (15% of acetonitrile, Fig. 2). This made it possible to record the mass spectrum of each oligomer without significant contribution from other oligomers. However, this also increased the retention times and peak widths of high molecular weight PEG oligomers above acceptable limits.

The mass spectra of all PEG oligomers show ammoniated molecules at M + 18 as the base peaks and other adduct ions at M + 23 (MNa^+), M + 39 (MK^+), M + 59 (MNH_4^+ + acetonitrile) and at M + 81 (unknown). No significant fragmentation of PEG was observed under these conditions (Fig. 3). The molecular weight difference between two PEG oligomers is 44 mass units (C_2H_4O). The MNH_4^+ ions (M + 18) used for quantitation were diagnostic for each oligomer and there were no overlapping adduct or fragment ions. This means that the various oligomers are well resolved from one another, although chromatographic baseline separation was not achieved for all oligomers.

Selected ion recording of the ammoniated molecules MNH_4^+ was used for quantitation of the PEGs in *in vitro* permeability studies. The thermospray ionization response was linear up to concentrations which corresponded to 0.2% permeation of PEGs (3 μg of PEG 300, 6 μg PEG 600 and 10 μg of PEG 1000 per injection). Above this concentration the response was nonlinear (below the expected value), and samples containing higher concentrations should be diluted. The method is best for the analysis of PEGs in the molecular weight range 238–810. For this range good linearity of the regression curves was observed and the relative standard deviation (RSD) for replicate analyses was acceptable (Table 1).

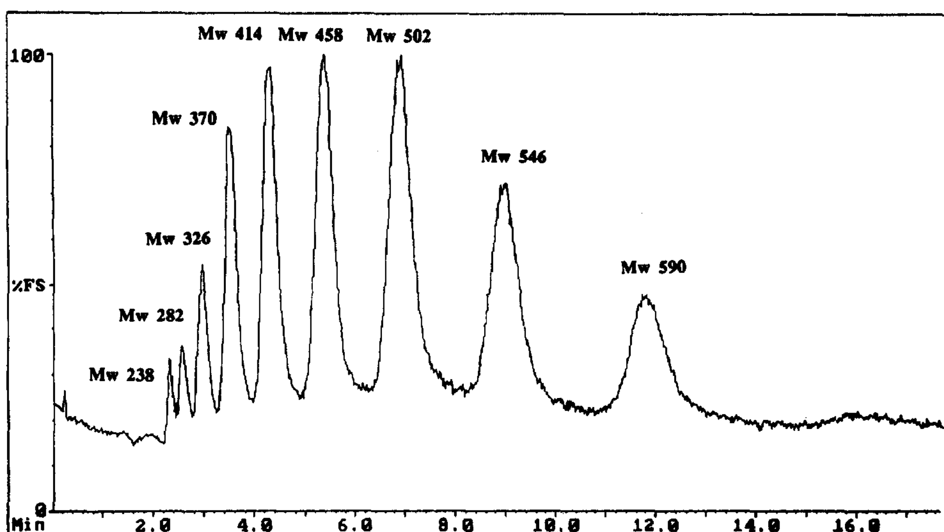

Figure 2
Total ion chromatogram of PEG 600 mixture. Conditions: as in Fig. 1, but eluent was ammonium acetate (0.1 M, pH 6.0)–acetonitrile (85:15, v/v). The molecular weights of the PEG oligomers in the chromatogram are 238 (at 2.3 min), 282 (2.5 min), 326 (2.9 min), 370 (3.5 min), 414 (4.2 min), 458 (5.3 min), 502 (6.9 min), 546 (8.9 min) and 590 (11.8 min).

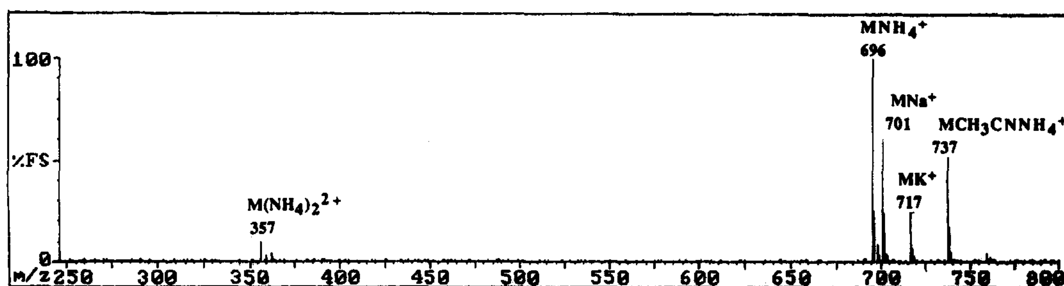

Figure 3
Mass spectrum of a PEG oligomer with molecular weight 678. Conditions as in Fig. 1.

Table 1
Quality parameters of the HPLC–TSP–MS assay of polyethylene glycols

Molecular weight	Curve equation	r^2	RSD (%)
238	$y = 0.99(\pm0.04)x + 0.003(\pm0.002)$	1.00	12.8
282	$y = 3.42(\pm0.11)x + 0.017(\pm0.005)$	1.00	9.5
326	$y = 5.71(\pm0.27)x + 0.020(\pm0.012)$	1.00	10.4
370	$y = 6.14(\pm0.33)x + 0.009(\pm0.017)$	1.00	8.8
414	$y = 5.49(\pm0.29)x + 0.001(\pm0.015)$	1.00	9.5
458	$y = 5.36(\pm0.26)x + 0.003(\pm0.010)$	1.00	8.1
502	$y = 4.99(\pm0.29)x + 0.001(\pm0.014)$	0.99	11.4
546	$y = 4.43(\pm0.19)x + 0.002(\pm0.009)$	1.00	11.0
590	$y = 3.59(\pm0.33)x + 0.009(\pm0.017)$	0.99	7.4
634	$y = 2.38(\pm0.10)x + 0.001(\pm0.005)$	1.00	13.8
678	$y = 2.08(\pm0.18)x + 0.001(\pm0.010)$	0.99	14.2
722	$y = 1.42(\pm0.05)x + 0.003(\pm0.002)$	1.00	13.4
766	$y = 0.95(\pm0.06)x + 0.004(\pm0.003)$	0.99	10.1
810	$y = 0.46(\pm0.05)x + 0.004(\pm0.003)$	0.97	10.0
854	$y = 0.29(\pm0.04)x + 0.003(\pm0.002)$	0.97	18.6
898	$y = 0.23(\pm0.04)x + 0.002(\pm0.002)$	0.95	18.4
942	$y = 0.15(\pm0.05)x + 0.004(\pm0.004)$	0.89	24.2
986	$y = 0.07(\pm0.02)x + 0.002(\pm0.002)$	0.88	34.9

y = Peak area ratio of the analyte and internal standard (polypropylene glycol); x = the percentage of the PEGs transferred to the receptor side of the *in vitro* apparatus; the confidence limits of the slope and intercept of the regression equations are given in parentheses ($P = 0.05$); r^2 = coefficient of determination; RSD = relative standard deviation ($n = 7$, 750 ng of PEG 300, 1.5 μg of PEG 600 and 2.5 μg of PEG 1000 per injection).

The signal-to-noise ratio at a concentration level corresponding 0.002% permeation of PEGs was between 10 and 50 for each individual molecular weight of PEG. This level of dilution (permeation) corresponds to 30 ng of PEG 300, 60 ng of PEG 600 and 100 ng of PEG 1000 polydisperse mixture per injection. When 60 ng of polydisperse PEG 600 was analysed the signal-to-noise ratio for MNH_4^+ at m/z 608 was 20. This means that the TSP LC–MS method is at least one hundred times more sensitive than the previously described HPLC methods using refractive index detection [3] or UV-detection [4]. This method can also be used for detection of high molecular weight PEGs up to the molecular weight 986, but both the reproducibility and linearity of the method is quite limited (Table 1).

The suitability of the quantitation range for the *in vitro* permeation studies was tested with two conjunctival experiments. The levels of PEGs 238–810 exceeded the quantitation limit (0.002%) in samples collected from the acceptor side of the apparatus at 15 min. The samples collected after 150 min had to be diluted with buffer (1:5), because the PEG levels exceeded the linear calibration range. The chromatogram presented in Fig. 1 was obtained with a 45 min sample.

In conclusion, TSP LC–MS method allows determination of permeability coefficients for a range of PEGs with different molecular sizes. This method may prove useful in the studies of molecular weight dependence in membrane penetration and effects of penetration enhancers on biomembrane leakiness. Highly purified PEG with a narrow molecular weight distribution is not needed, because the method separately analyses each molecular weight of PEG in the sample. This may decrease the need of animals in research, because in each penetration experiment the whole series of PEGs can be used and determined simultaneously.

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References

[1] W.D. Stein, *Transport and Diffusion Across Cell Membranes*, pp. 86–91. Academic Press, San Diego (1986).
[2] T.Y. Ma, D. Hollander, P. Krugliak and K. Katz, *Gastroenterology* **98**, 39–46 (1990).
[3] G.O. Young, D. Ruttenberg and J.P. Wright, *Clin. Chem.* **36**, 1800–1802 (1990).
[4] I.M. Kinahan and M.R. Smyth, *J. Chromatogr.* **565**, 297–307 (1991).
[5] M.D. Donovan, G.L. Flynn and G.L. Amidon, *Pharm. Res.* **7**, 863–868 (1990).
[6] R.E.A. Escott and N. Mortimer, *J. Chromatogr.* **553**, 423–432 (1991).
[7] T. Delahunty and D. Hollander, *Clin. Chem.* **32**, 351–353 (1986).
[8] L. Yang and G.J. Fergusson, Proceedings of the 33rd ASMS Conference on Mass Spectrometry and Allied

Topics, San Diego, CA, 26–31 May pp. 775–776 (1985).
[9] R.H. Robbins and F.W. Crow, *Rapid Commun. Mass Spectrom.* **2**, 30–34 (1988).
[10] H.F. Schröder, *Gewaesserschutz, Wasser, Abwasser* **112**, 351–384 (1990).
[11] R.D. Schoenwald and H.S. Huang, *J. Pharm. Sci.* **72**, 1266–1272 (1983).
[12] S. Fink and R.B. Freas, *Anal. Chem.* **61**, 2050–2054 (1989).
[13] C.E.M. Heeremans, R.A.M. van der Hoeven, W.M.A. Niessen, U.R. Tjaden and J. van der Greef, *Org. Mass Spectrom.* **24**, 109–112 (1989).

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