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Short Communication

Gas–liquid chromatographic quantitation of polyethylene glycol 400 in pharmaceutical preparations

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Introduction

A new process technology to enhance *in vitro* dissolution rates and bioavailability of poorly water soluble compounds has recently been developed [1]. Drug is solubilized in polyethylene glycol 400 (PEG 400) prior to its incorporation into pellets (approximately 0.6–1.0 mm in diameter) made by an extrusion/spheronization process. These pellets can contain as much as 30% by weight of PEG 400, and remain solid, non-cohesive and suitable for encapsulation in hard gelatin capsules. Formulations based on this technology can provide an alternate delivery system to liquid-filled capsules. Since the presence of PEG 400 in the final product is critical to its performance, an accurate methodology for the determination of PEG 400 in these pellets is essential. Loss of PEG 400 during processing or storage could change the PEG 400 to drug ratio, greatly affecting both the *in vitro* and *in vivo* performance of the dosage form. Although a number of analytical techniques have been reported for the measurement of PEG 400 [2–7] in biological fluids, none are easily adaptable to dosage form testing. This report describes a GLC method for the determination of PEG 400 in pellets during the optimization of the extrusion/spheronization process. Furthermore, with minor modification to the procedure, the method also determines the dissolution release profile of the pellets encapsulated in a hard gelatin capsule without employing extensive sample pre-treatment as described in the literature [2–7]. The availability of this procedure will assist in defining product performance and evaluating critical process parameters such as distribution of the drug and PEG-400 in different particle size ranges.

Experimental

Reagents and materials

Acetone (high purity solvent) was obtained from Burdick & Jackson (Mississauga, ON, Canada). Chloroform (glass-distilled Omni Solv) was purchased from BDH Inc. Toronto, ON, Canada) and PEG-400 (range of 380–420) (Carbowax Sentry®) NF grade was from Union Carbide Corporation (Danbury, CT, USA). Sodium dodecyl sulphate (sodium lauryl sulphate, SDS) was obtained from the Sigma Chemical Co. (St Louis, MO, USA). Acro-Disc CR PTFE syringe filter was supplied by Gelman Sciences (Ann Arbor, MI, USA). The ultrasonic bath (Model Branson 8200) and Polytron® Tissue homogenizer equipped with a 90 Polytron® 10-S blade (Brinkmann Instrument, Rexdale, ON, Canada) were used. The centrifuge was a CENTRA-4B benchtop

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model manufactured by International Equipment Company (IEC) and the shaker was a Type 50000 Maxi-Mix III® Thermolyne Sybron mixer (Fisher Scientific, PQ, Canada). A Hewlett–Packard, model 5890 series II gas chromatograph equipped with an HP-1 (methyl silicone gum) 5 m × 0.53 mm × 2.65 μm film thickness) column, a hydrogen FID, an automatic injector (model 7673) and an integrator (model 3390A) was used in all studies.

Standard preparations

For the pellets assay, stock solutions of PEG 400 were prepared by disolving 1.0 g of PEG 400 in 10 ml of acetone. Working standard solutions at concentrations from 5 to 30 mg ml^{-1} were prepared by appropriate dilutions of the stock solution. For the dissolution tests, stock solutions of PEG 400 were prepared by adding 1 g of PEG 400 in 100 ml of 0.5% SDS in water in a volumetric flask. Working standard solutions at concentrations from 0.1 to 0.5 mg ml^{-1} were prepared and treated as described in the extraction procedure for dissolution samples.

Sample preparations

For the pellets assay, 600-mg portions of pellets were suspended in 10 ml of acetone, the pellets were crushed by using the Polytron® tissue homogenizer and PEG 400 was extracted by sonication with three 5-ml portions of acetone. The combined acetone extract was filtered through a syringe filter and collected into a 25-ml volumetric flask and made up to volume with acetone. The filtrate was transferred to a GC vial for analysis. Dissolution tests were performed on 400 mg of pellets encapsulated in a #0 white opaque hard gelatin capsule containing 30% by weight of PEG 400 and 20 mg of drug. USP Apparatus II was used with a paddle speed of 75 rpm. The dissolution medium used was 900 ml of 0.5% SDS in water. The SDS is required to maintain the drug in solution. A 5-ml sample aliquot was withdrawn at 5, 10, 15, 20 and 30 min using a glass syringe. The samples were centrifuged at 2000*g* for 3 min. Each sample was then extracted as follows: a sample (2 ml) was pipetted into separate 13 × 100 mm glass screw-capped tubes. A sufficient amount of sodium chloride (about 500 mg) was added to each tube to saturate the sample, improve extraction efficiency and reduce emulsion formation at the chloroform/aqueous interface. The sample was extracted with 2 ml of chloroform for 30 min on a shaker set at a speed of 1500 rpm. The tubes were then centrifuged at 2000*g* for 10 min to separate the phases. The lower organic phase was transferred in to a GC vial for injection.

Instrumentation and chromatographic conditions

For the pellets assay the instrumental condition used were as follows: helium was used as the carrier gas (constant pressure 40 PSI, 8 ml min^{-1}) and nitrogen was used as the make-up gas for the detector (12 ml min^{-1}). The injector temperature and detector temperature were both set at 250°C. The temperature program started at 150°C and was isothermal for the first 3 min. Temperature was then increased at 10°C min^{-1} until a final temperature of 300°C was reached and maintained for 5 min. A 1 μl volume of the sample extract was injected splitless into the column. After 0.5 min the carrier gas splitting was started. For the dissolution tests the chromatographic conditions were the same as described above except for the oven temperature program. The program was started initially at 50°C and increased at 50°C min^{-1} immediately to 150°C and held at this temperature for 3 min, and then ramped at 10°C min^{-1} to 300°C and maintained at this final temperature for 5 min. These changes were made to accommodate the lower sample concentrations by concentrating the sample at the head of the column and to minimize band broadening.

Results and Discussion

In the formulation described elsewhere [1] drug dissolved in PEG 400 is made into pellets with microcrystalline cellulose but maintains the drug as a solution. This provides the handling advantages of a solid that can be filled into conventional hard gelatin capsules while at the same time achieving the *in vivo* performance of a liquid filled capsule. To maximize drug loading, the drug content in the pellets is usually close to its maximum solubility in PEG 400, consequently, any loss of PEG 400 due to the manufacturing process or to an uneven distribution in pellets with different mesh sizes could greatly affect both the *in vitro* and *in vivo* performance of the dosage form. Two assays were developed; one to

measure PEG 400 concentrations in pellets from different sieve fractions, the other to measure the much lower amounts of PEG 400 typically found in the dissolution media.

Since PEG 400 is comprised of a group of oligomers with an average molecular weight of approximately 400 each, the chromatogram contains a number of peaks with varying intensities. Each peak represents one oligomer and the peak intensity is proportional to its concentration in the mixture. Due to batch-to-batch variation in the molecular weight distribution of PEG 400, the number and intensity of these peaks will vary depending on the makeup of each lot. Therefore, this may require that the assay reference standard be taken from the same lot of PEG 400 used in the manufacturing process. For the particular lot of PEG 400 used in this study a total of nine peaks each representing a particular molecular weight fraction could be measured. Figure 1 shows a representative chromatogram for a 5 mg ml^{-1} solution of PEG 400 in acetone used in this study. Quantitation for the pellets and sieve fraction assays were carried out by summing the area of the four major molecular weight fractions represented by peaks with retention times of 5.7, 8.5, 10.8 and 12.9 min. Extraction recovery of PEG 400 from formulations was assessed by extracting a placebo mixture containing microcrystalline cellulose spiked with PEG 400 at four different levels (n = 3) in the relevant concentration range (see Table 1). Inter-assay precision was assessed by replicate measurements of a homogeneous sample on different days (n = 4). Without an internal standard the coefficient of variation was found to be less than 4%. Linear calibration plots of PEG 400 were

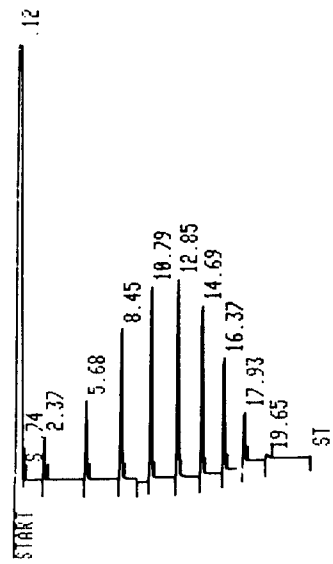

Figure 1
Typical GC Chromatogram of a standard solution of PEG 400 in acetone at 5 mg ml^{-1} concentration using a 5 m × 0.53 mm HP-1 megabore column. Detector and injector temperature was 250°C. The initial temperature of 150°C was increased after 3 min at a rate of 10°C min^{-1} to 300°C and held at this temperature for 5 min.

Table 1
Recoveries studies (mean ± SD) of PEG 400 and inter-assay precision (%RSD) in pellets

Concentration (mg ml^{-1})	Recovery (%) (n = 3)	RSD % (n = 4)
2	96.9 ± 0.5	3.6
5	99.9 ± 0.8	3.0
10	95.8 ± 0.4	1.1
20	94.5 ± 0.8	1.5

Table 2
Contents of PEG 400 in pellets (mean ± SD)

Pellet size	% PEG 400 claim	% PEG 400 found (n = 4)
>14 mesh (>1400 μm)	30	30.8 ± 0.2
14–18 mesh (1000–1400 μm)	20	19.1 ± 0.1
	25	23.6 ± 0.4
	30	28.4 ± 0.3
18–20 mesh (840–1000 μm)	20	19.1 ± 0.7
	25	23.6 ± 0.5
	30	27.9 ± 0.3
20–30 mesh (590–840 μm)	20	19.5 ± 0.2
	25	23.4 ± 0.7
	30	27.2 ± 0.2
30–40 mesh (420–590 μm)	20	19.4 ± 0.07
	25	24.0 ± 0.3
	30	28.7 ± 0.5
40–60 mesh (250–420 μm)	20	20.6 ± 0.2
	30	27.1 ± 0.4

generated by carrying out a least squares regression of the sum of the four peak areas vs the standard concentrations and this was then used to calculate the concentration of PEG 400 in the test samples after extraction.

Table 2 summarizes the results obtained from pellets of various sizes and manufactured with 20, 25 and 30% by weight of PEG 400. The size range of pellets shown represents a typical size distribution for a given batch. The amounts of PEG 400 measured were in close agreement with the theoretical value. The extraction recovery of PEG 400 from the pellets and standard deviation of the recovery were independent of particle size.

In vitro dissolution testing is used routinely to monitor solid dosage form performance. A profile of drug released vs time under specified conditions is usually adequate to ensure manufacturing reproducibility. However, in the development of this unique dosage form, monitoring the release rate of both drug and PEG 400 were considered important factors in optimizing its performance. Since dissolution media are mainly aqueous by definition, liquid–liquid extraction of the dissolution samples into an organic solvent was required: (1) to eliminate the direct introduction of aqueous sample into the column, (2) to increase detectability and (3) to remove possible interferences from other components of the dissolution medium.

In a typical dissolution test, one capsule is dissolved in 900 ml of media which gives samples with relatively low levels of PEG 400, consequently only four of the molecular weight fractions were detectable at these low levels and the quantitation of PEG 400 in the dissolution studies was based on three major molecular weight fractions with retention times of 11.4, 13.4 and 15.3 min (see Fig. 2).

Extraction recovery of PEG 400 from dissolution samples ($n = 3$) was assessed by extracting a sample of dissolution medium spiked with PEG 400 at four different levels in the relevant concentration range (see Table 3). Reproducibility of the assay was assessed by replicate measurements of a homogeneous sample on different days ($n = 4$). Without an internal standard the coefficient of variation was found to be less than 4%. Linear calibration plots of PEG 400 were generated by carrying out a least squares regression of the

Table 3
PEG 400 recovery studies (mean ± SD) and intra-assay precision in dissolution medium

Concentration (mg ml^{-1})	% Recovery ($n = 3$)	RSD % ($n = 4$)
0.1	85.6 ± 3.0	3.8
0.2	84.5 ± 2.2	2.8
0.3	95.4 ± 2.0	2.7
0.5	100 ± 2.5	3.1

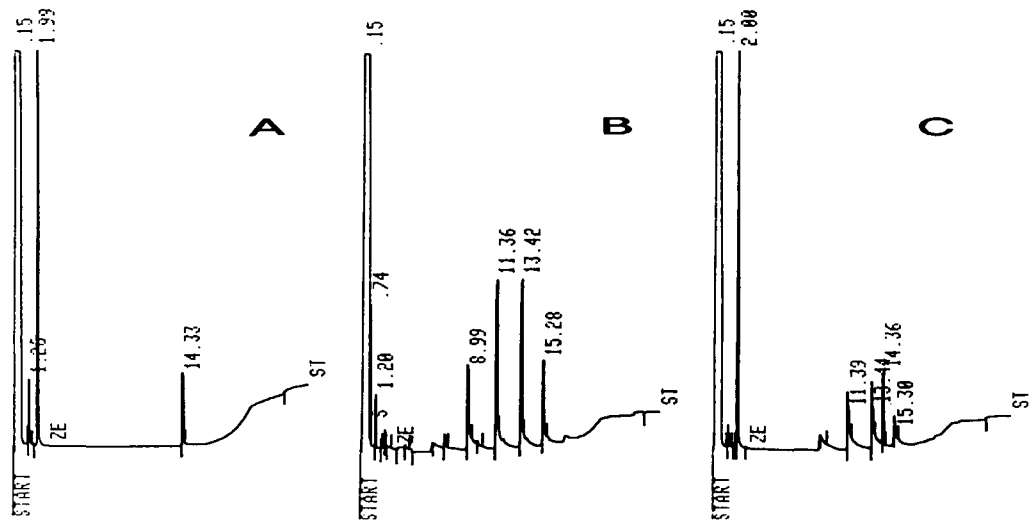

Figure 2
Chromatograms obtained from extracts of dissolution medium (a), 0.2 mg ml^{-1} standard solution (b) and an actual dissolution sample (10 min) with PEG 400 at 0.14 mg ml^{-1} concentration (c) using a 5 m × 0.53 mm HP-1 megabore column. Detector and injector temperature was 250°C. The initial temperature of 50°C was increased at 50°C min^{-1} immediately to 150°C and after 3 min increased to 300°C at 10°C min^{-1} and held at this temperature for 5 min.

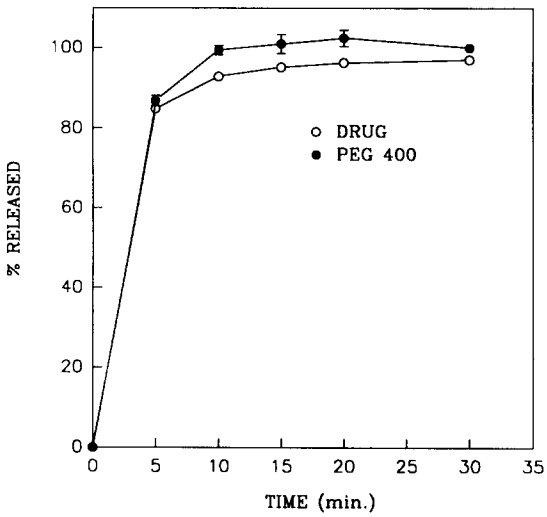

Figure 3
In vitro drug release and PEG 400 release profiles of pellets in 0.5% SDS at 37°C and 75 rpm paddle speed (n = 6) (mean ± SD).

sum of the three peak areas vs the standard concentrations and this was then used to calculate the concentration of PEG 400 in the test samples after extraction.

Representative chromatograms of a typical 2 ml blank extract, a standard solution containing 0.2 mg ml^{-1} PEG 400 and a 10 min sample obtained from an actual dissolution study are shown in Fig. 2. Figure 3 shows the comparative dissolution profiles of drug and PEG 400 from capsules where in this case the drug is released at the same rate as the PEG 400.

In conclusion, the methods described in this paper provide a simple, sensitive and reproducible means of assessing the PEG 400 content and its dissolution release rate from a unique pelletized dosage form.

References

[1] L. Baert, E. Kwong and S. Béchard, *Pharm. Res.*, in press.
[2] V.S. Chadwick, S.F. Phillips and A.F. Hofmann, *Gastroenterology* **73**, 241–246 (1977).
[3] J.B. Bouska and S.F. Phillips, *J. Chromatogr.* **183**, 72–77 (1980).
[4] T. Sivakumaran, R.T. Jenkins, W.H.C. Walker and R.L. Goodacre, *Clin. Chem.* **28**, 2452–2453 (1982).
[5] H.J. McClung, P.A. Powers, H.R. Sloan and B. Kerzner, *Clin. Chim. Acta* **134**, 245–254 (1983).
[6] T. Delahunty and D. Hollander, *Clin. Chem.* **32**, 351–353 (1986).
[7] H.A. Schwertner, W.R. Patterson and J.H. Cissik, *J. Chromatogr.* **578**, 297–301 (1992).
[8] N.H. Shah, D. Stiel, M.H. Infeld, A.S. Ralikar, A.W. Malick and M. Patrawala, *Pharm. Tech.* **16**, 126–131 (1992).

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