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Short Communication

Size exclusion chromatography of poloxalene poloxamers: polyethylene glycol–polypropylene glycol co-polymers used to control cattle bloat

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Introduction

Poloxalene poloxamers are synthetic block copolymers of ethylene oxide and propylene oxide with an average molecular weight of 3000 and the following formula [1]:

$$HO\text{-}[C_2H_4O]_a\text{-}[CH_3H_6O]_b\text{-}[C_2H_4O]_a\text{-}H,$$
average $a = 12$, average $b = 34$.

Poloxalene is a non-ionic surfactant that reduces froth produced in the rumen of cattle afflicted with rumenal tympany (bloat), thus, treating the disorder. It is formulated into the products Bloat Guard® and Thera Bloat® for treatment of affected cattle. A wet chemical analysis involving reaction of the poloxalene with phthalic anhydride in a pyridine solution, followed by acid–base titration, is used to determine average molecular weight [2]. The method is time-consuming and cannot calculate molecular weight distributions.

A size exclusion method was developed to determine both average molecular weight and molecular weight distributions of poloxalene. Both an aqueous and a non-aqueous chromatographic system were tested. The non-aqueous system used a THF mobile phase and a 500A Hewlett–Packard PLgel column. Correlation of this system with the wet chemical method was good. The aqueous system used a water–methanol (70:30, v/v) mobile phase and a 250A Beckman Spherogel TSK 2000SW column. Correlation of this system with the wet chemical method was not acceptable. The size exclusion columns were calibrated with polyethylene glycol molecular weight standards and a refractive index detector was used. The standard curves were used without Mark–Houwink constant corrections [3].

Experimental

Chromatographic system

Two model 501 (Waters, Milford, MA) HPLC pumps were used. One pump was connected to a model 712 (Waters) autosampler, the column and the sample side of the refractive index detector (Knauer differential refractometer, Rainin, Woburn, MA). The second pump was connected to the reference side of the detector. Flow rates were set to 1 ml

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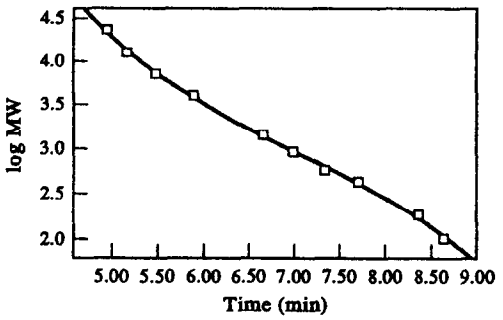

Figure 1
Calibration curve for a 7.5 mm × 30 cm HP PLgel 10 μm 500A column. THF mobile phase at 1 ml min^{-1} flow rate and RI detection using polyethylene glycol molecular weight standards.

min^{-1}. Home-built pulse dampeners were connected to each pump. Data were collected and analysed by a model 860 system (Waters). The non-aqueous chromatographic system utilized a 7.5 mm × 30 cm HP PLgel 10 μm 500A stainless steel column (Hewlett–Packard, Avondale, PA) and an HPLC grade tetrahydrofuran mobile phase. The aqueous chromatographic system utilized a 7.5 mm × 30 cm Spherogel TSK2000SW 10 μm 250A stainless steel column (Beckman, San Ramon, CA) and a water–methanol (70:30, v/v) (both HPLC grade) mobile phase.

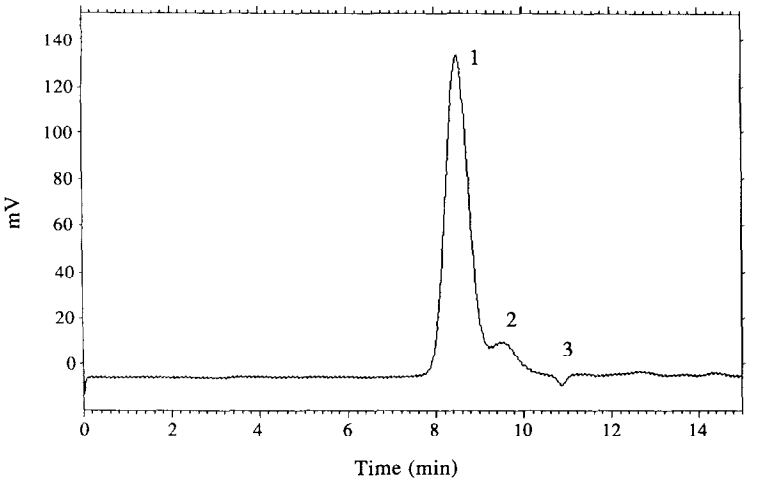

Figure 2
Chromatogram of poloxalene using a 7.5 mm × 30 cm HP PLgel 10 μm 500A column, a THF mobile phase at 1 ml min^{-1} flow rate and RI detection. Peak 1 contains a high molecular weight distribution (peak MW ~3000), Peak 2 contains a lower molecular weight distribution (peak molecular weight ~1000), Peak 3 contains low molecular weight oxidation inhibitors.

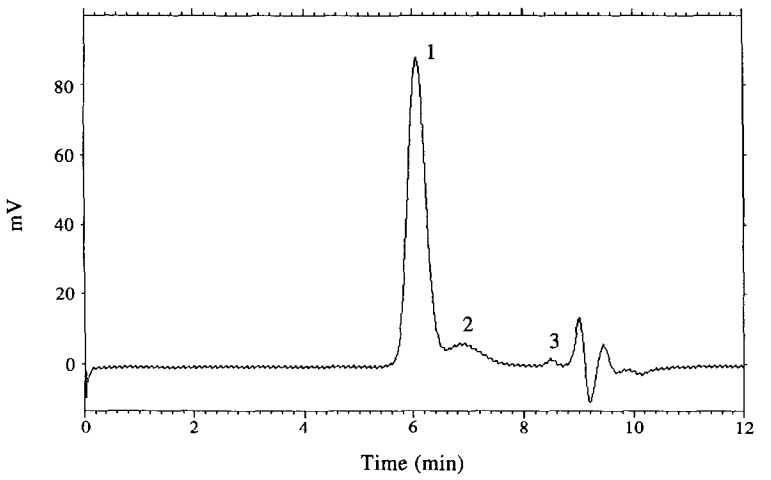

Figure 3
Chromatogram of poloxalene using a 7.5 mm × 30 cm Spherogel TSK2000SW 10 μm 250 A column, a water–methanol (70:30, v/v) mobile phase at 1 ml min^{-1} flow rate and RI detection. Peak 1 contains a high molecular weight distribution (peak MW ~3000), Peak 2 contains a lower molecular weight distribution (peak molecular weight ~1000), Peak 3 contains low molecular weight oxidation inhibitors.

Sample and standard preparation

Samples and standards were prepared in mobile phase at approximately 3% (w/v) and a 20 μl aliquot injected. The poloxalene samples were obtained from SmithKline Beecham Animal Health (West Chester, PA). The narrow distribution polyethylene glycol standards covered the molecular weight range from 106 to 23000 (Hewlett–Packard, Avondale, PA).

Results and Discussion

The size exclusion method developed for analysing poloxalene poloxamers used a high performance stainless steel column packed with porous (500A) cross-linked polystyrene–divinylbenzene spheres and a tetrahydrofuran mobile phase. The system was able to resolve 10 narrow distribution polyethylene glycol molecular weight standards ranging from 106 to 23000. The standard retention times were used to calibrate the system without Mark–Houwink constant corrections [3] (Fig. 1) because of the structural similarity to the samples.

Poloxalene samples were analysed immediately after calibration. The chromatograms clearly show that poloxalene is composed of three parts (Fig. 2): a high molecular weight distribution component (~90%) with a peak molecular weight ~3000, a lower molecular weight distribution component (~10%) with a peak molecular weight ~1000, and low molecular weight oxidation inhibitors. A second chromatographic system was used to confirm our conclusions about the composition of poloxalene and to insure that the second peak

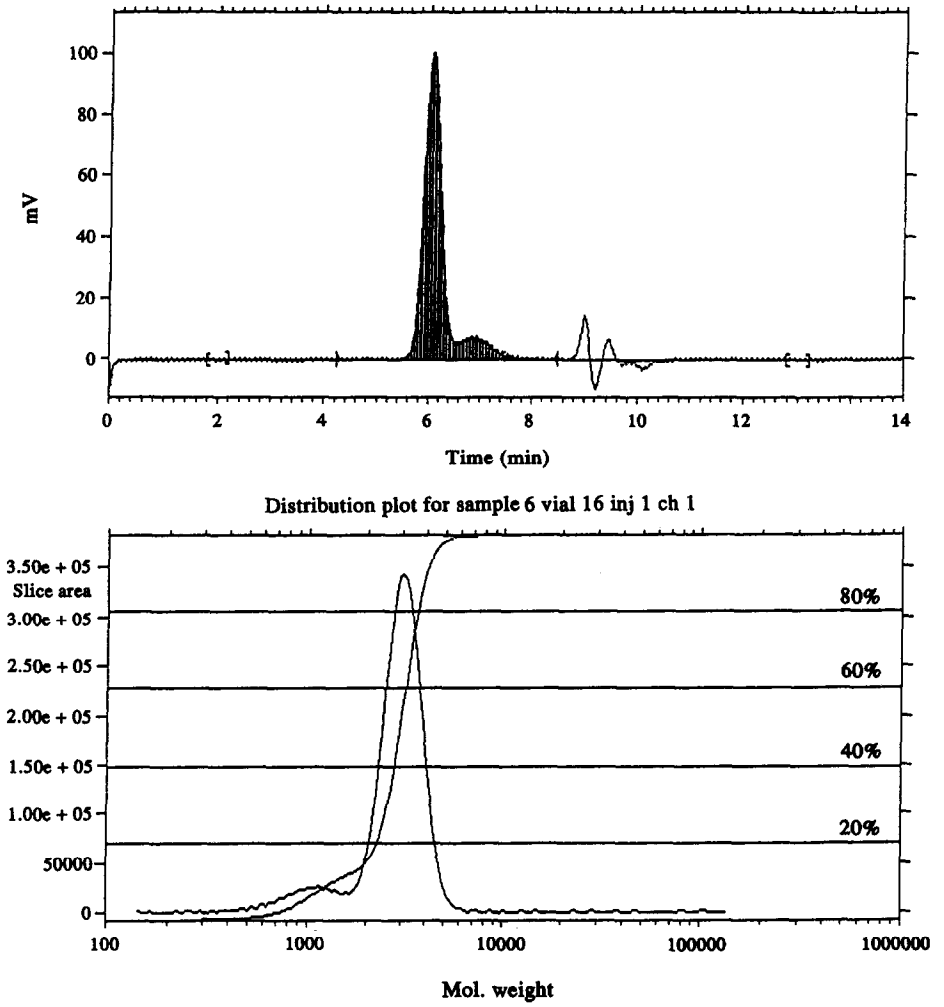

Figure 4
Time-slice integration and molecular weight profile of poloxalene generated using a 7.5 mm × 30 cm HP PLgel 10 μm 500A column, THF mobile phase at 1 ml min^{-1} flow rate and RI detection.

Table 1

Sample no.	Titration wet chem. Mw	SEC chromatography THF mobile phase* Mw (%RSD)	Mn (%RSD)	SEC chromatography aqueous mobile phase† Mw (%RSD)	Mn (%RSD)
1	2892	2876 (0.64)	2246 (0.27)	2449 (0.12)	2003 (0.35)
2	2930	2911 (2.10)	2073 (1.79)	2438 (0.15)	2005 (0.39)
3	3008	2854 (1.15)	2134 (1.61)	2445 (0.17)	2006 (0.53)
4	2944	2980 (2.65)	2265 (0.67)	2462 (0.46)	2019 (0.32)

* Average of three replicates.
† Average of two replicates.

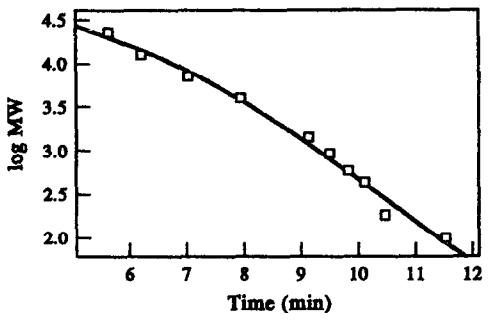

Figure 5
Calibration curve for a Spherogel TSK2000SW 10 μm 250 A column using a water–methanol (70:30, v/v) phase at 1 ml min^{-1} flow rate and RI detection using polyethylene glycol molecular weight standards.

was not a band-broadening problem. This system showed the same three components (Fig. 3).

Time-slice integration was used to generate molecular weight profiles for the samples. The integration was stopped just before elution of the inhibitor peak to allow characterization of the polymeric molecular weight distribution (Fig. 4). The weight average molecular weight, Mw, and the number average molecular weight, Mn, were calculated using both chromatographic systems. Results were compared to the weight average molecular weight determined by titration [2] as shown in Table 1.

Precision of both chromatographic systems was quite good (average %RSD <2). Accuracy for the non-aqueous system was good as shown by close agreement with the titration method. Accuracy for the aqueous system was poor, most likely due to adsorption of the poloxalene by the stationary phase [4–6]. The increase in retention time caused by adsorption produces low molecular weight information. This mixed mechanism theory is supported by unusual retention characteristics of the polyethylene standards as shown in the calibration curve (Fig. 5).

Acknowledgements — Bloat Guard® and Thera Bloat® are registered trademarks of SmithKline Beecham Animal Health.

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