Characterization of Pharmaceutical Polymers by HPLC and GPC

Application Note

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Introduction

For synthetic polymers used in the pharmaceutical industry, the characterization of both chemical composition and molecular weight distribution is important to the final product application. Liquid chromatographic techniques can be applied to investigate the purity and stability of some polymers typically used in pharmaceutical formulations.

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Analysis of diol content in monofunctional poly(ethylene oxide)

Monofunctional polyethylene oxides (PEO), bearing a single terminal hydroxyl moiety, have found increasing application in drug therapy because of their ability to shield administered proteinaceous drugs from the immune system. Such actions prolong the molecule's therapeutic lifetime and reduce the frequency of administration, with consequent cost and compliance benefits. For applications in drug therapy, the quality and purity of the PEO is of paramount importance. In particular, the presence of 'diol', ie impurities bearing two terminal hydroxyl groups in PEO samples, can cause problems in both processing and therapeutic usage where it can dramatically affect the behavior of the 'pegylated' protein. Determination of the diol content in PEO samples of low molecular weight (5000) has been achieved by measurement of the end group ratios by NMR. Although this technique has proved accurate for lower molecular weight materials, with the increasing usage of high molecular weight polymers, the intrinsic errors of the technique have become unacceptable.

Reversed-phase HPLC analysis of polymers has shown increased interest over recent years for the characterization of polymer composition. A HPLC method for the analysis of PEO has been developed using a polystyrene/divinylbenzene stationary phase using PLRP-S and gradient elution with an acetonitrile/water eluent. The Agilent evaporative light scattering detector is ideally suited to this application since the solutes are relatively high molecular weight and non-volatile, do not possess a UV chromophore and the separation requires gradient elution.

Conditions

Column: PLRP-S 300Å 5 µm, 150 x 4.6 mm (p/n PL1512-3501)
Eluent A: 99% Water, 1% Acetonitrile
Eluent B: 100% Acetonitrile
Gradient: 30-50% B in 20 min
Flow Rate: 0.5 mL/min
Detection: Agilent ELSD (neb=85 °C, evap=80 °C, gas=1.0 SLM)

Results and Discussion

Figures 1a and 1b show the separation of compounds that illustrate the technique. Peak 2 is a monofunctional PEO with a molecular weight of 31,500 and a polydispersity of 1.03, as determined by size exclusion chromatography (SEC). This sample was spiked with 5% of a difunctional PEO of similar molecular weight distribution (peak 1).

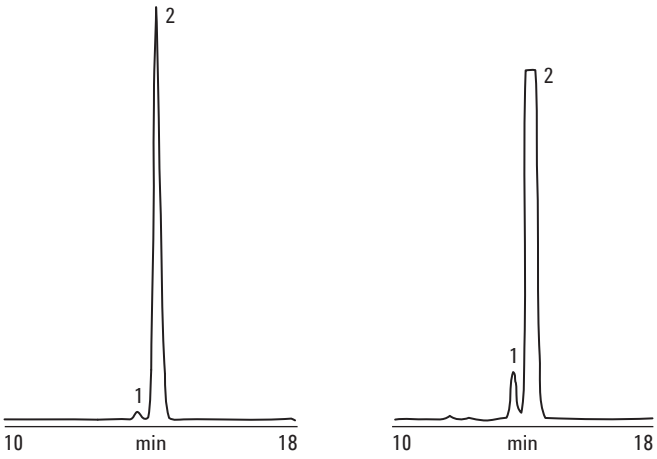

Figure 1a (left) and 1b (right). Separation of difunctional PEO (1) and monofunctional PEO (2).

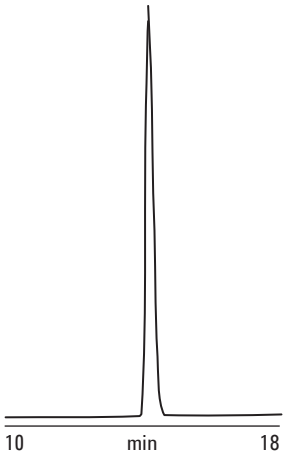

Figure 2. Chromatogram of a polymer under investigation.

This separation is based on a slight difference in hydrophobicity between the mono and difunctional species. Figure 2 shows the chromatogram of a polymer under investigation. In this case, the minor component is not detected, illustrating that this HPLC technique can be used to screen PEO for the presence of <5 % diol, which is the acceptable limit for use in therapeutic applications.

Analysis of polymer stability - poloxamer 188

Poloxamer 188 is one of a series of polyethylene oxide block copolymers that are used in pharmaceutical applications as surfactants in drug formulations. The effectiveness of the polymer is dependent upon its molecular weight distribution, and a major consideration is the stability of the polymer when exposed to sterilization processes, eg heat and gamma radiation. Gel permeation chromatography is an ideal technique for the determination of polymer molecular weight distribution. The eluent selected should fully solvate the polymer while minimizing non-size exclusion effects, mainly adsorption, which would undermine reliable molecular weight determination. For poloxamer 188, dimethylformamide (DMF) is a good GPC solvent, although its high viscosity means that elevated temperature operation is recommended for optimum resolution. The PL-GPC 120 is an integrated GPC system ideally suited to this type of application. GPC column selection is based mainly on the molecular weight resolving range and, in this case, PLgel 5 µm 10^3Å columns are ideal.

Conditions

Columns:	2 x PLgel 5 µm 10^3Å, 300 x 7.5 mm (p/n PL1110-6530)
Eluent:	DMF + 0.1% LiBr
Flow Rate:	1.0 mL/min
Temperature:	70 °C
Detection:	RI, PL-GPC 120

Results and Discussion

Figure 3 shows the raw data chromatogram for poloxamer 188, two peaks indicating an impure material. After irradiating the polymer, the chromatogram shows a different pattern. This visual comparison reveals the effectiveness of GPC in monitoring changes in polymer molecular weight after exposure to extreme conditions.

In order to quantify this further, a GPC calibration can be performed using narrow polydispersity PEO standards (Figure 4), which, using Cirrus GPC software, can transform raw data into molecular weight distribution information.

Figure 5 illustrates the dramatic decrease in molecular weight undergone as a result of irradiation.

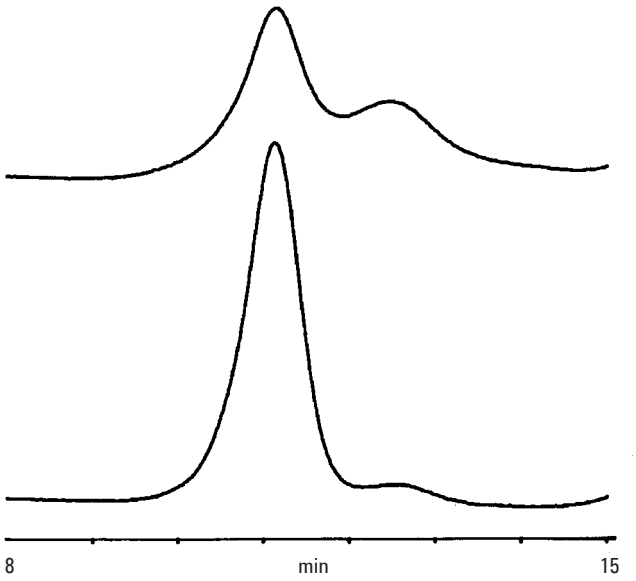

Figure 3. Raw data chromatograms of poloxamer 188 irradiated (below) and non-irradiated (above).

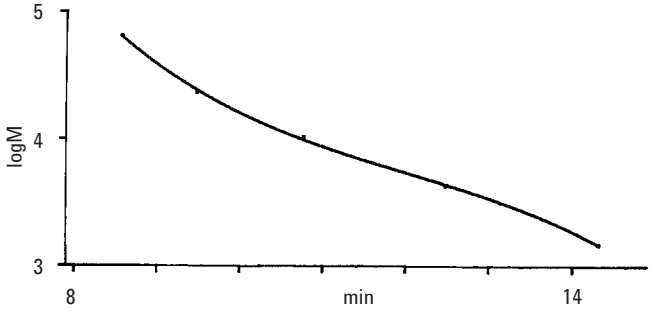

Figure 4. GPC calibration graph.

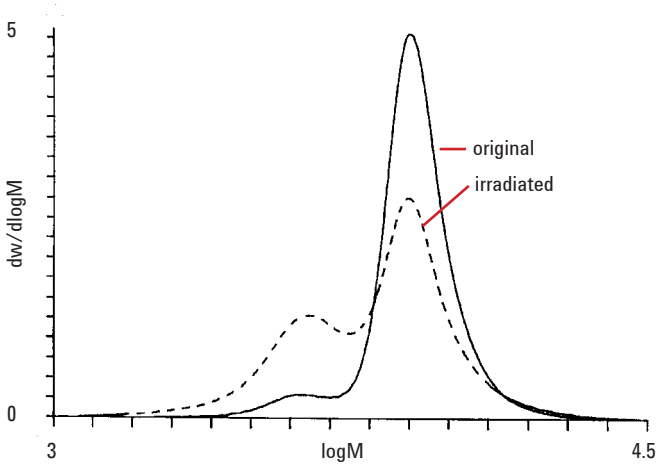

Figure 5. Molecular weight overlay of original (continuous line) and irradiated (dotted line) poloxamer 188.

Conclusion

HPLC and GPC liquid chromatography successfully investigated the purity and stability of some polymers typically used in pharmaceutical formulations. Detection of impurities and measurement of the effects of sterilizing radiation on molecular weight allows manufacturers to manipulate pharmaceutical polymers to maximize their performance.

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Published in UK, May 23, 2011
5990-8296EN

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