ANALYSIS AND PHYSICOCHEMICAL ASPECTS OF BIOCOMPATIBLE POLYMER CONJUGATES

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Biocompatible polymeric drug-conjugates are employed in drug targeting. The synthesis of such conjugates necessitates a rigorous analytical and physicochemical characterization strategy. Due to the large molecules (>40 kD) often used as the carrier polymer such analysis is now dominated by modern analytical and preparative chromatographic techniques. We have demonstrated such an approach in the preparation and analysis of conjugates of methotrexate (MTX) linked to amino-modified dextrans.

Amino-modified dextran-MTX polymeric conjugates and intermediates have been purified using two types of gel filtration column: Sephadex-G10 and Bio-Gel P2. The gels were swollen in water at 90°C, degassed and then packed into a glass column (100 x 2.65cm) using a gel applicator. The column was then eluted with degassed, distilled water (flow rate from 0.6 to 1 ml/min). The eluant was monitored by refractive index detection. Samples of dextran T40 and derivatives, and of dextran 1000 and its derivatives, were applied to Sephadex-G10 and Bio-Gel P2 columns respectively. The conjugates were analyzed on a TSK G3000 PW gel filtration column (Toya Soda) eluted with 0.067M aqueous Sorensen's phosphate buffer, pH 7.1, and the eluant monitored sequentially by UV absorbance (300nm) followed by refractive index detection. These measurements were used to quantify the levels of MTX covalently bound to the polymers. The broad distribution of dextran T40, as shown by its polydispersity index of 2.02, was a distinct disadvantage in the production of effectively characterized polymeric conjugates. Bio-Gel P2, was used as an alternative to Sephadex-G10 for the preparation of lower molecular weight dextran-MTX conjugates. It gave significantly improved resolution and excellent separation of the monomer and the linked glucose residues which constitute dextran 1000 and its derivatives. Chromatography on polyacrylamide gels is one of the major analytical techniques with ready application in the separation and analysis of oligosaccharides (Heyraud and Rinaudo 1981; John et al 1982). Each sample was spiked with glucose to enable an accurate identification of the number of sugar residues corresponding to each resolved peak. The amino-modified dextran 1000-MTX conjugate was eluted from the Bio-Gel column significantly earlier than the unconjugated dextran due to the increased size of the conjugate.

TSK analytical gel chromatography was used to demonstrate the molecular weight distribution of the dextran T40 polymers at each stage of the conjugate synthesis. The retention volume of the polymers increased after amino-modification, indicative of a decrease in molecular weight of the polymer or of an interaction with the stationary phase of the column, similar in nature to that observed with free MTX. Absorbance of the conjugate corresponded with a change in the observed refractive index i.e. an effect due to dextran. A previous calibration of the TSK column, with respect to MTX, enabled an accurate determination of bound MTX levels.

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