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Electrophoresis of polymeric dyes in macroporous polymer

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Summary

Macroporous poly(styrene-co-divinylbenzene), with poly(vinyl alcohol) (PVA)-coated pores, was investigated as a novel medium for thin-layer electrophoresis. The system was tested using polymeric dyes having polyelectrolyte character, which were prepared by reaction of a vinylsulphone reactive dye (CI Reactive Blue 19) with PVA. Higher molar mass samples exhibited higher mobility on electrophoresis in the macroporous medium.

Introduction

Polyelectrolytes pose particular problems of characterization with respect to molar mass [1,2]. Electrophoresis is widely employed in the analysis of biological macromolecules, but has been much less used for synthetic polyelectrolytes. Molar mass distributions of polyelectrolytes have been determined by electrophoresis in polyacrylamide gels [3] and agarose gels [4] and separations have been achieved in capillaries containing entangled polymer solutions [5,6] or dilute solutions of neutral polymers [7].

Electrophoresis of macromolecules is generally carried out in sieving media that impede the migration of larger molecules. The aim of the present work is to develop an electrophoresis medium that operates in a manner analogous to size exclusion chromatography (SEC), with larger molecules exhibiting higher mobility. The medium investigated was a macroporous crosslinked polystyrene in which the pores were coated with a hydrophilic polymer, poly(vinyl alcohol) (PVA). The system was tested utilizing polymeric dyes prepared by the reaction of a vinylsulphone reactive dye (CI Reactive Blue 19, commercially available as Remazol Brilliant Blue R) with samples of PVA of varying molar mass (Figure 1). The polymer-dye adducts exhibit polyelectrolyte character, from sulphonate and amine groups on the dye moieties, and are readily visible in electrophoresis, because of the presence of the chromophores.

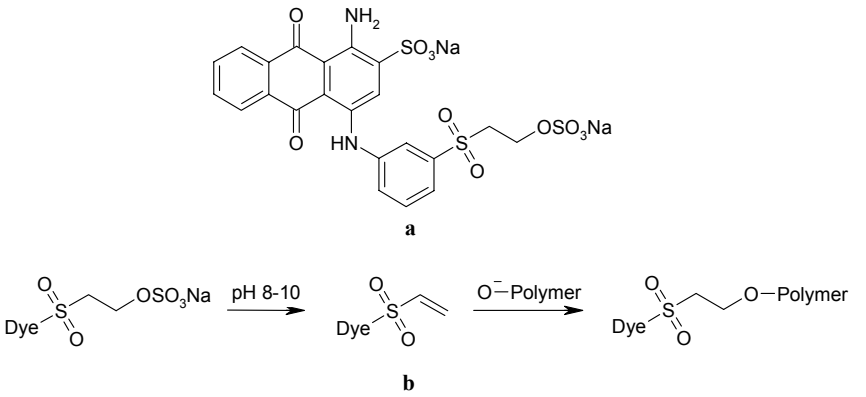

Figure 1. (a) Chemical structure of Reactive Blue 19 in sulphatoethylsulphone form. (b) Conversion to vinylsulphone form and reaction with deprotonated polymer.

Experimental

Preparation and characterization of polymer-dye adducts

A specially purified sample of Reactive Blue 19 in the sulphatoethylsulphone form was kindly provided by BASF. Analysis of the sample indicated that it comprised about 80 wt.% dye, together with some residual coupling agent and inorganic salts. Commercial samples of Reactive Blue 19 were found to be only about 45% dye, the remainder being largely anticoagulant. A sample of anticoagulant (Tamol NN9104, a naphthalene sulphonate formaldehyde condensate) was provided by BASF. Samples of PVA (≤2% residual acetate) were obtained from Fluka ($M \approx$ 72,000 g mol^{-1}) and Aldrich ($M \approx$ 124,000-186,000 g mol^{-1}).

The reaction conditions employed in the preparation of the polymer-dye adducts used in the present work are summarised in Table 1. Other experiments were also carried out, with different values of reaction variables. PVA was dissolved in distilled water, with heating to 80 °C, in a flask fitted with a condenser, thermometer and pH electrode. An aqueous solution of dye was added with stirring and the mixture brought to the reaction temperature. A 10% w/v aqueous solution of K_2CO_3 was added dropwise to adjust the pH and the solution then stirred for the required time. After allowing to cool briefly, the solution was transferred to Visking dialysis tubing and dialysed exhaustively against distilled water, to remove salt, unreacted dye and other low molar mass impurities. The polymer-dye adduct was recovered by freeze drying (Edwards Modulyo freeze dryer). Redissolution of adducts in aqueous media required the addition of anticoagulant, to inhibit hydrophobic association of dye moieties in water, but good solubility in dimethylsulphoxide was achieved without anticoagulant.

Polymer-dye adducts were characterized by UV/visible spectroscopy (sample concentrations: 0.05 to 0.1 g dm^{-3} in water, with 0.015 g dm^{-3} anticoagulant), aqueous gel permeation chromatography (GPC) (columns: TSKgel PW_{XL} 3000 & 4000; eluent: 0.2M $NaNO_3$, 0.02% w/v NaN_3; sample concentration: 0.2% w/v, with 0.05%

w/v anticoagulant), nuclear magnetic resonance spectroscopy (NMR) (Varian Unity 500; solvent: deuterated dimethylsulphoxide) and elemental analysis.

Table 1. Reaction conditions employed in the preparation of polymer-dye adducts

Sample	PVA $M/(10^3$ g mol$^{-1})$	Dye:OH (mol)	Dye % w/v	Reaction Temp./°C	pH	Reaction Time/h
D1	72	1:1	2	50	8	1
D2	124-180	1:1	5	50	8	4

Preparation of thin-layer macroporous PVA-coated poly(styrene-co-divinylbenzene)

Styrene monomer (98%, Aldrich) and divinylbenzene crosslinker (80%, Fluka) were vacuum distilled prior to use to remove inhibitor. 1-Dodecanol (Aldrich) was used as a porogenic solvent and benzoyl peroxide (Fisher) as initiator. A mixture of solvent, monomers (40% v/v, 20% crosslinker) and initiator were added to an upright mould comprising two glass plates (12 × 5 cm) separated by a rubber spacer (2 mm thickness) and polymerized at 50 ºC. The product, still attached to one glass plate, was dried under vacuum at 60 ºC for 24 h, washed with acetonitrile, dried, washed with methanol and dried again. Sample wells were engraved into the material. The macroporous poly(styrene-co-divinylbenzene) was coated with poly(vinyl alcohol) (M ≈ 72,000 g mol^{-1}, Fluka) by immersing under vacuum in an aqueous PVA solution (5% w/v) for 24 h [8]. Excess PVA solution was removed from the surface and the material dried overnight under vacuum at 60 °C.

Electrophoresis

The thin-layer electrophoresis medium was saturated under vacuum with pH 11 buffer (0.05M $NaHCO_3$, 0.01M NaOH) before use. The medium absorbed over twice its own mass of buffer. Samples (1 g dm^{-3}, with 2 g dm^{-3} Tamol NN9104 anticoagulant) were placed in sample wells with a 2 μL capillary micropipette. At high pH the samples are anionic. Electrophoresis was carried out at a field strength of 50 V cm^{-1} for 1 h using a Pharmacia LKB Multiphor horizontal electrophoresis system. After drying, plates were scanned using a Mikrotek E3 flatbed scanner and analysed using densitometer software (Biosoft Scan Analysis) on a Macintosh computer.

Results and Discussion

Polymer-dye adducts

Dye contents of the polymer-dye adducts, as determined by calibrated UV/Visible spectroscopy, are indicated in Table 2. Microanalysis gave similar results. We have found that strong non-covalent interactions between a reactive dye and a multifunctional polymer may compete with the desired reaction, and can lead to the presence of significant amounts of non-covalently bound dye in samples, even following extensive dialysis. An attempt was made to estimate the proportion of covalently bound dye from NMR data, and these results are included in Table 2. Gel Permeation chromatography with both refractive index and UV detection showed some dye to migrate with the polymer and some to contribute to a low molar mass peak.

Table 2. Dye content, dye loading per polymer hydroxyl and probable proportion of covalently bound dye for polymer-dye adducts

Sample	Dye content (wt. %)	Dye loading (mol %)	% covalently bound
D1	29	3.5	36
D2	48	7.9	90

Electrophoresis

A variety of conventional media were investigated for thin-layer electrophoresis of polymer-dye adducts, but poor results were obtained. For example, with polyacrylamide gels there was low sample mobility, whilst with agarose gels there was poor resolution. A reasonable electrophoretic separation was achieved, however, using macroporous PVA-coated poly(styrene-co-divinylbenzene), as can be seen from the densitometer data shown in Figure 2. Unlike sieving media, the highest molar mass sample (D2) exhibited the highest mobility, whilst the low molar mass dye showed much lower mobility. The breadth of the polymer peaks reflects the molar mass distributions of the samples.

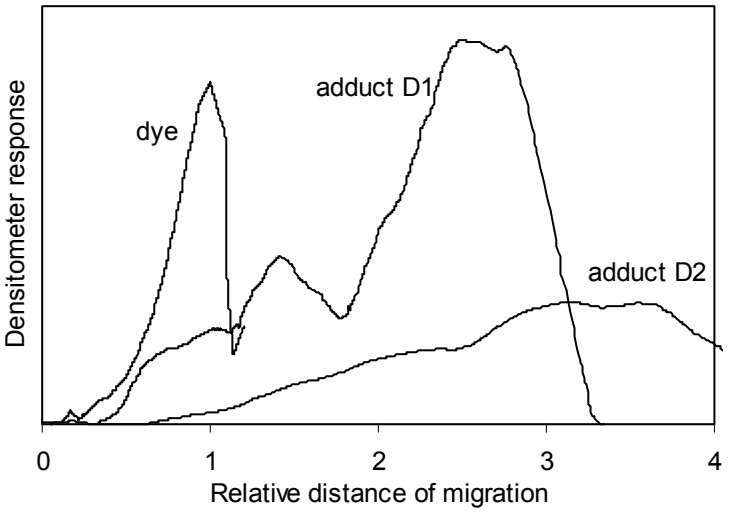

Figure 2. Electropherograms of low molar mass dye and poly(vinyl alcohol)-dye adducts in pH 11 buffer, showing higher mobility of higher molar mass samples.

The separation may arise through a size exclusion mechanism; a reduced pore volume is available to larger molecules, because their physical bulk excludes their centre of mass from a region close to the pore wall, and so they follow a less tortuous path. However, the results may also be explained in other ways, e.g. in terms of interactions with the PVA molecules used to coat the macroporous polystyrene. Further work is required to improve the system and clarify the mechanism of separation, but these initial results indicate the potential of macroporous media for electrophoretic separations.

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