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Field programming in frit inlet asymmetrical flow field-flow fractionation/multiangle light scattering: Application to sodium hyaluronate

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Abstract

The capability of field-programmed separation in frit inlet asymmetrical flow field-flow fractionation (FI-AFlFFF) has been examined for separating a high molecular weight sodium hyaluronate (NaHA) by varying the field programming parameters. Experiments were performed with on-line coupling of the field programming FI-AFlFFF and multiangle light scattering (MALS) detection. Sample relaxation, a pre-requisite step to establish equilibrium states of sample materials prior to the beginning of separation in most forms of FFF techniques, is obtained by hydrodynamically in FI-AFlFFF without stopping the migration flow. Thus, the procedures of sample injection – hydrodynamic relaxation – separation in FI-AFlFFF are continuously achieved without halting the sample migration. In this study, field programming in FI-AFlFFF was investigated for the separation of NaHA, water-soluble polysaccharides, by examining the influence of field decay pattern, initial field strength condition, and ionic strength of carrier solution on the successful separation of a degraded NaHA sample. Results were compared with molecular weight calculations of eluting materials among different field programming conditions from multiangle light scattering (MALS) signals. It was found that when the field programming was utilized in FI-AFlFFF, a proper selection of initial cross-flow rate, the field decay pattern, and an appropriate control of final field strength needed to be carefully selected in achieving a successful separation of a broad molecular weight water-soluble polymer sample.

Keywords: Sodium hyaluronate; Frit inlet asymmetrical flow field-flow fractionation; Field programming; Multiangle light scattering; Molecular weight determination

1. Introduction

Flow field-flow fractionation (FlFFF) is an elution-based technique that is capable of separating and characterizing colloidal particles, proteins, and macromolecules according to the differences in hydrodynamic size of sample components [1]. In FlFFF, separation is carried out in a thin, empty channel with the use of migration flow, while cross-flow, a driving force to retain sample components within the channel, is simultaneously applied to the direction perpendicular to the axial migration flow (or channel flow) [2–5]. Before sample components begin migration, they are required to achieve equilibrium states in advance at certain distances away from the channel accumulation wall. At the equilibrium positions, the field force (from cross-flow movement) exerted to sample materials and the diffusion of sample components are counterbalanced. This process is referred as the relaxation process, which is essential in most FFF techniques and it is achieved by applying cross-flow only with the temporary halt of the migration flow. When sample components achieve equilibrium states, they are differentially distributed against the accumulation wall according to sizes and therefore, they will migrate at different velocities when axial flow is then applied. Polymers with lower molecular weight (or smaller hydrodynamic diameter) have larger diffusion coefficient val-

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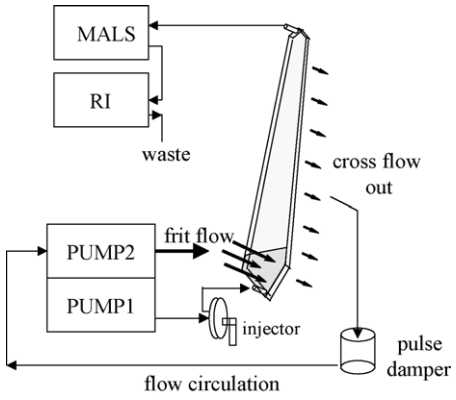

Fig. 1. Schematic diagram of frit inlet asymmetrical flow field-flow fractionation/multiangle light scattering/refractive index (FI-AFlFFF/MALS/RI) with the cross-flow circulation for programmed field operation.

ues and migrate faster at equilibrium positions that are higher than those reached by the larger ones. This is due to the differences in the flow velocities within the parabolic flow profile of the mobile phase that moves through a thin empty channel. Thus, separation in FlFFF is achieved in the order of increasing diameter or molecular weight of sample components.

Frit inlet asymmetrical flow field-flow fractionation (FI-AFlFFF) utilizes a modified channel that was designed to bypass the stop-flow relaxation procedure [5–8]. In FI-AFlFFF, sample materials are introduced through the channel inlet while a relatively high flow rate is applied through the inlet frit, as it is schematically represented in Fig. 1. Due to the compression role of the frit flow, sample materials injected at low injection flow rate (usually ca. 20 times lower than the frit flow rate) are quickly pushed toward the accumulation wall by incoming frit flow, and then they achieve hydrodynamic relaxation. Thus, sample relaxation and separation processes are continuously achieved without halting the sample migration that is normally required for the conventional FlFFF methods. While earlier studies on FI-AFlFFF channel showed that hydrodynamic relaxation can be successfully utilized to separate particles or macromolecules with a stopless flow operation, it always required the use of a high speed frit flow relative to the sample flow in order to assure sample relaxation. It might however reduce the flexibility of selecting separation conditions for highly retaining materials, such as very high molecular weight materials. The lack of flexibility can be overcome by applying field programming to FI-AFlFFF in which an initial field strength or cross-flow rate (normally set to be the same as frit flow rate) is applied sufficiently high enough to assure a successful hydrodynamic relaxation and then it is decreased gradually to fasten the elution. Field programming in FlFFF was not as popular as in other FFF techniques such as sedimentation FFF and thermal FFF but the potential of incorporating programming was already demonstrated in a few studies [4,5,9–12]. Field programming in FI-AFlFFF can be very easily employed by circulating the cross-flow to the frit flow and reducing the circulation flow rate with time. Since highly retaining components (large molecular weight) at a constant field strength condition may not elute properly when separating a broad molecular weight sample in FI-AFlFFF, a subsequent decrease in field strength during elution will be useful to elevate sample components from the vicinity of channel wall toward the fast flow streamline during run. This makes sample components eluting in a reduced time. In a previous study, it was demonstrated that programmed FI-AFlFFF can successfully expand the dynamic separation range of molecular weight utilizing polystyrene sulfonate standards in the molecular weight range of 4–1000 KDa [5].

In this study, field programming in FI-AFlFFF was applied for the separation of sodium hyaluronate, a sodium salt of hyaluronic acid, which is a natural and very high molecular weight linear polysaccharide composed of a disaccharide repeating unit (D-gluconic acid and *N*-acetyl-D-gluconsamine). NaHA has been found in various body tissues and fluids such as vitreous humour or umbilical cord, and utilized pharmaceutically for hydrogel formation or as a substitute for vitreous humor after opthalmic surgery [13–16]. The molecular weight of NaHA has been known as a few millions in Daltons, and the size characterization of NaHA in aqueous solution is important for certain desired applications. The analysis of NaHA molecules has been carried out mostly using size exclusion chromatography (SEC) [13,17]. However, when using SEC, a traditional size separation technique for polymers, difficulties in obtaining an accurate molecular weight arise from the followings such as a lack of suitable calibration standards for NaHA, a loss of resolution above the exclusion limit due to the nature of the ultra-high molecular weight of NaHA, a possible polymeric chain degradation due to the shear, and a possible sample adsorption at the surface of packing materials. Recently, a study was reported on the size characterization of NaHA by FlFFF-MALS but it was carried out with a conventional asymmetrical flow FFF channel [18]. Since the sample relaxation in the conventional asymmetrical FlFFF channel is achieved by focusing the two flows from both the channel inlet and outlet for a certain period of time, sample migration during the focusing procedure is temporarily stopped and then after the completion the separation begins by inverting the flow direction to the channel outlet. In the current work, FI-AFlFFF has been employed for the separation of high molecular weight NaHA with the use of field programming. This study focused on the evaluation of the programming conditions such as the initial field strength and field decay patterns on NaHA separation. It was also examined with the effect of ionic strength of carrier solution and injection amount on the retention of sodium hyaluronate by field programmed FI-AFlFFF. For the evaluation of separation effectiveness, on-line coupling of multiangle light scattering (MALS) detection along with refractometer (RI detector) was utilized for FI-AFlFFF and the resulting calculation in the molecular weight values or root-mean-square (RMS) radius of eluting NaHA materials were compared when varying FI-AFlFFF run conditions. By applying the field programming technique to FI-AFlFFF and on-line cou-