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Contents

Flow of Red Blood Cells in Capillary Networks

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Abstract

In the present work we have studied the flow of red blood cells (RBCs) through a column packed with soda lime glass spheres with diameter of 337.5 μm (pore diameter of 150 μm). The ratio between the average velocity of the RBCs and average velocity of the carrying fluid (physiological saline) was close to 0.9. The RBCs migrated faster through the column than the carrying fluid mainly due to a hydrodynamic chromatography effect.

1. Introduction

The size fractionation of polymers or particles by hydrodynamic chromatography (HDC) was suggested four decades ago by DiMarzio and Guttman [1] using theoretical approach. In the meanwhile, variety of applications for HDC was found in a broad range of separations, such as polymer latexes [2,3], rigid and flexible polymers [4-13], silica particles[14], zeolites [15], viruses [5], bacteria and yeasts [16], proteins [17], plasmid DNAs [17,18], starch [19,20], gold colloids [21], liposomes [21] and nanocapsules [22]. Those separations are being performed using columns packed with nonporous particles (packed-column hydrodynamic chromatography - PCHDC) or open capillaries (capillary hydrodynamic chromatography - CHDC).

Most of the theories for PCHDC have been derived from the model, which is used to describe the migration of polymers or particles in CHDC under laminar regime (Fig. 1).

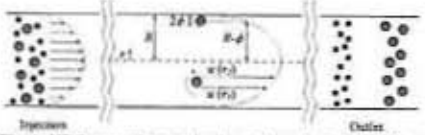

Fig. 1. HDC separation mechanism. The cylindrical capillary has a radius R and $u(r)$ represents the local velocity for a stream line at a radial position r.

As a result of Brownian motion, analytes (molecules or particles) with effective diameter 2ϕ will diffuse in all possible radial directions. This radial diffusion is fast enough to transport the analytes many times over the capillary cross section during their residence time in the tube [1, 6].

Due to their finite size, the analyte centre of the mass cannot approach the capillary wall closer than their own effective radius ϕ, see Fig. 1. While large analytes are exposed to higher velocities observed in the range of R-ϕ, infinite small particles or molecules can experience all the stream lines available in the column cross section. As a result, large analytes will migrate faster through the column than small analytes, if the ratio $\lambda = \phi / R$ is not too large [1, 6].

The ratio $\lambda = \phi / R$ is the key parameter in the HDC separations, since molecules/particles with different values of λ will migrate at different velocities. That effect is allowing size fractionation. Despite of this, it is important to note, that when the analytes are immersed in a parabolic flow, the side of the analyte that is closer to the capillary centre is located on a streamline with higher velocity ($u(r_2)$) comparing to the side closer to the capillary wall ($u(r_1)$), see Fig. 1. Therefore, the analyte rotates and this rotational movement affects the translational velocity [1, 6].

The different effects mentioned above are included in the following model [1, 6-8]:

$$RRT = \frac{1}{1 + 2\lambda - C\lambda^2} \qquad (1)$$

where RRT is a relative retention time and is always ≤ 1. It is defined as the ratio between the average residence time of the analyte with effective radius ϕ and the average residence time of the molecule or particle with infinite small size (the infinite small sized marker). For cylindrical capillaries, constant C varies between 1 and 5, obtaining a value of 2.7 for different flexible polymers and a value of 4.89 for solid spherical particles [23].

The modelling of transport of flexible polymers is much more complex than that of rigid spherical particles, since the size and shape of the polymer is not well defined. Hence, it is difficult to predict the distance to which the centre of the mass of the polymer can approach the wall of the capillary [7].

Using CHDC columns with internal diameters around 1 μm, Tijssen et al. (1986) [6] separated flexible linear random coil polystyrenes with different molecular weight (Mw). Stegeman et al. (1993) [7] used PCHDC columns filled with non-porous glass spheres with diameter of 1.5 μm and separated different Mw flexible polymers (polystyrene, polyisoprene, polyisobutadiene). Non-porous glass spheres with diameter of 1 μm were used by Venema et al. [8] in order to separate different Mw polysterenes and poly(methyl methacrylate). In all those works authors found, that the effective radius ϕ is well defined by the expression $(\sqrt{\pi}/2)R_g$, where R_g is the gyration radius of the polymer.

Using this effective radius definition and $C = 2.7$, the different researchers [6-8] reported, that the experimental values of RRT are well described by the Eq. (1). Therefore, this equation could be used as a universal calibration curve for the studied flexible polymers (see Fig. 2), similar to the procedure used in size exclusion chromatography [7].

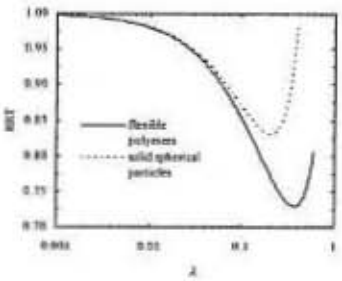

Fig. 2. RRT for different values of λ. The curves for flexible polymers and solid spherical particles were obtained using $C = 2.7$ and $C = 4.89$ [23] in Eq. (1), respectively.

In the Fig. 2 it can also be observed, that the RRT value starts to increase with the increase of λ when this parameter is higher than ~0.35. This fact is reported in different experimental works [6-8]. For values of λ large enough, the polymers will be trapped in the separation medium.

The relative retention times for solid spherical particles and flexible polymers start to be significantly different for values of λ higher than ~0.1. The value of C for micelles is not known but it is expected to be between $C = 2.7$ and $C = 4.89$ [23].

In works [7, 8, 11, 14] where PCHDC was used, it was assumed, that the interstitial channels could be represented by a bundle of cylindrical capillaries with average radius R, given by expression $(d_p/3)(\varepsilon/(1-\varepsilon))$, where d_p is the particle diameter and ε is the packing porosity.

2. Materials and Methods

The samples used in the present study were sheep's red blood cells (RBCs) suspended in a physiological saline (PS), containing 0.1 to 1% of hematocrit (Hct). The blood was collected from a healthy adult sheep, where heparin was added to prevent coagulation. The RBCs where separated from the bulk blood by centrifugation (3000 RPM for 15 min) and aspiration of the plasma and buffy coat. Afterwards the RBCs were washed twice with PS. The washed RBCs where diluted with PS to make required RBCs concentration by volume. All the blood samples were stored hermetical at 4ºC until the experiment was performed at room temperature around 20ºC. Different blood samples are shown in Fig. 3.

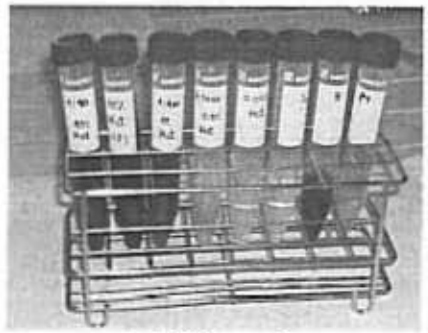

Fig. 3. RBC's suspensions.

The apparatus used in this work is shown in Fig. 4 and contains: a pump (1); injector (2); a chromatographic column packed with glass spheres (3); refractive index detector (4) and data acquisition system (5a and 5b). The mobile phase was PS, the flow rates varied between 0.2 ml/min and 1 ml/min and the chromatographic column was packed with glass spheres with diameter of 0.3375 mm. The infinite small sized marker was a water solution of sucrose with concentration of 1 g/ml.

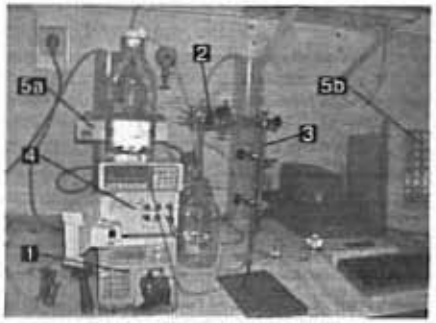

Figure 4. Chromatographic apparatus.

3. Results

The RRT values obtained for the different flow rates (0.2 – 1 ml/min) and different RBC's concentrations (0.1 – 1 %) were very similar and varied between 0.9 and 0.92. Figs. 5 and 6 show the results for flow rates of 1 ml/min and 0.5 ml/min, respectively, using a RBC concentration of 1%. In the referred figures, it can be seen that the obtained RRTs were 0.900 (81.5/90.5) and 0.916 (164/179), respectively.

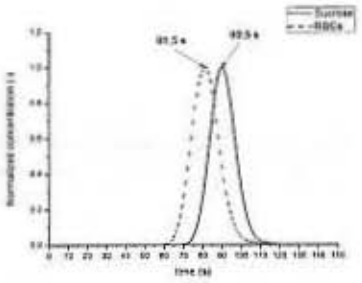

Fig. 5. Chromatogram for 1 ml/min.

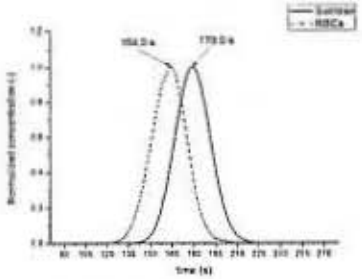

Fig. 6. Chromatogram for 0.5 ml/min.

In Fig. 2 we may see, that for ratios λ lower than 0.05 to 0.1, the RRT values predicted by Eq. (1), using C = 2.7 (flexible polymers) and C = 4.89 (solid spherical particles) are very similar. If we assume, that for RBCs the value of C is located between C = 4.89 and C = 2.7, it is possible to estimate (using Eq. (1) and the known values of the RRT for RBCs) that during the flow, the effective diameter 2ϕ of the RBCs is located in the approximate range of 6.96 - 9.95 μm.

4. Concluding Remarks

Preliminary experiments using different flow rates and RBCs concentrations have shown, that in the studied ranges these parameters had no detectable influence in the obtained RRTs.

Future works will include the use of human blood and experiments with higher range of λ in order to clarify the behavior of the RRT with λ for RBCs.

We will also try to find if the obtained RRTs are concomitantly influenced by the HDC and plasma layer effects since the obtained RRTs seems to be very low to be explained by a pure HDC mechanism.

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