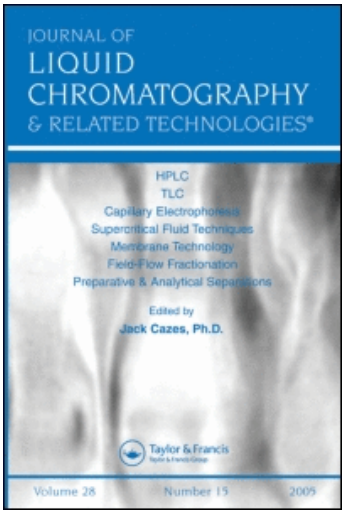

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Viscosity-Temperature Dependence on DNA Stretching: Slalom Chromatography Study

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ABSTRACT

Slalom chromatography (SC) is a chromatographic procedure for the separation of polymers, which is based on a non equilibrium principle. A novel equation was recently developed to model the retention variation of linear double stranded DNA molecules with the mobile phase velocity under SC. This paper analyzes the effect of mobile phase viscosity and

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temperature on the constants of this equation and confirms that these two factors play a great role on the DNA fragment stretching.

Key Words: Chromatography; DNA; Slalom; Model; Viscosity; Temperature.

INTRODUCTION

The study of methods to analyze the genomes of different biological systems is being undertaken in laboratories worldwide. The first complete sequence of a genome, a living organism, a bacterium called *Haemophilus influenza*, was carried out in 1953. The main technique used to separate the DNA fragments is electrophoresis. When used on agarose gel, for example, it separates fragments of chromosomes cut by restriction enzymes. Capillary electrophoresis uses techniques that are closer to biological conditions, are faster, and give efficient resolution. Electrophoresis is a separation technique based on the difference of migration speed of the DNA fragments under an electric charge. The use of capillaries with a small internal diameter($<10\,\mu m$) led to the use of high electric fields ($<1000\,V/cm$). This technique was then miniaturized and gave high resolution efficiency. With pulsed fields the method can separate up to 40,000 base pairs.

As well as these electrophoresis separation techniques, there are those using chromatography. The more usual modes are based on the affinity differences of the DNA fragments between the stationary and mobile[1–8] phases. On column chromatography, the stationary phase filling the column is a liquid impregnating a support. In the case of ion exchange chromatography, the retention mechanism for the DNA fragments is related to the electrostatic interactions between the phosphate groups of DNA fragments and the stationary phase cationic groups. In the case of hydrophobic-interaction chromatography, the retention of the DNA fragment is linked to the intensity of the hydrophobic interactions that it establishes with the stationary phase. Finally, with gel permeation chromatography, the separation criterion of the fragments is their size. The separation is based on their ability to penetrate the pores of a gel forming the stationary phase. At the present time, alternative non equilibrium chromatographic techniques such as slalom chromatography (SC), are being investigated, to separate flexible biological molecules. Recently, a novel empirical mathematical equation[9] was developed to model DNA fragment retention with mobile phase velocity in SC. In order to gain further insight into this mathematical model, the effect of mobile phase viscosity and column temperature on some constants of this equation was studied.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Merck Hitachi pump L7100 (Nogent-Sur-Marne, France), an Interchim Rheodyne injection model 7125 (Montluçon, France) fitted with a 20 μL sample loop, and a Merck L4500 diode array detector. A C_1 Kromasil column (particle size: 5 μm column size: 150 mm × 4.6 mm) was supplied by Interchim. All the experiments were carried out using a TM N° 701 Interchim column oven.

Reagents

Circular double-stranded DNA P_3, P_5, . . . , P_{10} and λ DNA (48.5 kbp) and restriction enzyme BamHI, KpnI, HindIII and ApaI were supplied by New England Biolabs (Gagny, France). Ethanol, EDTA, acetonitrile, and sodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from Prolabo (Paris, France). Water was obtained from an Elgastat option water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge.

Digestion of λ DNA

Restriction enzymes were used for cleavage of the λ DNA and plasmids into linear DNA fragments of different sizes: 17.09 kbp, 23.13 kbp, 29.95 kbp, 38.42 kbp. Two micrograms of λ DNA or circular DNA was treated with 3U of restriction enzymes in 16 μL of the reaction mixture at 37°C for 3 h, precipitated by ethanol, dissolved in 20 μL of water, and stored at −20°C until used.

Chromatographic Conditions

The mobile phase consisted of a sodium phosphate buffer 0.01 M pH = 6.8-EDTA/0.001 M-acetonitrile, 80/20 (v/v) mixture with different concentrations of glycerol varying from 0 to 1 M. Hirabayashi and Kasai[20] have previously shown that columns developed for reversed chromatography (the column used in this study) are useful for SC. In order to eliminate a possible hydrophobic interaction, which could interfere with the hydrodynamic principle, these authors used an aqueous mobile phase containing a 5–20% organic modifier such as acetonitrile. It was found that the hydrophobic interaction was negligible in such conditions. Thus, our experiments were carried out with a larger proportion of acetonitrile in the mobile

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phase (20%). The column temperature varied from 5 to 55°C. Twenty milligrams per liter of the DNA solutions were injected in triplicate. The mobile phase flow-rate varied from 0.02 to 1.5 mL/min.

RESULTS AND DISCUSSION

DNA Conformation in Solution

A model of the Gaussian chain with a persistent chain length ρ is used to describe the DNA conformation in solution.[11–13] The DNA is represented by N_k statistical chains with a length b or $b.$, the Kuhn length is equal to twice the persistence length of the molecule. The DNA molecule adopts the random coil conformation. Its contour length is $L_c = b_0 N_0$ where N_0 is the number of bases and b_0 the distance between the bases. Neglecting the excluded volume interactions, its gyration radius is given by the relationship $R_g = 1/3(\rho/L_c)\ \{1 - 3\rho/L_c + 6\rho^2/{L_c}^2 - 6\rho^3/{L_c}^2\ [1 - \exp(-L_c/\rho)]\}$. If the contour length is longer than the persistence length ($L_c \gg \rho$), the polymer is described by a random step of pitch b, the chain is Gaussian, and $R_g = {L_c}^{1/2}$. If, however, $L_c \ll \rho$, the chain conformation is the measurement of the contour distance on which there is still a direction correlation between the units of the chain; it is linked to the free energy of the surrounding deformation of the chain. All these considerations show that DNA, in certain well defined physical–chemical conditions, can adopt an elongated conformation.

Theoretical Models Describing Non Equilibrium DNA Chromatography

Non equilibrium chromatographic techniques, particularly SC, are currently used for the separation of DNA fragments with a length of between 15 kb and 50 kb.[14–28] Our laboratory has developed mathematical models to try and elucidate the retention and separation mechanisms.[9,23,25,27,28] In these models, the stationary phase filling the column is made up of silica particles with a diameter d_p, forming a network of three dimensional pores. The mean pore diameter is l. The number of pores (or segments) occupied by the DNA chain is p. The progression of the DNA fragments through this network will obviously depend on the mean pore diameter, as well as the gyration radius of the DNA molecule. As the DNA chain was aligned in the flow direction, it was assumed to be a linear stretching of the DNA fragment in one pore. The sequence of pores followed by the chain is called the tube with a total length, pl. To model the DNA fragment retention inside the column, a parameter τ[9]

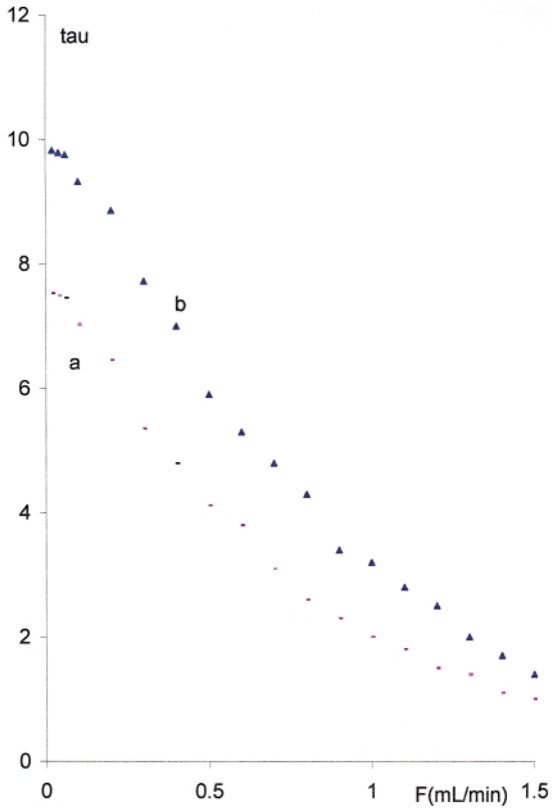

Figure 1. Curve τ vs. F (mL/min), for the 48.5 kbp DNA fragment size, for two different glycerol concentration in the mobile phase: (a) 0 M, (b) 0.1 M at $T = 25°C$.

was introduced and defined by the equation $\tau = t(v)/t_\infty$, where $t(v)$ is the DNA fragment retention time at the v mobile phase velocity and t_∞ its retention time when it is completely stretched. Recently, it was demonstrated that τ could be given by the equation:[9]

$$\tau = \psi(e^{-kv} + kv - 1) + \breve{\tau} \tag{1}$$

where ψ was an empirical constant depending on some geometrical characteristics of the DNA fragment. $\breve{\tau}$ was the τ value at the lowest mobile phase

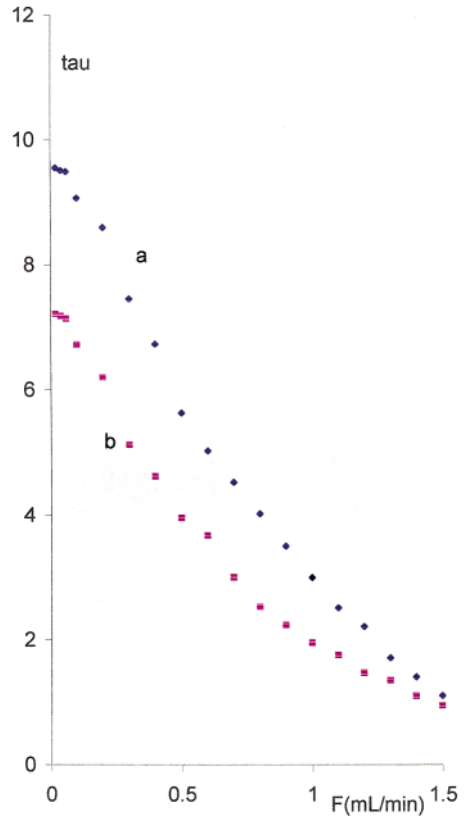

Figure 2. Curve τ vs. F (mL/min), for the 48.5 kbp DNA fragment size, for two different temperatures: (a) 5°C, (b) 20°C; the glycerol concentration in the mobile phase was nil.

velocity (for this value, the stretching fraction of the DNA fragment $\rightarrow 0$). k was the constant of the following equation:[9]

$$\frac{dp}{dv} = k(p_\infty - p) \tag{2}$$

where p_∞ was the number of pores occupied by the DNA chain at its stretching maximum. The value $\ln 2/k$ can also be defined as the value of the mobile phase velocity for which the p_∞ value was divided by two. A first series of experiments were carried out for which the column temperature was equal to 25°C, the glycerol concentration c in the mobile phase varied from

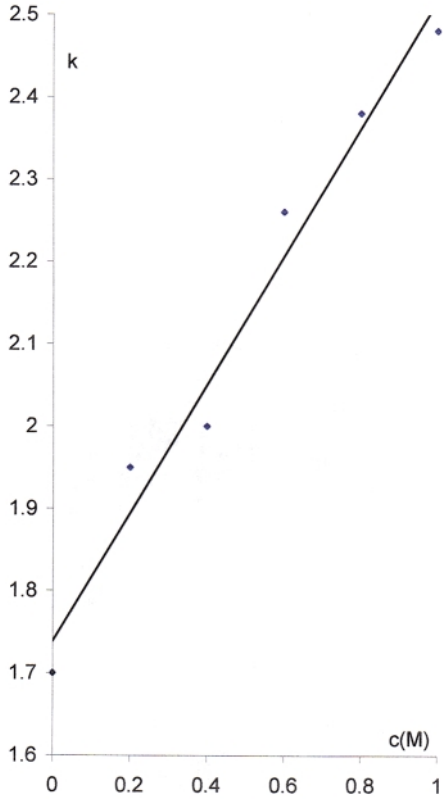

Figure 3. Curve k vs. c (M) for the 48.5 DNA fragment at $T = 25°C$.

0 to 1 M, and the mobile phase flow-rate from 0.02 to 1.5 mL/min. A second series of experiments were carried out for which the glycerol concentration in the mobile phase was nil, the column temperature varied from 5 to 55°C, and the mobile phase flow-rate from 0.02 to 1.5 mL/min.

All the experiments were repeated three times. For each DNA fragment, the RSDs of the τ values were less than 3% in most cases, indicating a high reproducibility and good stability for the chromatographic system. With a non linear regression procedure, which was used in earlier chromatographic studies,[9,27] the data obtained in the two series of experiments were fitted to Eq. (1). After the non linear regression procedure, the calculated ψ, k, and $\check{\tau}$ values, obtained for the two series of experiments and for each DNA fragment were used to estimate the τ values with the measured values. The correlation between all the predicted and experimental τ values exhibited slopes equal to

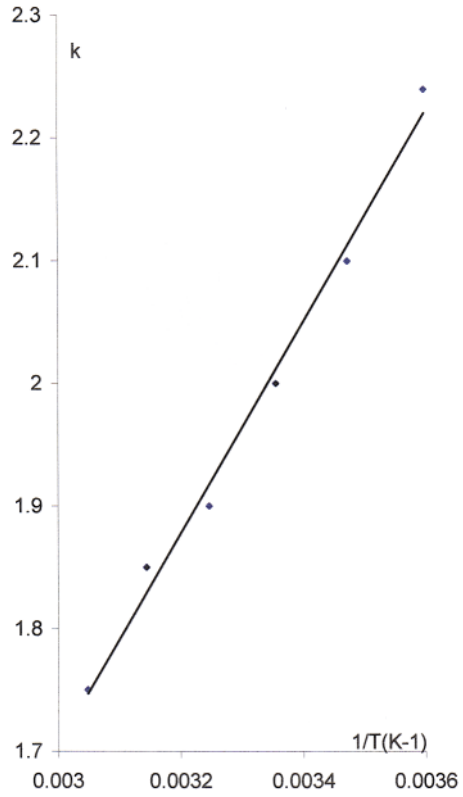

Figure 4. Curve k vs. $1/T$ (K^{-1}) for the 48.5 DNA fragment; the glycerol concentration in the mobile phase was nil.

0.98 with $r^2 > 0.97$. Figures 1 and 2 showed the τ dependence on v at two glycerol concentrations, c, and two column temperatures (T) for the 48.5 kbp DNA fragment. A similar variation was observed for the other c or T values and DNA fragments. As explained previously,[9] the liquid velocity dependence on τ was a sigmoidal like curve. It has been demonstrated that the glycerol effect on the DNA fragment retention in SC was a result of a change in the mobile phase viscosity η.[25] The dependence of the acetonitrile–water mobile phase viscosity was estimated by the use of the empirical relationship reported by Christ et al.[29]

$$\eta = \eta_{25^\circ C}\left(\frac{298}{T}\right)^6$$

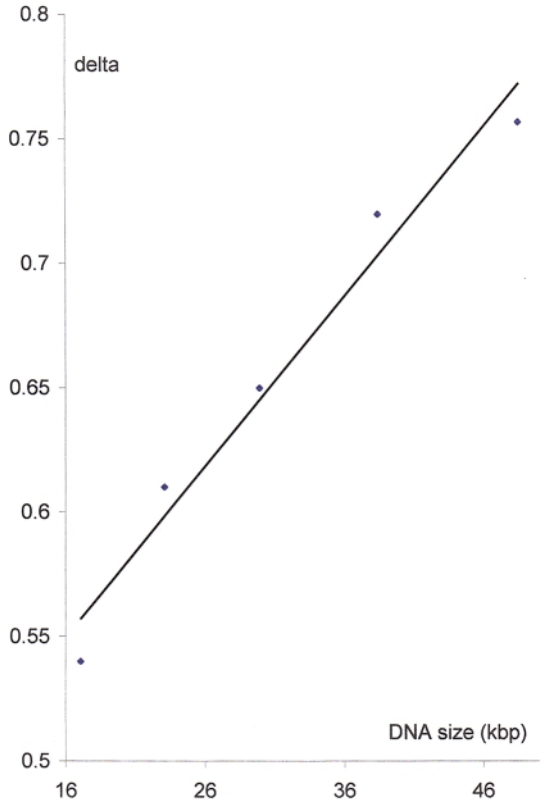

Figure 5. Curve $\delta = dk/dc$ vs. DNA fragment size at $T = 25°C$.

where $\eta_{25°C}$ is the viscosity at 25°C. For a given DNA fragment, when η; i.e. c increased or T decreased, the hydrodynamic force generated by the mobile phase and applied across the ends of the DNA chain increased. Therefore, the number of pores occupied and, thus, the k constant, increased (Figs. 3 and 4). The delaying factors

$$\delta = \frac{dk}{dc} \quad \text{or} \quad \delta' = \frac{dk}{d(1/T)} \text{I}$$

increased as the DNA length increased (Figs. 5 and 6). These δ and δ' variations can be explained by the fact that a greater stretching of the DNA fragment was observed for large DNA fragments rather than small DNA fragments. All these variations showed that in SC, the hydrodynamic force

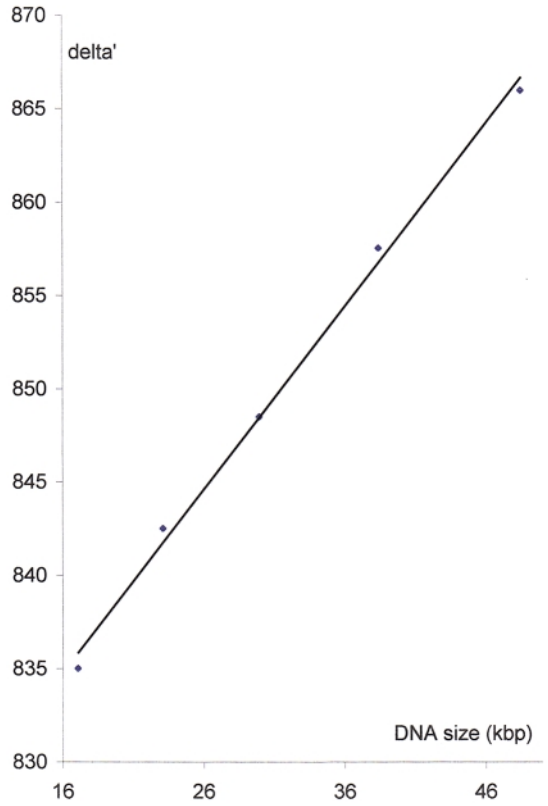

Figure 6. Curve $\delta' = dk/d(1/T)$ vs. DNA fragment size; the glycerol concentration in the mobile phase was nil.

played a major role on the slalom chromatographic mode. In summary, this paper confirmed, with the use of a novel mathematical model, that the retention mechanism in SC is strongly dependent on the DNA stretching, governed by the hydrodynamic force generated by the mobile phase.

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