INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Review Article

SLALOM CHROMATOGRAPHY: AN OVERVIEW

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ABSTRACT

Various techniques like gel electrophoresis, gel permeation chromatography, ion-exchange chromatography have been employed for analysis and separation of large biomolecules such as double-stranded DNA and RNA. A technique called Slalom chromatography was discovered in 1988 by Boyes et al and Hirabayashi and Kasai independently. This size-fractionation method includes reptation of DNA fragments through the column packing which resembles a person on skis going down a slope and turning quickly around flags. The principle mode of separation is based on hydrodynamic phenomenon rather than an equilibrium one. The reorientation time of DNA determines the orders of elution i.e. larger strands are eluted after the smaller ones. The article reviews about the chromatographic conditions used for slalom chromatography and various factors affecting the DNA separation. The physicochemical factors have been shown to have a critical effect on the separation DNA topology, temperature, mobile phase viscosity and particle size of packing material. The separation technique reviewed provides a new effective tool for physicochemical and hydrodynamic studies of DNA.

Keywords: Slalom chromatography, size-fractionation, DNA, hydrodynamic phenomenon.

INTRODUCTION

Biochemical analysis techniques refer to a set of methods, assays, and procedures that enable scientists to analyze the substances found in living organisms and the chemical reactions underlying life processes. To perform a comprehensive biochemical analysis of a biomolecule in a biological process or system, the biochemist typically needs to design a strategy to detect that biomolecule, isolate it in pure form from among thousands of molecules that can be found in an extracts from a biological sample, characterize it, and analyze its function. For the analysis and separation of large biological macromolecules such as DNA and RNA, various techniques have been developed over the past few years. Chromatographic modes so far used are divided into two groups: purely size-dependent modes and modes that eventually result in size-dependent separation. Ion-exchange chromatography is categorized under first group whereas size exclusion and slalom chromatography are truly size-dependent modes. Chromatography is versatile as a research tool for both preparation and analysis because as soon as a separation process is complete, valuable information is also obtained on aspects such as quantity, quality and composition. Therefore, the more sophisticated the chromatographic mode, the higher the quality of the information obtained.

Size of the nucleic acids

The term "size" can have a variety of meanings. In biochemistry, it is generally used to mean either molecular mass or degree of polymerization of macromolecules, The size of polynucleotides has been exclusively expressed

by the degree of polymerization, *e.g.* number of base pairs or kilobasepairs (abbreviated as bp or kbp) in the case of double-stranded DNA. Polynucleotides take different shapes depending on their degree of polymerization. A small double-stranded DNA fragment, *e.g.* 20 bp (2 x 6.4 nm), has a rigid and globular shape. Large double-stranded DNA fragments are fibrous, and it is becoming difficult to treat them as globular molecules. A fragment with *ca.* 100 bp (2 x 34 nm) should be treated as a rod with a certain degree of elasticity. A long double-stranded DNA molecule has a kink at every *ca.* 50 nm (150 bp) and consequently forms a random coil, which is the most favourable shape[1,2] .The random coil is very flexible, and its shape changes without a break between the contracted and slightly extended forms by an external force provided by Brownian motion of water molecules.

Chromatographic modes currently applicable for size-dependent separation of nucleic acids

1) Gel electrophoresis: Nucleic acids are separated by applying an electric field to move the negatively charged molecules through an agarose matrix. Shorter molecules move faster and migrate farther than longer ones because shorter can migrate more easily through the pores of gel. Disadvantages: It takes longer for both separation and recovery, and the recovered sample is usually contaminated with impurities contained in the Agarose.

2) Ion-exchange chromatography: Separation of nucleic acids by ion–exchange chromatography is mainly based on electrostatic interactions between the phosphate groups of the former and the positively charged groups of the ion exchanger. Adsorbed oligonucleotides were eluted by a salt gradient in the order of the degree of polymerization. Ion exchange HPLC separates oligonucleotides according to number of charged groups (phosphate linkages)[3]. The separation decreases with oligonucleotide length. Anion-exchange chromatography with a proper gradient is also widely used for purification of RNA and DNA, with plasmid DNA[4].

Disadvantages: The separation of double-stranded DNA fragments larger than several hundred base pairs by ion-exchange chromatography on macroporous supports has been difficult. Most DNA fragments cannot permeate the pores of the ion ex-changer and this resulted in too low an operational capacity. It remains inefficient because of poor resolution and slow speed. The amount of sample that can be used is rather limited, because the capacity of non-porous adsorbents is generally not so high hence low loading capacity[5].

3) Size-exclusion (gel-permeation) chromatography: It separates biomolecules on the basis of true size difference. The column consists of a hollow tube tightly packed with extremely small porous polymer beads designed to have pores of different sizes. Small molecules of analyte can enter the pores of gel more easily and therefore spend more time in these pores increasing their retention time. Conversely, large analytes spend little time in the pores and elute quickly.

Disadvantages: The size of the nucleic acids of interest is usually too large to be applicable to a gel-permeation column. The resolution attainable by gel permeation chromatography is usually inferior to that of other modes, such as ion-exchange and reversed-phase.It is difficult to speed up the operation because it is essential to maintain an equilibrium state of distribution of the target molecules between two phases, *i.e.* stationary phase retained in intrapore spaces and the mobile phase[6].

The comparison of chromatographic modes available for size fractionation of nucleic acids is shown in table 1.

SLALOM CHROMATOGRAPHY

Slalom chromatography (SC) was discovered independently by Boyes et al[7] and Hirabayashi and Kasai in 1988. It is a chromatographic mode for separation of flexible biological molecules. In this technique, the reptation of the DNA fragments through the column packing follows the flow direction and its like a snake edging is way into long grass. This new mode of separation was named "slalom chromatography", because the proposed model reminds us of a person on skis going down a slope and turning quickly around flags[8] and can be illustrated as figure. 1. The separation of molecules by liquid chromatography is classically based on an equilibrium phenomenon (determined by the distribution coefficient) between the eluent and the stationary phase[9]. In this method, the separation occurs via a hydrodynamic phenomenon rather than an

equilibrium one. Slalom chromatography can separate in a shorter space of time larger double-stranded DNA molecules (ranging from roughly 5 to 50 kbp) than the conventional chromatographic procedures by using a column for gel permeation with an ordinary HPLC system. The order of elution is opposite to that expected for gel permeation chromatography; the larger DNA fragments are eluted later than the smaller ones[10]. Theoretical background of SC is still giving its first steps and the transient condition between HDC and SC is under investigation[11].

Important characteristics of slalom chromatography revealed by extensive studies are summarized below:

1) DNA fragments do not interact with the column packing.
2) Only the particle size of the packing is important. Smaller particles can resolve smaller DNA fragments and larger particles can resolve larger DNA fragments.
3) Pore size and the chemical nature of packing particles are not important.
4) Separation depends largely on the flow-rate: the higher the flow-rate, the more the DNA fragments are retarded. The temperature also has an influence on the separation[1].

THEORY

The separation depends on the flow-rate and the particle size of the column packing and not on their pore size or chemical nature. In this case, the column packing is only used for the formation of the network of narrow interstices between spherical particles. When a DNA chain is applied to a chromatographic system, it frequently turns around the spherical obstacles; the larger the fragments or the smaller the particle size, the more difficult it is for the fragments to travel across the interstitial spaces created inside the column. According to this description, this separation mode cannot be explained in terms of an equilibrium constant between the mobile and the stationary phases. Another chromatographic technique i.e. column hydrodynamic chromatography (HDC), used for the separation of polymers or particles, shows the same characteristic. This technique is also based on the use of a laminar flow which occurs in the interstitial spaces created between the particles packed in the column[12-14]. When the DNA molecules are applied to the column, they are unfolded and extended owing to the laminar flow generated by the solvent passing through the narrow channels. For example, a 10 kbp fragment will become 3.4 µm at maximum. These extended molecules must flex quickly under a fast flow of mobile phase to pass through the openings. If we use a 30 cm column packed with particles of 10 µm diameter, DNA molecules should turn as many as 36,000 times, because the number of layers of particles reach 1200 per centimetre of column length. A fragment that has a retention time of 10 min will turn 60 times per second. It is quite possible that the longer the DNA molecule, the more difficulty it encounters in passing through the openings. Therefore size-dependent separation in the opposite order to gel chromatography should occur. However, the elution order in HDC is the same as in gel permeation chromatography due to the exclusion of the large polymer from the low velocity regions near the particle wall[15].

MODEL

In this model, the column packing is treated as a three-dimensional network of pores with an average diameter or length (l). This l diameter is linked to the average particle diameter d_p and the interparticle porosity n by

$$l = 0.42 d_p \, n / n-1$$

The DNA molecule is represented as in the reptation theory of de Gennes by a chain of p segments each of contour length l. The sequence of pores followed by the chain is called the tube with a total length equal to pl. Reptation theory describes effect of polymer chain entanglements on the relationship between molecular mass and chain relaxation time. The theory envisions that the movement of entangled polymer chains is analogous to snakes slithering through one another[16]. As the DNA fragment was aligned in the flow direction it was assumed to be a linear stretching of the DNA fragment in one pore. Δl_i is the linear progression length of the DNA fragment in pore i when the mobile phase velocity variation increases from v_i to v ($\Delta v_i = v - v_i$). When the mobile phase velocity increases by dv, Δl_i increases by d Δl_i and p increases by dp in accordance with SC mechanism.

The differential equations describing this phenomenon can be written as

$$dp / dv = k(p_\infty - p)$$

$$d\Delta l_i / d\Delta v_i = k'$$

where k and k' are positive constants and p_∞ is the value corresponding to the maximum DNA stretching.

To model the DNA retention inside the column, a parameter 'Relative retention Time (RRT)' was introduced and by the definition,

$$RRT = t_R / t_{R,0} \text{ or}$$

$$RRT = t_R / t_{NR}$$

Where t_R is the retention time of a particular DNA molecule and $t_{R,0}$ or t_{NR} is that corresponding to flowthrough fraction (non-retained molecules).

The resolution of a pair of DNA fragments can be measured from the chromatogram using the retention time t_R and peak width ω by the following equation,

$$R_S = 2(t_{RB} - t_{RA}) / (\omega_A + \omega_B)$$

Where the subscripts A and B refer to the first and the second eluting peaks, respectively. Assuming that the two peaks have a similar bandwidth, i.e. $\omega = \omega_A + \omega_B$, it can also be assumed to be related to the column plate number N determined on the B peak by the well known equation[17],

$$N = 16 (t_{RB}^2/\omega_B^2)$$

The progression of a fragment through the closed column packing can be modeled as shown in figure 2. There are two cases: 1) when the chain is short, it is aligned in the direction x_1 of the flow before it is switched from an average value of angle θ to the new direction of the flow x_2 , then the fragment progression is considered as a sequence of independent oriented paths with no or only weak retardation. 2) When the chain is sufficiently large, it is partially aligned along x_1 before the flow turns around the particles going in the x_2 direction and the DNA strand is strongly retarded[18].

This behavior can be analytically treated by introducing the notion of the reorientation time needed for macromolecules. The reorientation time $'l_0$ is defined as the time needed for a chain completely aligned along a direction to completely reorient itself and go in a new direction. Smaller molecules need less time for reorientation hence cover a longer distance than larger molecules[19]. The larger is the DNA molecules, the longer the reorientation time. If *J* is the curvilinear co-ordinate along the tube, the reduced curvilinear co-ordinate *j* is defined as

$$j = J/Pr$$

When j varies from 0 to 1, the fraction of j oriented along x is replaced by the same fraction along x. On average the tube is oriented along the diagonal z as reported by Viovy et al[20]. The DNA segment that leaves the tube aligns itself preferentially in the field direction to minimize its potential energy.

The flow rate dependence of macromolecules of different size can be explained in terms of deformation and orientation of simple macromolecular models in steady uniaxial elongation. The strength of the flow can often be correlated with Deborah number, D_e: a ratio of the hydrodynamic forces to the Brownian forces. Deborah number is defined as ratio of relaxation time characterizing the time it takes for a material to adjust to applied stresses or deformations[21]. Significant molecular stretching in steady flows occurs only when $D_e > 0.5$. A relaxation effect in the macromolecular flow is characterized by the Deborah number that can be presented as the ratio of a moving object relaxation time θ to the time when the object was exposed to deformation θ_p

$$D_e = \theta/\theta_p$$

For macromolecules θ is directly proportional to the boundary viscosity at zero shear velocity and to the molecular weight and is inversely proportional to the molecular concentration[22] whereas θ_p is defined by porous media properties:

$$\theta_p = a. \varepsilon. d_p/u$$

Where, ε is the bed porosity, d_p is the particle diameter and a is a numerical coefficient.

CHROMATOGRAPHIC CONDITIONS

As mentioned earlier, slalom chromatography requires a simple chromatographic system consisting of a column for gel permeation with an ordinary HPLC system.

Stationary phases

In slalom chromatography, the chromatographic separation column is packed with "slalom chromatography DNA separation particles". The term "slalom chromatography DNA separation particles" refers to any material which is capable of separating DNA fragments by slalom chromatography. Slalom chromatography separation particles can be inert organic polymers, inert inorganic polymers, silica, or cation exchange resin. The only requirement for the slalom chromatography DNA separation particles is that they must have little interaction with the DNA fragments[23]. In earlier experiments, commercially available packed columns for size-exclusion chromatography were mainly used: for example,

1) TSKG2000SW- TSK gel columns comprising of spherical silica or polymeric resins
2) Asahipak GS-320[24]
3) Macroporous polystyrene gels
4) Porous and non-porous packings originally developed for cation-exchange chromatography[25]
   - Porous packings e.g. Silica
   - Non-porous packings e.g. Ethylenedimethacrylate polymer.

Therefore recently a wider range of packing materials was examined. The results obtained on columns developed for reversed-phase chromatography were reported. Two types of microbeads (Capcell-Pak and Hypersil-3) were chosen for this purpose, with the aim of realizing the mode of separation, i.e., slalom mode and also expanding the separation range to smaller DNA molecules. The results improved the understanding of the actual separation mechanism in slalom chromatography.

➢ Experimental

Capcell-Pak columns (250 X 4.6 mm I.D.) of Cl (total carbon content including that used for silicon coating, 4.5%), Phe (carbon content, 8.1%) types were used.

5 types of Hypersil-3 packings which were packed into columns (250 *X* 6 mm I.D.) were also studied. Phe (phenyl, 5.0%), SAS (trimethylsilyl, 2.6%), CPS (cyanopropyl,4.0%) MOS (dimethyloctyl, 7.0%) and ODS (octadecyl, 10%) were obtained.

➢ Chromatographic separation

Various sized DNA fragments (l0-40 kbp) were prepared by digestion with the restriction endonucleases (ApaI, Xh0I and KpnI). Chromatography was performed essentially by using a Tosoh CCPD dual pump and a Tosoh UV-8011 detector coupled to a Shimadzu C-R4A integrator. The DNA solution was pre-heated at 65°C for 5 min and then cooled on ice until injection to prevent binding via cos sites. This heat-treated DNA was injected through a Rheodyne 20-~1 injector. When necessary, columns were heat-controlled (l0-60°C) by placing them, together with the solvent reservoir, in a water bath. DNA retardation under different conditions were compared in terms of relative retention time (RRT).

➢ Result:

1) Capcell-Pak C1: Large DNA molecules have a high tendency to bind to highly hydrophobic resins (4.5%)[26]. In addition, the Capcell-Pak packings consist of silicon-coated fine microbeads 5 µm in diameter that are chemically inert and stable even under alkaline conditions. Resistance to alkali is more favorable for DNA separation, because DNA is in general acid insoluble, and thus, usually dissolved in weak basic solvents such as Tris-HCl buffer, pH 7.5-8.0. The packing, having 5 µm particle diameter, allowed separation of the 6.6-kbp fragment from the flow-through fraction at a relatively fast flow-rate (e.g., ~1.0 ml/min). The chromatograms obtained were very similar to those obtained on size-exclusion columns packed with AsahipakGS-310 and GS-510 5 µm particles. Four peaks representing the 4.4, 6.6, 9.4 and 23.1-kbph/Hind111 fragments were eluted in that order, and retardation of the latter three fragments, i.e., 6.6, 9.4 and 23.1 kbp, increased when a higher flow-rate was applied. These observations indicate that the separation achieved on the Capcell-Pak Cl column is based on the slalom mode, and not on the hydrophobic-interaction mode.

2) Capcell-Pak phe: Another Capcell-Pak packing, Capcell-Pak Phe has the same particle diameter of 5 µm, but a significantly higher carbon content (S.l%,including that used for silicon coating). However,like Capcell-Pak Cl, the Phe column was also found to be useful for slalom chromatography. The Capcell-Pak Phe column also showed

size and flow-rate dependency under the normal low-salt conditions, suggesting that the separation is based on the slalom mode.

3) Hypersil-3 packings

It is of particular interest to utilize the smallest possible packing materials for applied slalom chromatography, since previous experiments demonstrated that packings having particles of 5, 9, 13 and 19 µm diameter resolved DNA fragments larger than 6, 9, 13 and 17 kbp respectively. This observation implies that the use of 3-pm particles might allow resolution of even smaller fragments, for instance, 4 kbp. Five Hypersil-3 packings having 3 µm diameter particles (i.e., those derivatized with trimethylsilyl, cyanopropyl, phenyl, dimethyloctyl or octadecyl groups) were used.

However, none of the λ /Hind111 fragments was recovered from the five Hypersil columns under the normal conditions. The poor recovery of the fragments is due to excessively strong or effective hydrophobic interaction between the Hypersil3 packings and DNA, although Capcell-Pak columns gave much more satisfactory results under the same conditions. Despite the significantly different carbon contents of the four Hypersil-3 packings, all of them showed almost the same retardations of λ/HindIII fragments in the presence of 10% (v/v) acetonitrile. This fact suggests that hydrophobic interaction is no longer significant when a hydrophobic solvent, such as acetonitrile, is included in the eluting solvent, and the slalom mode becomes predominant.

Stronger DNA retardation with Capcell-Pak Phe than with C1 seems to be associated with the stronger hydrophobicity of the former packing than the latter. However, all of the Hypersil-3 packings, differing in carbon content (2.6-10%) required the addition of more than 5% acetonitrile to the eluting solvent for adequate DNA recovery. This may suggest that either special features of the silicon coating of Capcell-Pak packings or the small 3 µm packing size of Hypersil packings accounted for the different results[27].

Mobile phase

The mobile phase consists of a sodium phosphate salt (0.01 M), EDTA (0.001 M) mixture at pH 6.8. Acetonitrile is used as a hydrophobicity modifying agent.

Compacting agents (CA) to improve oligonucleotide separation:

Sometimes there might be a small difference between the retention times of circular and linear DNA molecules. Hence, in order to get a better selective separation, Compacting Agents i.e. spermine, spermidine, hexamine cobalt are added. Addition of CA led to a DNA precipitation into a pellet form. The addition of these cations i.e. CA to a DNA solution leads first to the precipitation of the DNA and further addition resolubalizes the DNA pellet. The amount of compacting agents to resolubalize various DNA fragments corresponds approximately to 50 mM spermidine, 90 mM spermine, 220 mM hexamine cobalt[28].

In another experiment, the concentration range of compacting agents varying from 500 mM to 1 M was studied. 20 mg per litre of the DNA solutions were injected in triplicate. The mobile phase flow-rate varied from 0.02 to 1.5 ml/min. in this series of experiments, the column temperature was fixed at 10^0C. In these conditions, DNA fragments didn't precipitated. Above 0.1 M, the reptation time of DNA fragment was weaker to be determined with accurate precision.

At the highest mobile phase velocities, the DNA fragment was close to being fully elongated. The increasing extension of large DNA fragment increased the separation of oligonucleotide[29]. Spermine addition in the bulk solvent decreased the k value (representing the degree of the oligonucleotide stretching) for both plasmid and linear DNA fragment i.e. lead to a compaction of linear and circular DNA fragments. Increasing spermidine concentration in the mobile phase led to a decrease of stretching of circular DNA fragment (k decreased with x for plasmid) but not the one of linear DNA fragment (k value constant). In contrary, hexamine cobalt compacted only the linear DNA fragment (k decreased with x only for the linear DNA fragment. This CA difference effect can be

useful to separate linear and circular DNA fragments and plasmids[30].

FACTORS AFFECTING SLALOM CHROMATOGRAPHY

In case of slalom chromatography, the importance of the presence of narrow open spaces constructed by packaging microbeads (< 10 µm) developed for high-performance liquid chromatography (HPLC) and application of a relatively fast flow (0.3 ml/min) were emphasized. Studies showed that none of the (i) chemical nature (silica or synthetic polymer) of packings, (ii) pore size of packing material is important for separation in slalom chromatography. So far, some physicochemical factors closely related to hydrodynamics have been shown to have a critical effect on the separation, i.e.

1) DNA topology
2) Temperature
3) Flow rate and mobile phase viscosity
4) Particle size of packing material

Effect of DNA topology

In order to establish the concept that slalom chromatography is based on a hydrodynamic principle rather than an equilibrium one, the elution of DNAs having the same molecular weights but different topologies (i.e., linear and circular forms) was compared.

➢ **RESULT**

Both linear and circular forms of DNAs having different sizes (approximately 20, 28, 36, and 42 kbp) were prepared, and applied to AsahipakS-310 columns packed with various sizes of packing particles (5, 9, 13 and 19 µm in diameter). Extents of their retardations were compared in terms of RRT. Examples of elution profiles of circular (95%, super-coiled) and linear DNAs are shown in Figure 3.

In this experiment, both circular and linear forms were co-injected into a column of Asahipak GS-310 (particle size, 9 µm) and were eluted at a flow rate of 0.6 ml/min. In each case, circular (C) forms were eluted much faster than linear (L) forms; reasonable to speculate that the extent of retardation i.e., in terms of RRT 0.98 (20 kbp, C), 1.25 (20 kbp, L), 1.05 (28 kbp, C), 1.56 (28 kbp, L). However, circular forms were also size-fractionated, as were linear forms, in a size and flow-rate-dependent manner. The effect of particle size of the packings was also examined by using 5, 9, 13, and 19 particles of Asahipak GS-310. Apparently, retardation of each circular DNA became larger when smaller packings were used. The use of larger packings had little practical merit because most of the circular DNAs were not fully resolved from the fraction. The extent of retardation of a circular form is comparable to that of a linear form having half of the molecular size of the former. The elution profiles of four circular DNAs with those of linear DNA fragments, i.e., 10, 15, 17, 20, and 23 kbp derived from phage DNA. Evidently, the obtained curves were very similar for the following pairs: 10 kbp (L) and 20 kbp (C), 17 kbp (L) and 36 kbp (C), 20 kbp (L) and 42 kbp (C). This observation strongly suggests that DNA separation in slalom chromatography is based on extended ‘‘length’’, not on molecular ‘‘mass’’. These observations indicate that super-coiled circular DNAs take a relatively rigid conformation compared with linear forms having half of the molecular size of the former, probably because the former are stabilised by super-coil formation[31].

Effect of temperature

Column temperature is one of the main parameters which could influence DNA separation. In order to gain further insight into the behaviour of DNA in a hydrodynamic flux and enhance the efficiency of the technique, the retention of DNA fragments on a C1 stationary phase was analysed over a wide range of column temperature (3-60^{0}C).

➢ **Result**

The retention time values for the 17.05 and29.95 kb fragments (t_R) and for the 1.50 kb fragment which corresponded to the void volume marker (t_{NR}) were obtained at various column temperatures. From the t_R and t_{NR} values, the experimental RRT were calculated for the different chromatographic conditions.

The RRT values increased when T decreased. This result confirmed that the temperature acted on the DNA behaviour via two effects: The RRT value varied with the column temperature (T^7 function) more strongly that with the linear velocity. Lower the column temperature, greater the separation between non retained and retained molecules. Thus, the optimal conditions for the best separations between the void DNA fraction and other DNA fragments were represented by the lowest value of the column temperature at a constant flow rate which was compatible with a practicable back pressure and

the prevention of the physical degradation of DNA fragments[32].

Effect of flow rate and mobile phase viscosity

Since in slalom chromatography, DNA fragments move further in the direction of laminar flow of the mobile phase, the flow rate v and mobile phase viscosity η were some of the main parameters which could influence DNA separation. In order to gain further insight into the fractionation mechanism and enhance the efficiency of the technique, retention of DNA fragments on a stationary phase C1was analyzed over a wide range of glycerol concentrations (used as a viscosity modifier at concentrations of 0 to 1 *M*) and at various linear velocities *v* (i.e. flow rates values varying from 0.05 to 1.2ml/min).

➢ **Results**

Recoveries of the DNA fragments were calculated from the chromatographic areas. The DNA recovery at constant flow-rate was globally identical (difference <10%) whatever the value of glycerol concentration in the eluent. Thus, it can be concluded that the glycerol effect on relative retention time (RRT) values of the DNA fragments was the result of the change in the mobile-phase viscosity. Also, this showed that the destabilizing effect of polyol on DNA related to its capacity to interact with the polynucleotide solvation sites did not significantly affect the fragment integrity over the glycerol concentration range studied.

The parameters η and *v* acted in the same manner on DNA retardation (increase in RRT value). The main difference between these two factors was that the viscosity increase was associated with a concomitant enhancement of the analysis time, while the flow-rate increase was associated with the decrease in the analysis time. Higher the liquid velocity, greater is the separation between non-retained and retained molecules and shorter the analysis time. This fact shows the advantage of the slalom chromatography principle on the equilibrium principle of the classical chromatographic modes.

Thus, the optimal conditions for the best separations between the void DNA fraction and other DNA fragments were represented by the highest values of the linear velocity at a constant viscosity which was compatible with a practicable back pressure and the prevention of the physical degradation of DNA fragments[33].

Effect of particle size of packing material

In one of the experiments, DNA fragments ranging from 10 to 38 kbp were separated by 2 columns differing in particle size only. One was packed with 5 µm particles and the other with 9 µm particles. Both columns separated the fragments in the order of smaller to larger. Although the pore sizes are the same, the column packed with 5 µm particles was superior for the separation of smaller fragments(less than 20 kbp), while larger fragments (greater than 20 kbp), were better separated by the column packed with 9 µm particles. This result indicated that the fractionation range depends on the particle size of the column packing but not on the pore size.

In another experiment using 4 columns (average particle diameters 5, 9, 13.1, 19.1 µm) the RRT was plotted against DNA length i.e. no. of basepairs. These 4 columns showed different ranges of resolution: e.g.at a flow rate of 0.6 ml/min, the 5, 9, 13.1, 19.1 µm columns could separate DNA fragments greater than 7, 9, 13, 17 kbp, respectively. Smaller packings showed better resolution for smaller DNA fragments, whereas larger ones were better for larger fragments[8,10].

ADVANTAGES OF SLALOM CHROMATOGRAPHY

At present, it is the only chromatographic method applicable to the size-dependent separation of large DNA molecules. It is unique because it is based on the hydrodynamic principle that the mobility of fibrous molecules in a column is determined by their length and truly size-dependent separation occurs.

Its advantages are summarized below:

1) Both preparative and analytical uses are possible. As an analytical procedure, it provides information on the length of polynucleotides.
2) The experiment procedure is very simple and rapid. Ordinary HPLC apparatus and a gel permeation column are sufficient as equipment.
3) Results can be easily predicted.
4) Only isocratic elution programme is necessary and there is no need for column washing or reequilibration.
5) The separation and recovery of DNA fragments are very rapid in comparison

with gel electrophoresis. Recovered DNA fragments are free of undesirable contamination originating from the agarose gel.

6) DNA can be detected without the use of a harmful reagent, such as ethidium bromide.
7) This procedure provides a new effective tool for physicochemical and hydrodynamic studies of DNA.

LIMITATIONS OF SLALOM CHROMATOGRAPHY

1) At present, the range of separable sizes for DNA molecules is not wide; fragments of 5-50 kbp are separable using commercially available packing particles. However further extensive studies will improve the situation.
2) The resolution efficiency is still inferior to that of gel electrophoresis.
3) The flow-rate must be relatively high. This may cause physical degradation of extremely large DNA fragments. However DNA fragments less than 50 kbp proved to be generally very stable under the conditions of most experiments (e.g. flow-rates less than 1.2 ml/min).

APPLICATIONS

This principle will provide with a valuable tool for nucleic acid research. Although only application to DNA have been reported so far, it should be also useful for RNA research.

Some possible applications are listed below:

1) Size-dependent separation of DNA
2) Estimation of size of DNA
3) Monitoring and analysis of size change of DNA
4) Separation of DNA and RNA based on conformation or topology
5) Analysis of interaction of DNA with other molecules, such as DNA binding proteins
6) Distinction of types of circular DNA, e.g. super-coil, relaxed and single strand
7) Studies of the physicochemical properties of nucleic acids, e.g. rigidity, elasticity, bendability etc.
8) Hydrodynamic studies of nucleic acids[6].

CONCLUSION

Slalom chromatography provides us with a valuable tool for nucleic acid research. Unlike other chromatographic separation modes applied to biomolecules which are based on equilibrium phenomenon, this technique is based on a completely different principle of hydrodynamic phenomenon. The column packing serves only for the construction of spaces through which DNA passes. Hence this technique is important from the viewpoint of study of physiochemistry of macromolecules. Theoretical background of slalom chromatography is still giving its first steps therefore further investigations in this field are required to expand the horizons of slalom chromatography.

Table 1: Comparison of chromatographic modes available for size fractionation of nucleic acids

Mode	Size range for D.S. DNA (kbp)	Speed	Resolution
Ion-exchange	< 25	High	High
Gel-permeation	< 6	Low	Low
Gel electrophoresis	-	Low	High
Slalom	5-50	High	Medium

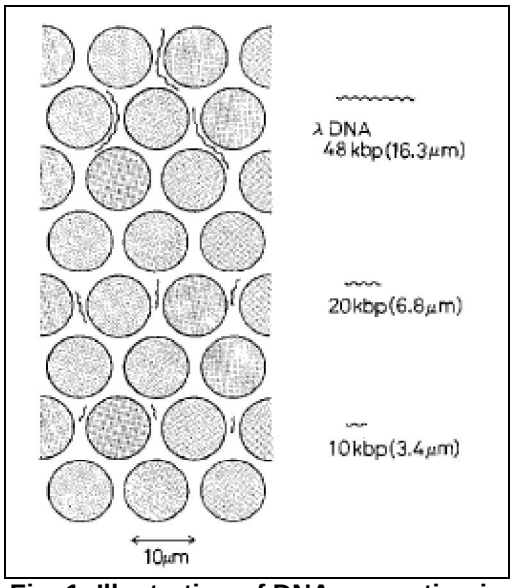

Fig. 1: Illustration of DNA separation in slalom chromatography

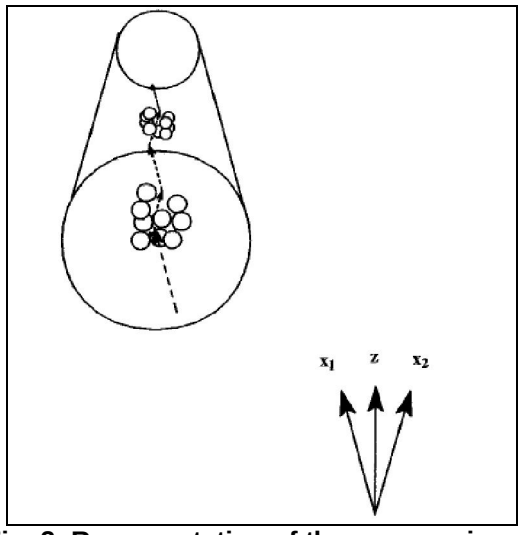

Fig. 2: Representation of the progression of the DNA chains (arrows) through the closed column packing particles

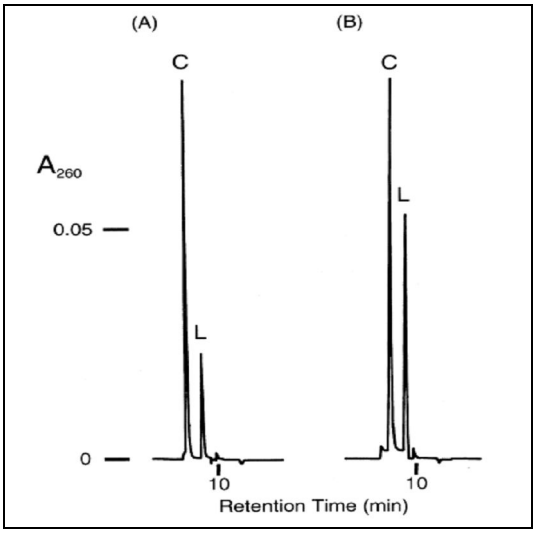

Fig. 3: Elution profiles of supercoiled and linearized DNA

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