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A Pinched Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation

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Abstract

The concept of a pinched inlet system for field-flow fractionation (FFF), in which the channel thickness at the inlet end is reduced to hasten relaxation, is introduced and its advantages in simplifying FFF operation and increasing analysis speed are noted. Three forms of FFF operation are described for taking advantage of the split inlet: stopless flow injection, slow flow injection, and stopflow injection. Stopless flow operation is the simplest because flow is neither stopped nor changed to accommodate relaxation. However, stopless flow operation causes band broadening. It is found that the time-based variance of band broadening for many FFF systems is proportional to the fourth power of channel thickness w. Therefore, by reducing w at the inlet end where relaxation occurs, this band broadening can be controlled. The implementation of this concept is discussed for different forms of FFF.

INTRODUCTION

When a sample first enters a field-flow fractionation (FFF) channel, it is generally distributed broadly over the channel cross-section. Before normal sample migration can occur, the components of the sample must undergo a relaxation process in which they approach a steady-state distribution within the channel, usually by accumulating near one channel wall (*1–3*). This process requires a finite time (typically from 10 s to 10 min) described by the relaxation time τ. Because a good deal of band broadening can occur during the relaxation process, a stopflow procedure is commonly used in which the downstream flow is halted for a period of time $> \tau$ in order to allow relaxation to occur under static conditions (*3*). The stopflow procedure, generally required to avoid losses in resolution, is particularly essential for high-speed runs. However, under any circumstances, stopflow is an experimental inconvenience and for high-speed operation it consumes additional time and often introduces baseline instabilities in sample detection. Sample losses due to adsorption at the accumulation wall are also greatest during the stopflow period. Attempts to reduce the stopflow time by increasing the field strength only magnify the sample loss problem. Clearly, the development of a method to reduce relaxation effects and, where possible, eliminate the stopflow procedure would represent an important advance in experimental FFF, particularly for high-speed operation.

One method proposed for reducing relaxation effects and thus eliminating the need for stopflow is the use of a split inlet system (*4*). In this method a thin flow splitter must be suspended evenly between the walls across the breadth of the channel near the inlet. By this means two independent inlet streams can be introduced into the channel (on opposite sides of the splitter) and merged uniformally into a single channel flowstream beyond the splitter. In this case the sample is introduced into the stream flowing along the accumulation wall where, in the absence of a splitter, it must eventually accumulate by virtue of the relaxation process. The opposite flowstream is carrier fluid; as it merges with the sample stream, it is devoid of sample. Since the object of relaxation is to remove sample from the vicinity of the depletion wall and concentrate it in the vicinity of the accumulation wall, the most lengthy part of the relaxation process is automatically achieved by such a splitflow process. However, the split inlet method has several disadvantages. First is the fact that two flowstreams must be generated and controlled. Even more importantly, the flow splitter must be precisely aligned within the narrow confines of the FFF channel. Such alignment is intrinsically difficult and becomes much more so as the channel becomes thinner, which is the direction often taken for high-speed FFF operation.

The pinched inlet method proposed here also involves a modification of the channel inlet region but it is much simpler to implement, particularly in thin channels. This method involves reducing the thickness of the channel at the inlet end. This ultrathin channel segment will preferably extend into the channel sufficiently far that the relaxation process can be essentially completed for the sample while it is in this segment. In some cases the extension of the ultrathin region into the channel may be substantial, involving 10 to 50% or more of the channel length.

Since full relaxation requires the displacement of sample particles across most of the thickness of the FFF channel, relaxation is hastened by a selective reduction of channel thickness in the region where relaxation takes place. Thus relaxation times can be reduced by appropriate reductions in channel thickness at the inlet end.

The reduction in relaxation times can be put to use in three principal ways. First, if τ is short enough, a stopflow procedure is not necessary; injection without stopping or reducing the flow rate is termed here stopless flow injection. Second, injection and relaxation can proceed at a reduced (but not zero) flow rate to reduce sample losses to the wall. This is termed slow flow injection. Third, even when stopflow is needed, the time delay before the initiation of FFF separation is reduced and the separation time is accordingly diminished.

It is important to note that a thin channel is generally desirable for the achievement of separation in FFF, as it is for the achievement of relaxation. However, separation and relaxation are two relatively independent processes, and the optimum channel thickness for one does not necessarily correspond to the optimum thickness for another. The proposed method allows the independent adjustment of channel thickness in two portions of the channel, one optimized for separation and the other optimized for relaxation.

THEORY

The relaxation time τ can be defined as the time required by a particle next to the depletion wall to reach the accumulation wall under the influence of the principal FFF driving force. For channel thickness w, this time is given by (*4*)

$$\tau = w/U \tag{1}$$

where U is the transverse velocity induced by the field or driving force. For the normal mode of FFF, U may be replaced by $D/\lambda w$ and τ becomes (*5*)

$$\tau = \frac{w^2\lambda}{D} \approx \frac{w^2R}{6D} \tag{2}$$

where λ is the retention parameter, $\lambda = D/wU$, D is the diffusion coefficient, and R is the retention ratio. The latter part of Eq. (2) is obtained from the limiting R expression, $R = 6\lambda$ (*2*).

The mechanism by which a narrow particle band is broadened in the course of relaxation with stopless flow injection has been discussed in several previous papers (*2, 3*). This mechanism is illustrated in Fig. 1. This figure shows the particle trajectories for a thin sample pulse introduced at the column inlet, $z = 0$. The particles starting near the depletion wall must traverse nearly the entire thickness of the channel to reach a steady-state condition. During this relaxation process, the particles are displaced downstream as shown by the upper trajectory in Fig. 1. Particles starting near the accumulation wall are already close to their steady-state position and suffer very little downstream displacement in the course of their brief relaxation. Consequently, by the time full relaxation is achieved, the particles starting at different elevations above the accumulation wall have become smeared out along the axial coordinate z. The initial distribution has been shown to be bimodal as illustrated by the shaded profile in Fig. 1.

After relaxation, the length of the starting zone is h_0, as shown in Fig. 1. This distance is given by

$$h_0 = \langle v \rangle \tau \tag{3}$$

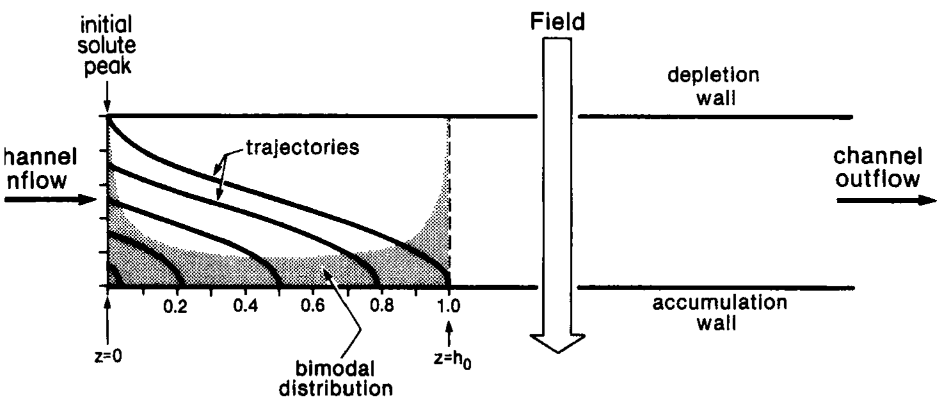

FIG. 1. Relaxation trajectories and the resultant bimodal distribution of particles near the inlet of an FFF channel with stopless flow injection. The figure shows an edge view of the channel.

where $\langle v \rangle$ is the mean cross-sectional flow velocity in the channel, related to the channel flow rate $\dot{V}$ by

$$\langle v \rangle = \dot{V}/bw \tag{4}$$

where b is the breadth of the channel. By substituting Eqs. (1) and (4) into Eq. (3), we get an alternate equation for h_0:

$$h_0 = \dot{V}/bU \tag{5}$$

This expression shows that h_0 is independent of thickness w for channels or channel segments with constant $\dot{V}$, b, and U. Thus the linkage of two segments of an FFF channel with the same b and U values and subject to the same flow rates $\dot{V}$ leads to equal values of h_0 in the two segments. This analysis would suggest that the zone is no less smeared out along the flow axis in a thin segment of a channel than in a thick segment. However, h_0 is not the sole criterion of zone broadening for an eluting peak. Consequently, a more complete analysis is necessary to ascertain the influence of segment thickness w on final peak width and resolution.

According to the original analysis of relaxation effects (*2*), the contribution of relaxation to the variance of a band of particles along axis z is given by

$$\sigma_z^2 = \frac{17}{140} h_0^2 \tag{6}$$

However, for our purposes we are more interested in the variance in the elution time of the emerging band which can be related to σ_z by (*6*)

$$\sigma_t^2 = \frac{\sigma_z^2}{(\text{zone velocity})^2} = \frac{17}{140} \frac{h_0^2}{R^2 \langle v \rangle^2} \tag{7}$$

The substitution of the h_0 from Eq. (3) into this equation yields

$$\sigma_t^2 = \frac{17}{140} \frac{\tau^2}{R^2} \tag{8}$$

This equation shows that the σ_t^2 contribution varies in proportion to τ^2; accordingly, the small τ associated with a thin channel segment will translate into a reduced σ_t^2. However, retention ratio R will generally vary as a function of channel thickness w. To account for this effect in normal

FFF, we substitute the last term of Eq. (2) for the τ value in Eq. (8), giving

$$\sigma_t^2 \approx \frac{17}{5040}\,\frac{w^4}{D^2} = 3.373 \times 10^{-3}\;\frac{w^4}{D^2} \tag{9}$$

which shows that the σ_t^2 contribution increases approximately with the fourth power of w. This high sensitivity of σ_t^2 to w shows the substantial improvement to be made by reducing the thickness of the segment in which relaxation occurs. A reduction of only 20% in w will reduce σ_t^2 by more than a factor of 2; a twofold reduction in w will reduce σ_t^2 by a factor of 16. This is especially important for high-speed analysis as shown by the following equations.

More specific values of σ_t^2 can be found by using the Stokes-Einstein equation (*7*), $D = kT/3\pi\eta d$, for D. This yields

$$\sigma_t^2 = 0.2996\left(\frac{w^2\eta d}{kT}\right)^2 \tag{10}$$

where d is particle diameter, η is viscosity, and kT is mean thermal energy. For a typical aqueous carrier we have $\eta = 10^{-2}$ poise and $T = 293$ K, which leads to

$$\sigma_t^2 = 0.0183 w^4 d^2 \tag{11}$$

In this expression σ_t is in seconds and w and d are in micrometers. One must be careful not to apply any of the last three equations to particles not conforming to $R = 6\lambda$ immediately after relaxation; larger particles, for example, may initially undergo a sterically dominated migration, thus reducing σ_t^2.

The σ_t^2 values calculated from the above equations represent the contribution of relaxation of the total time-based variance of an emerging component. These equations are valid as long as the zone velocity through the channel system is either constant or varies only as a function of axial coordinate position z. However, if zone velocity changes with time, as induced by field or flow programming or even by simple step changes in field or flow, the contribution of the σ_t^2 from relaxation to final variance is (*6*)

$$\sigma_t^2 = \sigma_t^2(\text{relax.})\left[\frac{\mathcal{V}(t_0)}{\mathcal{V}(t_r)}\right]^2 \tag{12}$$

where $\mathcal{V}(t_0)/\mathcal{V}(t_r)$ is the ratio of the zone velocity $\mathcal{V}$ at the time t_0 of completion of relaxation to that at the retention time t_r. This ratio must be calculated to account only for time-based changes in velocity; it does not include the effects of distance-based changes, such as those encountered in passing from the relaxation segment of the channel to the separation segment. In the present context this expression is most relevant in describing the effects of field programming and the effects of increasing the flow rate for separation following a reduced flow rate (slow flow) injection procedure.

We note that in the cases for which the σ_t^2 contribution due to relaxation is still large enough to perturb the results, a deconvolution procedure can be used to largely eliminate by mathematical means the band distortion due to the relaxation process. Effective deconvolution is made possible by the theoretical tractibility of FFF, specifically by the known mathematical form of the bimodal distribution shown in Fig. 1.

If relaxation were the only source of zone broadening, the largest
where $\mathcal{V}(t_0)/\mathcal{V}(t_r)$ is the ratio of the zone velocity $\mathcal{V}$ at the time t_0 of completion of relaxation to that at the retention time t_r. This ratio must be

$$N_r = (t_r/\sigma_t)^2 \tag{13}$$

The maximum number of plates, in turn, controls the achievable resolution in the system. This can be seen by writing the following expression for the difference in diameter δd of two particles of mean diameter d that can be separated at unit resolution (*8*),

$$\frac{\delta d}{d} = \frac{4}{N^{1/2}S_d} \tag{14}$$

where N is the number of theoretical plates and S_d is the diameter-based selectivity. Clearly some minimum N (usually a few hundred) is necessary to achieve the experimentally desired resolution of particles differing in diameter by δd. This requirement, combined with an analysis of the other contributions to σ_t, will fix some minimum value below which N_r should not fall. It is apparent from Eq. (13) that the minimum retention time of the component will be limited by σ_t:

$$t_r(\min) = N_r^{1/2}\sigma_t \tag{15}$$

A more complete optimization of the system requires a study of the other sources of band broadening, but the above equations show that the relaxation process establishes a floor below which retention time and thus analysis time cannot be driven.

IMPLEMENTATION

The implementation of the pinched inlet concept should be relatively simple as illustrated by Fig. 2. For channels having a sandwich construction in which the channel volume consists of a section cut out and removed from a spacer layer sandwiched between two wall layers, an appropriately segmented channel can be constructed by using two or more spacer layers from which volume elements of different lengths are removed. Thus a channel like that in Fig. 2 can be made by sandwiching together two equal spacer elements between the primary walls of the system. A section of one spacer (termed the blocking element) can be left intact while the corresponding region of the companion spacer can be removed to form a segment of the channel volume. (Alternately, part or all of the segmentation might be produced by machining, as will be discussed later.) The length L_r of this relaxation segment is chosen in general such that all the major components will undergo a major part, if

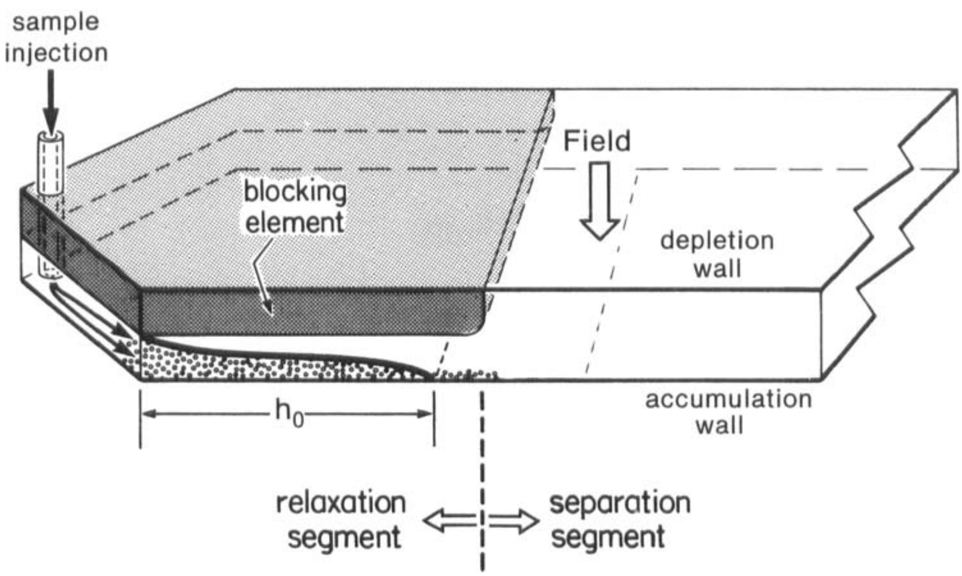

FIG. 2. Illustration of the structure of a pinched inlet channel for FFF. The relaxation process for one component is shown.

not all, of their relaxation within this segment; $L_r > h_0$. This relaxation is shown for one component in Fig. 2. Clearly, L_r and flow velocity are interrelated as made clear by Eq. (3). For high-speed stopless flow operation with correspondingly large h_0 values, we anticipate that the relaxation segment will occupy a substantial fraction of the total length of the channel system. Alternately, with slow flow injection, L_r can be greatly reduced.

Flow in thin FFF channels is almost universally laminar. It is important that the flow in the transition region between segments maintains these laminar characteristics despite the rather abrupt change in cross-section. Mixing currents at this point would have the potential to redistribute the component particles over the cross-section of the separation segment, in which case a second and less favorable relaxation process would be required. However, with smooth channel surfaces, a blocking element free of sharp edges and rough protrusions, and thin channel segments, effective flow laminarity should be achievable.

If the flow passing through the transition region were completely laminar, the transverse position of the blocking element would be immaterial. It could lie against the depletion wall, as shown in Fig. 2, or it could with equal effectiveness be layered against the accumulation wall or even divided into two sublayers, one adjacent to each wall. However, the arrangement shown in Fig. 2 is preferred because the sample components, once concentrated at the accumulation wall, will be likely to proceed without substantial perturbation even if flow disturbances are generated toward the interior of the channel.

We note that the two-segment channel system shown in Fig. 2 could be replaced by a tapered channel that is relatively thin toward the inlet and thicker toward the outlet. No distinct segments need exist. The general advantages of the method proposed here are expected from any such system no matter how the transition from the thin inlet region to the thicker outlet region is realized.

We also note that an abbreviated relaxation step will generally occur after the component particles pass through the transition region. The expansion of the flow channel will lead to a comparable fractional expansion of the particle-containing lamina upon passage through the transition region; the steady-state concentration profile of particles may also change at the transition point. Thus concentration reequilibration will be necessary after the transition region is passed. However, providing the particles are rather tightly confined to the accumulation wall through the transition region, the readjustment necessary to the new steady-state conditions should be relatively brief and nondisruptive to the separation.

While in Fig. 2 we show a channel with well-defined accumulation and depletion walls, under some circumstances different particles in the sample can go to opposing walls. This happens, for example, in sedimentation FFF when the carrier density is intermediate between that of two different particle populations (*9*). The pinched inlet concept will be equally applicable to this case providing proper attention is paid to the streamlining of the channel system in the transition region.

The pinched inlet method should constitute a useful modification to most any field-flow fractionation systems irrespective of field type, operating mode, or channel geometry. However, special considerations will apply for each individual system. Some of these special considerations will be examined below for several subtechniques of FFF carried out in thin rectangular channels.

Sedimentation FFF

The application of the pinched inlet concept to sedimentation FFF should be straightforward. For a channel having a sandwich construction, the single spacer element normally used would simply be replaced by two spacers, one of which would provide the blocking element as suggested in Fig. 2. However, in view of the strong centrifugal forces, it is important that the blocking element be sufficiently rigid or supported so that it does not substantially sag into the channel space of the relaxation segment. Alternately, if the density of the material in the blocking element is less than that of the carrier liquid, little channel distortion should be encountered. Denser blocking materials might best be placed adjacent to the outside wall providing smooth laminar flow can be maintained through the transition region. In some cases it might be preferable to machine part or all of the channel volume out of one of the channel walls so that mechanical stability would be assured.

Thermal FFF

The primary challenge of thermal FFF is the thinness of the channel space; state-of-the-art channels are now typically 75 μm thick (*10*). To implement the pinched inlet concept without sacrificing the thinness of the separation segment, it would be necessary to utilize a blocking segment of extraordinary thinness, ~25–50 μm. The thin spacer layer containing this blocking element could be made from a variety of materials. Both the uniformity of this layer and its thermal conductivity are important considerations. A highly conductive layer (made up of a film of metal) would lead to the highest temperature gradient in the relaxation segment of the channel and would thus give the fastest relaxation. Also, the relaxation segment could be shortened in proportion to the reduction of h_0 resulting from the increase in U as shown in Eq. (5). However, the precise speed of relaxation in this segment is unimportant to zone broadening, as shown by Eq. (9), as long as the driving force remains sufficient to drive the particles to the accumulation wall to achieve relaxation and uphold the validity of the expression $R = 6\lambda$. Since a high conductivity blocking element would tend to give a high heat flux through the relaxation segment and possibly distort the temperature distribution elsewhere in the channel system, there would be some advantages to constructing the blocking element from a low conductivity layer of material; for example, one made of Mylar. The material should not have a heat conductivity appreciably lower than that of the carrier liquid; a value too low would not provide an adequate temperature gradient across the channel spacer to drive the relaxation process.

Flow FFF

Somewhat greater difficulty is anticipated in applying the pinched inlet concept to flow FFF systems. The most uniform spacer materials for forming the blocking element are impermeable to flow, thus making normal crossflow difficult to realize in the relaxation segment. Without crossflow, relaxation would fail to occur. One solution might be to use a thin permeable membrane for the spacer forming the blocking element despite its potential nonuniformity. A second approach would involve using a tapered channel, perhaps formed around a spacer of continuously variable thickness. Alternately, a tapered channel or a channel with a blocking element like that shown in Fig. 2 might be partially or entirely machined out of the frit material forming the depletion wall, allowing a normal crossflow flux into the channel along its entire length. More specifically, the thickness of the relaxation segment might be provided by a spacer while the additional thickness of the separation segment could be machined from the depletion wall.

Another solution would entail using an asymmetric relaxation segment resembling the asymmetric flow FFF channels previously developed (*11*). Such a system is illustrated in Fig. 3. By making the inlet flow $\dot{V}_i$ much greater than the outlet flow $\dot{V}_o$ from the relaxation segment, most of the flow entering that segment can be diverted through the lower permeable

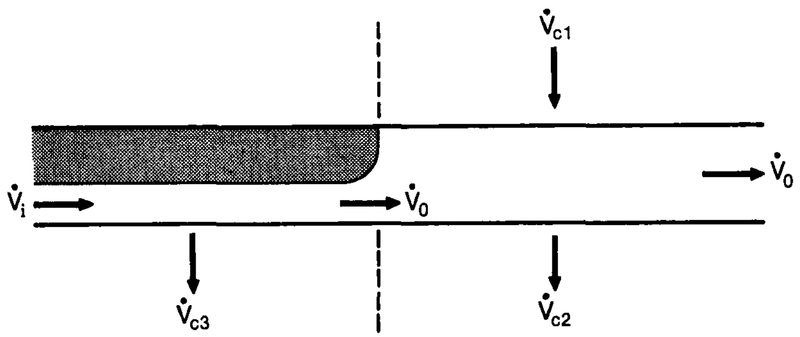

FIG. 3. One means for implementing the pinched inlet concept for flow FFF channel.

wall. This diverted flow is shown in the figures as $\dot{V}_{c3}$. With this flow arrangement most of the sample would be relaxed by the end of the relaxation segment although a small part of the sample (that must be ignored) would remain distributed over the cross-section. In the separation segment the crossflow could be uniform, $\dot{V}_{c1} = \dot{V}_{c2}$, and the axial flow constant throughout.

Steric FFF

The fast relaxation strategy proposed here should be generally applicable to steric FFF systems. Here, too, the sample must be forced to one wall before normal migration can occur. Hydrodynamic lift forces will have some influence on relaxation but the net effect should not change substantially because under steady-state conditions the sample particles still occupy a narrow region near the wall. However, for steric FFF, Eq. (9) cannot be expected to apply because we are unable to utilize the approximation $R = 6\lambda$. If instead we use $R = 3\gamma d/w$ (*12*) along with Eq. (1), Eq. (8) becomes

$$\sigma_t^2 = 0.0135 \frac{w^4}{\gamma^2 U^2 d^2} \tag{16}$$

which still increases with the fourth power of w.

By using stopless flow injection in steric FFF, continuous lift forces will be exerted on the sample particles. For the large particles used in steric systems, the flow velocity can easily be adjusted to a value high enough to prevent the particles from adhering at the wall. Thus a major problem of stopflow injection, namely, particle adhesion to the accumulation wall, should be possible to circumvent. In flow/steric FFF, for example, it should be possible not only to avoid particle adhesion but to dispense with the membrane normally used at the accumulation wall and employ only the rigid frit material supporting the membrane. Such a system would be simpler, more uniform in channel dimensions, and less prone to clogging.

Programmed Field FFF

The magnitude of relaxation effects in stopless flow FFF can be reduced by using programmed field FFF as noted in conjunction with Eq. (12). These advantages are complemented and amplified by a pinched inlet system. We note that relaxation tends to occur faster in the case of programming because generally a higher initial field strength is used than in the nonprogrammed (isocratic) case. (The disadvantage of this high initial field strength is that it can increase the adhesion of particles to the accumulation wall.) In addition, σ_r^2 is diminished by the acceleration of the particle zone down the channel resulting from the decreasing field strength (see Eq. 12). On the basis of these favorable characteristics of programmed field operation, it should be possible to use stopless flow or slow flow injection to advantage in a majority of programmed runs.

CONCLUSIONS

The cases cited above where the use of a pinched inlet system would be advantageous are simply examples of its general utility in FFF operation. The same general advantages could be stated for electrical FFF, magnetic FFF, cyclical-field FFF, and other subtechniques and operating modes.

While the pinched inlet geometry will often reduce relaxational zone broadening in stopless flow operation to acceptable levels, there are cases in which such zone broadening will still be excessive. Rather than using stopflow injection in these cases, the slow flow injection process (where relaxation occurs at reduced flow rates) could be used to bring relaxational broadening within acceptable limits. In this case σ_r^2 would be reduced in proportion to the reduction of the square of the flow rate, $\dot{V}_r^2$, used for relaxation (see Eq. 12), given a fixed postrelaxation (separation) flow rate $\dot{V}$. By using slow flow relaxation, a steady shear effect is available to combat particle adhesion. Since the shear rate increases with $\dot{V}/w^2$, the reduced flow rate during relaxation might still be adequate to avoid adhesion problems because of the $1/w^2$ dependence.

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