Published in final edited form as:

Anal Chem. 2008 September 15; 80(18): 7105–7115. doi:10.1021/ac800841q.

Characterization of Non-Specific Crossover in SPLITT

Fractionation

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Abstract

Split-flow thin channel (SPLITT) fractionation is a technique for continuous separation of particles or macromolecules in a fluid stream into fractions according to the lateral migration induced by application of a field perpendicular to the direction of flow. Typical applications have involved isolation of different fractions from a polydisperse sample. Some specialized applications involve the separation of the fraction influenced by the transverse field from the fraction that is not. For example, immuno-magnetically labeled biological cells may be separated from non-labeled cells with the application of a transverse magnetic field gradient. In such cases, it may be critically important to minimize contamination of the labeled cells with non-labeled cells while at the same time maximizing the throughput. Such contamination is known as non-specific crossover (NSC) and refers to the real or apparent migration of non-mobile particles or cells across streamlines with the mobile material. The possible mechanisms for NSC are discussed, and experimental results interpreted in terms of shear-induced diffusion (SID) caused by viscous interactions between particles in a sheared flow. It is concluded that SID may contribute to NSC, but that further experiments and mathematical modeling are necessary to more fully explore the phenomenon.

Split-flow thin channel fractionation (or SPLITT fractionation) is a separation technique particularly well suited to continuous sorting of micron size particles.^{1–4} It is also useful for selectively removing macromolecules or colloidal materials from a suspension of larger particles.^{5, 6} The SPLITT fractionation technique has much in common with crossflow filtration. The feed stream is introduced, using a sheath flow arrangement, adjacent to one wall of a thin channel. Differential transport of feed components occurs across the channel thickness either under the influence of an applied field or by diffusion. A division of flow at the channel outlet completes the separation. For submicron particles and macromolecules the transverse transport may occur by diffusion,^{5, 6} but if particles are larger than around a micron in diameter, so that diffusional transport is negligible, then some type of field must be applied across the channel thickness to induce migration. The rate of transverse transport is therefore either a function of the diffusion coefficients of the feed components, or the mobilities of the components under the influence of the applied field. In either case, the feed is selectively depleted of some of its components as it passes along the length of the channel.

The advantage of SPLITT fractionation over crossflow filtration is that the binary separation is obtained without the use of a membrane and therefore without all of the associated problems

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of membrane fouling and consequent flux reduction. The selective transport takes place across a thin fluid lamina which is defined by two virtual surfaces lying between the channel walls. This so-called transport lamina is constantly replaced with fresh fluid during the fractionation and has none of the disadvantages associated with a physical membrane. The virtual surfaces are the result of introducing two fluid streams at the channel inlet, the feed suspension stream and a stream of pure fluid, and dividing the fluid between two channel outlets. The inlet streams are generally introduced to each side of a stream splitter placed between, and parallel to, the channel walls. A similar stream splitting arrangement divides the fluid flow at the channel outlets. The virtual surfaces define the boundaries between the fluid elements derived from the two inlets and the fluid elements exiting at the two outlets, and are known as the inlet splitting surface (ISS) and outlet splitting surface (OSS), respectively. The positions of the ISS and OSS are determined by the ratios of volumetric flow rates at the inlets and outlets, respectively. These may be adjusted so that the transport lamina has a suitable thickness to obtain the desired fractionation during the sample residence time in the channel.

The study we present is specifically concerned with non-specific cross stream migration that interferes with selective fractionation under the action of an applied field, and is not concerned with simple diffusional transport of nanoparticles or macromolecules. Recent application of SPLITT fractionation to magnetic sorting of magnetic particles and immunomagnetically labeled biological cells has revealed the importance of non-specific cross stream transport. This is where effectively non-magnetic materials migrate across streamlines by some mechanism that cannot be attributed to their interaction with the applied field – they are non-mobile with respect to the field. When this migration takes place across the full thickness of the transport lamina it is known as nonspecific crossover (NSC). The importance of NSC became apparent because in the special case of magnetic SPLITT fractionation^{7–10} the mixture to be separated is often composed of a binary mixture of mobile species (for example, biological cells labeled with magnetic particles) and non-mobile species (un-labeled cells) and the objective is to separate mobile from non-mobile species. The NSC of non-mobile species is a source of contamination for the mobile fraction. Almost all other applications of SPLITT fractionation described in the literature have dealt with the fractionation of a feed suspension in which species have a broad mobility distribution and the goal is to deplete the feed of materials having mobilities higher than a certain *cut-off* mobility.^{3, 4, 11–25} (This does not preclude the possibility that the higher mobility fraction is the material of interest.) In these cases, the transport lamina must have a thickness appropriate to the desired mobility cut-off. Depending on the overall flow rate and field conditions, this may occupy a significant fraction of the channel thickness. However, when the goal is to separate mobile from non-mobile cells by magnetic SPLITT fractionation then the transport lamina may be very thin, or in the ideal case, of zero thickness. In the ideal case, cells which are truly non-mobile would not be expected to cross the transport lamina no matter how thin it was. A magnetically labeled cell population is not generally monodisperse in magnetophoretic mobility. A range of mobility is exhibited due to natural variation in antigen expression level on the cells and consequent variation in immunomagnetic labeling density.^{26–28} To completely isolate a population of mobile cells, it would be necessary to arrange conditions such that the least mobile cells have time to migrate across the thickness of the feed lamina as well as the thin transport lamina during their residence time in the channel. The non-mobile cells would then be expected to exit the channel at the outlet on the feed side of the channel, and the mobile cells at the opposite outlet. In practice, NSC detracts from the ideal specific fractionation and leads to contamination of the mobile fraction.

The occurrence of NSC is of particular importance in the isolation of relatively rare labeled cells such as embryonic stem cells in umbilical cord blood,²⁹ circulating breast cancer cells in the peripheral blood stream,³⁰ or hematopoietic progenitor cells in apheresis blood samples.^{31, 32} In such cases, a very small NSC can greatly reduce the purity of the isolated fraction.

³³ In fact, the crossover of a very small fraction of the non-mobile cells that constitute the vast majority of the feed can have a highly detrimental effect on the collected mobile cell purity. It has been demonstrated^{30–35} that when using zero or thin transport laminae in SPLITT fractionation, non-mobile particles or cells do tend to contaminate the mobile fraction of cells recovered at the far outlet. The degree of contamination may be reduced by arranging flow rate ratios to obtain thicker transport laminae. However, this would result in reduced throughput and/or reduced recovery of the rare mobile cells, neither of which are desirable. The control or prevention of NSC is therefore of critical importance for isolation of rare cells by SPLITT fractionation.

Most implementations of SPLITT fractionation have made use of gravitational or centrifugal fields. The conventional parallel-plate channel geometry is ideal for the exploitation of the uniform and essentially unidirectional gravitational field.^{2, 3, 11–25} An adaptation of the parallel-plate geometry is used for the centrifugal field by wrapping the channel around the inside of a centrifuge basket.⁴ Parallel-plate channels have also been used with electrical^{36, 37} and acoustic^{38–40} fields where the field acts uniformly between the channel walls. Parallel-plate geometry has also been used with some success for magnetic SPLITT fractionation,^{41–45} but it is less suited to this field type because of the variation of both field strength and gradient in field strength with position around the simple dipole magnets used in these studies. The quadrupole magnetic field is radially symmetric, with field strength increasing linearly from zero at the axis. The gradient in field strength is therefore constant within the aperture of the quadrupole and is directed away from the axis. This radially symmetric field has been exploited in the quadrupole magnetic flow sorter (QMS)^{7–10, 28, 30–35} using annular channels and cylindrical stream splitters.

Although NSC was found to be of particular importance for the fractionation of magnetically labeled biological cells using the annular SPLITT channel of QMS, with a radially symmetric magnetic field provided by a quadrupole magnet, the phenomenon is, however, common to all forms of SPLITT fractionation. The experiments reported in this work were performed using pulse-injection of small samples of a suspension of silica particles (diameter $4.7\ \mu\text{m} \pm 1.0\ \mu\text{m}$) into a parallel-plate SPLITT channel. The results of these experiments have relevance to QMS as well as the conventional parallel-plate systems.

PRINCIPLES OF SPLITT FRACTIONATION

Ideal SPLITT Fractionation

Several publications have already presented the principles of transport mode SPLITT fractionation for both parallel-plate channels,^{1–4} and annular channels.^{8, 28, 31, 34} These principles were later taken as the basis for development of approaches to optimization of separation conditions for the systems.^{46, 47} Only the essential concepts required for comprehension of this work are presented below.

An edge view of a parallel-plate SPLITT fractionation channel is shown in Figure 1 with thickness expanded for clarity. It consists of a Hele-Shaw cell provided with pairs of opposed inlets and outlets, each pair being separated by a stream splitter that serves to either smoothly merge or divide the fluid stream. The thickness w typically ranges from 50 to several hundred microns, the length L from 10 to 30 cm, and the breadth b from 1 to several centimeters. The particulate sample suspension and the particle-free sheath fluid are continuously introduced at the inlets labeled a' and b' , with volumetric flow rates $Q(a')$ and $Q(b')$, respectively. The total channel flow is divided between outlets a and b . Conservation of mass requires that the sum Q of the inlet flow rates $Q(a')$ and $Q(b')$ is equal to the sum of the outlet flow rates $Q(a)$ and $Q(b)$. Generally, the sample inlet flow rate $Q(a')$ is arranged to be smaller than the sheath flow rate $Q(b')$. This flow configuration causes the inlet splitting surface (ISS) to curve toward the

wall adjacent to the inlet a , wall A , as shown in the figure. The analogous virtual surface at the boundary between the outlet flow streams defines the outlet splitting surface (OSS). The position of the ISS is located at distance $w(a')$ from wall A and is related to sample flow rate $Q(a')$ by the relationship:⁵

$$\frac{Q(a')}{Q} = 3 \left(\frac{w(a')}{w} \right)^2 - 2 \left(\frac{w(a')}{w} \right)^3 \quad (1)$$

The OSS is located at distance $w(a)$ from wall A and is related to $Q(a)$ by an analogous equation. The distance between the two virtual surfaces ISS and OSS, $w(t) = w(a) - w(a')$, is called the transport lamina thickness. In the figure, filled circles represent non-mobile particles and unfilled circles mobile particles. All particles are initially compelled by the flow to start their axial migration in the vicinity of wall A , within the sample stream thickness $w(a')$. The mobile particles migrate across the transport lamina under the influence of a transverse field. Three different transport lamina thicknesses are drawn, corresponding to outlet flow rate ratios of 0.3, 0.5 and 0.7. The outlet flow rate ratio of 0.3, corresponding to the thinnest transport lamina allows the slowest of the mobile particles to be collected at outlet b . Note that some of the non-mobile particles are shown to have migrated a small distance above the ISS, and that a zero transport lamina thickness would have resulted in a contamination of the mobile fraction. Thicker transport laminae corresponding to outlet flow rate ratios of 0.5 and 0.7 would have resulted in incomplete collection of the mobile fraction. Fractional recoveries at the outlets a and b , F_a and F_b , are defined as the ratios of the mass of particles eluted at each outlet, $M(a)$ and $M(b)$, and the total mass of particles M injected at a' . Therefore, $F_a = M(a)/M$ and $F_b = M(b)/M$. In the ideal case where there is no loss of material in the channel, $M = M(a') = M(a) + M(b)$. Fractional recoveries may be calculated for different subgroups of the sample, such as the mobile or non-mobile particles.

As explained in the introduction, a *cut-off* mobility can be selected by adjusting the transport lamina thickness. In fact, due to the finite thickness $w(a')$ of the sample lamina, two critical mobilities define the cut-off. The first, m_0 , corresponds to the mobility of species that can migrate just across the transport lamina from the ISS.³ Species having lower mobilities will be fully recovered at outlet a , and therefore $F_b = 0$ for $m \leq m_0$. In the case of parallel-plate SPLITT with constant field-driven velocity, this mobility is given by:

$$m_0 = \frac{Q(a) - Q(a')}{bLS} \quad (2)$$

where S is a measure of the applied field strength, and the field-induced transverse migration velocity u is given by the product of mobility and field strength, mS . The second critical mobility m_1 corresponds to species that have sufficient time to migrate from wall A to the OSS, and therefore all species having higher mobilities will be fully retrieved at outlet b . (In this brief overview, it is assumed that species that migrate as far as wall B will exit the channel at outlet b , and will not be retained in the channel.) It follows that $F_b = 1$ for $m \geq m_1$, again assuming no loss of material in the system. This mobility is given by:

$$m_1 = \frac{Q(a)}{bLS} \quad (3)$$

These equations are general in that they apply to any uniform field applied to a parallel-plate channel. The same principles apply for the annular QMS system, but the equations are modified

to account for the non-uniform field and the annular geometry. In the case of gravitational SPLITT, S is the acceleration due to gravity and m is the sedimentation coefficient given by $V\Delta\rho/f$, in which V is the particle volume, $\Delta\rho$ is the density difference between the particle and the suspending fluid, and f is the friction coefficient given by $3\pi\eta d$ with η being the fluid viscosity and d being the hydrodynamic diameter of the particle. In the annular QMS system the local measure of field strength is given by $|\nabla B_0^2|/2\mu_0$, where B_0 is the local magnetic field strength, ∇ is the gradient operator, and μ_0 is the magnetic permeability of free space. For paramagnetic particles, the magnetophoretic mobility²⁸ is given by $V\Delta\chi/3\pi\eta d$, where $\Delta\chi$ is the difference in magnetic susceptibility between the particle and the suspending fluid.

Non-ideal SPLITT fractionation

The theory developed for ideal SPLITT fractionation suggests that it is possible to achieve a separation of mobile species from non-mobile species with mobile exiting at outlet b and non-mobile at outlet a . However, a performance test has been suggested¹² for checking the integrity of a parallel-plate SPLITT channel. The channel is positioned vertically with downward flow in order to eliminate transverse migration under gravity. A sample consisting of a dilute suspension of micron size particles (such as 10 μm calibrated latex beads) is introduced to inlet a' . The sample volume should be approximately equal to the channel volume between the flow splitters, V^0 . Pure carrier solution is introduced at inlet b' . Flow rates at the two inlets and two outlets are set equal so that the ISS and OSS should both be located at the channel midpoint and the transport lamina should have zero thickness. The total channel flow rate is set to obtain a channel void time of around 30 seconds (*i.e.*, $V^0/(Q(a') + Q(b')) \approx 30$ s). If it is found that $F_b < 0.1$ then the channel performance is considered acceptable. This criterion implicitly admits that in practical situations, a non-specific cross stream migration remains even for non-mobile species.

This seemingly arbitrary criterion for acceptable NSC of 10% under these test conditions may appear to be quite crude. However, this criterion was established after considerable practical experience in the use of SPLITT systems. We can examine the criterion more closely. Consider continuous operation of the SPLITT channel under the test flow conditions with the sample suspension entering the channel and initially occupying half the channel thickness. Suppose that the suspension expands in thickness as it passes along the channel and occupies a thickness of $(0.5w + \delta x)$ just before the outlet splitter. (Following the usual coordinate system of the SPLITT literature, the x -coordinate is directed across channel thickness from wall A, and the z -coordinate is directed along channel length from the inlet splitter.) Suppose also that the suspension remains uniformly distributed across this expanded region. The concentration of the suspension at this point may be determined by consideration of mass conservation. The integral of the product of concentration and local fluid velocity should therefore remain constant:

$$\int_0^{0.5} c_0 v d\xi = \int_0^{0.5+\delta\xi} c' v d\xi \quad (4)$$

where c_0 and c' are the initial and final concentrations, respectively, v is the parabolic velocity profile in the parallel-plate channel (given by $v = 6\langle v \rangle \xi(1 - \xi)$, in which $\langle v \rangle$ is the mean fluid velocity), and ξ is the reduced distance x/w from wall A. Solution of eq (4) shows that

$$c' = \frac{c_0}{(1+2\delta\xi)^2(1-\delta\xi)} \quad (5)$$

For $F_b = 0.1$ and $F_a = 0.9$ it follows that $c' = 0.9 c_0$ and eq (5) may be solved to obtain the result $\delta\xi = 0.037$. For a typical parallel-plate SPLITT channel of $380 \mu\text{m}$ thickness this amounts to an expansion of the sample zone by just $14 \mu\text{m}$, or 1.4 times a particle diameter of $10 \mu\text{m}$. It is therefore apparent that the criterion for acceptable performance is in fact quite stringent. The conditions are arranged for a void time of ~ 30 s, and the residence time along the channel center line would thus be ~ 20 s. The mean lateral velocity for the sample zone front would therefore be approximately $0.70 \mu\text{m/s}$, which is about 60% of the sedimentation velocity for $10 \mu\text{m}$ polystyrene particles under gravity. If the integrity test was carried out at the same total flow rate but with $Q(a')/Q = Q(a)/Q = 0.104$ so that the ISS and OSS lie at $\xi = 0.200$, the equivalent analysis shows that

$$c' = \frac{0.104c_0}{(0.2 + \delta\xi)^2(2.6 - 2\delta\xi)} \quad (6)$$

In this case, $F_b = 0.1$ when $\delta\xi = 0.012$. This corresponds to a lateral advance of only $4.6 \mu\text{m}$ and, accounting for the slower local longitudinal velocity, a lateral velocity of just $0.15 \mu\text{m/s}$.

We refer to NSC in SPLITT channels as the fraction $F_b > 0$ of non-mobile particles that exit at outlet b when $Q(a)/Q \geq Q(a')/Q$. For $Q(a)/Q < Q(a')/Q$, it is the F_b in excess of the ideal F_b given by

$$F_b(\text{Ideal}) = 1 - \frac{Q(a)/Q}{Q(a')/Q} \quad (7)$$

which is valid for $Q(a)/Q < Q(a')/Q$, that constitutes the NSC.

In practice, a critical transport lamina thickness $w(t)_{\text{NSC}}$ is defined as that just sufficient to eliminate NSC. For the model assumed above, this critical transport lamina thickness is equal to δx . Therefore, for a binary mixture of non-mobile and mobile species, the conditions for obtaining a separation such that only a pure mobile fraction is collected at outlet b corresponds to

$$\delta x \leq w(a) - w(a') \quad (8)$$

We may alternatively consider this zone expansion model in terms of an “*apparent transverse velocity*” u_{app} for the boundary of the zone occupied by non-mobile species as it is carried along the channel length. The condition for only mobile species to be collected at outlet b may then be written as:

$$u_{\text{app}} \leq \frac{Q(a) - Q(a')}{bL} \quad (9)$$

An alternative approach is to describe NSC in terms of an effective diffusivity for the particles that are not influenced by the field. Each of these approaches will be considered in the discussion of the experimental results.

CONTRIBUTIONS TO NSC

There are several phenomena that may contribute to NSC: 1) hydrodynamics at edges of splitters; 2) hydrodynamic lift forces; 3) hydrodynamic instabilities; 4) particle drafting; 5)

channel or splitter imperfections or deformations; and 6) shear-induced diffusion. All these phenomena can result in either real or apparent migration of particles across streamlines and potentially across the OSS to exit at outlet *b*.

Hydrodynamics at splitter edges

The flow patterns at the edge of an inlet splitter of a parallel-plate channel have been studied using computational fluid dynamics.⁴⁸ It was shown that when the inlet flow rate ratio $Q(b')/Q(a')$ was not strongly asymmetric (they considered $Q(b')/Q(a') = 4$), the two flow components merged smoothly up to a channel Reynolds number *Re* of 42. They found that fully developed channel flow, and therefore the approach of the ISS to its steady state position, required a greater distance to be established as *Re* increased. Even at *Re* = 42 this was accomplished within a distance of 5*w*. However, if the flow rate was increased further, or if the asymmetry of the inlet flows was increased, then a vortex was predicted to form on the side of the channel corresponding to the lower inlet flow. It was suggested that such vortices could result in some mixing between incoming laminae and thereby contribute to NSC. As inlet flow asymmetry was reduced the formation of a vortex required a higher channel *Re*, so that when $Q(a') = Q(b')$ a channel *Re* of about 70 was necessary. Such flow rates are far higher than those generally used in SPLITT fractionation, and far higher than those used in the experiments described in the present work. This mechanism may therefore be disregarded.

Hydrodynamic lift forces

In SPLITT fractionation, the suspension of particles is initially confined to a thin region adjacent to wall A, as shown in Figure 1. In this zone, shear rates can be high and in some cases, high enough to induce significant hydrodynamic lift forces upon individual particles. Studies have shown the possibility of fractionating species of different size using the balance between gravity and lift forces in gravitational SPLITT fractionation.^{49, 50} To achieve separation, the particles were necessarily relatively large (10 to 50 μm), and relatively high flow rates of 10 mL/min or more were required. We have computed the lateral migration induced by inertial lift forces in our experimental conditions, using the widely accepted Ho and Leal,⁵¹ and Vasseur and Cox⁵² (see also Williams⁵³) theory. We found that for particles initially adjacent to wall A, where shear rates and, consequently, lift forces are strongest, lateral displacement is predicted to be less than one particle diameter by the time they reach the channel outlet. The inertial lift force is predicted to fall to zero at around $\xi = 0.20$, and to be directed back toward wall A between $\xi = 0.20$ and 0.50. At the channel mid-point ($\xi = 0.50$), zero inertial lift force is again predicted. Therefore, under the proposed conditions for the performance test where ISS and OSS fall at the channel mid-point, inertial lift forces will not contribute to NSC. In fact, inertial lift forces may contribute to NSC only if both ISS and OSS are positioned at $\xi < 0.20$, and should reduce NSC if the OSS falls between ξ of 0.20 and 0.50. We note that inertial lift forces are well understood only for single isolated particles in viscous shear flows and no rigorous theoretical or experimental study has been carried out on a suspension of interacting particles. We also note that inertial lift forces on particles very close to a wall are not well understood. Experimental measurements of near-wall lift forces have revealed the presence of relatively strong forces directed away from the wall.^{54–57} However, these forces apparently rapidly attenuate with increasing distance from the wall. If we assume collective effects do not significantly influence hydrodynamic lift, we may consider that lift forces do not play a major role in NSC in our experiments.

Hydrodynamic instabilities

We are not concerned in this work with NSC caused by instabilities that are induced by curvature or rotation of centrifugal SPLITT channels.^{58, 59} There have been several studies of hydrodynamic instabilities caused by unstable stratification of density in gravitational SPLITT

fractionation,^{5, 60–62} and such instabilities undoubtedly reduce resolution. They would contribute to NSC only if a mobile species were present in the feed at sufficiently high concentration and fractionation was carried out under the influence of a transverse field. This type of transverse field-driven instability is not relevant to NSC under either the channel performance test conditions or the conditions employed in the experiments reported here. In each of these cases, a transverse field is not applied and only a single species is introduced to the channel. Of course, it is possible that hydrodynamic instabilities may be induced by gravity acting in the direction of flow and this may influence test condition results as well as our experimental results.

Particle drafting

In the present study we are not concerned with hydrodynamic particle drafting, which refers to the hydrodynamic drag of mobile species on non-mobile species. As in the case of hydrodynamic instabilities discussed above, drafting requires the mobile species to migrate under the influence of a transverse field and to cause non-mobile species to migrate by viscous drag. This mechanism is not relevant to channel performance test conditions or to the experiments reported here. In this study we are concerned with NSC of non-mobile species alone. The particle drafting effect will be the subject of a forthcoming paper.

Channel imperfections or deformations

These are difficult to quantify because splitter imperfections or misalignments of plates or cylinders cannot be easily determined *in situ*. The effects of splitter imperfections on NSC in annular channels have been simulated using computational fluid dynamics software.^{63, 64} An imperfection in an inlet splitter is predicted to result in a distortion of the ISS such that it advances across the channel thickness in some regions and retreats in others. Ignoring the influence of any other effects, the local particle concentration in the sample zone is unchanged. It was shown that a relatively small offset of 100 μm for a cylindrical splitter within a 2000 μm thick annulus can result in NSC of 19% at $Q(a)/Q = Q(a')/Q = 0.20$ and channel Reynolds number of 2.18. Very small imperfections, much smaller than those considered for the simulations, are almost certainly present in the most carefully constructed SPLITT channels, and these will give rise to a correspondingly small contribution to NSC. This contribution to NSC would be expected to be independent of sample suspension concentration and independent of Q under typical laminar flow conditions. The criterion for collection of all non-mobile species in the a outlet would correspond to the condition given by eq (8), with δx being independent of sample concentration and total flow rate. It is possible under conditions of highly imbalanced inlet or outlet flows and high total flow rate that splitters be deformed by the resulting hydrodynamic pressure difference on the two sides. Again, this effect would not arise under the balanced flow conditions of the performance test or the experiments reported here.

Shear-induced diffusion (SID)

The discovery of this phenomenon has an interesting history. In a study of the viscosities of concentrated suspensions of 40 to 50 μm polystyrene particles using a Couette device, Gadala-Maria and Acrivos⁶⁵ observed a “memory effect” on stopping and restarting rotation. When rotation was resumed in the same direction as that preceding an interruption, the torque required to attain the same shear rate immediately returned to the previous level. However, if the rotation was restarted in the reverse direction, the required torque jumped to an intermediate level (*i.e.*, a lower level than the previous steady-state), from which it gradually decreased a little before gradually rising again to the steady-state level required before interruption. This effect, which was observed over a period of seconds or fractions of a second, was interpreted as evidence for development of some structure in the suspension upon shearing. They also noticed

a longer term asymptotic approach to a reduced torque (corresponding to lower effective viscosity) on shearing a suspension for many hours. This latter effect was later explained by Leighton and Acrivos⁶⁶ as being due to migration of particles out of the sheared zone by a SID process normal to the plane of shear. A short time-scale increase in viscosity upon initial shearing of a suspension was also observed that was not reported by Gadala-Maria and Acrivos⁶⁵ who were more concerned with steady-state measurements and shear reversal effects. They attributed this effect to shear-induced diffusive migration of particles across the Couette gap thickness from regions of high shear stress (near the walls) to regions of lower stress.

A mechanism for self-diffusion of particles in a shear flow had been proposed by Eckstein, Bailey, and Shapiro.⁶⁷ They predicted that hydrodynamic interaction between three or more particles entrained in stream lines of differing velocity would result in random cross stream displacements. The macroscopic effect appears as a complex process of velocity fluctuations that constitute a random walk. These authors had studied shear-induced self-diffusion of non-colloidal particles in a shear flow in a Couette viscometer. Diffusion in the plane of shear (across the thickness of the gap) was studied by recording the lateral position and time for a marked particle to pass a narrow fixed window. The determination of self-diffusion coefficient was based on a one-dimensional random walk taking into account the unequal time intervals. The coefficient was predicted to increase with suspension concentration and to scale with $\dot{\gamma}a^2$ where $\dot{\gamma}$ is the shear rate and a is the particle radius. In the limit of low concentration, the coefficient was predicted by Leighton and Acrivos⁶⁸ to vary with ϕ^2 , where ϕ is the particle volume fraction, basing their argument on rates of three-particle interactions. Their experiments showed a higher migration of particles from regions of high concentration to regions of lower concentration than predicted by random self-diffusion and they suggested the action of an additional mechanism. They suggested that under constant shear rate a particle would be expected to experience a higher rate of interactions from the side of higher particle concentration and this would tend to drive it toward the region of lower concentration. Also, the displacements might be expected to be larger into regions of lower effective viscosity where concentration is lower. These effects would therefore be expected to operate in concert. However, for uniform applied shear stress, shear rate will be higher in regions of lower viscosity and this may attenuate the drift velocity to some extent. For a gradient in applied shear stress, the irreversible collision mechanism leads to a migration from regions of high shear stress to low shear stress for uniform concentration and viscosity.

Leighton and Acrivos⁶⁹ developed a model based on SID to explain the phenomenon they referred to as viscous resuspension. This process occurs when an initially settled layer of particles in a horizontal channel is subjected to a laminar shear flow. Particles are found to be resuspended and entrained in the flow. When opposed by gravity, the concentration profile approaches a steady state. The phenomenon had first been observed by Gadala-Maria⁷⁰ during the course of his graduate studies toward his Ph.D. Davis and Leighton⁷¹ adapted the model to cross-flow filtration where viscous resuspension is opposed by the cross flow.

The application of SID to the modelling of conditions in SPLITT fractionation is similar to the viscous resuspension model. There are some differences in that the viscous resuspension model, as initially presented, was concerned with a settled bed of particles that is subjected to a shear flow whereas in SPLITT fractionation the initial state is a uniform flowing suspension of particles occupying a fraction of the channel thickness. The initial volume fraction is therefore generally significantly lower in SPLITT fractionation. The viscous resuspension model also included an opposing transport, due to gravity or a cross flow, with an approach to steady state concentration profile. In SPLITT fractionation, the shear-induced diffusion may be opposed or enhanced by an imposed field, but in the special case of NSC the particles migrate without interaction with a field. In any case, the concentration profile does not generally

approach a steady state before the channel outlet, although in continuous operation the overall concentration distribution throughout the channel may be considered as stationary.

Most experimental studies of shear-induced diffusion have been carried out using relatively concentrated suspensions of $\phi > 10\%$. A recent study found much higher diffusivities than expected at low concentrations, and a limiting dependence on the first power of ϕ indicating irreversible interactions between pairs of particles.⁷² Such irreversible interactions have been predicted when the particles have a finite degree of surface roughness,^{73, 74} but this effect was not sufficient to explain the observed magnitude of diffusivity. The dependence of diffusivity on particle volume fraction is therefore not well established, although various expressions have been presented in the literature for different ranges of volume fraction.^{68, 69, 72}

EFFECTIVE DIFFUSIVITY MODEL

As a first approximation, the effect of SID may be modeled by a simple convection-diffusion approach with a shear-induced diffusivity, D , replacing the Brownian diffusion coefficient. For a parallel plate channel we have

$$\frac{\partial \phi}{\partial t} = \frac{\partial}{\partial x} \left(D \frac{\partial \phi}{\partial x} \right) - v(x) \frac{\partial \phi}{\partial z} \quad (10)$$

in which

$$D = \dot{\gamma} a^2 f(\phi) \quad (11)$$

where $f(\phi)$ is a function of the volume fraction ϕ . As is usual for such simulations of diffusional SPLITT fractionation, diffusion across the channel breadth is not relevant and transport along the length of the channel (in the z -direction) is dominated by convection. For exploratory purposes, this may be solved for an assumed constant effective value for D corresponding to conditions close to the ISS. (A more detailed approach will be taken in a later publication.) Eq. (10) then reduces to the form

$$\frac{\partial \phi}{\partial t} = D \frac{\partial^2 \phi}{\partial x^2} - v(x) \frac{\partial \phi}{\partial z} \quad (12)$$

which is solved in the same manner for continuous operation as the Brownian diffusion model.⁵ We effectively consider the particle concentration to be sufficiently low that gradients in the effective viscosity do not contribute significantly to SID, and also that the contribution due to gradient in shear rate is small over the range of advance of the sample zone. There is, however, a fundamental difference between SID and Brownian diffusion in that the particle-laden zone advances into a region which is devoid of particles, and where, according to the SID model, the diffusivity approaches zero. The front might be expected to advance with more of the character of a step function than is found with Brownian diffusion (where D is not a strong function of concentration). Nevertheless, we can assume that the diffusivity may be related to a mean diffusion distance l by the classic relationship for migration in one dimension:

$$l \cong \sqrt{2Dt} \quad (13)$$

where t a characteristic time, which may be approximated by the residence time of fluid close to the ISS. This distance l may be considered in the same light as δx of eq (8) for the prevention of NSC.

Another fundamental difference concerns the linear dependence of shear-induced diffusivity on shear rate as opposed to the independence of Brownian diffusion on shear rate. This means that a change in total flow rate Q will result in a proportional change in shear-induced diffusivity everywhere in the channel. However, there would be a compensatory reduction in residence time everywhere in the channel so that diffusion distances would be unchanged. Consequently, it is not possible to distinguish between the contributions of channel imperfections and SID by varying total channel flow rate alone. The strong dependence of NSC on particle size and sample volume fraction would indicate the presence of SID rather than channel imperfections. In fact, extrapolation to zero particle size and zero sample volume fraction could provide a means of extracting the individual contributions to NSC. Such experiments will be considered for a future study. Another aspect of the dependence of shear induced diffusivity on shear rate relates to the fact that shear rate falls to zero at the center of a parallel-plate channel. It follows that shear induced diffusion cannot result in transport of particles across the center line of the channel. For $Q(a')/Q \leq 0.5$, SID cannot therefore account for NSC when the outlet splitting plane lies beyond the center line, *i.e.*, for $Q(a)/Q > 0.5$.

The solution of the convection-diffusion model for the diffusion mode of SPLITT fractionation in continuous operation has been described in detail in a previous publication,⁵ and we need not describe the approach here except to say that the Crank-Nicholson method is used. As stated above, our approach for SID is equivalent except that a constant effective diffusivity is considered rather than a Brownian diffusion coefficient. Our phenomenological approach consists then in using the effective diffusivity D as an adjustable parameter to obtain a fit between theory and experiment. It is the fractional retrieval at outlet b , F_b , that must be matched to the experimental results by adjusting the assumed diffusivity. The predicted F_b is calculated from the simulated concentration profile at the channel outlet as follows

$$F_b = \frac{\int_0^{w(a)} \phi(x, L) v(x) dx}{\int_0^{w(a)} \phi_0 v(x) dx} = \frac{6Q}{\phi_0 Q(a')} \int_{\xi(a)}^1 \phi(\xi, L) \xi(1 - \xi) d\xi \quad (14)$$

where $\phi(x, L)$ is the volume fraction profile at the outlet splitter (at $z = L$), ϕ_0 is the initial sample volume fraction, and $\xi(a)$ represents $w(a)/w$.

EXPERIMENTAL

Materials and experimental set-up

The parallel plate SPLITT channel had the usual sandwich construction, and was composed of two glass plates, two Mylar spacers, and a stainless steel layer of 127 μm thickness which provided the stream splitters. The channel had a length L of 12 cm, breadth b of 1.5 cm, thickness w of 447 μm . The nominal volume between the splitter edges V^0 is therefore calculated to be 0.805 cm^3 .

Silica particles of mean diameter $4.7 \mu\text{m} \pm 1.0 \mu\text{m}$ (Spherocil particles, Rhône Poulenc, France) were used in the experiments. De-ionized water with 0.05% sodium dodecyl sulfate (SDS) and 0.02% sodium azide (a bactericide) was used as suspending fluid for the particles and as the pure fluid entering at inlet a' . The particles were suspended at a volume fraction of 0.5%.

The experimental set up was very similar to that employed in previous studies involving pulse injections of magnetic particles³⁴ and stem cells³² into an annular SPLITT system. Two syringe pumps (Precidor 5003-1, Bottmingen, Switzerland and KDS 100, KD Scientific, Holliston, MA, USA) were used for sample inlet a' and fluid inlet b' , respectively, and a single aspiration pump (KDS 100, KD Scientific) was used for one of the outlets, the second outlet being free to the atmosphere. Pulse injections were introduced using a Rheodyne (Rohnert Park, CA, USA) six way injection valve (Model 7725i) having a 50 μL loop, which is considerably less than the channel volume V^0 – the volume suggested for the channel integrity test. Two Model VUV-10 UV-detectors were used to monitor the two channel outlets (HyperQuan, Colorado Springs, CO, USA). The data acquisition was carried out using an analog-to-digital converter (DI-190, Dataq Instruments, Akron, OH, USA) and software (WinDaq Lite, Dataq Instruments) with a PC-compatible computer. The data were analyzed using a statistical software package including peak analysis chromatographic software (PeakFit, SPSS, Chicago, IL, USA).

Determination of fractional retrievals

Fractional retrievals at outlet b were obtained by determining the area A_b of the UV absorption peak for this channel outlet, and by applying the relationship:³⁴

$$F_b = \frac{Q(b) A_b}{Q A} \quad (15)$$

where $Q(b)$ is the volumetric flow rate at outlet b . Q is the total volumetric flow rate in the channel (equal to $Q(a) + Q(b)$). The area A is the area of the peak obtained at outlet b when the entire sample flow is directed into the detector b (by closing off outlet a).

The total volumetric flow rates used for the crossover experiments are listed in Table 1.

RESULTS AND DISCUSSION

Interpretation in Terms of Effective Diffusivity

Some results for the solution of the Brownian diffusion model for continuous operation are shown in Figure 2. In all cases, an inlet flow rate ratio $Q(a')/Q$ of 0.100 was assumed, corresponding to $w(a')/w$ of 0.196. Figure 2a shows the calculated concentration profiles at the outlet splitter (at $z = L$) for different values of the dimensionless combination of parameters Dt^0/w^2 , in which the void time t^0 is defined as V^0/Q . The profile for $Dt^0/w^2 = 0$ corresponds to the result for zero diffusion as well as to the initial profile at $z = 0$ for all of the simulations. Figure 2b shows the variation of F_b with outlet flow rate ratio $Q(a)/Q$ for each of the cases considered in Figure 2a. The values of F_b are in fact calculated as functions of $\xi(a)$ using eq (14), and the results plotted as functions of $Q(a)/Q$.

The mathematical model developed for Brownian diffusion mode in SPLITT fractionation predicts that, for a given diffusion coefficient, the fractional retrieval at the outlet b is a monotonically decreasing function of the position of the OSS (corresponding to $w(a)/w$), or of the outlet flow rate ratio $Q(a)/Q$. The OSS can be placed at any position across the channel thickness by adjusting the flow rate ratio $Q(a)/Q$. It was mentioned earlier that, because of the dependence of shear induced diffusivity on shear rate, this mechanism cannot predict migration beyond the channel center line. SID can not therefore predict a non-zero NSC for $Q(a)/Q > 0.5$. The Brownian mechanism can predict positive NSC under such conditions, however, as shown by the results for $Dt^0/w^2 = 3 \times 10^{-2}$ in Figures 2a and b.

Experimental results for the four different flow rates $Q = 1.5, 2, 3$, and 3.5 mL/min, are plotted as points with error bars ($\pm\sigma$) in Figures 3a, b, c, and d, respectively. (Experiments were typically carried out in triplicate.) In all cases, an inlet flow rate ratio $Q(a')/Q$ of 0.100 was used. In each case, the dashed line represents the ideal case in which there is no migration of sample particles (see eq (7)). As expected, the experimental fractional retrievals F_b representing the NSC are monotonically decreasing functions of the outlet flow rate ratio $Q(a)/Q$. This would be the case for any diffusion-like mechanism for particle migration across streamlines. A field-driven migration may, of course, result in a quite different variation of F_b on $Q(a)/Q$. The curves plotted in Figures 3a to d were obtained by least squares fitting of the Brownian diffusion model to the data using the value of Dt^0/w^2 as the single adjustable parameter. These best fit parameters are listed in Table 1 along with the corresponding calculated values for diffusivity D . Also listed in Table 1 are the shear rates at the position of the ISS. The curves fit the data reasonably well, but F_b data tend to be higher than the curves at higher $Q(a)/Q$. Small values of F_b are more difficult to measure than higher values, as reflected by the larger error bars in these regions. Nevertheless, it is expected that the SID mechanism would predict lower F_b at high $Q(a)/Q$ than the Brownian mechanism because of the dependence of shear induced diffusivity on local particle volume fraction. Transport into regions of lower volume fraction should therefore be curtailed. It has already been mentioned that F_b should fall to zero before $Q(a)/Q = 0.5$, but even before this point is reached, the local diffusivity should fall linearly with the local shear rate to zero at the mid-point from a maximum at the walls. It is also noted that the best fit values for Dt^0/w^2 increase with channel flow rate (see Table 1). This is not predicted for the SID mechanism, as explained earlier; the value for Dt^0/w^2 should be independent of flow rate because the dependence of D on shear rate should be exactly compensated by the change in t^0 . The same may be said of NSC caused by channel or splitter imperfections; the NSC should be independent of flow rate over such small relative changes. Figure 4 shows the best fit values for Dt^0/w^2 plotted as a function of shear rate, in units of s^{-1} , at the ISS. The linear least squares regression line is also included:

$$\frac{Dt^0}{w^2} = 0.000332\dot{\gamma} - 0.00687 \quad (16)$$

The fitted values for Dt^0/w^2 are seen to vary strongly with the shear rate at the ISS.

We can calculate effective mean diffusion distances corresponding to the best fit diffusivities using eq (13). As a first approximation, the characteristic times were set to the residence times at the position of the ISS at each channel flow rate. These residence times are assumed to correspond to the channel length between the splitters divided by the fluid velocity at the position of the ISS. Taking into account the parabolic velocity profile if the fluid in the thin channel, the characteristic times are given by the equation

$$t = \frac{V^0}{6Q \frac{w(a')}{w} \left(1 - \frac{w(a')}{w}\right)} \quad (17)$$

in which V^0 is fixed at 0.805 cm^3 , and $w(a')/w = 0.196$ for all experiments. We can carry this approach one step further, and assume that NSC might be reduced to sufficiently low levels if the outlet flow rate ratio $Q(a)/Q$ is set such that the transport lamina thickness is equal to some multiple of the calculated mean diffusion distance. The results of these calculations are given in Table 2 using the arbitrary multiplier of 1.5 for the mean diffusion distances. The calculated required outlet flow rate ratios are seen to be very high, and the transport lamina thicknesses are relatively thick. These results do reflect the degree of NSC shown by the experiments, however.

Interpretation in Terms of Sample Zone Expansion

It was pointed out earlier that there is a fundamental difference between the SID and the Brownian diffusion models. The particle-laden zone advances into a region devoid of particles where diffusivity, according to the SID model, must approach zero. The Brownian model assumes a diffusion coefficient that is not a strong function of particle concentration. We might therefore expect the particle-laden zone to advance under the influence of SID with more of the character of a step function than is predicted for the Brownian diffusion model. We can approximate the result by a sample zone expansion, as considered for the discussion of the SPLITT channel performance test. If the concentration remains constant across the expanded zone, then F_b should remain a linear function of $Q(a)/Q$, with F_b falling to zero at some $Q(a)/Q$ ($> Q(a')/Q$) that corresponds to the position of the sample zone front (SZF). In fact, for data corresponding to $Q(a)/Q < 0.25$ and $Q = 2.0, 3.0$, and 3.5 mL/min, linear dependencies of F_b on $Q(a)/Q$ are exhibited, as shown in Figure 5. The straight lines correspond to least squares fits to the data sets for which $Q(a)/Q < 0.25$, that are also forced through the point $F_b = 1.0$ at $Q(a)/Q = 0.0$. For $Q(a)/Q > 0.25$, the observed F_b are higher than predicted by the zone expansion model. These F_b are, however, relatively small, and therefore difficult to measure accurately. Consideration of eq (15) shows, for example, that for $Q(a)/Q = 0.25$ and $F_b = 0.1$, the area of the peak given by a detector on the a outlet would be 27 times the area of the peak for the b outlet. The peaks tend to be rather broad, and baseline subtraction becomes more problematic for lower peaks. There is also the possibility that more than one mechanism contributes to NSC, and the small NSC at these high values of $Q(a)/Q$ may be due to some other mechanism that becomes apparent once the contribution due to SID becomes insignificant. The data for $Q = 1.5$ mL/min do not fit so well, but the measured F_b are smaller and there are fewer data points in the relevant region (see Figure 3a).

Apparent positions of the SZF may be calculated from the $Q(a)/Q$ at which F_b extrapolate to zero. The intercepts on the $Q(a)/Q$ -axis are obtained directly from the least squares fits shown in Figure 5. These values are listed in Table 3, along with the apparent relative positions of the SZF (ξ_{SZF}) and the apparent distance of advancement of the SZF (δx). It can be seen that the apparent δx values increase with the channel flow rate, which is not predicted for the SID mechanism. Also listed are the calculated apparent diffusivities, based on eq (13), assuming the characteristic times listed in Table 2. The apparent diffusivities obtained by the two different approaches (Brownian model and sample zone expansion model) differ by an almost constant factor. Those obtained via the Brownian model are about 80% higher than those obtained by the sample zone expansion model. In both cases, the apparent diffusivities do not vary in direct proportion to shear rate at the ISS.

CONCLUSIONS

It cannot be concluded with certainty that shear induced diffusion dominates the phenomenon known as non-specific crossover. Further experiments must be carried out in order to distinguish between the different contributions that may contribute to this phenomenon. Experiments using larger sample volumes are called for to better approach continuous operation. The particle size and volume fraction must also be varied. It would be advisable to arrange for density equivalence between particles and suspending fluid in order to eliminate instabilities caused by the effects of gravity in the direction of fluid flow. It is also intended to model NSC by the mechanism of SID using a diffusivity dependent on particle size, local particle volume fraction, and local shear rate. This will help in the interpretation of experimental measurements of NSC. Experiments are also planned using a new splitter-less channel that may be less susceptible to channel imperfections.

Some experiments have recently been reported where the particle concentration was varied, and the degree of NSC was found to increase with particle concentration.⁷⁵ This does suggest

that SID plays a significant part, although instabilities in the direction of flow may have been induced at high particle concentrations. The proposed experiments should advance our knowledge in this area, and help characterize this interference to efficient binary separation by SPLITT fractionation.

Acknowledgments

The authors gratefully acknowledge support from the National Institutes of Health (CA62349 to MZ), the National Science Foundation – Centre National de la Recherche Scientifique (CNRS) Cooperative Research Program (Grants 3787 and 10631 to MH and MZ), and Centre National d'Etude Spatiales (CNES) (Grant 60241 to MH).

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Figure 1.

Edge view of a SPLITT fractionation channel showing the separation of a mobile species (vacant circles) from a non-mobile species (filled circles). The mobile species is shown to have a polydispersity in mobility, and the non-mobile species show a small cross stream migration under some unspecified mechanism.

Figure 2a

Figure 2b

Figure 2.

a) Concentration profiles predicted for the Brownian diffusion model for $Q(a')/Q$ of 0.10 and the indicated values of Dt^0/w^2 , b) Predicted curves of F_b versus $Q(a)/Q$ for the same values of Dt^0/w^2 .

Figure 3a

Figure 3b

Figure 3c

Figure 3d

Figure 3.

Experimentally measured crossover (F_b) plotted as points with error bars ($\pm\sigma$) as functions of outlet flow rate ratio $Q(a)/Q$, for $Q(a')/Q$ of 0.10 and total flow rates of a) 1.5, b) 2.0, c) 3.0, and d) 3.5 mL/min. The curves in each graph are the result of least squares fitting of the Brownian diffusion model to the data using Dt^0/w^2 as the single variable parameter. The dashed line in each graph represents ideal behavior where there is no non-specific crossover (see eq (7)).

Figure 4.

A plot of the best fit vales for Dt^0/w^2 versus the fluid shear rate at the position of the inlet splitting surface, ISS. The line corresponds to a linear least squares fit to the data.

Figure 5.

Experimentally measured crossover (F_b) plotted as points with error bars ($\pm\sigma$) as functions of outlet flow rate ratio $Q(a)/Q$, for $Q(a')/Q$ of 0.10 and total flow rates of 2.0, 3.0, and 3.5 mL/min ($\bullet$, $\blacktriangledown$, and $\blacktriangle$, respectively). The full lines represent predicted crossover for a simple expansion of the sample zone from 0.196 of the channel thickness (corresponding to $Q(a')/Q = 0.10$) to 0.30, 0.31, and 0.34 of the thickness (corresponding to $Q(a)/Q = 0.22$, 0.23, and 0.27, respectively). The dashed line represents ideal behavior where there is no non-specific crossover (see eq (7)).

Table 1

Results of crossover experiments at the different total flow rates. The values for Dt^0/w^2 were obtained by least squares fitting of the Brownian diffusion model to the experimental data. Effective diffusivities D are calculated from the Dt^0/w^2 values.

Flow Rate Q (mL/min)	Dt^0/w^2	D (cm ² /s) $\times 10^6$	Shear rate at ISS (s ⁻¹)
1.5	0.00224	0.139	30.4
2.0	0.00859	0.709	40.6
3.0	0.0113	1.40	60.8
3.5	0.0177	2.56	71.0

Table 2

Mean diffusion distances l calculated using eq (13) for the best fit diffusivities listed in Table 1. For all experiments $(wa')/w = 0.196$ and $w(a') = 87.6 \mu\text{m}$. The positions of the OSS ($w(a)/w$) and corresponding outlet flow rate ratios are calculated such that the transport lamina thickness is equal to each value of l multiplied by the arbitrary factor 1.5.

Flow Rate Q (mL/min)	Characteristic time (s)	Mean diffusion distance l (μm)	$w(a)/w$	$Q(a)/Q$
1.5	34.1	30.8	0.299	0.215
2.0	25.5	60.2	0.398	0.349
3.0	17.0	69.1	0.428	0.393
3.5	14.6	86.4	0.486	0.479

Table 3

Results of the application of the sample zone expansion model to the experimental data, as shown in Figure 5. The value of ξ_{SZF} corresponds to the apparent position of the sample zone front (SZF), and δx to the apparent distance of advance of the SZF. The apparent diffusivities D are calculated using eq (13) assuming $l = \delta x$.

Flow Rate Q (mL/min)	$Q(a)/Q$ where $F_b = 0$	ξ_{SZF}	δx (μm)	D (cm^2/s) $\times 10^6$
2.0	0.21	0.30	44	0.38
3.0	0.23	0.31	51	0.77
3.5	0.27	0.34	65	1.4