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J. Calvin Giddings[a]
[a] Department of Chemistry, University of Utah, Salt Lake City, Utah

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Crossflow Gradients in Thin Channels for Separation by Hyperlayer FFF, SPLITT Cells, Elutriation, and Related Methods

J. CALVIN GIDDINGS

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF UTAH
SALT LAKE CITY, UTAH 84112

Abstract

The formation and use of thin equilibrium layers or hyperlayers in separations are discussed and the advantages of developing such hyperlayers along the transverse coordinate of thin channels is described. It is shown how a new method for establishing crossflow gradients in thin permeable-wall channels can be combined with sedimentation or electrical forces to generate transverse hyperlayers free of gradient-forming materials. The sedimentation case is shown to be equivalent to elutriation, but with the advantages of increased speed and of flow uniformity and thus of improved resolution. A general theory describing the form of the transverse flow gradient is developed. This theory is applied to the special case of hyperlayer formation in a thin channel with one permeable and one impermeable wall.

INTRODUCTION

Several classes of separation processes are best carried out in thin rectangular flow channels, including field-flow fractionation (FFF) and continuous separation in split-flow thin (SPLITT) cells (*1*, *2*). Both of these approaches utilize some kind of field or gradient applied transversely across the thin dimension of the channel with the purpose of redistributing sample material in such a way as to implement separation. This redistribution can take many forms. In one of the forms, some force or combination of forces is used to focus individual species of sample

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material into thin layers suspended between the confining walls (*3*). The forces must act selectively such that the suspended layers (hyperlayers) of different species are centered at different positions. Following the formation of a series of thin hyperlayers in the channel, the FFF or SPLITT mechanisms can be utilized to separate one particle type from another. The FFF approach requires the differential displacement of different hyperlayers along the longitudinal (flow) axis while SPLITT methodology takes direct advantage of the distribution of hyperlayers along the transverse axis (*2, 3*).

Focusing forces of the type noted above have been applied (but not transversely across thin cells) to the development of two widely used separation systems: isopycnic sedimentation and isoelectric focusing (*4*). Isopycnic sedimentation hinges on the combination of sedimentation forces and density gradients, leading to the formation of hyperlayers based on different particle densities. Isoelectric focusing is based on the combination of an electrical field and a pH gradient, in which case sample zones focus at a position determined by the isoelectric point (*5*). Several other systems have been proposed (*6*). These methods are carried out in general in extended chambers in which the hyperlayers are distributed over a distance of several centimeters or more in order to facilitate component detection and collection.

The advantage of developing focused hyperlayers along the transverse coordinate in thin channels instead of along an extended coordinate in conventional chambers is the much higher speed of hyperlayer formation and thus of separation. In thin channel systems, hyperlayer formation generally requires sample transport over a path of less than 1 mm in extent, which can be accomplished rapidly. The multicentimeter transport in conventional systems is correspondingly slow. In addition, hyperlayer FFF has a potential resolution advantage over alternate focusing techniques because overlapping hyperlayers can be separated along the longitudinal axis but not along the transverse (field) axis (*3*).

Hyperlayers are generally formed (*3*) under circumstances in which the displacement forces on a sample particle vanish at a certain position, x_{eq}. If the particle is moved to either side of this "equilibrium" position, a force arises and acts in such a direction as to restore the particle to x_{eq}. Generally, such focusing forces cannot be generated in a homogeneous system solely by the application of a single external field. In isopycnic sedimentation, for example, a secondary density gradient is required to modulate buoyancy forces and thus act in conjunction with sedimentation to focus particles at their position of neutral buoyancy. Isoelectric focusing uses a similar combination of a primary electrical force and a secondary pH gradient.

In theory, focusing can be achieved by combining sedimentation (or other) forces acting in one direction and a variable flow acting in the opposite direction. In such a system it is possible to find a location at which the sedimentation forces on a given particle are exactly balanced by the position-dependent frictional forces of the fluid moving in the opposite direction. Particles larger than the designated particle will fall (sediment) away from this location toward a new equilibrium position because of the predominance of sedimentation forces, and smaller particles will rise because of the increasing weakness of sedimentation forces relative to frictional forces. Under ideal circumstances, many components (hyperlayers) could be simultaneously separated in a single run.

The phenomenon described above constitutes the basis of elutriation methods of separation (*7*). In theory, elutriation is a powerful separation method capable of yielding narrow sample bands or hyperlayers (*8*). However, in practice, one rarely achieves a fraction of the potential resolution because of the nearly ubiquitous nonuniformity of flow in conventional separation chambers. Such nonuniformities could be largely eliminated by transverse flow in thin channels.

In this paper we propose a method for introducing a transverse velocity gradient into a thin channel. To do this, we require a channel having walls permeable to a carrier fluid. A transverse flow can be established by the permeation of fluid through such walls, a technique utilized in flow FFF (*1, 9*). However, by adjusting flows such that the two walls experience different permeation rates, it is possible to impose a gradient on the transverse velocity of flow in the channel. It should be possible to combine this transverse velocity gradient with sedimentation to yield a thin channel elutriation system. (It should also be possible to use electrical and other forces in conjunction with the velocity gradient to develop "electrical elutriation" and other such systems.)

If the "elutriation" mechanism is transferred to thin channels, the potential advantages become significant. First of all is the major increase in speed as noted earlier. However, of equal importance is the fact that fluid flowing transversely across a thin channel does so in a manner that is extraordinarily uniform except for a very small region near the channel edges. Therefore, substantial advantages would appear to accrue to a thin channel elutriation system if it could be practically developed.

We note for completeness that even without a gradient in crossflow, one could utilize constant crossflow, as used in flow FFF channels, for effective elutriation providing only two fractions were required. The transverse flow velocity would then be adjusted to such a level that the large particles (above a certain critical size) would fall to the bottom of

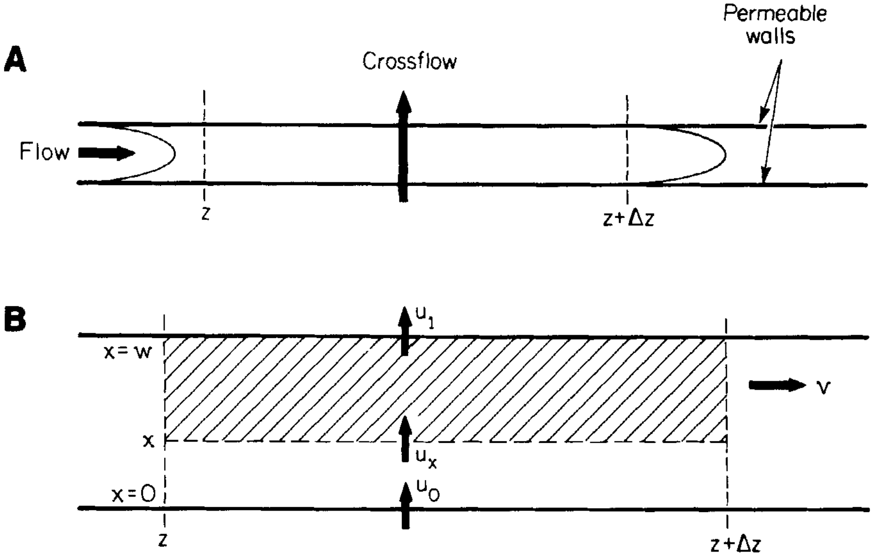

FIG. 1. Side view of thin channel with crossflow superimposed on longitudinal flow. (A) Schematic diagram of segment of length Δz. (B) Enlargement of segment showing crossflow velocities at different transverse positions.

the channel to be collected independently of the small particles carried to the top. These two particle populations could be collected at the outlet by a split-flow outlet system (*2*).

We note further that a gradient in transverse flow in thin channels may be used in cases where hyperlayers are not formed. Transverse flow gradients, for example, could be used in the operation of normal flow FFF. To account for all such possibilities, we have developed a theory applicable to any arbitrary inflow or outflow permeation rate through the two channel walls.

THEORY

We consider here a thin rectangular channel whose major side walls are permeable and thus subject to an inflow or outflow of fluid. The channel is thus essentially identical to that employed in flow FFF. A side view of such a channel with flow occurring along its length is shown in Fig. 1(A). In conventional flow FFF, a transverse flow is superimposed upon the longitudinal flow by allowing fluid to permeate in through one wall and permeate out through the opposite wall at an equal rate.

The case we consider here varies in one major respect: the rates of permeation through the two walls are considered to be generally unequal. By introducing unequal flow rates, a gradient in the transverse flow velocity is induced. This gradient can be controlled by varying the fluid fluxes through the two walls. In theory, as noted above, this velocity gradient can be combined with other forces such as sedimentation or electrical forces to form focused particle hyperlayers between the channel walls.

In order to treat the problem generally, we allow for flow of any magnitude in either direction through the two permeable walls. This situation is illustrated in Fig. 1(B). We choose an arbitrary x axis for the transverse coordinate; in the figure the x direction proceeds from the bottom wall at $x = 0$ to the top wall at $x = w$. The transverse fluid velocity along this axis is u. A u value that is negative will denote a flow in the negative direction along the x axis. The control of the volumetric flow rates of the fluid streams penetrating the lower and upper walls will lead to readily calculable values for transverse velocities u_0 and u_1 found just inside the channel walls.

We assume that the length L of the channel is very large compared to the thickness w and that the walls are uniform geometrically and homogeneously permeable. The flow along the length of the channel will be considered to be parabolic; this approximation requires that the transverse flow velocity u at any point be much smaller than the average longitudinal flow velocity $\langle v\rangle$. We will require the arbitrary segment of channel of length Δz illustrated in Fig. 1(B) to be very short compared to channel length L but very long compared to the channel thickness w.

We now do a mass balance for the above-mentioned segment lying between z and $z + \Delta z$. As shown in Fig. 1, flow can enter and exit the segment from the two ends and through the two permeable walls. Considering the fluxes through all four of the "open" boundaries of the segment, we can write the following equation for the net inflow into the segment:

$$I = b\Delta z(u_0 - u_1) + bw(\langle v\rangle_z - \langle v\rangle_{z+\Delta z}) = 0 \tag{1}$$

in which dimension b is the breadth of the channel measured from edge to edge. The net volumetric flow rate I must, of course, equal zero for a segment of fixed boundaries with incompressible fluid flow. Because the segment is short compared to L, we can write

$$\langle v\rangle_{z+\Delta z} - \langle v\rangle_z = \frac{d\langle v\rangle}{dz}\Delta z \tag{2}$$

When this is substituted back into Eq. (1), we obtain for the gradient in the mean longitudinal velocity

$$\frac{d\langle v\rangle}{dz} = \frac{u_0 - u_1}{w} \tag{3}$$

In order to obtain the transverse velocity u_x as a function of x, we must now write a mass balance for the shaded area of Fig. 1(B). The net influx into the shaded area is

$$I_s = b\Delta z(u_x - u_1) + b\int_x^w (v_z - v_{z+\Delta z})dx = 0 \tag{4}$$

where $v = v(x,z)$ is the longitudinal component of flow velocity in the channel. Equation (4) is like Eq. (1) except that u_x substitutes for u_0 and only the flow contributed by the parabolic flow profile lying above plane x is considered in the flow balance. If we treat the velocity difference at the two segment ends in the same way as we treated mean velocities in Eq. (2), we can write Eq. (4) in the following form:

$$I_s = b\Delta z(u_x - u_1) - b\int_x^w \left(\frac{\partial v}{\partial z}\Delta z\right)dx = 0 \tag{5}$$

When this equation is solved for u_x, we get

$$u_x = u_1 + \int_x^w \left(\frac{\partial v}{\partial z}\right)dx \tag{6}$$

If, as assumed, the longitudinal velocity is constrained to a parabolic profile, we can write

$$v = 6\langle v\rangle\left(\frac{x}{w} - \frac{x^2}{w^2}\right) \tag{7}$$

Since mean velocity $\langle v\rangle$ has no dependence on x, we can write for the partial derivative under the integral sign in Eq. (6) the following expression:

$$\frac{\partial v}{\partial z} = 6\,\frac{d\langle v\rangle}{dz}\left(\frac{x}{w} - \frac{x^2}{w^2}\right) = \frac{v}{\langle v\rangle}\,\frac{d\langle v\rangle}{dz} \tag{8}$$

When this is substituted back into the integral of Eq. (6), velocity u_x assumes the form

$$u_x = u_1 + \frac{1}{\langle v \rangle} \frac{d\langle v \rangle}{dz} \int_0^w v dx \tag{9}$$

If we now substitute Eqs. (3) and (7) into this expression, and follow this by integration, we obtain

$$u_x = u_1 + (u_0 - u_1)\left(1 - \frac{3x^2}{w^2} + \frac{2x^3}{w^3}\right) \tag{10}$$

or, equivalently,

$$u_x = u_0 + (u_1 - u_0)\left(\frac{3x^2}{w^2} - \frac{2x^3}{w^3}\right) \tag{11}$$

For both of these expressions $u_x = u_0$ when $x = 0$ and $u_x = u_1$ when $x = w$. We note that the constituent u terms can be either positive or negative.

The foregoing equations confirm our expectations in showing that the velocity increases with axial position z when the inflow, proportional to u_0, exceeds the outflow, proportional to u_1 (see Eq. 3). When the velocities u_0 and u_1 are constant throughout the channel, as dictated by a condition of uniform permeability, then Eq. (3) shows that the mean velocity gradient along the channel is constant and therefore that the velocity is linear in coordinate position z as expressed by

$$\langle v \rangle = \langle v \rangle_0 + \frac{u_0 - u_1}{w} z \tag{12}$$

While the variation of $\langle v \rangle$ down the channel makes the calculation of separation parameters somewhat more difficult than when $\langle v \rangle$ is constant, the simple linear relationship keeps those difficulties within reasonable bounds.

Another simplifying feature, illustrated by Eqs. (10) and (11), is that the transverse velocity u_x at any point x depends only upon coordinate position x and is independent of axial position z. Therefore, if we superimpose the variable velocity u_x on a second displacement velocity U induced by an externally applied field in order to create a particle hyperlayer, the position of the hyperlayer will remain the same throughout the length of the channel.

Any number of special cases can be treated by applying the general equations derived above. The simplest example is that in which inflow velocity u_0 equals outflow velocity u_l. This condition, generally imposed in the operation of conventional flow FFF, leads to the result $u_x = u_l = u_0$ as shown by Eqs. (10) and (11), and $\langle v \rangle = \langle v \rangle_0$ following Eq. (12).

HYPERLAYER EXAMPLE

Another special case is that in which no fluid passes through one of the walls. In this case the wall could, if desired, be impermeable. A system of this type might have some advantages because impermeable walls (e.g., glass plates) can be made more uniform than permeable walls (frits and membranes). If, for example, the upper wall in Fig. 1 is impermeable, then u_l goes to zero and Eqs. (11) and (12) assume the forms

$$u_x = u_0\left(1 - \frac{3x^2}{w^2} + \frac{2x^3}{w^3}\right) \tag{13}$$

$$\langle v \rangle = \langle v \rangle_0 + \frac{u_0}{w} z \tag{14}$$

A thin channel with one impermeable wall could be operated in much the same way as conventional flow FFF. With the upper wall impermeable, u_0 would be made negative by outflow from the lower wall, and flow FFF could be realized at the lower wall. In this case the mean velocity $\langle v \rangle$ would decrease linearly with axial position z, as shown by Eq. (12).

An inflow rather than outflow mode of operation would lead to hyperlayer formation. When u_0 is positive (inflow condition), then u_x decreases from u_0 at the lower wall to 0 at the upper wall (see Eq. 13). In this case the mean velocity would increase linearly with z. A sedimentation or other field impelling the particle at a fixed velocity U (generally negative) transversely through the fluid would therefore lead to the development of an equilibrium layer or hyperlayer at a position where the net velocity U^* vanishes; that is, where

$$U^* = u_x + U = 0 \tag{15}$$

We note that in the case of superimposed velocities described by Eq. (15), a zero particle velocity can only be found if u_x (or U) varies with x

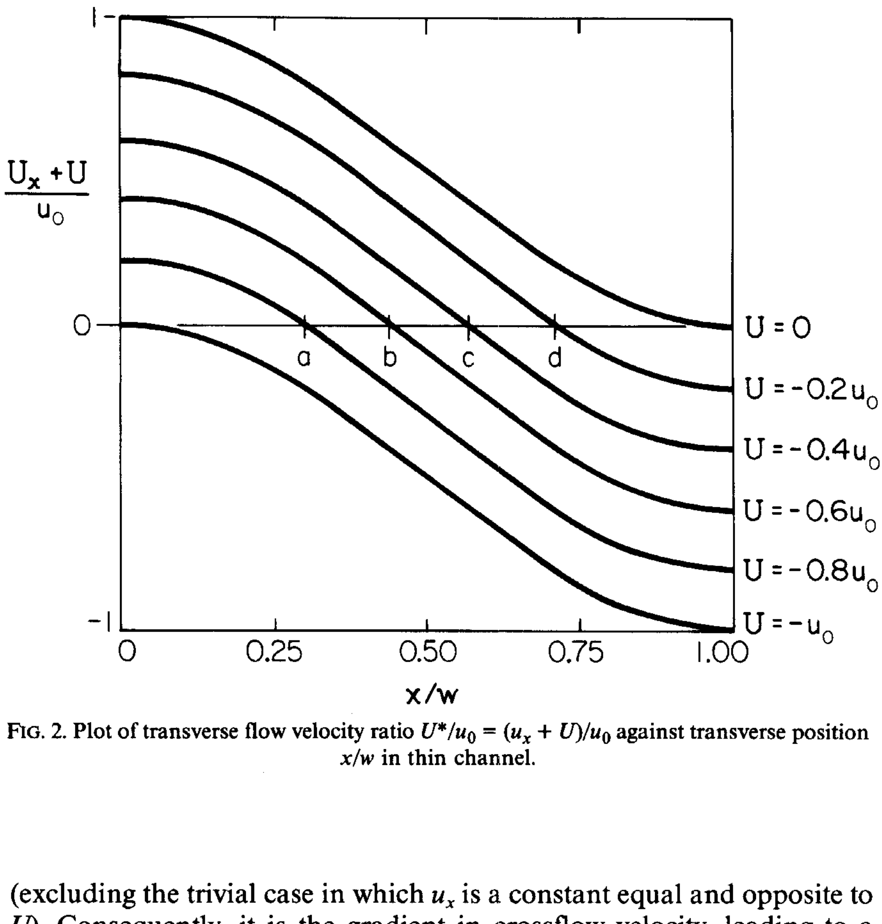

FIG. 2. Plot of transverse flow velocity ratio $U^*/u_0 = (u_x + U)/u_0$ against transverse position x/w in thin channel.

(excluding the trivial case in which u_x is a constant equal and opposite to U). Consequently, it is the gradient in crossflow velocity, leading to a variable u_x, that permits the formation of hyperlayers in the presence of uniform transport (represented by U) induced by an external field.

The hyperlayer case is further illustrated in Fig. 2. Here we have plotted the net velocity ratio $U^*/u_0 = (u_x + U)/u_0$ (with u_0 used for normalization) against x/w for different relative U values. The top curve applies when $U = 0$ and therefore represents the dependence of the crossflow velocity, expressed as the ratio u_x/u_0, on transverse channel coordinate. We see that this curve drops smoothly from 1 to 0 across the channel thickness, thus providing the desired gradient in transverse flow velocity. The curves beneath this represent the influence of increasingly negative U values; each successive curve is displaced downward by a fixed amount due to the superposition of increasing negative increments in U. The position at which each curve crosses the zero line represents the position x_{eq}/w

around which the hyperlayers are centered. These positions are indicated by the letters a, b, c, and d in the figure.

The series of curves in Fig. 2 can be interpreted in two ways. First, successive curves can be considered as representing the shift in the relative net velocity of a single particle type at different positions with increasing increments in field strength. Second, the different curves alternatively represent the relative net velocities of different types of particles, each subject to a different unique U value. The latter case represents a multicomponent separation by elutriation. In order to realize the benefits of such a separation, it is necessary to divide the hyperlayers into separate substreams for collection using a split-flow outlet (*2*).

The position x_{eq} of a component hyperlayer is related to the force F exerted on a component particle and the friction coefficient f of the particle. If we substitute $U = F/f$ and eq. (13) into Eq. (15), we get

$$\frac{F}{fu_0} = \left(1 - \frac{3x_{eq}}{w^2} + \frac{2x_{eq}^3}{w^3}\right) \qquad (16)$$

where coordinate x_{eq} replaces x because Eq. (15) defines the equilibrium condition. When written in terms of the reduced distance $\Gamma = 1 - x_{eq}/w$ from the upper wall, this becomes

$$\frac{F}{fu_0} = 3\Gamma^2 - 2\Gamma^3 \qquad (17)$$

Near that wall, where retention ratio R for hyperlayer FFF can be expressed by $R = 6\Gamma$ (*3*), we have

$$R = 6\left(\frac{F}{3fu_0}\right)^{1/2} \qquad (18)$$

For spherical particles of diameter d, where

$$F = \pi d^3 \Delta\rho G/6 \qquad (19)$$

and for which Stokes' law gives

$$f = 3\pi\eta d \qquad (20)$$

we have

$$R = d\left(\frac{2}{3}\frac{\Delta\rho G}{u_0\eta}\right)^{1/2} \tag{21}$$

where η is viscosity, $\Delta\rho$ is the density difference between the particles and the fluid, and G is the sedimentation field strength expressed as acceleration. This expression, in which R is proportional to d, shows that the form of crossflow gradient hyperlayer FFF under consideration here is approximately equivalent to steric FFF in its dependence on d and thus in its selectivity *(10)*.

If in Eq. (21) we write velocity u_0 as the volumetric flow rate $\dot{V}_c$ entering through the lower wall divided by wall area bL, we get

$$R = d\left(\frac{2}{3}\frac{\Delta\rho GbL}{\eta\dot{V}_c}\right)^{1/2} \tag{22}$$

which shows that retention can be increased (R decreased) by increasing $\dot{V}_c$. However, there are limits to this procedure.

The transverse flow velocities generated in thin permeable-wall cells are not generally large. In typical flow FFF channels, these velocities range around 1 μm/s *(11)*. While somewhat higher velocities can be obtained by increasing flow rate $\dot{V}_c$, as noted above, these gains will ultimately be at the expense of excessive longitudinal flow velocities simply because all of the fluid flow $\dot{V}_c$ entering through the channel wall(s) must be accommodated by the longitudinal outflow of the thin channel.

By way of example, a 1-μm diameter sphere with $\Delta\rho = 1$ g/cm^3 suspended in water at 20°C with a relatively high crossflow per unit area ($\dot{V}_c/bL$) or velocity u_0 of 1 cm/min = 167 μm/s would have $R \sim 0.2$ when acted on by one gravity. For a wall surface area bL of 20 cm^2, the channel outflow would be 20 cm^3/min plus any initial inflow. Larger particles would have correspondingly larger R's until Γ reached 0.5, then R would decrease. The largest particle of this density suspendible above the bottom wall would have d = 17 μm.

If the crossflow velocities cannot be made high enough to offset U and thus to suspend the desired particle hyperlayers (as is the case illustrated by the lower curve of Fig. 1), several adjustments can be made to restore the balance between flow- and field-induced transport. Most simply, the field-induced transport velocity can be reduced; in the case of gravitational fields, the channel can simply be tilted on one end in order to reduce the transverse component of the gravitational field. Another approach would be to reintroduce a permeable upper wall and allow sufficient flow to exit through that wall to lead to particle suspension without incurring extremely high longitudinal flow rates.

CONCLUSIONS

The independent adjustment of flow rates through the two walls of a permeable-wall thin channel shows the potential of providing several new or modified forms of separation. One new separation system would be a form of hyperlayer FFF in which sedimentation or electrophoresis would be balanced against a variable transverse flow velocity. This approach would have an advantage over existing hyperlayer techniques where gradient-forming materials (such as densifiers in isopycnic sedimentation), which must be introduced into the chamber, sometimes contaminate the product.

A second new class of separation systems would directly utilize transverse separation by employing split-flow (SPLITT) methods at the end of the channel. These methods would largely resemble continuous elutriation systems. However, rapid transport and the promise of uniform flow over the transverse flow cross-section promises considerable advantage over conventional systems.

One requirement necessary to realize the above separation techniques is the availability of wall material of uniform permeability. Recent work in our laboratory suggests significant nonuniformities in the permeation of various frit materials and membranes (*12*). This problem would have to be solved before one could expect substantial progress in developing the proposed separation systems.

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