MAGNIFICATION OF RESOLVING POWER OF COLLECTORS IN FREE FLOW ELECTROPHORESIS*

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The applicability of the device described in this paper is not limited to the serpentine flow bed method[1,2] and to the flow column stabilized by magnetic rotation[3,4] to which it has been applied so far. The following discussion will make it obvious how the proposed collection device can be used wherever the separated components of a mixture are distributed transversely to the fluid flow which carries them.

The problem to be solved is as follows: Let us assume that the flowing buffer carrying n separated components forms a thin fluid ribbon, say 2 mm in thickness and 5 cm in width. Let us suppose that the separated fractions form thin streaks in the buffer stream about $^1/_4$ mm in diameter. The buffer solution enters through the tube B (Fig. 1a) into the manifold M from where it is admitted to the separation chamber SC which it leaves through the collector tubes CT. IN is a hypodermic needle (gauge No. 22) through which the mixture to be fractionated is injected in a continuous stream which breaks up into the individual streaks S_1–S_4 carrying the components to be collected separately**.

It is obvious from inspection of Fig. 1a that one can get excellent resolution in the visible separation pattern without obtaining adequate resolution in the collection pattern. For example, if the streaks S_3 and S_4, each being $^1/_4$ mm in diameter, are separated by a gap of $^1/_4$ mm, they will be very clearly resolved in a photograph of the separation pattern, but they could easily pass out of the apparatus through one single exit tube CT of the collector if the latter were, for instance, 1 mm in internal diameter. Thus, there will be no separation of these components in the collection pattern.

In principle, resolution could be improved by making the adjacent collector tubes CT much narrower, spacing them as closely as possible, but this becomes practically very difficult. We have chosen, therefore, a different path to solve this problem. Instead of altering the lumen and the density of spacing of the collector tubes, the separation of the streaks was magnified by purely hydrodynamic means after completion of the electrophoretic separation. Fig. 1b shows the exit plate E of the buffer flow channel of Fig. 1a modified by omitting the exit tubes CT in the section Q_1. At this point, the flow channel is modified as shown in Figs. 1b and 1c.

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** This separation could, for instance, have been established electrophoretically or by any other method. For the sake of generality, the details of the separation apparatus are not indicated.

The buffer continues to flow between sections Q_1 and Q_2 through a trapezoidal channel at the end of which the exit tubes CT (of equal diameter and spacing as in Fig. 1a) are spaced at the same intervals as in Fig. 1a. This deformation of the flow channel leads to the deformation of the flow pattern indicated by the streaks S_1–S_4 in Fig. 1b. The intervals between the streaks are magnified approximately in the ratio of the cross-sections Q_2/Q_1 and the thickness of the streaks is similarly magnified. The latter magnification is no disadvantage since there is no objection to a separated fraction escaping through more than one collection tube. The collection of two ad-

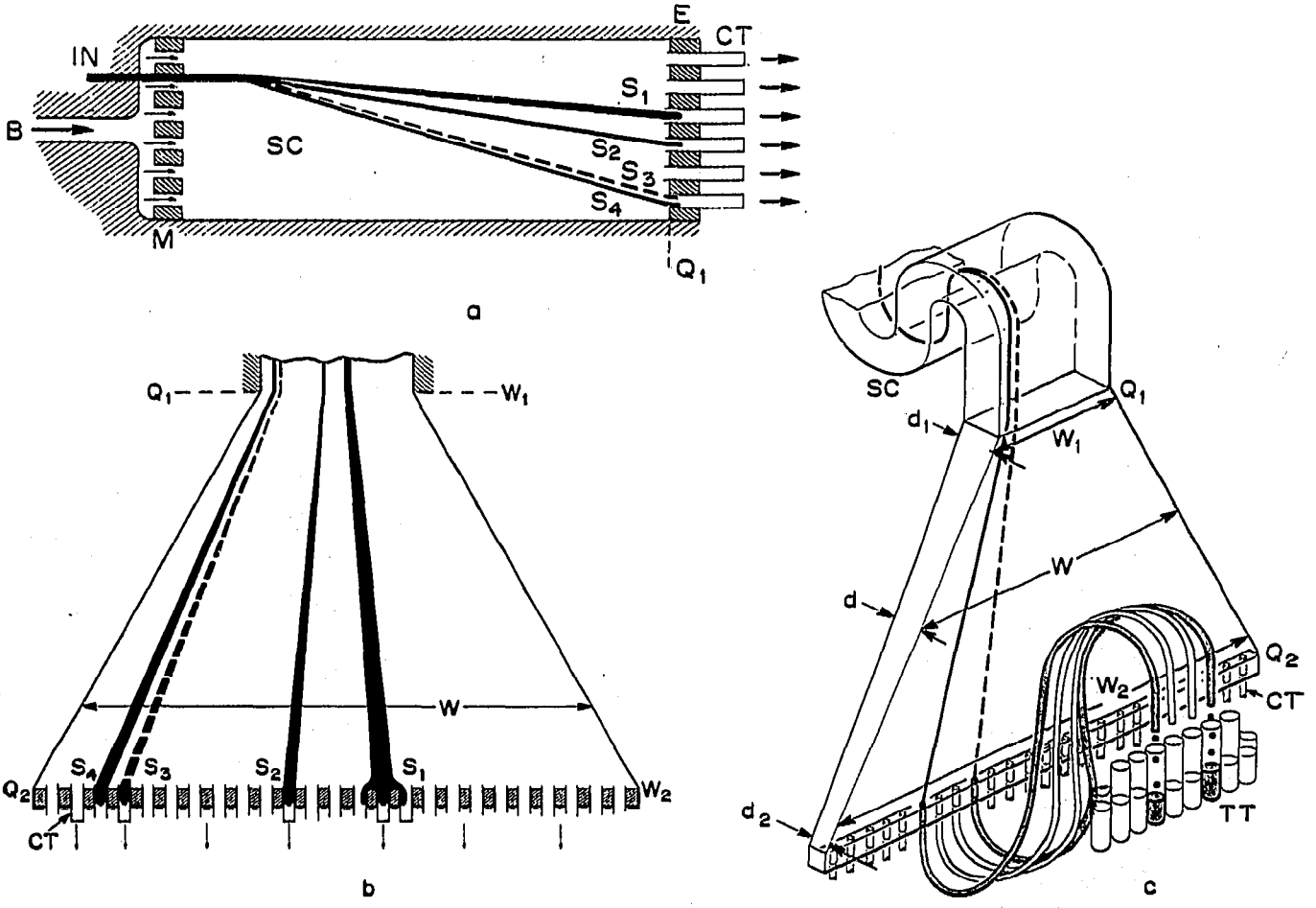


Fig. 1a. Continuous flow separation of four components S_1–S_4 entering from injector IN into the separation cell SC and leaving the cell through collector tubes CT. M = manifold. B = tube conveying the buffer solution. Q_1 = section at the location of the end plate E. The width at this point is 7 cm for the apparatus shown in Fig. 3.

Fig. 1b. The "fan" replacing the end plate E of Fig. 1a. Q_1 and Q_2 are the sections defining the origin and the end plate of the "fan", respectively. W_1 and W_2 are the widths of these sections. W = width of "fan" at an arbitrary point. S_1–S_4 = separated fractions. The separations between the fractions entering at section Q_1 are magnified purely by change of the flow pattern as they move toward section Q_2. At the same time, the streaks become wider. Components S_3 and S_4 which leave through the same collector tube in Fig. 1a, leave through separate collector tubes in Fig. 1b.

Fig. 1c. Perspective view of the "fan" with collector. SC = end section of the serpentine separation cell attached to the fan at section Q_1. The fan narrows down from depth d_1 at section Q_1 to depth d_2 at section Q_2. W and d = width and depth of the fan at an arbitrary location. CT = collector tubes. TT = test tubes of collector.

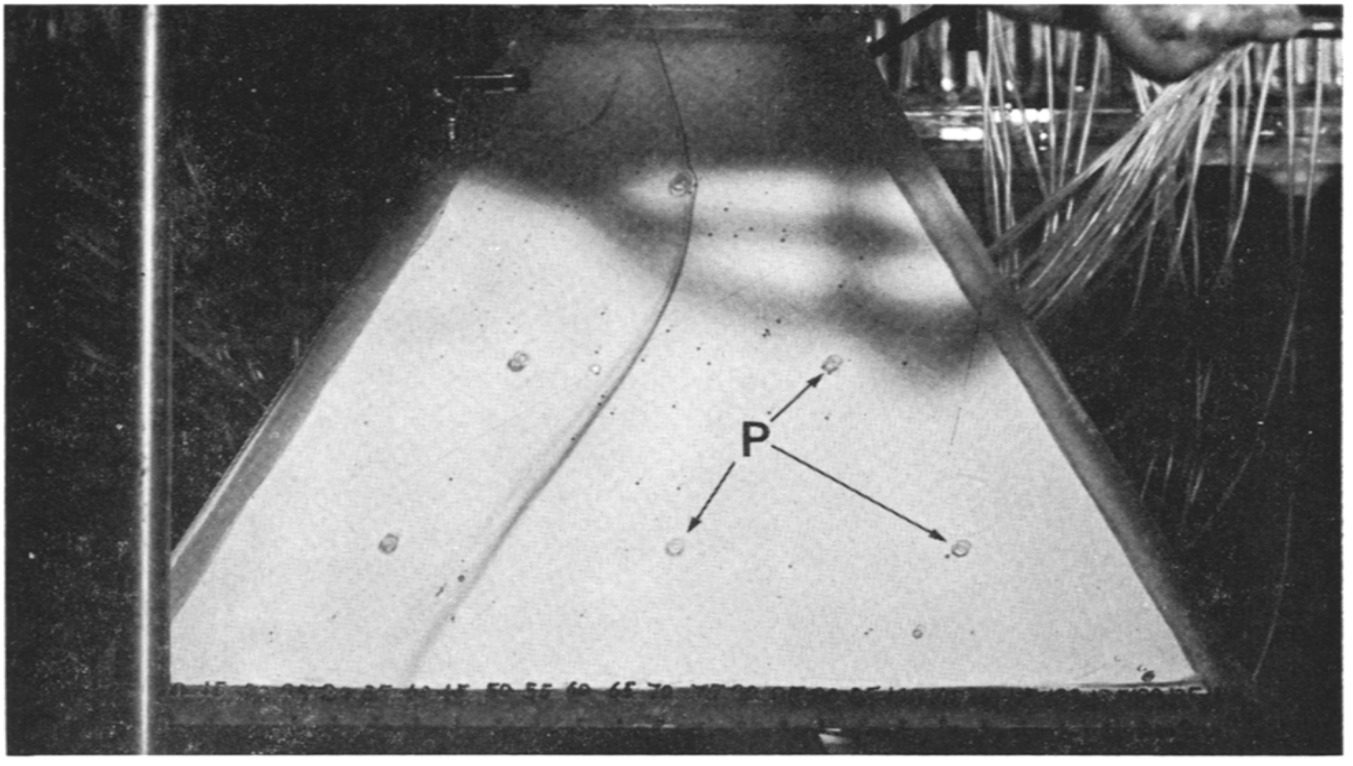
jacent fractions through one collection tube is, however, effectively avoided as shown by magnification of the gap between streaks S_3 and S_4 in Fig. 1b as compared to Fig. 1a. They are now far enough apart to be collected through different collector tubes.

To insure sufficient rigidity and parallelism between the trapezoidal plastic plates, small plastic pellets P, 2 mm in thickness, are cemented at several points between them as seen in Figs. 2a and 2b. These pellets do not disturb the separation process, they merely cause a deformation of streaks passing very close to them, which is of no consequence. This deformation is noticeable as a slight increase in curvature of the streaks seen in Figs. 2a and 2b at points of nearest approach to the pellets P.

We have to decide now what orientation of the trapezoidal channel will be best. A horizontal orientation is not recommended, especially with particulate components, because of gravitational sedimentation in the flow channel. Among the two possible vertical orientations, the one allowing the buffer to flow downward in the trapezoidal channel is preferable in electrophoretic separators since the buffer entering at Q_1 is still warm from exposure to the electrophoretic current; its temperature drops as it moves down in the trapezoidal channel and greater thermal stability is assured by an upward temperature gradient thus achieved. Nevertheless, if the current is large, thermal convection may still occur and may curve the streaks as shown in Fig. 2a. This can be avoided, as shown in Fig. 2b, and greater stability of the collection pattern can be achieved by submerging the trapezoidal "fan" into a cooling bath kept at a constant temperature (preferably 4°). An alternative method to cooling for suppression of thermal convection would be to use a serpentine "fan", *i.e.*, to use a serpentine flow bed as described in reference 1 allowing the width of the bed to increase in the downstream direction. Fig. 3 shows the apparatus without the cooling bath, which is merely a plastic box large enough to contain the "fan" and the collector tubes issuing from it.

The velocity of flow in the trapezoidal channel should be large as compared to velocities of possible thermal convection disturbances. The flow velocity decreases as we progress from the small cross-section Q_1 toward the wider and larger cross-section Q_2. One can achieve a uniform flow velocity in the trapezoidal channel by diminishing its depth as it becomes wider (*cf.* Fig. 1c). If we designate the depth of the channel by d, and its width in any cross-section by W, then the product $d \cdot W = d_1W_1 = d_2W_2 = C$ should remain constant to obtain the desired constancy of the flow velocity. It is not always practical to adjust the depth of the flow channel to safeguard constancy of the flow velocity. For instance, in the case of the apparatus shown in Figs. 1c and 3, assuming a ratio $W_2/W_1 = 4$ and $d_1 = 2$ mm, the channel exit should be reduced in depth to $^1/_2$ mm, which makes the construction of the device more difficult. Thus, as a compromise solution, the depth $d_2 = 1$ mm was chosen in the illustrated case with $d_1 = 2$ mm.

Figs. 1c and 3 show a trapezoidal collection "fan" used in conjunction with a serpentine separation cell[2]. Application to the magnetically stabilized rotating separation column will be described in a subsequent publication[4]. It is seen in Fig. 2b how the streaks of Evans blue (faster component on the left) and Rose Bengal, which are barely discernible at the entry into the "fan", are spread far enough apart at the exit to issue through non-adjacent collector tubes: No. 27 for Rose Bengal and No. 31 for Evans blue in a typical run (with slight amounts of dye entering the test tubes



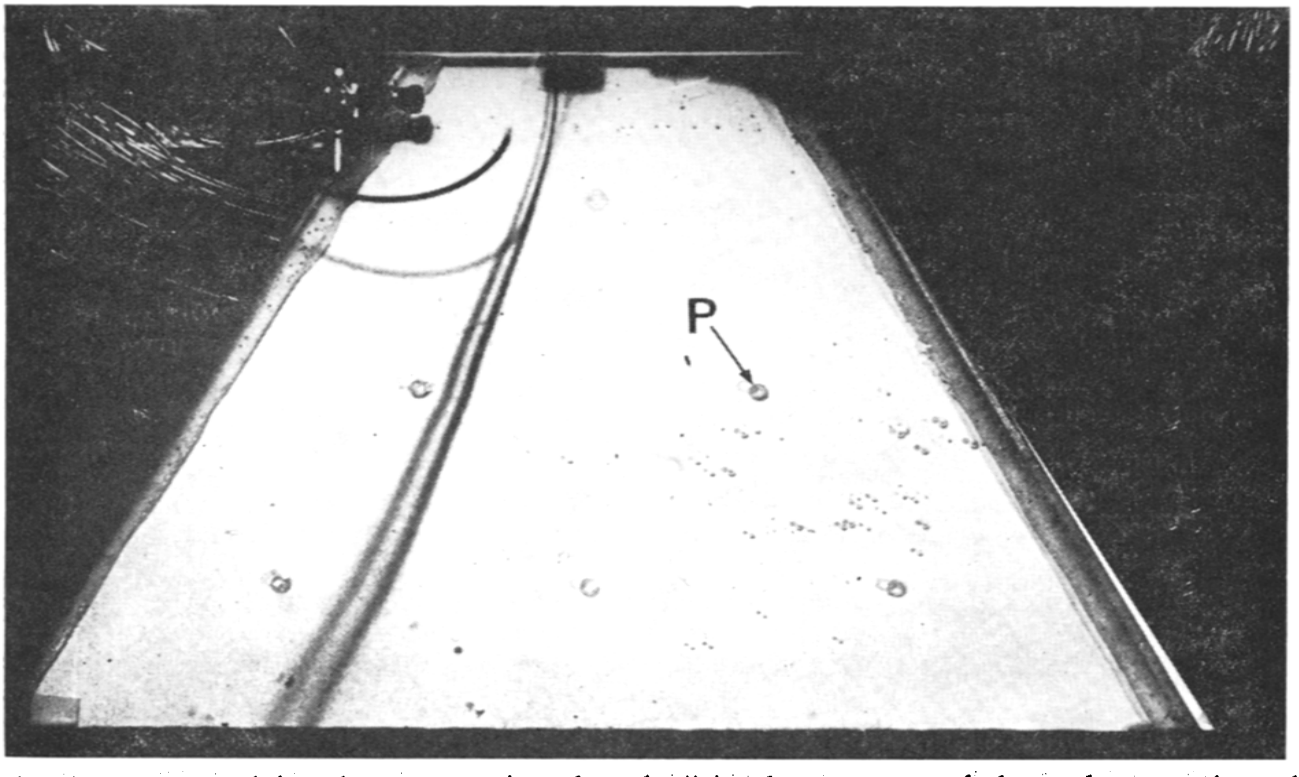


Fig. 2a. Separated fractions traversing the "fan" in the presence of thermal convection which causes the curvature of the streaks. P = lucite pellets safeguarding proper spacing between the walls of the "fan". The numbers at the bottom label the collector's exit tubes.

Fig. 2b. Inhibition of the thermal convection, shown in Fig. 2a, by immersion of the "fan" into a cooling bath (water temp. 16°). P = pellet (see Fig. 2a). The curvature of the streaks is slightly increased at the points of closest approach to a pellet. Left streak: Evans blue (faster component); right streak: Rose Bengal.

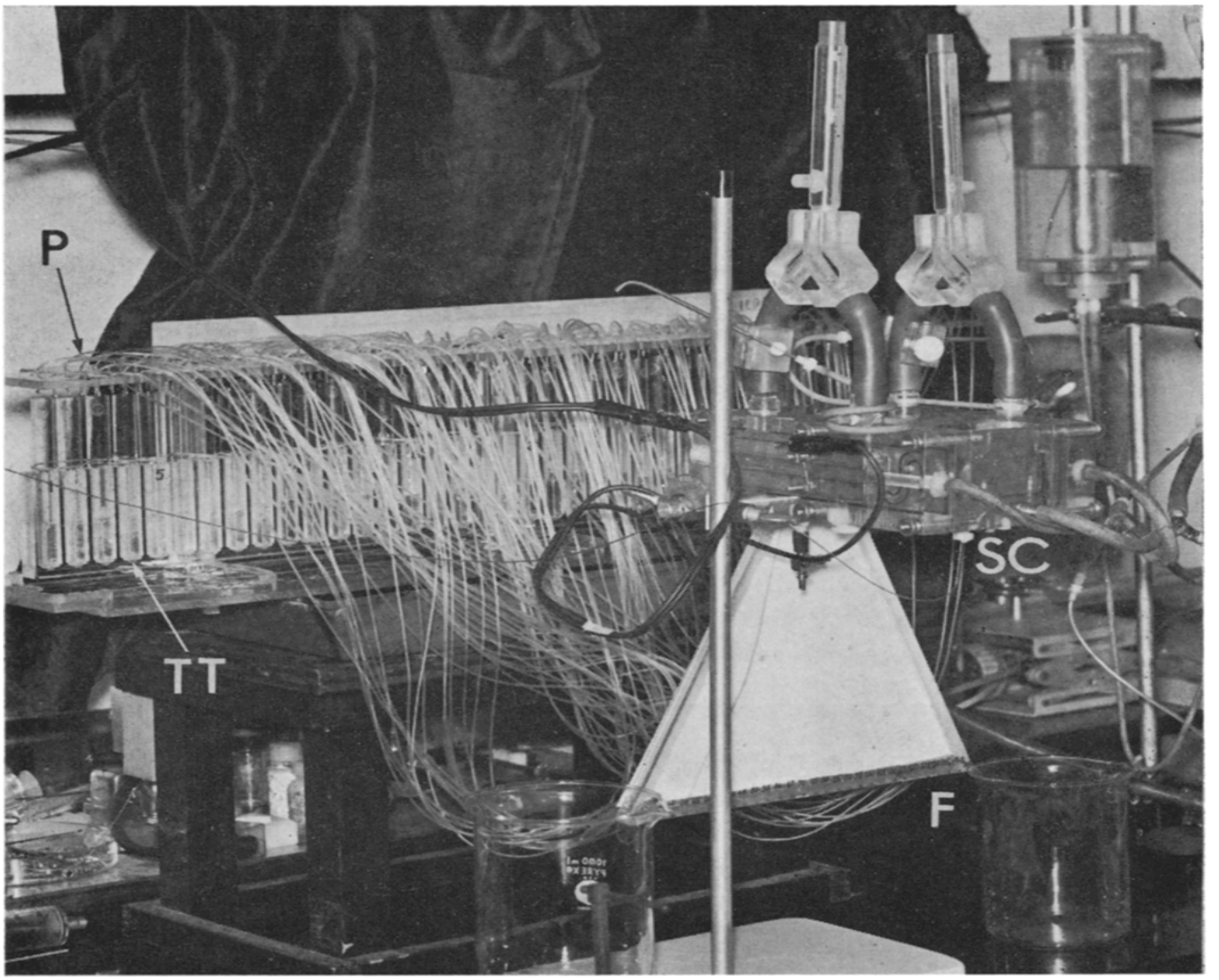


Fig. 3. View of a serpentine electrophoretic separator SC attached to the "fan" F. P = plate fixing the end points of plastic tubes collecting the outflow from the fan which is distributed among the test tubes TT. There are four such rows of tube endings. Only one row of test tubes is shown.

adjacent to No. 27 and No. 31). The total number of exit tubes is 143. They are linked by PVC tubing (approximately 1 mm I.D. and 2 mm O.D.) to 4 rows of holes in a plastic strip (P of Fig. 3) through which the tubes are passed so as to guide drops of issuing buffer into 4 rows of test tubes underneath. Only enough test tubes are used in practice to collect the desired components, the remaining buffer outflow is discarded. Only one row of test tubes is shown in Fig. 3.

Instead of inserting the exit tubes CT parallel to the flow into the terminal plate at Q_2 of Fig. 1c, they can be mounted at right angles to a wall of the "fan". This construction has been used in the device shown in Fig. 3. The tubes CT are mounted on the back side facing the test tube rack. This mode of construction is advantageous where the terminal interspace between the "fan" walls becomes too narrow as compared to the diameter of the tubes CT.

The general advantage of the device described above lies in the fact that one

can aim in practice at achieving only very narrow separation patterns by any type of method yielding continuous flow fractionation and spread this pattern rapidly by purely hydrodynamic means to dimensions permitting separate collection of the fractions.

I wish to acknowledge the technical assistance of Mr. PAUL COX in fabricating the "fan".

SUMMARY

By means of expansion of the width of the flow channel, it is possible to augment the resolving power of a collecting system in continuous flow fractionations. The device is applicable to any method of continuous flow fractionation based on injection of the mixture as a narrow streak. The operation of the method is demonstrated in the case of serpentine channel electrophoresis.

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