United States Patent

[11] 3,586,296

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[54] **APPARATUS FOR PRODUCING A FLOW OF LIQUID OF LINEARLY VARYING DENSITY**
7 Claims, 4 Drawing Figs.

[52] U.S. Cl. 259/66
[51] Int. Cl. B01f 7/16
[50] Field of Search 259/5, 6, 7, 8, 9, 10, 23, 24, 48, 60, 61, 64, 65, 66, 67, 68, 69, 70, 71, 95, 97

[56] **References Cited**
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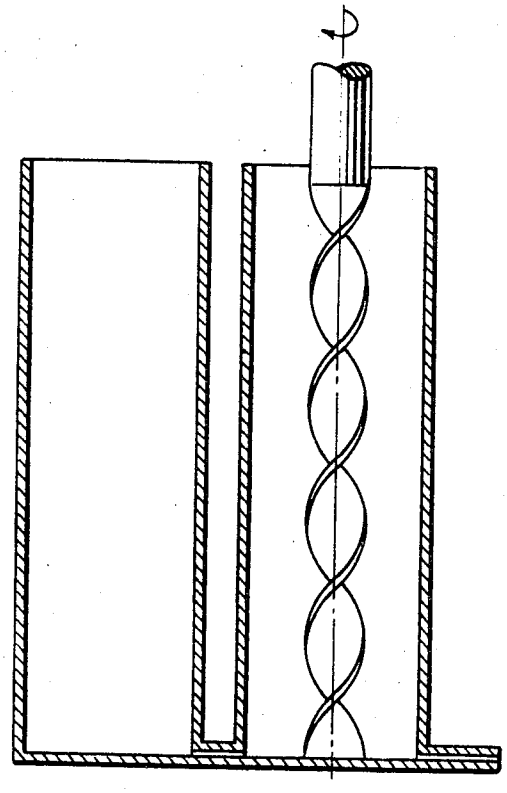
ABSTRACT: An improved constant density gradient mixer comprises two open vessels communicating at their bottoms, one of which vessels having a constant free cross-sectional area "A" and being equipped with stirring means and an exit tube. The other vessel has a variable free cross-sectional area $A/f(x)$ the function f varying with the height x essentially as by the equation

$$f(x) = \sqrt{1 - x/a}$$

where the distance a is defined by the equation

$$a = V_1 P_0 / 8A(\Delta P)_1,$$

in which V_1 is the total initial volume.
P_0 is the density in the vessel without stirrer, and
P_1 is the density in the vessel with stirrer.



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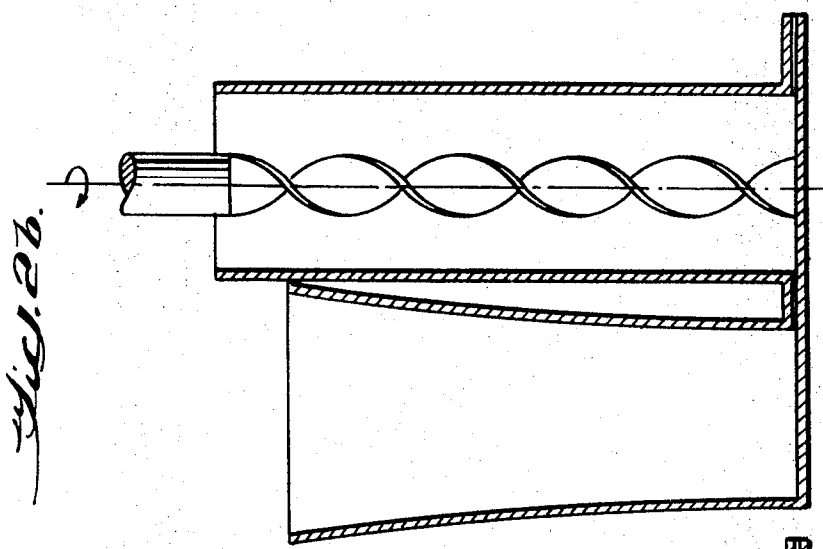

Fig. 2b.

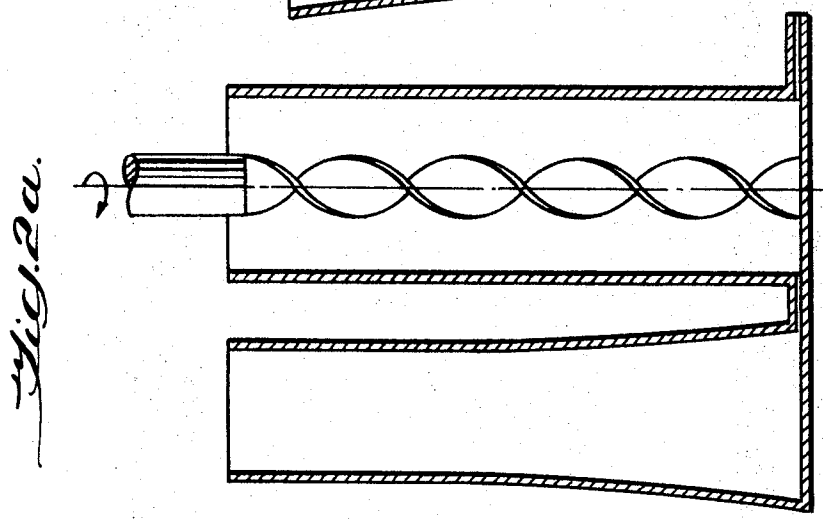

Fig. 2a.

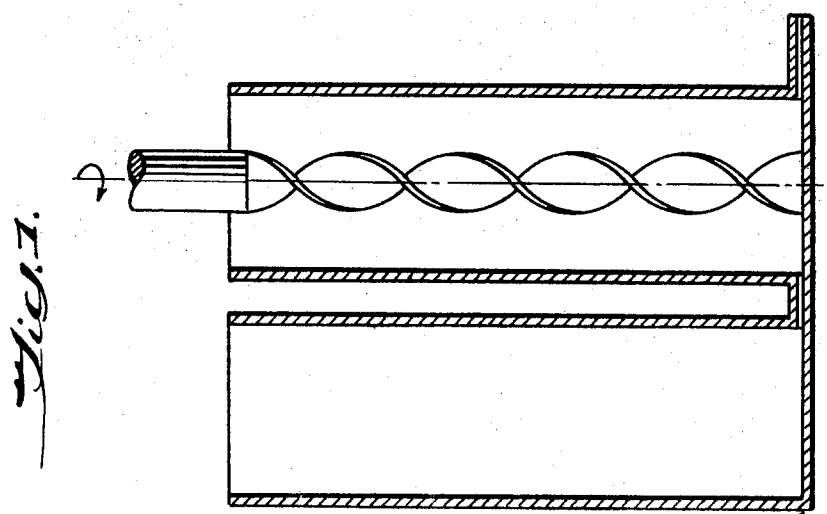

Fig. 1.

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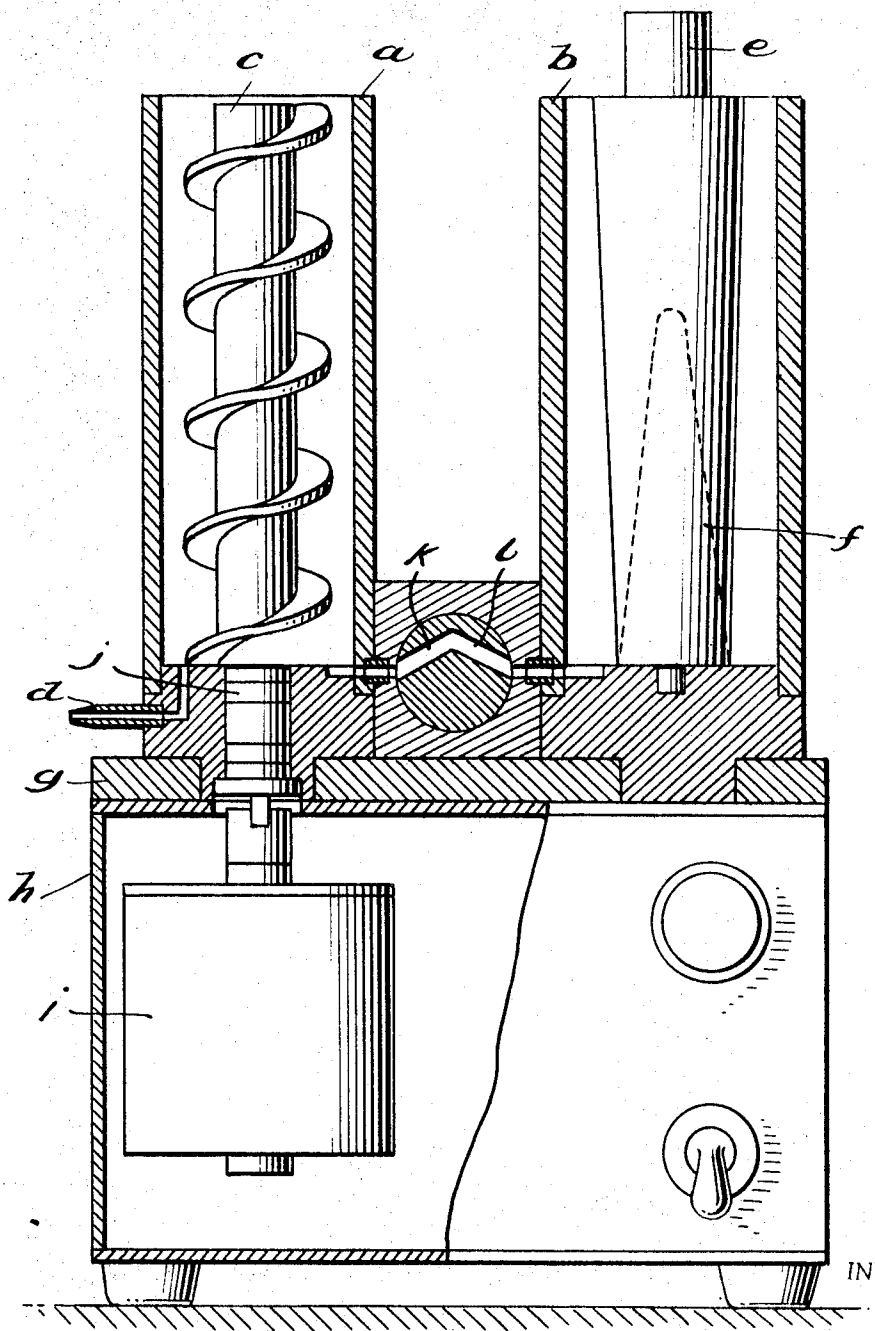
Fig. 3.



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APPARATUS FOR PRODUCING A FLOW OF LIQUID OF LINEARLY VARYING DENSITY

In chromatographic and electrophoretic work and at preparative centrifugation there is an ever increasing demand for simple means for production of liquid flows with continuously variable composition. Hitherto known arrangements, so-called gradient mixers, are often complicated and expensive types of apparatus, in certain cases of great flexibility with regard to the composition of the liquid flow.

Very simple gradient mixers are, however, also known. Most of them have a rather limited flexibility and must consequently be constructed separately for every specific demand concerning total volume and the way in which the composition of the liquid flow shall vary. One known and simple arrangement with unusually great flexibility consists of two open vessels communicating at the bottom, one of them being equipped with stirrer and exit tube (FIG. 1). When the vessels are filled with either of two different, mutually miscible liquids, the liquid flow leaving the system will get a continuously variable composition which can be calculated from the compositions of the two initial solutions and from the dimensions of the vessels. It is further known that the exit flow from the arrangement gets a linearly varying composition provided that (1) the two vessels (2) the same constant cross-sectional area; (2) the two liquids to be mixed have the same density; (3) the rate of flow is low enough to maintain hydrostatic equilibrium during operation.

The invention will be described hereinbelow with reference to the accompanying drawing, in which

FIG. 1 is a vertical sectional view of a simple gradient mixer of the prior art;

FIG. 2*a* is a vertical sectional view of a gradient mixer embodying one specific application of the principles of the present invention, according to which one of the vessels of the gradient mixer has an upwardly progressively diminishing cross section;

FIG. 2*b* is similar to FIG. 2*a* but illustrates the possibility of having the cross section of one of the vessels progressively increase from bottom to top; and

FIG. 3 is a vertical sectional view through a specific gradient mixer apparatus, illustrating a still further species of the invention.

Density gradients have gained an ever increasing use in centrifugation as well as in electrophoresis techniques. It is then in general desirable to have constant density gradients, that is, a linearly increasing or decreasing density in the liquid flow that is allowed to run into and fill the centrifuge tube or electrophoresis column. When using the simple device according to FIG. 1 for preparation of a density gradient, the demand for equal densities of the two liquids obviously cannot be satisfied. To secure hydrostatic equilibrium, the two vessels must be filled to unequal levels, in reciprocal proportion to the densities, and the density gradient then obtained from the arrangement will not be constant. The present invention is concerned with such modifications of the device in FIG. 1 that the exit flow gets a linearly varying density.

In a forcibly guided mixer one can obtain a linear density course by taking out a liquid flow from the exit tube twice as large as the flow between the chambers, which has recently been shown by Ayad, Bonsall, and Hunt in Science Tools 14 (1967), p.40. The two vessels then continuously contribute equal volumes to the exit flow, and, if they were initially filled with equal volumes of dense and less dense solutions, equal volumes will always be left in them. A flow with a linearly varying density obeys the equation:

$$\rho=\rho_o+(\rho_i\rho_o)\ V/R^1{}_i \qquad (1)$$

where ρ is the variable density of the exit flow, ρ_o the constant density in the vessel without stirrer, ρ_i the initial density in the compartment with stirrer, ${}_iV_i$ the initial total volume and V the variable remaining volume. The constant density gradient becomes:

$$d\rho/dV=(\Delta\rho)_i/V_i \qquad (2)$$

where $(\Delta\rho)_i$ is the initial density difference as defined by:

$$(\Delta\rho)_i=\rho_i\rho_o \qquad (2)$$

According to the present invention, the constant density gradient (2) is accomplished by such a modification of the device in FIG. 1 that the compartment without stirrer is given a variable free cross-sectional area A/f, where A is the constant free cross-sectional area in the vessel with stirrer and f is a dimensionless shape factor varying in a certain way with the height x over the bottom of the vessel: the same symbol denotes the variable liquid level in the container without stirrer. If the corresponding liquid level in the container with stirrer is called V, the hydrostatic law gives the equation:

$$\rho_o x=\rho y \qquad (4)$$

which on differentiation gives:

$$\rho_0 dx=\rho dy+y\,d\rho \qquad (5)$$

Further, on account of the above-mentioned volume balance between the containers, one has the equations:

$$Ay=V/2 \qquad (6)$$

$$2A\,dy=dV \qquad (7)$$

$$(2A/f)\,dx=dV \qquad (8)$$

Solving Eqn. (5) for $d\rho$ and insertion of dx and dy from (7) and (8) gives the following expression for the density gradient:

$$d\rho 1\ dV=f\rho_o-\rho 12Ay \qquad (9)$$

According to (6) however, the denominator is equal to V, and with the aid of (2) one obtains the equation:

$$f\rho_o-\rho 1V=(\Delta\rho)_i/V_i \qquad (10)$$

Insertion of ρ from Eqn. (1) now gives:

$$V_i\,\rho_o f\,df=4A\,(\Delta\rho)_i \qquad (11)$$

Differentiation and use of Eqn. (8) gives the differential equation:

$$V_i\rho\delta\,\gamma\delta=4\,A\,(\Delta\rho)_i\,dx \qquad (12)$$

which beside constants contains only the shape factor f and the height x. It is easily integrated to give:

$$V_i\,\rho_o\,(f^2-k)=8A(\Delta\rho)_i x \qquad (13)$$

where k is the integration constant. Its value must be 1 because the flow comes to an end for $x=0$, the density ρ_o then prevails in both containers, and their base areas consequently must be the same. Thus one puts $k=1$, and if for increased convenience the parameter:

$$a=\frac{V_i\rho_0}{8A(\Delta\rho)_i} \qquad (14)$$

is introduced, Eqn. (13) may be solved for f to give:

$$\sqrt{f=\ 1+x/a} \qquad (15)$$

This equation describes how the container without stirrer has to vary with height for the arrangement to give a constant density gradient according to Eqn. (2).

For ρ_i greater than ρ_o one has in the exit flow a falling density, $(\Delta\rho)_i$ and the distance a become positive, the parameter f becomes greater than 1, and the container without stirrer has to narrow off upwards. For ρ_i smaller than ρ_o one has a rising density, $(\Delta\rho)_i$ and the distance a become negative, the parameter f becomes smaller than 1, and the container without stirrer has to widen upwards.

Herewith the leading idea of the invention has been described and worked through mathematically, and Eqn. (15) in conjunction with (14) can be said to constitute the exact description of how the device in FIG. 1 has to be modified to give an accurately constant density gradient between the limiting densities ρ_i and ρ_o and within the total volume V_i. A container with a free cross-sectional area varying with height essentially, although not exactly, as demanded by Eqn. (15) gives of course an essentially constant density gradient. The present invention can thus be described as an arrangement with two containers, open at the top and communicating at the bottom, one with a constant free cross-sectional area A and equipped with stirrer and exit opening, the other characterized by a free cross-sectional area A/f that varies with the height x essentially as demanded by Eqn. (15) in conjunction with (14).

In a special embodiment of the invention the free cross section of the container without stirrer is circular. Then the radius r, varying with the height x must satisfy the equation:

$$\nu r = \bar{A}_i \rho \bar{f} \qquad (16)$$

Examples of such containers are shown in FIG 2, *a* and *b*, the former for a falling, the latter for a rising density in the exit flow

In another embodiment of the invention the two containers and the stirrer have constant cross-sectional area, whereas a plunger with a variable cross-sectional area P satisfying the equation:

$$P = C - A/f \qquad (17)$$

is used in the container without stirrer, C being the constant cross-sectional area of the container without stirrer.

In a special case of the design defined above both containers are cylindrical in shape and have the same cross-sectional area c, whereas the plunger is characterized by circular horizontal sections with a variable radius b satisfying the equation:

$$\pi b^2 = \frac{C(f-1)+S}{f} \qquad (18)$$

where S is the constant cross-sectional area of the stirrer.

Arrangements with plungers in uniformly thick containers have the advantage of great flexibility. In one and the same arrangement one can choose a plunger out of a set of plungers made for various, positive and negative, values of the distance a, Eqn. (14). In this way one has the possibility of producing constant density gradients within different total volumes, with rising and falling density, and with different values of the limiting densities.

Since $f=1$ for $x=0$, the two containers must obviously have equal free base areas. When using plungers in uniformly thick containers, this is achieved by giving all plungers the same base area, equal to the constant cross-sectional area of the stirrer. If this condition is satisfied, the stirrer need never be changed, one and the same stirrer being usable for all occurring density gradients. Such a stirrer has to be relatively thick since it has to be used also in conjunction with plungers which are thickest at the base and narrow-off upwards.

The theory of the invention presupposes hydrostatic equilibrium at every moment during operation. In order to avoid an excessively long time for preparing a density gradient, the duct between the containers, must consequently be thick enough for essentially instantaneous achievement of equilibrium, even in the case of a high viscosity, which sometimes prevails in one of the solutions. A thick duct between unequally dense solutions involves, however, new problems concerning hydrostatic stability since the two solutions must be prevented from intermixing within or through this duct. A thick horizontal duct thus cannot be used.

According to the present invention this problem is solved by making the duct in the form of a V or U for a rising density and in the form of an inverted V or U for a falling density in the exit flow. On charging the device for a rising density the denser solution is allowed to enter the downward bend of the duct. On charging it for a falling density, the less dense solution is allowed to enter the upward bend of the duct. In both cases hydrostatic stability prevails initially as well as during operation.

In a special embodiment the communicating duct is constructed as a stop-cock with a bent flow-through channel, the stopcock being changeable from an upward to a downward bend and vice versa by turning through 180°.

As an example, but not in any restricting sense, FIG. 3 shows a mixing arrangement according to the invention for a falling density. It comprises two equal cylinders (*a*,*b*) one with stirrer (*c*) and exit tube (*d*), the other with a plunger (*e*) calculated with the aid of Eqns. (14) and (15). That plunger (*f*) which has to be used instead for a rising density between the same limits and within the same volume is depicted beside. The cylinders are mounted on a baseplate (*g*) on top of a chassis (*h*) containing a synchronous motor (*i*) driving the stirrer (*c*) by way of an O-ring sealed slide bearing (*i*). The containers communicate through a stopcock (*k*) with a V-shaped flow-through channel (1).

For a falling density the mixing arrangement is used in the following way. The stopcock is first filled with the less dense liquid and is then closed by turning through 90°. Surplus of the less dense liquid is removed from both containers. A plunger widening upwards and calculated with an a value according to Eqn. (14) corresponding to the desired total volume V_t and limiting densities is placed on the bottom of the cylinder without stirrer, and the volume $V_t/2$ of the less dense liquid is introduced into this cylinder. The same volume of the denser liquid is introduced into the cylinder with stirrer after temporary closing of the exit tube. A capillary tube is used to connect this tube with the upper end of the column in which a density gradient is to be prepared. The capillary tube ends in contact with the internal wall of the column. In work under self-pressure, mixing device and column are placed at a suitable level difference, otherwise a liquid pump is mounted between them. The flow is initiated by starting the stirrer, opening the stopcock (*k*) between cylinders to an upward bend, and opening the exit tube to the column. The liquid with the falling density runs down the column along its internal wall, every new portion stratifying on top of the preceding one.

For a rising density the mixing device is used in the following way. The stopcock is first filled with the denser liquid and is then closed by turning through 90°. Surplus of the denser liquid is removed from both cylinders. A plunger narrowing off upwards and calculated with an a value according to Eqn. (14) corresponding to the desired total volume and limiting densities is placed on the bottom of the cylinder without stirrer, and the volume $V_t/2$ of the denser liquid is introduced into this cylinder. With the exit tube (*d*) closed, the same volume of the less dense liquid is poured into the cylinder with stirrer. The exit tube is connected to the bottom stopcock of the column in which the density gradient is to be prepared by way of a capillary tube. In work under self-pressure, mixing device and column are placed at a suitable level difference, otherwise a liquid pump is mounted between them. The flow is initiated by starting the stirrer, opening the stopcock between cylinders to a downward bend, and opening the exit duct to the column. The liquid with a rising density rises through the bottom stopcock, every new portion stratifying beneath the preceding one.

In a test run with this mixing device in order to ascertain the degree of linearity of the density of the liquid flow, a constant rate liquid pump and a recording light absorption meter were coupled in series between mixer and column. A solution containing 500 g. of pure sucrose, 0.86 millimol of potassium chromate, and 0.5 millimol of potassium hydroxide per liter was used as the denser liquid, whereas the less dense liquid consisted of pure water. Since the sugar concentration in this way always becomes proportional to the chromate concentration, and because the sugar concentration almost exclusively determines the density of the solution, the density course can be followed by measuring the light absorption of the chromate. The experiment gave data given in TAble 1. Its first column gives the delivered volume, its second column the corresponding chromate concentration. An exactly linear concentration course satisfying the equation:

$$c = -0.00373 + 0.008226\,V \qquad (19)$$

is given in the third column, and the 1000 times enlarged deviations from linearity are found in the fourth column. The relative deviations expressed in 10^{13} of the total concentration, 0.86 millimol/liter, have been introduced into the fifth column. As can be seen, the maximum deviation from the linearity is 0.55 percent The mean deviation is found to be 0.27 percent, which must be regarded as very satisfactory.

TABLE 1

V	c	$c_{lin.}$	1,000 Δc	1,000 $(\Delta c)_{rel.}$
2.64	0.0189	0.0180	+0.9	+1.0
10.90	0.0865	0.0859	+0.6	+0.7
19.10	0.1550	0.1534	+1.6	+1.9
27.50	0.2219	0.2225	−0.6	−0.7
35.80	0.2898	0.2908	−1.0	−1.2
44.00	0.3535	0.3582	−4.7	−5.5

TABLE 1-Continued

V	c	$c_{lin.}$	1,000 Δc	1,000 $(\Delta c)_{rel.}$
52.30	0.4251	0.4265	−1.4	−1.6
60.60	0.4957	0.4948	+0.9	+1.0
68.80	0.5656	0.5622	+3.4	+4.0
77.10	0.6293	0.6305	−1.2	−1.4
85.40	0.7004	0.6988	+1.6	+1.9
93.60	0.7691	0.7662	+2.9	+3.4
102.00	0.8322	0.8353	−3.1	−3.6

We claim:

1. A device for producing a flow of liquid of continuously and linearly varying density with the aid of two unequally dense, mutually miscible liquids, comprising two vessels for each one of the two liquids, open at the top and communicating at the bottom, one vessel being equipped with a stirrer and, at the bottom, with an exit tube and having a constant free cross-sectional area A, the other vessel being characterized by a variable free cross-sectional area $A/f(x)$, the function f of the height x above the bottom essentially satisfying the equation:

$$f(x) \sqrt{1+x/a}$$

with the distance a defined as:

$$a=\frac{V_i\rho_0}{8A(\rho_i-\rho_0)}$$

where V_i is the total initial volume, ρ_0 the density in the vessel without stirrer and ρ_i the initial density in the vessel with stirrer.

2. A device according to claim 1 characterized in that the variable free cross section is circular with the radius $r=\sqrt{A/\pi f}$

3. A device according to claim 1 in which the two vessels and the stirrer have constant cross-sectional areas, characterized by a plunger submerged into the vessel without stirrer, its variable cross-sectional area P essentially satisfying the equation:

$$P=C-A/f$$

where C is the cross-sectional area without stirrer.

4. A device according to claim 3 comprising two vessels of the same cross-sectional area C, in one vessel a stirrer of a constant cross-sectional area S, in the other a plunger characterized by circular horizontal sections with a radius b satisfying the equation:

$$\pi_b^2=\frac{C(f-1)+S}{f}$$

5. A device according to claim 3 comprising alternate plungers for different positive as well as negative, values of the distance a, characterized by a constant base area identical with the constant cross-sectional area of the stirrer.

6. A device according to claim 1 characterized by the communicating duct between the vessels having an upward bend in the case of a falling and a downward bend in the case of a rising density in the outflowing liquid.

7. A device according to claim 6 characterized by a communicating duct between the vessels comprising a stopcock with a bent flow-through channel, this stopcock being turnable between an upward and a downward bend.

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(5/65)

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

Patent No. 3,586,296 Dated June 22, 1971

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It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Claim 5, line 1, - the word "alternate" should be

-- alternative --

Signed and sealed this 26th day of October 1971.

(SEAL)
Attest:

EDWARD M.FLETCHER,JR.
Attesting Officer

ROBERT GOTTSCHALK
Acting Commissioner of Patents