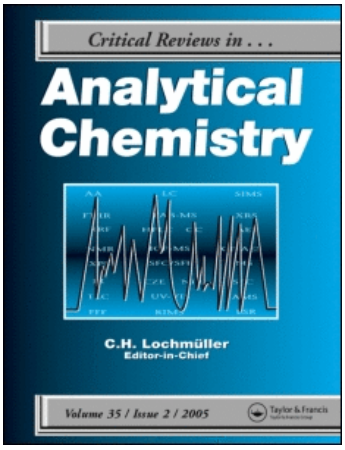

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Phase Segmentation in Liquid-Liquid Extraction Continuous Flow Analysis

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KEY WORDS: liquid-liquid extraction, flow injection analysis, continuous flow analysis, theory of phase segmentation, phase segmentors.

I. INTRODUCTION

Several research groups have been actively designing flow systems for automatic or semiautomatic liquid-liquid extraction, most of them using air-segmented flow systems. The first application of liquid-liquid extraction in flow injection analysis (FIA) was carried out simultaneously by Karlberg et al.[1] and Bergamin et al.[2] in 1978. Since then, a large number of analytical procedures have been devised in combination with all common detection systems.[3-6]

Nowadays liquid-liquid extraction is the most frequent used separation/preconcentration method in FIA.[3,4] Approximately 50% of all preconcentration methods in FIA involve liquid-liquid extraction. The advantages and difficulties of this very useful method, together with some analytical applications, have been discussed in several comprehensive reviews and monographs[3-6] and the feasibility of the method has been repeatedly demonstrated.

Regardless of the way of performing liquid-liquid extraction, by a manual batch procedure or by use of some kind of mechanized or automated system, defined volumes of the immiscible organic and aqueous phases must first be brought together. The phases must then be brought into intensive contact with each other to facilitate the extraction to take place, and finally physically separated from each other after the extraction in order to make the chemical separation meaningful.

In conventional FIA extraction (see manifold flow chart in Figure 1) aqueous sample solutions are, in the simplest version, usually introduced continuously or in definite volumes (100 to 200 μl) into a continuous aqueous carrier stream, serving both as reagent and carrier stream. The aqueous sample solution can also be merged and mixed with another aqueous stream containing an analytical reagent. Appropriate chemical reactions as well as solution homogenization take place in a reaction and mixing coil before entering the phase segmentor.

The continuous (ideally pulseless) streams of the two immiscible solvents meet at the segmentor. A more or less regular segmented flow results, containing independent segments of the two phases. The extraction takes place mainly in the extraction coil but to some extent also in the segmentor and the phase separator. The segments of aqueous and organic phase are separated in a

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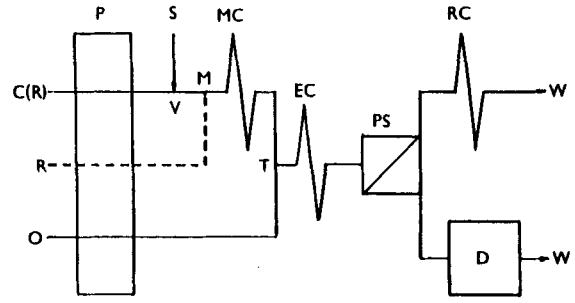


FIGURE 1. Liquid-liquid extraction FIA manifold. C — carrier stream, R — reagent stream, O - organic phase stream, P - peristaltic pump, S - sample solution, V - injection valve, M - mixing point, MC - reaction and mixing coil, T - phase segmentor, EC - extraction coil, PS - phase separator, RC - restriction coil, D - flow through detector, W - waste.

phase separator into two or more streams, at least one of them containing only one solvent. The extractable component in at least one stream is sensed by a flow through detection system. The analytical signal is treated in a customary manner, the concentration of analyte being calculated from the peak height, the peak width or the peak area. The other stream goes to waste through a restrictor which also controls the separation efficiency of the phase separator.

These three principal operations of liquid-liquid extraction also characterize the use of three basic liquid-liquid extraction FIA units —(1) a segmentor or confluence point for the organic and aqueous phase streams providing alternate and regular segments of the two immiscible solvents into one uniform segmented flow in a single channel, (2) an extraction coil, in which the solute is transferred from one phase to the other, and (3) a phase separator, with the purpose of continuously and quantitatively separating the segmented stream into two or more parts, at least one of them consisting of only one phase.

Each particular operation of liquid-liquid extraction FIA is important for obtaining high sensitivity of determination with the lowest possible sample zone broadening. Each step has been studied theoretically, including the segmentation,[7] separation of the phases by a phase separator,[8,9] peak height and peak area depression,[10,11] sample dispersion and peak broadening,[12-14] and the mechanism of the extraction process.[15-18]

Segmentation and separation of the two immiscible phases are of crucial importance for the results obtained by liquid-liquid extraction of FIA. The whole segmentation process can negatively influence sample dispersion, extraction rate, and phase separation.[15,18] The possibilities to vary the segment length are limited with the available segmentors; the repeatability of segmentation is often not satisfactory and the applicability of the segmentors at the high flow rates and phase flow rate ratios often sought in connection with sample workup is limited.

Several segmentor types of varying efficiency have been described in the literature. The most common segmentor types are T-piece segmentors made of glass,[19,20] fluoroplastics,[8,21] stainless steel,[22] or glass lined T-pieces of stainless steel and combinations of hydrophobic and hydrophilic materials.[23,24] Also, improved glass A8-T and A10-T fittings,[1,25,26] and T-pieces made of fluoroplastics with inserts of fluoropolymer tubing,[7,11,21] or with an enlarged inner diameter of the outflow channel[15,26] have found wide use. Different configurations of Y-[27,30] or W-pieces[31-34] made of glass or fluoroplastics, and four-way fittings[35,36] have also been recommended. Lately, a coaxial (falling drop) segmentor has been introduced[37,38] in order to overcome some disadvantages of the other types.

The geometry of the inner capillary system of the T-shape segmentor[19,39,40] and the geometry of the confluence chamber of the coaxial segmentors have also been carefully investigated.[38] Flow systems without phase segmentation have been used.[35,36,41-46] Sample introduction downstream from the segmentor has been suggested,[47] because extraction efficiency, extraction rate, and sample dispersion (peak broadening) are all influenced by the segmentation pattern. A multichannel dropping segmentor has also been used for homogenization and instantaneous introduction of sample and reagent aqueous solutions into the continuous flow of the other immiscible solvent[48] directly inside the segmentor. Conventional loop injectors operated by a cycling motor with adjustable filling/draining times and a brief intermittent period can be used at low flow rates or for introducing very long segments[49] of an immiscible solvent into the continuous flow of the other phase.

There is an increasing need to classify the existing segmentors and to compare their characteristics. In the end there is also an increasing need for detailed fundamental studies of the segmentation process with the aim to develop more efficient phase segmentors and to explore the possibilities offered by improvements in segmentation repeatability. The accurate performance of the segmentor is a key to successful development of continuous liquid-liquid extraction. Reproducible segmentation can improve the precision of signal measurements and may frequently simplify signal evolution. Finally, it may even be possible to eliminate phase separation altogether if the segmentation repeatability is good enough to allow precise timing of the measurement intervals.

II. PHASE SEGMENTATION IN LIQUID-LIQUID EXTRACTION

A. Principles of Segmentation

Segmentation may result from two different mechanisms. First, droplets or plugs of one phase may be formed in a continuous flow of the other solvent, immiscible with the first. This takes place in a small mixing chamber in the segmentor, at the end of a single inlet tubing capillary or at the junction of a multichannel inlet capillary system. The second principle leading to segment formation is the "ripple" process, resulting from destruction of the thick layer of one of the solvents, which is formed at high pumping rates on the wall of the outlet tubing or on the walls of the mixing chamber in the segmentor.

The first mechanism controls the segmentation in a mixing compartment made of hydrophilic material (a glass T-piece or an A8-T fitting) at moderate flow rates, the second dominates at very high flow rates of both phases (Q_a and Q_o) or at very high flow rate ratios, or when the walls of the mixing compartment are constructed from lipophilic material (fluoroplastics). Also, the walls of the mixing compartment made of hydrophilic material may be covered by a layer of lipophilic impurities, thus changing their hydrophilic character.

Although segmentation is very important for every individual step of liquid-liquid extraction FIA, no detailed study of the factors controlling segment size and reproducibility of the segmentation has been done so far. From experience, some conclusions can be drawn about segmentors of the gravity/density and hydrodynamic types.[7,38,40,50]

There are two major variables to examine in connection with segmentation: segmentation reproducibility and segment size. While segment size may not affect the extraction efficiency of a fast extraction process or when large sample volumes are introduced into the system, it could theoretically affect the efficiency of slower extraction systems.[15,18] The maximum segment size is determined by the interfacial tension of the organic and aqueous phase, both between each other and between a phase and the tubing material, such that the segment size decreases with decreasing value of $\gamma_{o/a}$. The choice of tubing material, tubing dimensions and geometry of the mixing chamber critically influence the final results.

The segment length in the extraction coil is the principal parameter controlling the size of the contact area between the phases. An irregular segmentation pattern results in losses of the solvent by the wetting process because of a varying film thickness. Serious coalescence of segments of different size is due to differences in the linear velocity of the segment, resulting in different geometries (due to differences in viscous drag of the wetting phase). This means that the segmentation pattern has to be under control and constant during the entire analytical procedure. Thus, during manifold optimization it is necessary to consider kinetic efficiency, total extraction yield and peak (sample zone) broadening.

The enrichment factor, which equals the ratio between the flow of the aqueous phase containing the sample and that of the organic phase, is influenced by the segmentation process. The enrichment factor rarely exceeds 20 in commonly used manifolds. The length of the organic and the aqueous segments cannot be reduced indefinitely; the lower limit of organic phase segments has experimentally been estimated to be 1.5 times the internal diameter of the extraction coil tube.[13] The extraction efficiency decreases drastically if the droplets of organic phase become too small to form a continuous film on the tubing wall. Also, the segmentation may be unstable due to

coalescence of very small segments during transport through the extraction coil.

B. Continuous "Droplet-Form" Segmentation

Several assumptions are necessary before a quantitative description of segmentation in a gravity/density segmentor becomes possible: (1) four forces direct the droplet formation — gravity, density (uplift), interfacial, and hydrodynamic forces. The orientation of the forces will determine the value of the resulting force vector and the equivalence of the forces. (2) Perpendicular and vertical/horizontal orientation of inlet/outlet flows is assumed for simplicity. (3) The droplet volume grows linearly with time and droplet forming phase flow rate ($V = Q_d t$). (4) The skewing process is fast enough to allow one to neglect meantime processes. (5) It is assumed that no contact exists between the wall surface of the outflow capillary and the confluence chamber wall (this is questionable in the case of a narrow inner diameter chamber made of fluoroplastics). (6) The droplet and the profile of the confluence chamber are spherical and circular, respectively. (7) No phase losses due to film formation are observed. (8) No jet effect occurs at the droplet forming phase flow rate used.

The droplet-forming solvent flows into the stream of the other immiscible phase in the form of droplets or small plugs. Their sides are in contact with the side walls of the inlet tubing at the junction of the mixing chamber of the segmentor (see Figure 2). The gravity and density forces are oriented vertically and the resulting gravity/density force F_{GD} is vertical and parallel to F_G and F_D, its value equaling[51]

$$F_{GD} = F_G + F_D = V \cdot \Delta\rho \cdot g = (4/3) \cdot \pi \cdot r_d^3 \cdot \Delta\rho \cdot g \quad (1)$$

where V is the droplet volume, $\Delta\rho$ is the density difference between the two solvents, r_d is the droplet radius, and g is the gravitational constant.

The interfacial force F_I can be expressed by the following form of the Tate[51] equation

$$F_I = 2 \cdot \pi \cdot r_i \cdot \sigma = 2 \cdot \pi \cdot (d_i/2) \cdot \gamma_{o/a} \cdot (1 - \cos\Theta) \quad (2)$$

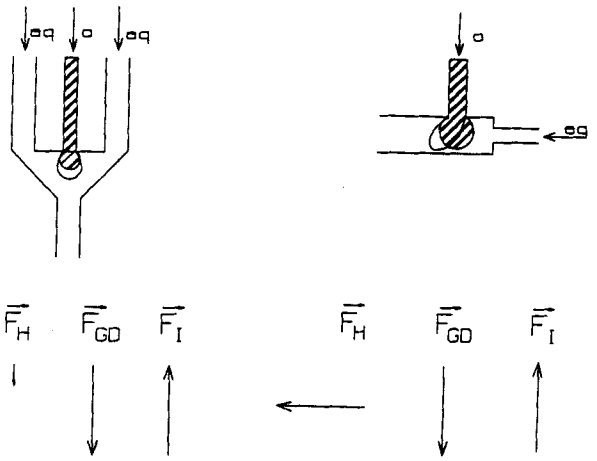


FIGURE 2. Schematic diagram of segmentation in a coaxial (left) and noncoaxial (right) segmentor with hydrodynamic (F_H), gravity/density (F_{GD}), and interfacial (F_I) force orientation (below).

where σ is the interfacial tension, $\gamma_{o/a}$ is the interfacial tension between organic and aqueous phase and Θ is the solid-liquid-liquid contact angle ($\cos\Theta = \Delta\rho \cdot h \cdot g \cdot (d/2)/2 \cdot \gamma_{o/a}$) related to the height of the capillary rise h or to the droplet shape on the surface of the appropriate solid material, and d_i and r_i denote the inner diameter and radius of the inlet capillary tubing.

The spherical shape of the droplet is deformed to the resulting distorted spherical or elliptical form by a hydrodynamic force F_H active on the drop area facing the other immiscible phase flow, as a result of the continuous flow Q_n of the phase that does not form droplets (see Figure 2). The hydrodynamic force F_H may be written as $F_H = \Delta P \cdot A_f$, where A_f is the front area of the droplet facing the flow of the other phase, and $\Delta P = P_1 - P_2$ is the pressure difference across the profile of the confluence chamber, which is due to the continuous phase flow.[51]

The pressure difference ΔP consists of two terms arising from viscous drag on the flow of the phase that does not form droplets (the "Poiseuille" term P_P), and from changes of kinetic energy of the same phase as it flows through the narrowing profile of the tubing beneath the drop of the droplet forming phase (the "Bernoulli" term P_B). Both terms are linear and quadratic functions of the total flow rate Q_n of the phase not forming droplets. The value of ΔP (assuming a circular chamber cross section and a spherical droplet profile) is given by the expression

$$F_H = \Delta P \cdot A_f = (P_P + P_B) \cdot A_f = k_p \cdot Q_n + k_B \cdot Q_n^2 \quad (3)$$

where k_P and k_B are the constant "Poiseuille" and "Bernoulli" terms consisting of the viscosity and density of the phase not forming the droplet and the geometry factors of the confluence chamber and droplet.

This force tends to dislodge (skew off) the droplet from the end of the inlet tubing at the moment when the sum (F_R) of the forces pushing it (F_H, F_G, and F_D) is equal to the interfacial force F_I holding the droplet onto the end of the segmentor inlet capillary surface. The resulting force vector has to equal zero at the moment when the droplet is cut off. This equilibrium of forces can be expressed by the following equation:

$$F_G(\alpha_s) + F_D(\alpha_s) + F_I(\alpha_s,\alpha_i) + F_H(\alpha_s,\alpha_i) = 0 \quad (4)$$

where symbols α_s and α_i express the influence of the force orientation in space or among themselves.

One generalized model for the segmentation process can be used to describe all different kinds of continuous segmentors. The four differently oriented forces (with respect to the vertical axis and to the geometry of the inlet/outlet tubing system) affect the skewing process. The forces can be oriented axially (coaxial, falling drop, gravity/density segmentors), perpendicularly (T-type segmentors), and/or at different angles among the inlet and outlet tubes (30, 45, 60, 120, 150°, etc., for W or Y type segmentors). Several extreme cases can be solved where the gravity/-density F_{GD} and hydrodynamic forces F_H are oriented axially or perpendicularly to each other.

1. Single Channel Coaxial Segmentors

Singe channel coaxial segmentors consist of two basic parts: a screw with an inlet capillary channel for introduction of the droplet forming phase, organic or aqueous; and a segmentor body with inlet and outlet capillary channels for delivery of the other immiscible phase and drainage of the segmented flow stream. When assembled, the screw inlet capillary ends in the conical chamber of the segmentor body. All resulting phase streams are oriented axially among themselves, and the segmentor main axis is vertical.

At low flow rates and a low flow rate ratio (Q_a and Q_a/Q_o or Q_o and $Q_o/Q_a \rightarrow 0$, respectively), the gravity and density forces predominate in gravity/density coaxial segmentors. The droplets are spherical. Their size depends primarily on the inner diameter of the inlet capillary d_i, the interfacial tension σ, the density difference between the two liquids $\Delta\rho$, and the value of the gravitational acceleration g. The total droplet volume V grows linearly with the droplet forming phase flow rate Q_d (organic or aqueous) and time t as the droplet is formed. The resulting total droplet volume V can, under ideal conditions (neglecting the influence of hydrodynamic forces and assuming the equivalence of gravity/density and interfacial forces $F_{GD} = F_I$), be expressed by the following equation, valid at the moment when the droplet is cut off, t_{max}

$$V = Q_d \cdot t_{max} = \pi \cdot d_i \cdot \sigma/g \cdot \Delta\rho \quad (5)$$

The total droplet volume V and also the total segment length L_s (neglecting the film formation process or assuming a stable film thickness) are independent of the flow rate Q_n of the phase that does not form droplets. They are influenced only by the $(1 - \cos \Theta)$ term when the values of the other parameters are kept constant.

$$V = V_{max} \cdot (1 - \cos \Theta) \quad (6)$$

The maximum droplet volume V_{max} (at equal interfacial and gravity forces) and the corresponding segment length L_{max} are given by the following expressions.

$$V_{max} = (4/3) \cdot \pi \cdot r_d^3 = \pi \cdot d_i \cdot \sigma/g \cdot \Delta\rho = \pi \cdot d_i \cdot \gamma_{o/a}/g \cdot \Delta\rho \quad (7)$$

$$L_{max} = V_{max}/\pi \cdot (d_e/2)^2 \quad (8)$$

Here, r_d is the droplet radius and d_e is the inner diameter of the outflow measuring or ex-

traction capillary. The actual droplet volume and segment length vary from V_{max} or L_{max} to $V_{min} = L_{min} = 0$ for $\cos \Theta = 0$ or 1, respectively.

The normalized droplet volume $V_n = (V_{max} - V)/(V_{max} - V_{min})$ and the normalized segment length $L_n = (L_{max} - L)/(L_{max} - L_{min})$ vary from 0 to 1 independently of the other solvent properties, as can be shown from the dependence of V_n or L_n against the (s)-(1)-(1) interface contact angle Θ at the tubing end (see curve 4 in Figure 3 for different Θ values). Both values are completely independent of the flow rate Q_n of the phase not forming the droplet, and consequently the dependence of V_n or L_n on Q_a, as an example are depicted as straight lines (see curve 1 in Figure 3) for $V_n = L_n = 1$.

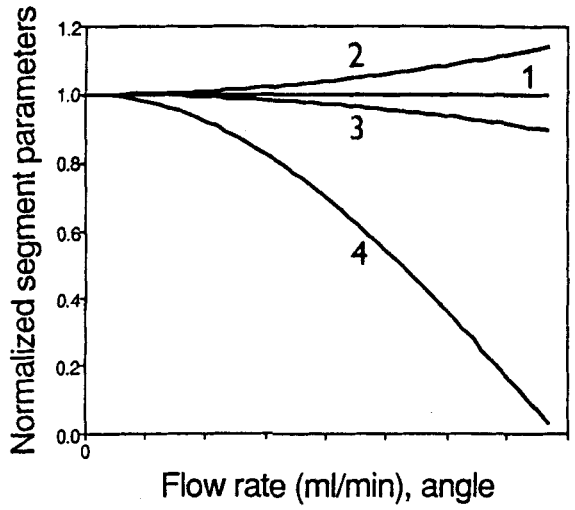


FIGURE 3. Normalized segment volume (V_n) and segment length (L_n) as functions of solid/liquid/liquid contact angle Θ (curve 4), and of the flow rate for $F_H = 0$ (curve 1) or assuming negligible influence of the hydrodynamic force in downstream or upstream orientation (curves 3 and 2).

The actual droplet volume of any defined two-phase, liquid-liquid extraction system can thus be easily varied by careful choice of material for the inlet tubing, as it changes the solid-liquid-liquid angle Θ, by choice of inner diameter for the inlet tubing, and, to a small extent, by adjusting the flow rates of the two solvents. The droplet volume is also affected by the change in surface tension and by the change in density difference between the two solvents. This can be demonstrated by the changes in segment length resulting from the presence of inorganic salts, surfactants, or methanol. The resultant force vector and the segment volume are affected by the spatial orientation of the end surface of the tubing.[38]

The influence of the hydrodynamic force cannot be neglected at high flow rate of the phase that does not form droplets (Q_a or Q_o) and/or at a high flow rate ratio (Q_a/Q_o or Q_o/Q_a). The reason for this is changes in the confluence chamber cross-section profile at the end of the inner inlet capillary (see Figure 2). The "Bernoulli" term of the continuously flowing phase stream is more important than the "Poiseuille" term, and the volume of the droplet is influenced mainly by the gravity/density force. To a smaller extent, pressure differences across this profile will also have an influence. The frontal and total area of the droplet-facing flow of the phase not forming droplets is very small, and consequently, the viscosity ("Poiseuille") term is less important or even negligible.

The volume of the resulting droplet is influenced by the flow rate Q_n of the phase that does not form droplets, the flow rate ratio between the phases, and by the construction of the inner capillary system. The force vector F_R is equal to $F_R = F_{GD} \pm F_H$ and is vertically oriented. As a result, a graph depicting the dependence of V_n and L_n on Q_n (see Figure 3, curves 2 and 3) exhibits a small curvature, depending on the orientation of the hydrodynamic force with respect to the axis of the flow of the organic phase forming droplets (counter-current, upstream), because of the quadratic term $k_B \cdot Q_n^2$ in Equations 3 and 4. The same conclusions are also valid for a segmentor with the flow rates oriented in opposite directions, with the aqueous phase forming the droplets. The geometry of the inner capillary system will thus also affect the influence of the flow rate in both cases.

The segment length of the wetting phase not forming droplets is regulated by its total flow rate and the droplet time t_{max} ($V_s = Q_n \cdot t_{max}$) or dropping frequency $f_d = 1/t_{max} = Q_d/V_s$. It grows linearly with the flow rate because the influence of the hydrodynamic forces can be omitted. A small curvature of the dependence $V_s = f(Q_n)$ appears, and a power relationship better fits for higher flow rates and flow rate ratios.

2. Multichannel Dropping Segmentor/ Injector[48]

A multichannel dropping segmentor consists of two basic parts, a fluoropolymer screw with a multiple capillary inlet channel and a segmentor body with a conical confluence chamber containing inlet and outlet capillary channels for delivery of organic phase and drainage of the segmented flow stream, respectively.

The multichannel dropping segmentor can be used for simultaneous introduction of aqueous solutions of a sample and reagent(s), in the form of droplets or small plugs, directly into a continuous flow of an organic solvent, immiscible with the aqueous phase. Each aqueous phase stream containing sample, reagent(s), and other components of the reaction mixture flows through a separate capillary channel. Droplets of the reaction mixture of precisely defined volume are formed at the point where the inlet capillaries are joined together. The droplet sides are in contact with the side walls of the inlet capillary tubing system at the junction of the mixing chamber of the multichannel dropping segmentor (see Figure 4).

Several additional assumptions are needed in order to make possible a quantitative description of the multichannel dropping segmentor: (1) the droplet volume is assumed to grow linearly with time and total aqueous phase flow rate ($V = t \cdot \Sigma(Q_a)_i = t \cdot Q_a$). (2) The concentration of each individual component of the reaction mixture is assumed to depend on the flow rate ratio between the stream of aqueous phase in question and the total flow rate of the aqueous phase $c_i \cdot (Q_a)_i/Q_a$, or on the dosing time at a constant flow rate ratio. (3) For simplicity, the inlet and outlet flows are assumed to be vertical and horizontal, respectively.

At low flow rates of the organic phase (as preferred in this segmentor type) the gravity and density forces predominate and the influence of the hydrodynamic force can be neglected. The droplets are spherical. Their size depends primarily on the inner diameter of the junction of the inlet capillary system d_i, the interfacial tension σ, the difference in density between the two liquids $\Delta\rho$, and the value of the gravity acceleration g. During droplet formation, there is a linear relationship between the total droplet volume V and the total aqueous phase flow rate $Q_a = \Sigma(Q_a)_i$ and time t. The resulting total droplet volume V can, under ideal conditions (neglecting the influence of hydrodynamic force, and assuming equivalence between the gravity/density and interfacial forces $F_{GD} = F_I$), be expressed by an equation similar to Equation 5, valid at the moment when the droplet is cut off, t_{max}:

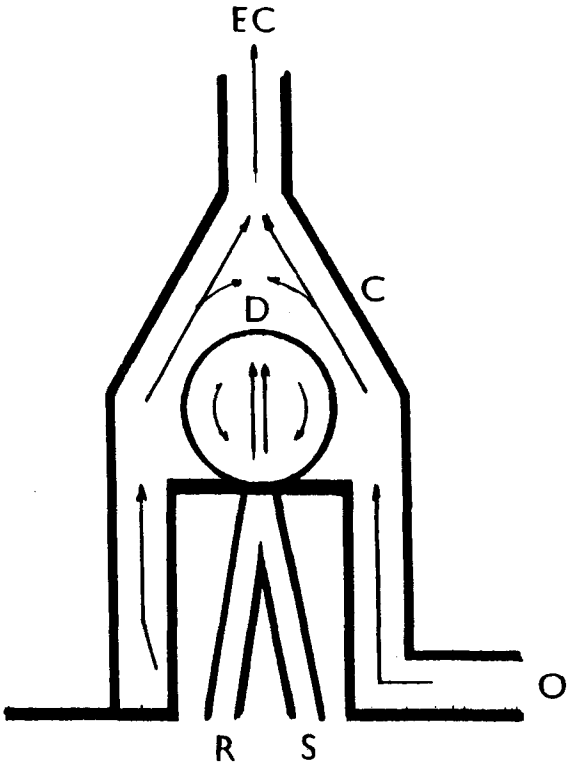


FIGURE 4. Schematic diagram of the segmentation process in multichannel coaxial segmentor. R — reagent stream, S — sample stream, O — organic phase, D — droplet of the reaction mixture, C — conical compartment of the segmentor, EC — outflow channel to an extraction coil.

$$V = \Sigma(Q_a)_i \cdot t_{max} = \pi \cdot d_i \cdot \sigma/g \cdot \Delta\rho = \pi \cdot d_i \cdot \gamma_{o/a} \cdot (1 - \cos\Theta)/g \cdot \Delta\rho \quad (9)$$

The total segment volume V of the reaction mixture in the aqueous medium as well as the total segment length L_s are independent of the flow rate of the organic phase Q_o, and influenced only by the $(1 - \cos\Theta)$ term at constant values of the other parameters. The maximum droplet volume V_{max}, valid when the interfacial force is

equal to the gravity force, the corresponding segment length L_{max}, the actual droplet volume, and the actual segment length are all governed by the equations given for the single channel coaxial segmentor.

The concentration of a particular component in the aqueous reaction mixture, c_i, is governed by the flow rate ratio between the aqueous stream in question and the sum of the flow rates of all streams of aqueous phase, $(Q_a)_i/\Sigma(Q_a)_i$, and by the actual concentration of the component in each solution $(c_o)_i$

$$\begin{aligned} c_i &= \Sigma[(c_o)_i \cdot (Q_a)_i/\Sigma(Q_a)_i \\ &= \Sigma[(c_o)_i \cdot V_i]/\Sigma(V_i) \\ &= \Sigma[(c_o)_i \cdot V_i]/V \end{aligned} \quad (10)$$

and is independent of the total segment volume V. It can also be varied by changing the dosing time at constant flow rate ratio of the aqueous phase component streams, as the partial volume of each solution in a particular segment, V_i, depends on its flow rate and dosing time $V_i = t_i \cdot (Q_a)_i$.

The reaction mixture is homogenized during the droplet formation period due to the very intensive mixing pattern produced by the flows of aqueous phase meeting at the end of the inlet capillary system. The homogenization process is forced by the secondary flows occurring due to the viscous drag of the organic phase flow on the front area of the droplet of aqueous phase. Thus, a practically homogeneous reaction mixture is usually obtained before the droplet is cut off from the capillary system.

The droplets move into the outflow channel after cutting off, together with the continuous flow of the organic phase which is continuously wetting the walls of the mixing compartment and conical housing. Additional mixing takes place in the conical outflow housing of the multichannel dropping segmentor, and while the spherical shape of the droplet is being transformed into a distorted spherical or cylindrical shape inside the reaction coil. A more or less regular segmented flow consisting of independent segments of the two phases is obtained.

The homogeneous reaction mixture is transported through the fluoropolymer capillary system of the FIA analyzer in the form of individual segments of aqueous phase. It is exposed to continuous mixing during passage through the reaction/extraction coil due to an intensive intrasegmental flow, forced by the velocity distribution in a laminar flow profile. Diffusion also aids in distributing the analyte evenly within the segment. The reaction takes place in each segment of aqueous phase, forming a closed reaction system completely isolated from the other aqueous segments by the film of organic phase which prevents analyte carry over. An instantaneous extraction of the solute follows the reaction, increasing the reaction/extraction rate in many cases.

As indicated earlier, the segment length of the organic phase is regulated by its total flow rate and droplet time t_{max} ($V_s = Q_o \cdot t_{max}$) or dropping frequency $f_d = 1/t_{max} = Q_o/V_s$; it grows linearly with the flow rate because the influence of the hydrodynamic forces becomes negligible.

3. Noncoaxial Segmentors

This type of a segmentor consists mostly of a compact segmentor body made of hydrophilic, lipophilic, or combined hydrophilic and lipophilic materials, with an inner triple channel capillary system. The channels can be oriented perpendicularly to each other (T-type segmentors) and/or at different angles among the inlet and outlet tubes (30, 45, 60, 120, or 150° etc., for W or Y type segmentors), the immiscible phase flows entering the segmentor horizontally, vertically from the bottom or the top, and/or at different angles to the main segmentor axis. Segmentation processes of hydrodynamic and interfacial origin in T-shape segmentors have been described in detail,[7] but overlooking the important role played by a "ripple" segmentation pattern in narrow bore fluoroplastics segmentors; thus, only the basic conclusions are given here.

At very low flow rates and low flow rate ratios (Q_a and Q_a/Q_o, or Q_o and Q_o/Q_a, respectively) the gravity and density forces predominate and the influence of the hydrodynamic force F_H can be neglected when F_H and $F_G + F_D$ are perpendicular to each other (and also at any different angles). The total segment volume and the segment length are governed by Equations 5 to 9.

The resulting total droplet volume V can, under ideal conditions (neglecting the influence of the hydrodynamic force and assuming that the gravity/density and interfacial forces are equal, $F_{GD} = F_I$), be expressed by Equation 5 at the moment t_{max}, when the droplet is cut off. The total droplet volume V and the total segment length L_s (neglecting the film formation process or assuming a stable film thickness) are practically independent of the flow rate Q_n of the phase not forming droplets, and consequently, the dependence of V_n or L_n on Q_a are depicted as straight lines parallel to the x-axis (similar to curve 1 in Figure 3).

At low flow rates the gravity force still predominates, but the influence of the hydrodynamic force F_H cannot be neglected. The resulting force vector can be calculated from the condition that the forces acting on the droplet must be equal, $F_R = F_I$, where $F_R = (F_{GD}^2 + F_H^2)^{1/2}$. The frontal area A_f and the total surface area A_t of the droplet can be expressed from the droplet volume, assuming a spherical shape ($A_f = \pi \cdot r_d^2 = 3 \cdot V / 4 \cdot r_d$). The normalized droplet volume V_n and the normalized segment length L_n can be expressed for several hydrodynamic/gravity force rates Q_n or F_H/F_{GD} and several preselected cos Θ values as functions of the flow rate Q_n of the phase not forming droplets, using Equations 1 to 5. The resulting curves are nearly linear and parallel to the X-axis, or at higher Q_n are slightly deformed towards lower V_n values (similar to curve 2 in Figure 3). This results from the decrease in droplet volume due to the hydrodynamic force acting on the droplet.

At moderate flow rates Q_n the influence of the hydrodynamic force F_H is quite markedly expressed and is comparable with the influence of the gravity and density forces. The resulting curves are strictly nonlinear for different cos Θ values, the curvature depending mainly on the F_H/F_{GD} ratio. At high F_H/F_{GD} ratios the hydrodynamic force predominates and the curves are distinctly nonlinear and close to parabolic. When F_H/F_{GD} is close to unity both forces are approximately equal, and the curves are slightly concave or convex but may also be linear.

The hydrodynamic force predominates at very high flow rates of the phase not forming droplets, high flow rate ratios (Q_a and Q_a/Q_o or Q_o and Q_o/Q_a, respectively), and perpendicular orientation of F_H and F_{GD}. The segmentation is regular at the segmentor over a wide range of flow rates, but it often becomes irregular at the measurement point (as a result of several small segments joining together to a bigger aggregate during transport through the extraction coil). Shorter segments are formed at higher flow rates and, at a given flow rate, increasing interfacial tension $\gamma_{o/a}$ results in longer segments. The influence of gravity and density forces is negligible and the volume of the droplet is influenced mainly by the condition that F_H and F_I must be equal when the droplet is cut off (Equation 4).

Depending on the total flow rate and the droplet volume, the droplet shape becomes deformed spherical or ellipsoidal (see Figure 2). The hydrodynamic force F_H grows with increasing total droplet volume as the total droplet area increases and the frontal area of the droplet decreases. Also, the deformation of the spherical shape of the droplet increases at the same conditions, depending on droplet deformability. The frontal area of the droplet A_f (expressed in terms of droplet volume assuming an elliptical shape[7]) and the "Bernoulli" term of Equation 3 are influenced both by the total droplet volume and by the flow rate. As the volume increases, the deformation also increases and the frontal area decreases with time. The total surface area increases when the segment shape becomes increasingly ellipsoidal.

The influence of the two terms in Equation 3, combining all the above-mentioned equations, can be expressed as a dependence of the total volume of the droplet V or the total segment length L_s (see Figure 5) on the flow rate Q_n of the phase not forming droplets. The resulting curves for different values of the constant "Bernoulli" and/or "Poiseuille" terms k_B and k_P are clearly nonlinear and very similar to curves presented in literature.[7] The reproducibility of total droplet volume measurements decreases sharply with increasing flow rate at very low Q_n values due to the very strong dependence of the droplet volume on the flow rate Q_n of the phase that does not form droplets (see Figure 5, curve on the left for organic phase as a droplet forming solvent).

Quite similar curve shapes can be derived for the Y or W continuous segmentor types because

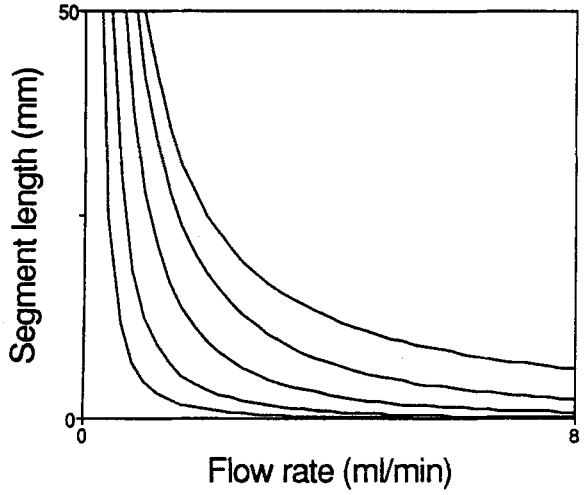


FIGURE 5. The dependence of the segment length on the flow rate of the phase that does not form droplets for different values of F_{GD}/F_H ratio.

the resulting hydrodynamic force can be expressed in the same way. The resulting force vector predominantly influences the droplet volume or the segment length. An exact mathematical treatment of the curves is difficult since the change in the radial velocity profile is complex in nonlinear tubing.

The segment length of the wetting phase (the phase that does not form droplets) is regulated by its total flow rate and droplet time t_{max} ($V_s = Q_n \cdot t_{max}$) or dropping frequency $f_d = 1/t_{max} = Q_d/V_s$. It grows nearly linearly with the flow rate when the influence of the hydrodynamic forces is negligible, and power relationship is valid for higher flow rates and flow rate ratios.

C. The "Ripple" Segmentation Process

The following parameters influence the skewing process and (usually) the segmentation mode:

- The ability of one of the phases to wet the wall material
- Possible contamination on the walls of the segmentor chamber
- The distance between inlet/outlet tubes, which should approach zero
- The flow rate of the phase not forming the droplet and the flow rate ratio (Q_a and Q_a/Q_o or Q_o and Q_o/Q_a, respectively), which should both be high
- The viscosity ratio η_a/η_o and interfacial tension $\gamma_{o/a}$ of both phases
- The changes in the geometry of the inner capillary system

Both phases flow continuously into the outlet tube, forming two independent axial laminar flows, one of them having higher affinity towards the material, thus wetting the walls of the mixing compartment of the segmentor and the outflow tube. The walls are covered by a thin layer of the solvent forming a more or less stationary film of the wetting phase. The driving force for the film formation is the minimization of the interfacial energy at the solid/liquid interface which is determined by the relative magnitudes of the surface tension of the inner wall surface of the tubing to the liquids (wetting ability) and the interfacial tension of the liquids. The thickness of the film depends on the flow rate, the flow rate ratio, and the alternation frequency and segment length ratio $L_{s(org)}/L_{s(aq)}$. It can, for practical purposes, be expressed by an exponential function in the form[51]

$$d_f = k \cdot r_o \cdot (u \cdot \eta/\gamma_{o/a})^a \qquad (11)$$

where, r_o is the inner diameter of the tubing, u is the linear velocity of the flowing stream (in cm s^{-1}), η and $\gamma_{o/a}$ are viscosity (in poise) and surface tension (in dyn cm^{-1}), and a and k are constant terms which both are usually equal to 0.5 or to 0.67.

Once a thick film of wetting phase is formed on the inner wall surface of the two cylindrical parts of the segmentor, it is not stable with time. This is quite independent of the wettability, and any film of a fluid deposited on the inner surface of a narrow cylindrical tube is inherently unstable. It will rearrange so as to decrease the interfacial energy at the liquid/liquid interface, (i.e., to reduce the interfacial area), and will eventually produce ripple, lenses, and finally droplets or plugs of the phases.

The film rearrangement process is forced by many factors. The importance of each of them will depend on the wall material, physical properties of the two solvents, total flow rate, and the character of the flow and its velocity distri-

bution. It is also influenced by changes in the flow rate, cross sectional area, etc. Yet another property affecting film rearrangement is the Rayleigh instability of the film on the inner wall of the tube.[52]

A relatively smooth and very thick film of organic phase is formed at the experimental conditions discussed previously. The film thickness is relatively uniform at the beginning of the fluoropolymer compartment or the outlet tube (see Figure 6). The film will, however, rapidly rearrange (partly inside the mixing chamber) into waves of different amplitudes and will finally break down into droplets or plugs. The instability with time for the most rapidly growing perturbations of the film thickness can be expressed[52] by the equation

$$\ln[(\delta_f)_t/(\delta_f)_o] = (\gamma_{o/a} \cdot d_f^3 \cdot t)/(12 \cdot \eta \cdot d_e^4) \quad (12)$$

where $(\delta_f)_t/(\delta_f)_o$ is the growth of the film waves in time t, η is the viscosity of the organic phase, d_f is the film thickness $(\delta_1)_o$ and $(\delta_f)_t$ are the amplitudes of the wave at times zero and t, respectively. According to this theory,[52] when the film is subjected to an infinitesimally small perturbation, a standing wave of the length $\lambda = 2 \cdot \pi \cdot d_e \cdot \sqrt{2}$ will start to grow in amplitude until droplets are formed.

Another important aspect of the film rearrangement and of the segment formation is its centricity. Under influence of the gravity and density forces, together with the hydrodynamic force, the organic flow tends to wet preferably the bottom or top part of the tube, depending on the direction of the resulting force vector and the spatial orientation of the inlet/outlet tube axis. The asymmetry in centricity does force ripple formation and results in rapid droplet formation, especially in horizontally positioned outlet tubes. Horizontal or vertical orientation of the axis of the outflow channel and/or the axis of the main axial flow influences the resultant force vector, which impedes or forces droplet formation.

The geometrical shapes of the outlet tube and the segmentor compartment strongly influence the "ripple" formation during the segmentation process. Coiling the outlet tube will change the parabolic flow velocity distribution across the tubing profile into an asymmetrical one, which is deflected towards the outer wall.[3]

The changes of the inner diameter of the outflow branch of the segmentor mixing chamber, together with the short fluoropolymer tubing inserts inside the outflow channel of the segmentor, also change the crossectional area of the outflow tube. As a result, the flow of both phases will be speeded up and oriented into the center of the tube. A secondary flow, perpendicular oriented to the main axial laminar flow, is thus introduced. The secondary flow inflicts changes in the flow velocity distribution across the tubing profile, forces the formation of ripple on the film, and increases the radial mass transport in the outflow

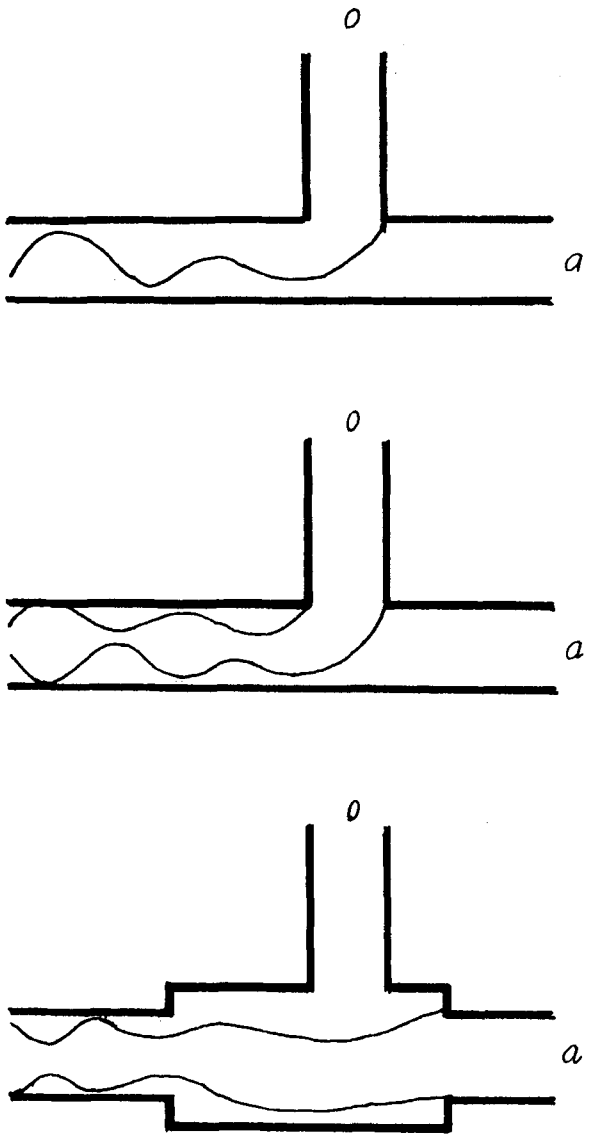


FIGURE 6. The "ripple" segmentation mechanism in a T-shaped segmentor.

tube. It also increases the influence of the "Poiseuille" and "Bernoulli" forces.

All these factors introduce secondary forces which speed up the ripple formation process. When the segmentor is in a horizontal position, segments are formed in the segmentor mixing chamber or immediately after reaching the beginning of the fluoropolymer outflow tube; whereas, in a vertical position (with the outflow axis oriented from the top to the bottom of the chamber) segments are formed approximately 1 to 20 cm from the segmentor. Thus, the ripple segmentation mode is faster and less reproducible with a horizontal outflow axis than with a vertical outflow from top to bottom. Also, coiling the outflow tube, especially near to the segmentor outflow channel, has a positive effect on segment formation and its reproducibility.

III. SEGMENTORS

The existing segmentors can be divided into two general groups depending on the principle of segmentation, (post-segmentor introduction of the sample being a special case):

- Continuous flow segmentors (including those which operate on the basis of differences in gravity or density)
- Mechanical types of segmentors

A. Continuous Flow (Gravity/Density, Hydrodynamic) Segmentors

1. Noncoaxial Segmentors

a. Classical T, Y, and W Segmentors

T, Y, and W pieces made of homogeneous materials such as fluoroplastics, stainless steel or glass, glass-lined T-pieces made of stainless steel, or metal capillaries with fluoropolymer coating (in T-shaped segmentors) are the most widely used segmentor types. Capillary tubes made of glass, stainless steel and other materials in various geometrical configurations and combinations of materials have also been used for phase segmentation. The segmentors are commonly used with the aqueous phase entering through a horizontal inlet capillary axially oriented to the main segmentor axis, and the organic through the upper or lower capillary, depending on the density difference between the two solvents; however, other orientations also are often used (Figure 7).

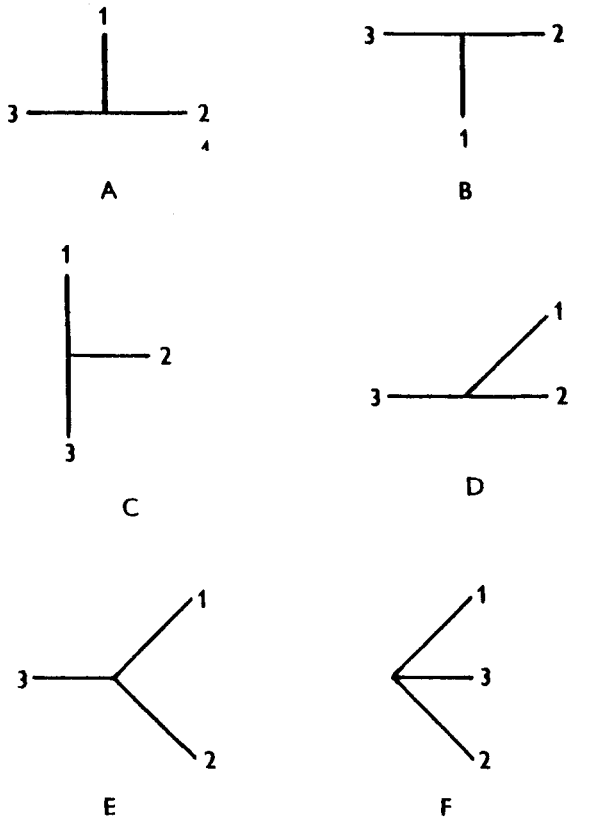


FIGURE 7. Classical T-piece (A, B, C), Y-piece (D, E), and W-piece (F) segmentors with the most often used orientation of the aqueous (1), organic (2), and segmented flows (3), but also other orientations are possible.

Segmentors made of hydrophilic material work by the wetting and skewing principle at low or moderate flow rates. The segment volume is controlled by the position and orientation of the input and output channels and by the inner diameter of the input channels, and, to a great extent, the output channel (at constant inner diameter of the input channels, 0.3 to 1.0 mm i.d.). The resulting force vector, controlling the segment size, is influenced by the angle between the two inlet tubes and also by the orientation of the outlet tube. The maximum droplet size is limited by the resulting force vector and by the volume

of the compartment at the junction of the glass T-piece.

With Q_o/Q_a near to unity, the lengths of the organic and aqueous phase segments are approximately equal when segmentation is regular. The segment lengths depend on the total flow rate Q_t over a limited range and decrease with increasing Q_t. Above some limiting value $Q_{t(lim)}$ the segmentation pattern is usually regular at the segmentor but irregular in the extraction coil (as a result of ripple segmentation or combination of segments during passage through the extraction coil[40]). At a given Q_t, longer segments are obtained for the MIBK (methylisobutyl ketone)/water system than for the chloroform/water system, depending on the aqueous interfacial tension $\gamma_{o/a}$ and the viscosity ratio η_o/η_a for both organic solvents.

The T-shaped segmentors made of fluoroplastics are in many cases able to produce small and reproducible segments, assuming optimum geometry of the inner capillary system (0.3 to 1.0 mm i.d.). They work best at low flow rates and at phase flow rate ratios Q_a/Q_o close to unity. At higher flow rates and flow rate ratios the repeatability is not satisfactory. An ordinary commercial T-piece made of Kel-F gives segments of a repeatable size.[7] The simple Teflon T-shaped segmentor at $Q_a = Q_o = 0.3$ ml min^{-1} gives 1 μl organic phase segments.[53]

The mutual positions of the inlet and outlet tubes can influence the efficiency of the segmentation process. Type B (see Table 1) was found to be best[39] but the influence was not very marked and could, at some higher flow rates, not be confirmed at all.[26,38] Other combinations of organic and aqueous phase inlet orientations gave an uneven flow. The relative segment length changes only negligibly with the angle between the vertical axis and the axis of the organic phase inlet channel. However, the repeatability of the segment formation decreases when the angle is increased.

Several geometries and dimensions were investigated for stainless steel phase segmentors[54] with the smallest segment possible being approximately 20 μl. The optimum design was found to consist of three 0.18 mm i.d. capillaries joined as close as possible in a stainless steel block (0.25 mm i.d. bore) at 30°/30° angles among them, with the aqueous phase entering through the upper capillary, the organic through the center capillary, and the segmented flow exiting through the lower one. Using larger capillaries (0.25 and 0.30 mm i.d.), other geometries (45, 90, and 120°), and larger bore (0.7 mm i.d.) of the steel block produced 2- to 3-fold increases in segment size.

The W-piece Teflon segmentor with 45° angles between the inlet and outlet channels has also been shown[31] to be able to give repeatable segmentation.[18] A W-piece segmentor made of PVDF (polyvinyldifluoride) with channels of 0.7 mm i.d.,[34] and an Y-type segmentor at $Q_a = 1$ ml min^{-1} and $Q_o = 0.75$ ml min^{-1}, have also been used with very good results.[28,29]

TABLE 1
Comparison of Segmentation Efficiency for Different Types of Segmentors and Different Inlet Flow Orientations[39]

Type	B	B[a]	B[b]	F	E
H_p	16.03	16.30	15.70	16.60	14.75
W_p	55.0	56.2	55.8	55.5	54.8

Note: The peaks widths, W_p, are measured at 1% of the maximum peak height, H_p.

[a] Orientation 2, 3, and 1 (aqueous phase, segmented flow, and organic phase).
[b] Orientation 2, 1, and 3 (aqueous and organic phases, and segmented flow).

b. Modified T-Segmentor

This type of segmentor is realized by inserting one or more concentric fluoropolymer tubes into the outlet (and sometimes also into the inlet) tubing of a conventional glass T-piece. The inner diameter of the fluoropolymer tubes as well as their distance from the position of the inlet tubing positively influence[40] the repeatability of the segmentation process. The maximum droplet size is limited by the volume of the compartment formed by introducing fluoropolymer tube inserts into all branches of the glass T-piece. The volume of the droplets is, in this case, influenced by the wetting ability of the organic solvent at the moment when

the distorted droplet surface contacts the end of the fluoropolymer tubing. In this case, irreproducible droplet formation can be partly eliminated in a similar way as for the modified A8-T fitting.

The glass T-segmentor (2 mm i.d.) with fluoropolymer tubing inserted into the outflow channel at 3 to 15 mm distance from the T-joint yields repeatable segmentation over a wide range of flow rates (up to 6 ml min^{-1}, $s_r < 10\%$) at organic phase flow rates down to 1 ml min^{-1}. The length of the organic segments is reduced with increasing flow rate of the aqueous phase (up to 30%), and reaches a limiting value at higher aqueous phase flow rates ($Q_a > 4$ ml min^{-1}). The aqueous segment length increases at low Q_a and at high total flow rates. The organic segment length increases nearly linearly with increasing organic phase flow rate. Both decrease with increasing total flow rate at a constant flow rate ratio.[40]

The organic segment length shows a nearly linear dependence on the distance of the fluoropolymer tubing end from the joint of the T-piece at constant total flow rate in the interval from 3 to 15 mm. At shorter distances, the segment lengths of both phases and the segmentation repeatability are drastically or markedly decreased, respectively, as a result of ripple segmentation.

The segmentation repeatability is satisfactory in the Q_a interval from 1 to 5 ml min^{-1} with Q_o equaling 0.5 or 1 ml min^{-1}, and in the Q_o interval from 0.2 to 1.5 ml min^{-1} with Q_a equaling 1.5 or 3 ml min^{-1}. The best repeatability is achieved for Q_a-values between 1 and 2 ml min^{-1} or Q_o-values ranging from 0.25 to 1.2 ml min^{-1} at phase flow ratios Q_a/Q_o from 0.7 to 4. However, values of $Q_a/Q_o < 6$ can be used. The segmentation repeatability is rapidly decreased at higher flow rates due to the formation of very small droplets at the end of the fluoropolymer tubing insert.

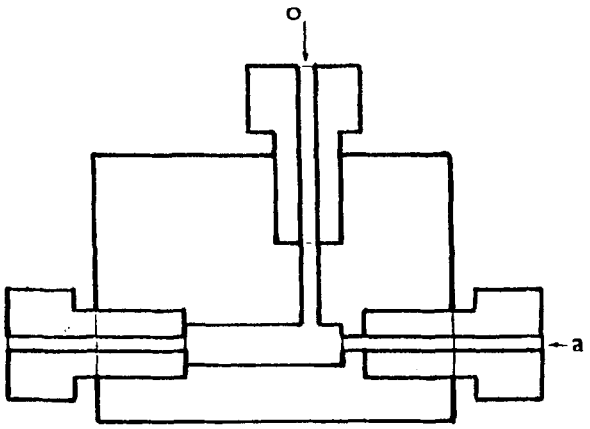
This type of phase segmentor has also been made by drilling out the narrow bore cylindrical chamber of a commercially available Kel-F tee-piece to 1/16 in. i.d. and inserting 2 mm long flared pieces of 0.8 mm i.d. × 1/16 in. o.d. fluoropolymer tubing into all three branches. In the absence of tubing, an irregular segmentation pattern sometimes developed, usually displaying both long and short segments. This was observed to be due to creeping of the organic phase along the walls into the aqueous inlet capillary of the T-piece, where it formed an adherent droplet located approximately 1 or 2 mm into the branch.[21]

The droplet grew in size until it formed a constriction large enough to occasionally cause breakage of the aqueous stream at the junction of the branches where it normally occurs. Installing the fluoropolymer tubing inserts into the capillaries eliminated the problem over a wide range of Q_t. At given Q_t, longer segments are obtained for the MIBK/water system than for the chloroform/water system, depending on the aqueous interfacial tension $\gamma_{o/a}$ and the viscosity ratio η_o/η_a of the two organic solvents.[7]

The ability of the organic solvent to wet the wall material of the segmentor is important in the case of segmentors made of fluoroplastics, where the ripple segmentation mechanism is the predominating one. The segments are formed in the extraction coil after the segmentor, and segmentation is facilitated by a somewhat pulsating flow. A pulsating flow might cause the segmentation pattern to be in phase with pulsation. Excessive pulsation or irregular flow give rise to uneven segmentation for both classical and modified T shape segmentors.

Increasing the inner diameter of the outflow channel (0.3 to 0.7 mm i.d. for inlet and 0.5 to 2 mm i.d. for outlet tubes) result in longer segments with this kind of segmentor, a prerequisite being that the organic phase can form a very thick film on the walls of the compartment or that it can fill the whole diameter of the channel before the organic segment is skewed off by the aqueous flow. At high phase flow rate ratios this phenomenon can not occur and the segments are actually formed in the extraction coil (see Figure 8).

The length of the segments is determined by the inner diameter of the outlet tubing so that, for the chloroform/water system, an 0.8 mm i.d. T-piece in its original shape gives approximately 2 mm long segments in a 0.7 mm i.d. tube, whereas the segment length is around 7 mm when the outlet is enlarged to 1.2 mm. A suitable size for practical purposes was found to be around 4 mm at the flow rate ratio $Q_a/Q_o = 2.5$, and the sizes corresponding to the outlet tube diameters 0.5, 0.7, 1.0, and 1.2 mm were 1, 3-5, 5-10,



T piece - PVDF

FIGURE 8. Improved fluoropolymer T-shape segmentor with an enlarged outflow channel.

and 6-14 mm, respectively, for 0.5 mm i.d. inlet tubes and the chloroform/water system.[26]

Refined versions of this design are commercially available, e.g., the Tecator design, where the outlet channel volume can be varied by exchanging fittings. In this way segment lengths between 2 and 15 mm can be obtained. Neither of these segmentor types use materials displaying different wetting characteristics. Thus, segment size control is limited in comparison with the A8-T fitting.

The relative segment length changes with the angle between the vertical axis and the axis of the organic phase inlet channel, and reaches a maximum between 135 and 180° for both T-segmentors used, in combination with 1.2 or 2 mm i.d. outlet tubes and 0.7 mm i.d. inlet channels. The repeatability of the segment formation decreases when the angle is increased.

The maximum relative segment length was achieved when the aqueous phase was flowing through the T-segmentor from the bottom to the top along the longer axis, while the organic phase was flowing horizontally. The minimum segment length was achieved in the reverse position. The difference in segment length between the vertical organic flow stream inlets (segmentors A and B in Figure 7, respectively) was less evident (only from 2 to 8%) with the aqueous phase flowing horizontally along the main segmentor axis.

Irregular segmentation due to breakup of some of the segments as a result of irregularities in the walls of the drilled channels has also been observed. In fact, smoothness of the walls was as important for obtaining even segments as was avoidance of impurities or precipitates causing deposits. The geometry of the segmentation point was found to be critical with respect to the size of the mixing cavity and its proximity to turbulent points: i.e., points at which the flow direction changes abruptly had to be optimized so that segments of uniform size resulted.[35]

c. A8-T Modified Fitting

A modified standard A8-T glass Technicon connector having glass and platinum inlet capillaries and glass outlet tubing with two concentric pieces of fluoropolymer tubing inserted into the outflow channel has often been used as a segmentor. The working principle is based on the formation of a droplet of the organic phase in the aqueous phase stream when the organic stream is led into the platinum capillary at right angles to the aqueous stream which enters the glass capillary (Figure 9).

The droplet grows in size until it comes into contact with the fluoropolymer tubes or until it reaches the maximal volume V_{max} (when the distance between the platinum and fluoropolymer capillaries is long enough to prevent any contact with fluoropolymer). The droplet is then dislodged to form a segment, since it does not wet the glass surface. The volume of the droplet and the segment length are therefore determined by

- The volume of the glass compartment between the platinum capillary and the edges of the two concentric fluoropolymer tubes
- The inner diameter of the platinum capillary
- The flow rate ratio
- The viscosities of the fluids
- The interfacial tension $\gamma_{o/a}$
- The gravity force
- The hydrodynamic force of the flow of aqueous phase

In such a case, the segment size can easily be adjusted by changing the position of the edge

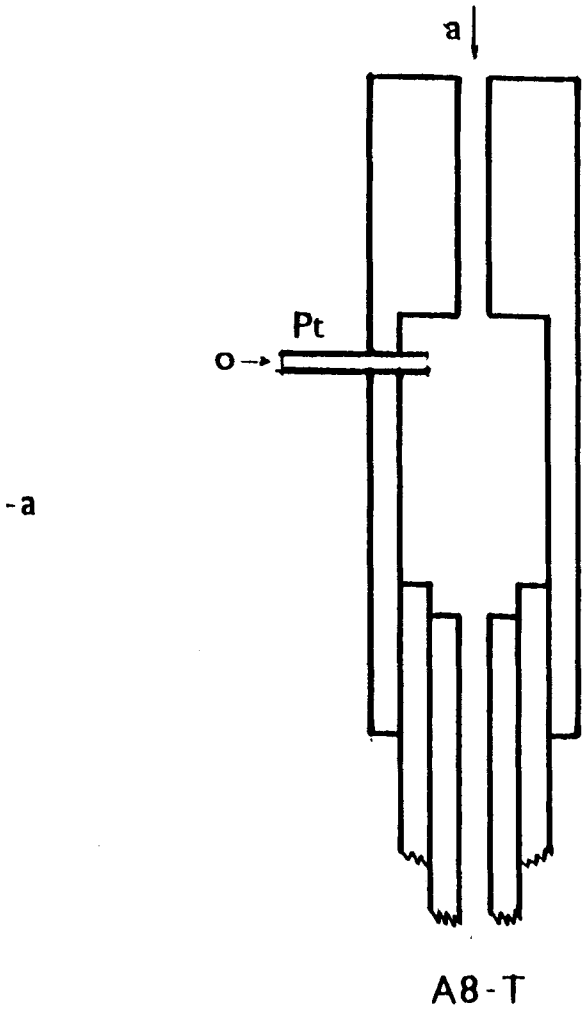


FIGURE 9. Improved A8-T fitting with the glass and platinum inlet capillaries and two fluoropolymer tubing inserts in an outflow channel.

of the inner tube when using a certain solvent system. Appropriate positioning is necessary in order to obtain a regular segmentation pattern. Adjusting the mutual positions of both tubes will change the segment length.[55] The segment length can be thus varied from 3 up to 40 or 50 mm for chloroform.

The maximum size of the segments is determined by the interfacial tension between the organic and aqueous phases in such a way that a low $\gamma_{o/a}$ makes it difficult to form larger segments. For example, regular segment lengths up to 50 mm can be produced using an A8-T fitting in the chloroform/water system. In the pentanol/water system having a lower $\gamma_{o/a}$, only 10 to 20 mm segments are formed, and going to the even lower $\gamma_{o/a}$ value for the n-butanol/water system, the maximum segment size is 2 to 3 mm.[8]

Increasing the flow rate Q_a of the aqueous stream at constant flow rate Q_o of the organic phase using a very long distance between the platinum and fluoropolymer capillaries (1 to 10 mm) results in a decrease of the organic segment length, the distance preventing any influence of the wetting properties of the lipophilic capillaries on the segment formation process.[40] The segment length reaches some limiting value at higher aqueous flow rates, $Q_a > 4$ ml min^{-1}. This value depends mainly on the inner diameter of the tubing and on the aqueous phase flow rate Q_a, as droplet deformation increases with increasing Q_a.

In contrast to this, the length of the aqueous segments increases rapidly, and varies exponentially or nearly linearly with the flow rate of the aqueous phase at low and high total flow rates, respectively. Also, the sum of the lengths of the organic and aqueous segments increases at higher aqueous flow rates.

The segment length in the organic phase is practically not influenced by the flow rate Q_o of the organic phase up to 1.6 ml min^{-1} at a constant aqueous flow rate Q_a, or is only slightly increased at shorter distances between the platinum and fluoropolymer capillaries (1 to 3 or 5 mm). The length of the aqueous segments decreases with increasing organic flow rate Q_o, and reaches a limiting value at a very high Q_o, which depends principally on the glass compartment size. The influence of the total flow rate is negligible in the interval $Q_t = 0.8$ to 7.5 ml min^{-1}.

The repeatability of segmentation is satisfactory ($s_r < 10\%$) over a relatively wide Q_a range (0.3 to 10 ml min^{-1}) and Q_a/Q_o range (0.3 to 12.5). It decreases at very low and very high flow rates and flow rate ratios. The best repeatability (s_r 1 to 3%) is achieved in the flow rate ratio interval $Q_a/Q_o = 1$ to 5.

Small changes in spatial orientation do not influence the segment length or the repeatability of segmentation, especially not at high flow rate Q_a of the aqueous phase. Turning the axis of the inlet/outlet glass capillary around the horizontal axis of the platinum capillary increases the segment length, which will reach its maximum value after 180°. The segment length will then be 10 to 20% longer compared to the 0° orientation,

due to a decreased influence of the gravity/density force. The irreproducibility is decreased from 5 to 10%.

Lipophilic impurities on the glass wall surface or other changes of the hydrophilic properties of the walls, a short (< 2 mm) distance between the platinum and fluoropolymer capillary ends, and a very high total flow rate (Q_t > 5 ml min^{-1}) or flow rate ratio ($Q_a/Q_o > 10$) cause a change from the regular droplet formation segmentation to a "ripple" segmentation using the A8-T segmentor. In such cases, the glass surface is covered by organic phase, or a small droplet adheres to the end of the platinum capillary, and a short bridge of a hydrophobic interface results. Also, it was reported that relatively large Freon-113 droplets can cling to the entrance and cause undue dispersion.[27] The stream of organic phase then flows continuously into the fluoropolymer capillary without the regular droplet formation inside the glass compartment.

Segmentation takes place inside the extraction coil at some distance from the edge of the capillary (from 0 to 20 cm for vertical orientation). This distance depends on the orientation of the main segmentor axis, the coiling of the outflow capillary, its position, etc. The segment length is decreased to one half or less of the ordinary segment length and the segmentation pattern becomes irregular. The segment lengths of the organic and aqueous phases remain unchanged or decrease with increasing total flow rate. This is in contrast to the regular segmentation mode, where the segment lengths remain constant or increase with increasing total flow rate. The segmentation repeatability decreases to 20 to 50%.

Any changes in the laminar character of the flow due to changes in the inner diameter of the tube, fluctuations in the flow rate, insertion of sharp edges, etc., destroys the organic layer formed on the fluoropolymer tube walls in a laminar flow of both phases. Instead, small droplets or plugs are formed. This "ripple" process of droplet formation is quite sensitive to any irregularities in the experimental conditions than is the classical droplet formation process, and results in impaired segment formation repeatability. The process can be partly overcome by insertion of a second fluoropolymer tube.

This phenomenon rarely occurs at low aqueous flow rates, but frequently occurs at very high Q_a/Q_o or at very high back pressures. The perpendicular flow of aqueous phase will then deform the droplet shape and slide it along the surface of the walls of the glass compartment. The stream of organic phase can finally bridge over this hydrophilic surface, forming a continuous film of organic phase which changes the mode of segmentation.

The spreading of the organic droplet along the walls of the glass compartment is very important at higher Q_a. The droplet bends somewhat with the stream and becomes distorted to some extent at all flow rates. At very high flow rates, this, together with the gravity force, will control the droplet size. This phenomenon becomes more expressed for a fluoropolymer compartment in the common T-segmentor than for the glass compartment of the A8-T segmentor.[40]

The modified A8-T segmentor cannot be used with standard tubing connectors of the low pressure HPLC type, which is a drawback in routine work. Serious leakage often occurs at high total flow rates or at high overpressure (due to the phase separator or the restrictor coil), since this type of segmentor was designed for low pressure segmented continuous flow analyzer systems. It can be readily used with a T-shape phase separator or in systems without phase separation, but difficulties are encountered in systems employing phase separators of the membrane type.

The segment size is influenced by surface active substances such as anionic surfactants.[54] The segment length is constant with no surfactant present, but with increasing surfactant concentration the segment length decreases significantly and peak height decreases more rapidly for shorter segments than for longer ones. This phenomenon is probably depending on the amount of surfactant at the interface between the phases, changing the interfacial tension.

In aqueous reagent systems using methanol, the segment length increases with increasing methanol content and peak tailing is reduced as a result of increases in extraction efficiency and rate, and due to a reduction of the lipophilic nature of the fluoropolymer tubing walls, causing adsorption of ion associates from the aqueous stream. The repeatability also decreases with increasing methanol content in the reagent stream.

The modified A8-T fitting has the advantage

of providing a variable segment length over a wide range and of being very good at maintaining the direction of flow. It can tolerate even high back pressures if tightly fitted to the extraction system. However, the separation between segments is sometimes not consistent.[18]

The dead volume at the confluence point is too large for organic flow rates that are much smaller than the aqueous flow rate. When segmentation is controlled by droplet formation, segment length can be easily varied by the Q_a/Q_o flow rate ratio, or better, by the distance of the fluoropolymer tubes from the platinum capillary end. The segmentation was shown to become irregular at higher phase flow rate ratios, and, even with careful adjustment, occasional droplets of larger size would be dislodged.

Fluctuations in solvent flow rate or solvent flow rate ratio caused by segmentation has the adverse effect of forcing some of the phase intended for resampling out to waste at the phase separator. Hence, uneven segmentation leads to a decrease in sample recovery.[18] The segmentation in the back extraction step is not as critical because the organic and aqueous phase segments are often about the same size.[27,37] Irregular segmentation increases pulsation.

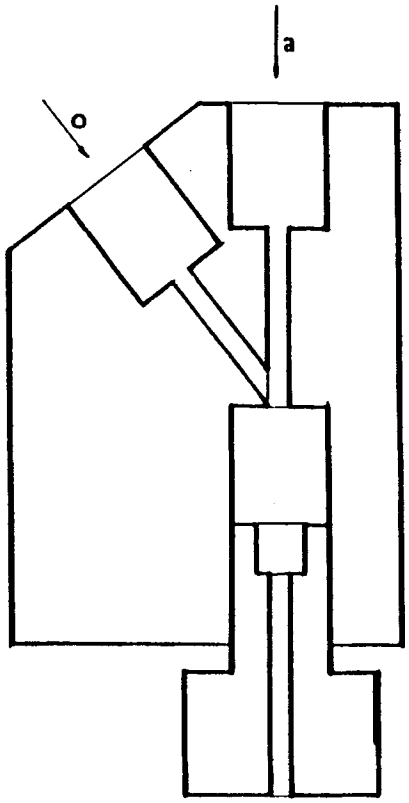


FIGURE 10. Improved Y-piece with the outflow channel made of a fluoropolymer screw.

d. Modified Y-Piece[27]

Most of the segmentors mentioned above are not well suited to cope with the high ratios of aqueous to organic phase flow rate ratios which are needed in some applications. In the modified Y-piece (see Figure 10), the organic segments are formed on the hydrophobic surface of a fluoropolymer piece, which is screwed into a Perspex block containing connectors for the aqueous and organic solvent lines. The segmentation is regular and does not change when the segmentor is turned by 180°. Adsorbed solids may, however, cause irregularities in the segment formation and make the segmentation position dependent.

A model having a continuously adjustable length of the confluence chamber was also used and could, at certain settings, produce segments of widely different sizes. When the flow rate was increased at such a setting, the forming chamber tended to switch to modes yielding segments of one half to one third of the previous size. A large hysteresis was observed when the flow rate was decreased. The dimensions of the forming chamber may be critical, and the optimum may possibly be different for different solvents.

e. A Four-Way Fitting[35,36]

A four-way fitting has also been used as a segmentor, allowing a small "makeup" stream of the organic phase to be continuously introduced into the segmented stream as a constant fraction of the total flow. In the MIBK/water system, this additional stream was essential to compensate for the loss of MIBK in the phase separator and for the solubility of the organic phase in the aqueous phase.[35] In order to prevent dilution in a "closed-loop" liquid-liquid extraction FIA system, a very low flow rate of the "makeup" stream was used.

3. The Coaxial (Gravity/Density) Segmentor

a. Single Channel Segmentor

The coaxial segmentor (falling drop) consists of two basic parts (see Figure 11): a glass capillary inlet channel for introduction of organic phase; and a segmentor body with inlet and outlet capillary channels for delivery of aqueous phase and drainage of the segmented flow stream. The glass capillary inlet tube of an inner diameter ranging between 0.1 and 0.35 mm is pressed into a PVDF screw. When assembled, the capillary ends in the conical housing of the compact segmentor body, made of Perspex or PVDF. The outflow capillary channel is drilled at the bottom of the conical segmentation chamber (Figure 11A, B).

An improved PVDF segmentor body with the confluence chamber made of a thick wall glass tube and a conical PVDF insert, and an outlet channel of fluoropolymer capillary at the bottom of the conical chamber, was also used (Figure 11C). The all-glass coaxial segmentor with integral conical confluence chamber (Figure 11D) and the glass segmentor having a PVDF conical insert in the straight glass tube (Figure 11E, F) were compared with the above mentioned segmentors.

The organic phase flows though the glass capillary, forming small droplets at its end. The droplet size depends predominantly on the gravity/density forces, since the influence of hydrodynamic forces is negligible. The droplets, of defined volume, either descend or ascend (depending on the density difference $\Delta\rho_{o/a}$) through a small conical chamber filled with flowing aqueous phase. The aqueous flow has a negligible influence on the droplet size due to the formation of a whirl, depending on the inner/outer diameter of the glass capillary. The droplet is then transported by the aqueous stream through the outlet channel, where the segment develops, and is passed into the extraction coil.

The glass segmentor[38] with an integral glass conical housing works properly ($s_r < 10\%$) over a wide range of flow rates of the two phases ($Q_o \leq 1.6$ ml min^{-1} of Freon-113 and chloroform, $Q_a \leq 10$ ml min^{-1}) and over a range of flow rate

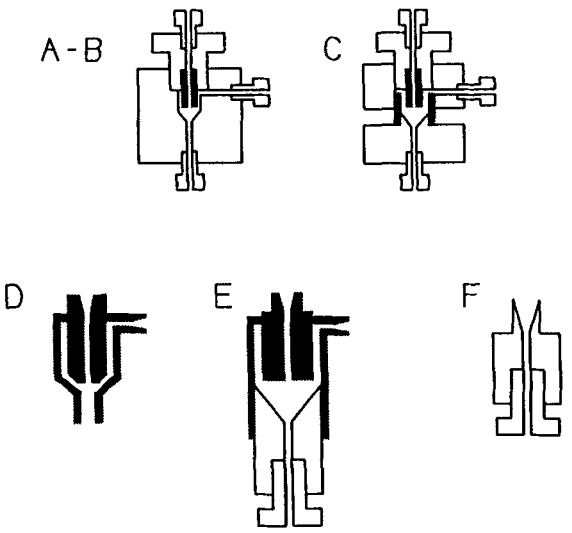


FIGURE 11. Coaxial segmentors of different geometries made of PVDF or Perspex (A, B), PVDF body with a thick wall glass confluence chamber and PVDF conical compartment (C), an all-glass segmentor, (D) and a glass segmentor with the two different geometries of the PVDF conical compartment (E, F).

ratios (from 2 to 35 to 40). Similar segmentation repeatabilities were obtained for the compact PVDF segmentor and for the PVDF/glass segmentor with a PVDF conical insert inside the glass confluence compartment ($s_r < 15\%$ at Q_a down 10, 8, 6, and 5 ml min^{-1} for Freon 113, chloroform, CCl_4 and MIBK, respectively). Less repeatable segmentation ($s_r \leq 15\%$) of Freon-113 was achieved for the Perspex compact segmentor body.

The organic segment length increases linearly with the inner diameter of the glass inlet capillary at constant flow rates of both phases $Q_a = 3.0$ and $Q_o \leq 0.5$ ml min^{-1}. The best segmentation repeatability ($s_r < 5\%$) is mainly achieved for glass capillaries of inner diameter from 0.1 to 0.3 mm for all tested organic solvents, and it decreases for other inner diameters.

The length of the organic segments is only slightly influenced over a wide range of aqueous phase flow rate Q_a (up to 10.0 ml min^{-1}) at the constant organic flow rate $Q_o \leq 0.6$ ml min^{-1}. It is not changed with the organic phase flow rate Q_o at constant aqueous phase flow rate up to the limiting value $Q_{o(lim)}$ at which the jet effect appears, depending on the inner capillary diameter and character of the solvent. When the flow rate

ratio Q_a/Q_o is varied, the length of the organic segments decreases slightly, to 95% of the original value.

The length of the aqueous segments increases nearly linearly with the flow rate Q_a of the aqueous phase and with the total flow rate Q_t (slopes of segment length vs. flow rate of aqueous phase are close to unity), and decreases rapidly with increasing flow rate of the organic phase Q_o, at all the above-mentioned experimental conditions, according to the changes in the dropping frequency of the organic phase.

The segmentation repeatability of the compact PVDF segmentor is acceptable over a relatively wide range of Q_a up to 8 or 10 ml min^{-1} for an inner diameter of the inlet tube of 0.25 and 0.35 mm, respectively, using Freon-113 and chloroform as organic solvents. The segmentation repeatability decreases in the order Freon-113, chloroform, MIBK, and carbon tetrachloride. Uneven segmentation rarely occurred with MIBK and only exceptionally with carbon tetrachloride at low organic flow rates where small droplets of the organic solvent were cut off at the end of the segments.

The values of the flow rate and the flow rate ratio have to be established for each solvent at a given inlet capillary inner diameter and defined experimental conditions. Better segmentation repeatability is usually achieved at a medium total flow rate and a flow rate ratio between 5 and 15, when the flow rate of the organic phase Q_o is in its optimal range between 0.3 and 0.7 ml min^{-1} for all solvents tested. The best diameter of the organic phase inlet tubing varies between 0.10 and 0.35 mm i.d., depending on the character of the solvent and its flow rate, but generally, 0.25 $\pm$ 0.05 mm i.d. can be used for most organic solvents.

For all tested solvents, an uneven segmentation pattern is obtained at a very low organic flow rate, $Q_o < 0.2$ ml min^{-1}, and a constant aqueous flow rate Q_a in the range from 1 to 3 ml min^{-1}. The segmentation repeatability decreases from units up to tens of percent. At higher organic phase flow rates, a jet effect appears, depending on the solvent character, and the segmentation repeatability decreases sharply, giving $s_r > 50\%$. Segmentation breaks down completely as a result of this.

Changing the orientation of the main axis of the coaxial segmentor from the horizontal position has a negligible effect on the segment length ($d(L_s) < 4\%$) up to an inclination of 10°, but over 10° the segment length decreases by between 25 and 50% for both phases. No significant difference between the lengths of the organic segments in straight and coiled fluoropolymer tubing (30 mm coil diameter) was found at moderate flow rates, but the segmentation repeatability was better for coiled than for straight tubing.

Up to a 5% content of inorganic salts such as NaCl in the aqueous phase increases the length of the organic segments by 4%, due to the increase in density of the aqueous phase. The segmentation repeatability remains constant or increases slightly. The presence of a methanol content between 0 and 15%, or content of a nonionic surfactant up to 1%, cause a decrease in segment length by less than 10%. The segmen tation pattern and segmentation repeatability are strongly impaired by a higher content of either of these substances, and segmentation breaks down completely at a methanol concentration over 30% or a surfactant concentration over 1.2% due to the change in surface tension.

Larger segments are produced by the coaxial segmentor than by classical segmentors. It works better than these at high phase flow rates and phase flow rate ratios (best at $Q_a/Q_o > 1$ and $Q_o < 1.2$ ml min^{-1}). At very high flow rates and phase flow rate ratios there is a tendency for small organic segments to decrease in size and for larger organic segments to grow as they pass through a fluoropolymer extraction coil propelled by a peristaltic pump. In order for the segments to survive, it is necessary for them to have a certain minimum volume, which may be ascertained by using a 0.25 to 0.50 mm i.d. glass capillary.[38] To prevent the influence of aqueous flow stream pulsation and formation of a whirl, the inner glass capillary can be screened by a thin wall tubing.

b. Multichannel Segmentor/Injector[48]

The multichannel dropping segmentor consists of two basic parts: a PVDF screw with a multiple capillary inlet channel; and a segmentor body with a conical confluence chamber con-

taining inlet and outlet capillary channels for the organic phase delivery and drainage of the segmented flow stream, respectively.

The working principle of this segmentor is the formation of small droplets (usually from units to tens of microlitres) of an aqueous reaction mixture in a continuous flow of another fluid, immiscible with water. Droplets of the homogeneous reaction mixture are transported through the fluoropolymer capillary system of the analyzer in the form of separate segments of aqueous phase, forming a closed reaction system, completely isolated from each other by a film of organic phase which prevents analyte carry over. An analytical signal can be measured on the aqueous, organic or both segments by an "on-tube" fast reading detector and treated mathematically or by the classical procedure, after separation of the phases.

Each component in the reaction mixture is transported through one of the capillary channels in the body of the dropping segmentor, and they meet at the common confluence point at the junction of the inlet tubing capillary system (see Figure 4). The droplet formation process takes place in a coaxial mixing compartment made of hydrophilic (glass) or lipophilic material (fluoroplastics) at a moderate flow rate. More or less reproducible droplets of aqueous phase are obtained, with their size controlled by the equivalence of the gravity, density, interfacial tension, and mainly hydrodynamic forces. The droplet size also depends on the material of the inner part of the compartment and on the geometry of the inner capillary system of the dropping segmentor.

The best repeatability of the signal parameters was obtained using the PVDF compact body device ($s_r < 4.5\%$) and the PVDF body device, with the confluence compartment made of glass tube with a conical PVDF insert ($s_r < 3\%$) when measuring at a moderate flow rates. Slightly better results were obtained for Freon-113 than for chloroform. Also, the baseline noise was worse by one order of magnitude than when the carrier fluid was Freon-113.

Both PVDF body devices produced their most repeatable segmentation at a moderate flow rate of aqueous phase (Q_a between 0.4 and 3.2 ml min^{-1}) and a rather low flow rate of the organic phase ($Q_o < 1$ ml^{-1}). The repeatability of the segment length decreased toward lower flow rate ratios Q_a/Q_o and higher flow rates of the organic phase ($Q_o < 1$ ml min^{-1}). Serious pulsation of the segmented phase stream appeared when the flow rate of the organic phase exceeded 5 ml min^{-1}, and the results were not satisfactory. The reason for this was a serious pulsation of the segmented stream due to differences in viscous drag of the two solvents moving into the conical housing and outlet capillary.

The relatively large volume of the aqueous phase droplet (ca. 50 μl) is important in this context, because it has a relatively long residence time in the mouth of the conical housing and outflow fluoropolymer capillary. The segmentation repeatability obtained using peristaltic pumping of the droplet forming phase is generally worse than that obtained using liquid chromatographic pumps, as the dislodging process is negatively influenced by pulsating flow. This is in agreement with results obtained earlier with droplets formed by the organic phase.[38]

The repeatability of the segmentation was improved by using a narrower measuring tube, because longer segments were then obtained. The PVDF device with a confluence compartment made of glass and a conical PVDF housing is preferable because it allows visual checking of the droplet forming process and the homogenization process of the reaction mixture. Finally, it also produces higher measurement precision and sensitivity.

B. Mechanical Segmentors

1. Loop Injector

In some experiments (especially when it is preferable not to use a phase separator) very precise and repeatable segments are needed. In such cases it is crucially important that the segment length remains unaffected by experimental factors.[48,49] Segmentation can then be implemented using a pneumatic or motor-driven loop injector (Figure 12). Very precise and repeatable volumes of one phase can be introduced into a continuous flow of the other phase, the segment length being controlled by the preselected volume of the injector loop only.[48]

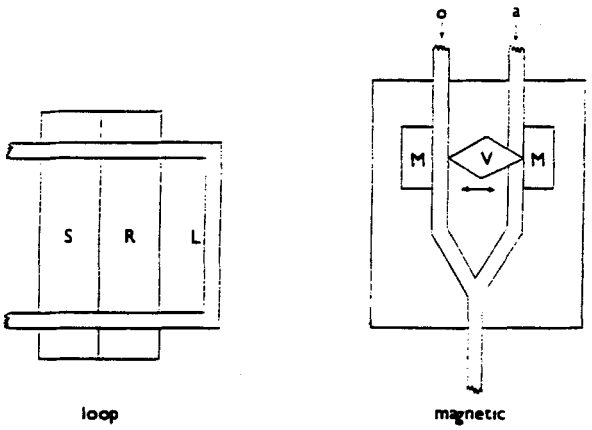


FIGURE 12. A loop injector with a stator (S), a rotor (R) and a capillary loop (L), and a magnetic valve with two electromagnets (M) and a magnetic valve (V).

The segmentation repeatability decreases with increasing length of the extraction capillary (L_{EC}) and increasing flow rate (Q_t). Inadequate segmentation repeatibility often results at very low flow rates, below 0.15 ml min^{-1}. Small droplets of organic or aqueous phase are in these cases formed at either end of the segments as a result of incomplete washing out of the sample loop at the low flow rates. The same phenomenon may be observed at high flow rates, when the narrow (relative to their length) aqueous segments split off small droplets. Alternatively, the thick film of organic solvent formed on the fluoropolymer tubing wall at high flow rates may split off droplets by the "ripple" process.

The segmentation is quite regular ($s_r < 10\%$) over a wide range of $Q_a < 6$ ml min^{-1}, $Q_o/Q_a < 30$, and total length of the extraction coil. A mechanical segmentor, allowing precise timing of alternating segments of organic and aqueous segments, may make it possible to eliminate phase separation altogether.

2. Intermittent Pumping

The introduction of repeatable volumes of one phase into a continuous flow of the other can also be realized by the intermittent pumping principle.[3] In that case, two microcomputer controlled peristaltic pumps equipped with stepper motors can produce a repeatable segmentation with a variable segment volume ratio, provided that large segment volumes are acceptable. Unlike classical liquid-liquid extraction FIA in which the immiscible phases are segmented by continuous segmentors, leading to the formation of segments with random size, a well-defined segmentation pattern was generated through the use of alternately operated stepper-motor driven micro syringe pumps.[56] The resulting uniform segmented flow with approximately 14 μl volume segments allowed subsequent digital "phase separation" using a computer.

3. Magnetic Valve

A magnetic valve system (Figure 12) controlled by a cycle timer can also be used to segment two immiscible solvents. The segment length is controlled by the flow rate and by the cycling frequency, and it can be easily varied over a wide range from millimeters to tens of millimeters by changing these parameters.

The system works repeatable at moderate flow rates up to 2.5 ml min^{-1}, and in a narrow interval of flow rate ratios close to unity (up to $Q_a/Q_o < 2$). The best repeatability ($s_r < 10\%$) is usually achieved in the flow rate range $Q_o = Q_a = 0.2$ to 0.5 ml min^{-1} at a cycling frequency ranging from units to tens per minute. At higher flow rates or higher flow rate ratios, especially when solvents of different viscosity and interfacial tension are used, serious pulsation occurs and the segmentation repeatability decreases rapidly up to tens of percent, compared to a repeatability of units of percent found at $Q_a/Q_o = 1$. Serious pulsation may also occur when the magnetic valve is cycled at a slow cycling frequency.

C. Postsegmentor Introduction of the Sample

A relatively high dispersion occurs in all conventional liquid-liquid extraction FIA systems where samples are introduced into an aqueous carrier stream. Lower dispersion results when samples are introduced into the segmented rather than the unsegmented flow, because the dispersion to which the sample is subjected during transport throught the reaction tube and phase segmentor is eliminated.[47] The sensitivity is bet-

ter and the sampling frequency higher than when samples are introduced prior to segmentation. Also, the calibration graphs are linear over a slightly wider range because the peaks are sharper and higher. The peak shape is also sharper than that obtained in the conventional way, ensuring that dispersion both before reaching the segmentor and in it is one of the major contributors to peak broadening.

IV. CONCLUSIONS

Comparison of several parameters of the tested segmentors shows that the best segmentation pattern and the best segmentation repeatability are produced by the coaxial segmentor, the modified A8-T fitting, and the conventional loop injector (see Table 2).

The T-shape segmentors made of hydrophilic material work according to the wetting and skewing principle at low or moderate flow rates. The length of the segments is governed by the position and orientation of the input and output channels, and by the inner diameter of the input channels and, mainly, of the output channel (at constant inner diameter of the input channels). When fluoropolymer tube inserts are introduced into all branches of the T-piece, the maximum droplet size is limited by the volume of the compartment formed. With Q_a/Q_o near to unity, the length of the aqueous and organic segments are approximately equal when segmentation is regular. The segment lengths depend on the total flow rate Q_t (within a limited range), and decrease with increasing Q_t.

The T-shaped segmentors made of fluoroplastics are in many cases able to produce small and reproducible segments, assuming optimum geometry of the inner capillary system. The length of the segments is determined by the inner diameter of the inlet and outlet tubing. An increase of the inner diameter of the outflow channel results in longer segments with these kinds of segmentors, a prerequisite being that the organic phase can fill the entire diameter of the channel before the organic segment is skewed off by the aqueous flow. At high phase flow rate ratios this is not the case, and the segments are actually formed in the extraction coil by "ripple" segmentation.

T-segmentors work best at low flow rates and at flow ratios Q_a/Q_o close to unity. At higher flow rate ratios the repeatability is inadequate. The ability of the organic solvent to wet the wall material of the segmentor is important in the case of fluoropolymer segmentors in which "ripple" segmentation predominates. The segments are formed in the extraction coil downstream from the segmentor, and segmentation is facilitated by a somewhat pulsating flow.

The major advantages of the fluoropolymer

TABLE 2
Comparison of the Basic Parameters of Different Types of Segmentors

Parameters	A8-T glass	T-glass	T-PVDF	Coaxial	Loop injector	Magnetic valve
Q_a(ml min^{-1})	<4	<4	<3	<8—10	>8	2.5
Q_o(ml min^{-1})	<1.5	<1.6	<1.5	<0.8,1.5[a]	3	2
Q_a/Q_o	<15	<15	<3—5	<40	—	2
L_s(org)	<38	<45	<8	<50	≫	>1
s_r(5)[b]	2.02,11.95[c]	9.05,12.44[c]	12.4	3.88,3.84[d]	—	6[e]
s_r(10)[b]	7.21,24.57[c]	?, 33.1[c]	?, ?	7.46,12.19[d]	—	—

[a] Freon-113, see text for other solvents, 1.2 ml min^{-1} for Freon-113 (Ref. 37).
[b] Relative standard deviation at Q_o = 0.5 ml min^{-1} and at Q_a/Q_o 5 and 10, respectively.
[c] "Ripple" forming principle.
[d] 0.35 and 0.25 mm inlet glass tubing.
[e] At $Q_a = Q_o$ = 0.5 ml min^{-1}, 19% at Q_o/Q_a = 0.5/1.5 ml min^{-1}.

T-piece segmentor are that it is inexpensive and readily available. These segmentors offer relatively consistent segmentation, but the segments are fixed in size. The size is large compared to the smallest segments obtainable with an A8-T fitting. A disadvantage of this segmentor is that it does not direct the flow well; i.e., too high an overpressure applied to the outlet of the segmentor may result in the direction of the flow being reversed in one of the solvent delivery channels. The choice of material is most important (particularly important are the wetting properties of the organic solvents used in liquid-liquid extraction FIA). Teflon is not ideal because changes of the segmentation mode often cause irregularities in the segmentation.[38]

Although the modified A8-T segmentor works very well it cannot be used with standard tubing connectors of the low pressure HPLC type, which is a drawback in routine work. Leakage often occurs at high total flow rates or at high overpressure caused by the phase separator or a restrictor coil, since this segmentor type was designed for low pressure segmented continuous flow analyzer systems. The segmentor can conveniently be used with a T-separator or in a separatorless system, but problems are encountered in systems equipped with membrane phase separators.

The dead volume at the confluence point may be excessive when the organic flow is much smaller than the aqueous flow. The segmentation pattern becomes irregular at high phase flow rate ratios, and, even with careful adjustment, occasional droplets of larger size would be dislodged. Also, relatively large droplets of the organic phase would cling to the entrance and cause undue dispersion.

The modified A8-T fitting has the advantage of producing a variable segment length over a wide range, and of maintaining the flow direction very well. The problems are that the separation between consecutive segments is sometimes not consistent. The segment length can be easily varied by the Q_a/Q_o flow rate ratio, or (better and simpler) by the distance of the fluoropolymer tubes from the end of the platinum capillary, when segmentation is controlled by droplet formation.

All the above-mentioned segmentor types work relatively well at a wide range of flow rates and phase flow rate ratios, but the total volume of the droplets and the segment length are strongly influenced by the flow rate of the aqueous phase. The segment size is also influenced by surface active substances such as anionic surfactants. In some experiments (especially when a phase separator is not desirable), very precise and repeatable segmentation is needed. In such cases it is very important that the segment length remains unaffected by experimental factors.

Segmentation may be achieved by using a loop injector, driven pneumatically or by a motor. With such a device, very precise and repeatable volumes of one phase can be introduced into a continuous flow of the other phase. The volume and the length of the segments are, in principle, controlled by the preselected volume of the injector loop only. Conventional loop injectors operated by a cycling motor with adjustable filling/draining times and a brief intermittent period can be used at low flow rate ratios or for producing long segments. Phase separation may be eliminated by the use of a specially designed mechanical segmentor, making possible precise timing of the segmented stream passing through the detector cell.

A coaxial segmentor seems to be more convenient in these applications, offering, in general, comparable segmentation repeatability to the loop injector over a wide range of flow rates of the two solvents. The use of such a segmentor does not introduce any additional moving parts into the liquid-liquid extraction FIA system. The results so far show that a considerable improvement in performance can be obtained with this new design. Apart from giving a two- to threefold rise in the segmentation repeatability, a coaxial segmentor can be operated over a wider range of flow rates and phase flow rate ratios than previous segmentor designs. A further advantage is that it behaves according to a simple and well established theory, making it possible to calculate in, beforehand, the parameters of the segmentation pattern.

The segment lengths of the two immiscible phases can be varied over a relatively wide range from units to tens of millimeters (from 3 to 50 mm for the organic phase and from 10 to 300 mm for aqueous phase), simply by changing the

inner diameter of the inlet glass capillary and by changing the flow rates of the two solvents. The segment length of the organic phase can easily be predicted, and is independent of the majority of the factors influencing segmentation in the other segmentor types, such as the flow rates of the two solvents, the flow rate ratio, etc.[38]

The segmentation repeatability depends on how clean the walls of the mixing compartment and the flat end of the inlet capillary are. The segmentation pattern is changed by lipophilic substances present on the walls of the confluence compartment. From time to time the walls of the conical housing become covered by a thin film of organic phase, due to irregularities of the segmentation produced by small droplet formation at the end of the regular segment, or to the unrepeatable volume of the organic solvent clinging on the surface of the conical part of the outlet channel. Segmentation repeatability may be decreased by as much as several tens of a percent, relative to the usual repeatability s_r. Careful washing of these parts with ethanol and grinding the capillary end from time to time are important for obtaining regular segmentation, especially after a long dry storage period. Also, air bubbles must be removed from all parts of the segmentor, especially from the space between the glass capillary and the mixing compartment walls.

The relatively large dead volume of the conical chamber (around 0.1 ml) increases sample and reagent consumption, and the risk of contamination of the samples by carry over. These factors are less serious at high aqueous flow rates and high flow rate ratios, preferable in some practical applications such as AAS and when high enrichment factors are desirable.[37] The dead volume can obviously be minimized by miniaturization of the confluence compartment. The distance of the inlet glass capillary from the conical housing and the inner and outer cross sectional diameters of the confluence chamber have to be optimized for the desired droplet size, as they also greatly influence the cutoff process. The nominal volume of the conical part has to be greater than the actual droplet volume in order to avoid contaminating the walls of the confluence compartment with organic phase.

The compact PVDF body segmentor has the disadvantage of being opaque, and, consequently, visual checking of the segmentation process is impossible. Such checks are important when the walls of the confluence chamber can become covered by lipophilic substances. Perspex is attacked by most common organic solvents and the conical compartment is difficult to machine to the desirable wall smoothness. Both compact body segmentors are less sensitive to leakage at higher back overpressure, and thus are more suitable for systems with membrane phase separators.

Coaxial segmentors usually work better at moderate or high than at low flow rates, because the droplets are often destroyed on the walls of the conical chamber, resulting in decreased segmentation repeatability. A hydrophilic construction material such as glass can be recommended for the confluence chamber (particularly the conical part of the chamber).

The geometry of the confluence chamber and especially of the conical part should be optimized to decrease the dead volume of the segmentor and to increase the segmentation repeatability. A narrow angle in the conical part of the confluence chamber increases the risk that the droplet may split off small particles, which may then cling on the walls of the compartment or the conical housing. This phenomenon occurs more frequently in conical chambers made of Perspex, probably because they are difficult to machine to the desirable wall smoothness of the conical part, and because the wetting ability of water is less on Perspex than on glass. The phenomenon was rarely observed in the PVDF conical housing, and never in the integral conical compartment made of glass.

The use of the multichannel dropping segmentor in liquid-liquid extraction FIA does not introduce any additional moving part into the completely closed FIA system. The segmentation repeatability and segment volumes are mostly comparable with the repeatability of the liquid sample introduction using a loop injector. The segmentor works reliably over a wide range of flow rates and flow rate ratios of both phases, and is easy to handle.

The analytical signal obtained using the multichannel dropping segmentor is comparable with those obtained by steady state measurements in which aqueous solutions of extractable reaction

product are introduced directly into the flow of organic phase. An instantaneous extraction of the reaction product speeds up the reaction rate when an extractable product is formed from nonextractable reaction components. No serious lengthening of the extraction/reaction coil is needed when a dropping segmentor is used for the instantaneous preparation of the reaction mixture and for the direct introduction of the preprepared extractable product solution into the system.

Direct "in-segmentor" introduction of the sample and organic analytical reagent by a multichannel segmentor can eliminate the reaction/mixing coil, and thus most problems connected with its use. The application of the multichannel dropping segmentor in liquid-liquid extraction FIA can also eliminate a phase separator and a sample injector, and thus significantly simplify the manifold.

Several separate inlet capillaries meeting at a common confluence point of very small diameter can thus be used for dilution (changing the Q_1/Q_2 for sample and water), reagent introduction, titration (changing the Q_1/Q_2 for sample and titrand), calibration (changing the concentration or the Q_1/Q_2), gradient formation (using two pumps for gradient, or a gradient pump), kinetic measurements (different Q_t or L_{EC}), etc.

The high degree of reproducibility of the segmentation obtainable with this kind of segmentor can improve the precision of signal measurement, and may frequently simplify signal treatment.

Recent improvements in phase segmentors open a whole new set of possibilities where studies of liquid-liquid extraction systems can be performed directly on each individual segment of the organic and aqueous phase, thus avoiding the problems connected with phase separation. Finally, it may even be possible to eliminate the phase separation process altogether if the segmentation reproducibility is sufficient to allow precise timing of the measurement periods.

V. SUMMARY

The paper gives an overview of the theory and praxis of phase segmentation and phase segmentors used to segment two immiscible liquid phases in continuous flow analysis. Two different segmentation mechanisms have been used depending on the experimental conditions, the segmentor geometry, and the material of the inner capillary system of the segmentor. Segmentors of T-shape made of fluoroplastics and glass (A4-T fitting), a modified glass A8-T fitting, W- and Y-pieces of a different geometry, a four way fitting, and a recently introduced coaxial (dropping) segmentor of different geometries are discussed. A single loop injector, stepper-motor driven syringe pumps, a magnetic valve and multichannel coaxial segmentor have also been suggested as devices for introducing definite volumes of one phase into the continuous flow of the other one. The segment length can be varied over a wide range from units to tens of millimeters using different segmentors.

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REFERENCES

1. **Karlberg, B. and Thelander, S.,** Extraction based on flow injection principle. I. Description of the extraction system, *Anal. Chim. Acta,* 98, 1, 1978.
2. **Bergamin, F. H., Madeiros, J. X., Reis, B. F., and Zagatto, E. A. G.,** Solvent extraction in continuous flow injection analysis. Determination of molybdenum in plant material, *Anal. Chim. Acta,* 101, 9, 1978.
3. **Růžička, J. and Hansen, E. H.,** *Flow Injection Analysis,* J. Wiley, New York, 1988, pp. 186-192.
4. **Valcarcel, M. and Luque de Castro, M. D.,** *Flow Injection Analysis. Principles and Applications,* J. Wiley, New York, 1978.
5. **Valcarcel, M. and Luque de Castro, M. D.,** Continuous separation techniques in flow injection analysis (A review), *J. Chromatogr.,* 393, 3, 1987.
6. **Karlberg, B.,** The theoretical and practical development of flow injection extraction, *Anal. Chim. Acta,* 214, 29, 1988.
7. **Cantwell, F. F. and Sweileh, J. A.,** Hydrodynamic and interfacial origin of phase segmentation in solvent extraction/flow injection analysis, *Anal. Chem.,* 57, 329, 1985.
8. **Nord, L. and Karlberg, B.,** Extraction based on the flow injection principle. V. Assessment with a mem-

brane phase separator for different organic solvents, *Anal. Chim. Acta,* 118, 285, 1980.
9. **Persaud, G., Xiu-min, T., and Cantwell, F. F.,** Behaviour of solute adsorbed at the liquid-liquid interface during solvent extraction with porous-membrane phase separators, *Anal. Chem.*, 59, 2, 1987.
10. **Fossey, L. and Cantwell, F. F.,** Characterization of solvent extraction/flow injection analysis with constant pressure pumping and determination of procyclidine hydrochloride in tablets, *Anal. Chem.*, 54, 1693, 1982.
11. **Sweileh, J. A. and Cantwell, F. F.,** Use of peak height for quantification in solvent extraction/flow injection analysis, *Can. J. Chem.*, 63, 2559, 1985.
12. **Bäckström, K., Danielsson, L.-G., and Nord, L.,** Dispersion in phase separators for flow injection extraction systems, *Anal. Chim. Acta,* 187, 255, 1986.
13. **Nord, L. and Karlberg, B.,** Extraction based on the flow injection principle. VI. Film formation and dispersion in liquid-liquid segmented flow extraction systems, *Anal. Chim. Acta,* 164, 233, 1984.
14. **Lucy, C. A. and Cantwell, F. F.,** Kinetics of solvent extraction-flow injection analysis, *Anal. Chem.*, 61, 107, 1989.
15. **Nord, L., Bäckström, K., Danielsson, L.-G., Ingman, F., and Karlberg, B.,** Extraction rate in liquid-liquid segmented flow injection analysis, *Anal. Chim. Acta,* 194, 221, 1987.
16. **Lucy, C. A. and Cantwell, F. F.,** Mechanism of extraction and band broadening in solvent extraction-flow injection analysis, *Anal. Chem.*, 61, 101, 1989.
17. **Fossey, L. and Cantwell, F. F.,** Determination of acidity constants by solvent extraction/flow injection analysis using a dual-membrane phase separator, *Anal. Chem.*, 57, 922, 1985.
18. **Rossi, T. M., Shelly, D. C., and Warner, I. M.,** Optimization of a flow injection analysis system for multiple solvent extraction, *Anal. Chem.*, 54, 2056, 1982.
19. **Kawase, J., Nakae, A., and Yamanaka, M.,** Determination of anionic surfactants by flow injection analysis based on ion-pair extraction, *Anal. Chem.*, 51, 1640, 1979.
20. **Kawase, J.,** Automated determination of cationic surfactants by flow injection analysis based on ion-pair extraction, *Anal. Chem.*, 52, 2124, 1980.
21. **Lucy, C. A. and Cantwell, F. F.,** Simultaneous determination of phenylephrine hydrochloride and pheniramine maleate in nasal spray by solvent extraction flow injection analysis using two porous membrane phase separators and one photometric detector, *Anal. Chem.*, 58, 2727, 1986.
22. **Fujiwara, K.,** Application of a wave-guide capillary cell in the determination of copper by flow injection analysis, *Anal. Chim. Acta,* 212, 245, 1988.
23. **Gallego, M., Silva, M., and Valcarcel, M.,** Determination of nitrate and nitrite by continuous liquid-liquid extraction with a flow-injection atomic-absorption detection system, *Fresenius' Z. Anal. Chem.*, 323, 50, 1986.
24. **Gallego, M. and Valcarcel, M.,** Indirect atomic absorption spectrometric determination of perchlorate by liquid-liquid extraction in a flow injection system, *Anal. Chim. Acta,* 169, 161, 1985.
25. **Martinez-Jimenez, P., Gallego, M., and Valcarcel, M.,** Indirect atomic absorption spectrometric determination of some cationic surfactants by continuous liquid-liquid extraction with tetrathiocyanatocobaltate, *Anal. Chim. Acta,* 215, 233, 1988.
26. **Sahleström, Y. and Karlberg, B.,** Flow injection extraction with a microvolume module based on integrated conduits, *Anal. Chim. Acta,* 185, 259, 1986.
27. **Bengtsson, M. and Johansson, G.,** Preconcentration and matrix isolation of heavy metals through a two-stage solvent extraction in a flow system, *Anal. Chim. Acta,* 158, 147, 1984.
28. **Farran, A. and De Pablo, J.,** Continuous-flow extraction of organophosphorus pesticides coupled on-line with high-performance liquid chromatography, *Anal. Chim. Acta,* 212, 123, 1988.
29. **Tyson, J. F., Adeeyinwo, C. E., Appleton, J. M. H., Bysouth, S. R., Idris, A. B., and Sarkissian, L. L.,** Flow injection techniques of method development for flame atomic absorption spectrometry, *Analyst (London),* 110, 487, 1985.
30. **Kubáň, V., Komárek, J., and Čajkova, D.,** Determination of copper by flow injection analysis with flame atomic absorption detection after liquid-liquid extraction preconcentration, *Collect. Czech. Chem. Commun.*, 54, 2683, 1989.
31. **Ogata, K., Taguchi, K., and Imanari, T.,** Phase separator for flow injection analysis, *Anal. Chem.*, 54, 2127, 1982.
32. **Ogata, K., Taguchi, K., and Imanari, T.,** Determination of orthophosphate by flow injection analysis based on solvent extraction (in Japanese), *Bunseki Kagaku,* 31, 641, 1982.
33. **Kato, T.,** Flow injection spectrophotometric determination of enalapril in pharmaceuticals with bromothymol blue, *Anal. Chim. Acta,* 175, 339, 1985.
34. **Del Valle, M., Alonso, J., Bartoli, J., and Marti, I.,** Spectrophotometric determination of low levels of anionic surfactants in water by solvent extraction in a flow injection system, *Analyst (London),* 113, 1677, 1988.
35. **Atallah, R. H., Christian, G. D., and Hartenstein, S. D.,** Continuous flow solvent extraction system for the determination of trace amounts of uranium in nuclear waste reprocessing solutions, *Analyst (London),* 113, 463, 1988.
36. **Atallah, R. H., Růžička, J., and Christian, G. D.,** Continuous solvent extraction in a closed-loop system, *Anal. Chem.*, 59, 2909, 1987.
37. **Bäckström, K. and Danielsson, L.-G.,** On the de-

sign of a continuous flow two step extraction work-up system for graphite furnace AAS, *Anal. Chim. Acta*, 232, 255, 1990.
38. **Kubáň, V., Danielsson, L.-G., and Ingman, F.,** The design of a coaxial segmentor for liquid-liquid extraction flow injection analysis, *Anal. Chem.*, 62, 2026, 1990.
39. **Motomizu, S. and Oshima, M.,** Spectrophotometric determination of phosphorus as orthophosphate based on solvent extraction of the ion associate of molybdophosphate with malachite green using flow injection, *Analyst (London)*, 112, 295, 1987.
40. **Kubáň, V., Danielsson, L.-G., and Ingman, F.,** Comparison of segmentors for liquid-liquid extraction flow injection analysis, *Talanta*, 37, 1169, 1990.
41. **Sahlestrom, Y. and Karlberg, B.,** An unsegmented extraction system for flow injection analysis, *Anal. Chim. Acta*, 179, 315, 1986.
42. **Hirai, Y. and Tomokumi, K.,** Extraction-spectrophotometric determination of anionic surfactants with a flow injection analysis, *Anal. Chim. Acta*, 167, 409, 1985.
43. **Imasaka, T., Harada, T., and Ishibashi, N.,** Fluorimetric determination of gallium with lumogallion by flow injection analysis based on solvent extraction, *Anal. Chim. Acta*, 129, 196, 1981.
44. **Fukamachi, K. and Ishibashi, N.,** Flow injection-atomic absorption spectrometry with organic solvents, *Anal. Chim. Acta*, 119, 383, 1980.
45. **Audunsson, G.,** Aqueous/aqueous extraction by means of a liquid membrane for sample cleanup and preconcentration of amines in a flow system, *Anal. Chem.*, 58, 2714, 1986.
46. **Kina, K., Shiraishi, K., and Ishibashi, N.,** Ultramicro solvent extraction and fluornimetry based on the flow injection method, *Talanta*, 25, 295, 1978.
47. **Toei, J.,** Segmented flow injection-solvent extraction analysis, *Analyst (London)*, 113, 1861, 1988.
48. **Kubán, V., Danielsson, I.-G., and Ingman, F.,** The Multichannel Dropping Dispenser, a New Tool for Stream Mixing in Segmented Continuous Flow Analysis (IFCCA), Talanta, submitted.
49. **Dasgupta, P. K. and Lei, W.,** Solvent extraction in continuous flow systems with intelligent zone sampling, *Anal. Chim. Acta*, 226, 255, 1989.
50. **Motomizu, S., Oshima, M., and Kuroda, T.,** Spectrophotometric determination of anionic surfactants in water after solvent extraction coupled with flow injection, *Analyst (London)*, 113, 747, 1988.
51. **Coulson, J. M. and Richardson, J. F.,** *Chemical Engineering*, 3th Ed., Vol. 1., Pergamon Press, 1976.
52. **Bartle, K. D., Wooley, C. L., Markides, K. E., Lee, M. L., and Hansen, R. S.,** *JHRaCC*, 10, 128, 1987.
53. **Nekimken, H. L., Smith, B. F., Jarvinen, G. D., Peterson, E. J., and Jones, M. M.,** Computer controlled flow injection analysis system for on-line determination of distribution ratios, *Anal. Chem.*, 60, 1390, 1988.
54. **Apffel, J. A., Brinkman, U. A. Th., and Frei, R. W.,** Design and application of a post-column extraction system compatible with miniaturized liquid chromatography, *Chromatographia*, 18, 5, 1984.
55. **Karlberg, B., Johansson, P. A., and Thelander, S.,** Extraction based on the flow injection principle. II. Determination of codeine as the picrate ion-pair in acetylsalicylic acid tablets, *Anal. Chim. Acta*, 104, 21, 1978.
56. **Thommen, C., Fromageat, A., Obergfell, P., and Widmer, H. M.,** Application of a capillary flow cell to sophisticated flow injection analysis, *Anal. Chim. Acta*, 234, 141, 1990.