

exits the fiber at end face 24, where it is collected by fluorimeter 7.

If the interspace between the fiber and tube is completely filled with sample fluid, at least adjacent activated region 36, the volume of sample instantaneously opposite active region 36 of the fiber is given by

$$V = L \pi (D^2 - d^2) / 4 \quad (2)$$

where L is the length of active region 36 of fiber 12, D is the inside diameter of the capillary, and d is the diameter of the fiber. The volume observed is given by a similar relation, with the quantity (D-d) replaced by the effective thickness of the observed zone. The incubation time, necessary to scavenge a static volume as given by equation (2) is

$$t = k [(D-d)/(2s)]^2 \quad (3)$$

where k is a dimensionless chemical reaction constant (depending, e.g., on the density of binding sites on the fiber, the probability a reacting species in the sample encounters a binding site, etc.) and s is the Brownian drift. If D and d are in cm and s is in cm sec⁻¹, then t will be in seconds. The value of k is between about 1 and 10 for all practical purposes.

If the sample is allowed to flow past the active region so as to move through the length of the active region for a period equal to the static incubation time, then, from equation (3), we find

$$v = L/t = 4Ls^2/k(D-d)^2 \quad (4)$$

where v is the flow velocity.

Combining equations (2) and (3), we find the volume scavenged per unit incubation time, V/t, to be

$$V/t = \pi L s^2 (D+d)/k(D-d) \quad (5)$$

Equation 5 defines the actual effective sample volume flow per unit time. A larger flow rate will not permit completely scavenging the sample, while a smaller volume flow rate will result in the sample being opposite the active region for a longer than the necessary time.

It may be seen from equation (5) that the value of V/t may be maximized by increasing the length of the active region, and by appropriately adjusting the difference between D and d to be as small as possible. It should be noted however that it is not necessary to constrict physically the capillary diameter. The volume due to additional diameter will then not be sampled, but waste of some sample is usually tolerable and preferable to having to build apparatus with extremely small tolerance requirements.

A large capillary volume may be used with a sample flow velocity larger than that given by equation (4) so as to produce an effective capillary diameter, D_{eff} , given by

$$D_{eff} = d + s (L/kv)^{1/2} \quad (6)$$

In such an arrangement, k will be smaller, and therefore the optimal volume to time ratio, V/t, will be larger because of the scavenging of sample outside of the effective diameter, D_{eff} , as well as the lack of a need to wait for the complete scavenging of the sample.

As an example, for $D=4d$, $(D+d)/(D-d)=1.66$, while for $D=1.1d$, $(D+d)/(D-d)=21$. The latter is a 12.6 fold improvement.

It will also be understood that fiber 12 and tube 14 might be of other than right circular cylindrical shape, and that, for instance, they might be a pair of parallel plates with a capillary spacing therebetween.

Since these and certain other changes may be made in the above apparatus and method without departing from the scope of the invention herein involved, it is intended that all matter contained in the above description or shown in the accompanying drawing shall be interpreted in an illustrative and not a limiting sense.

What is claimed is:

1. In a method for performing assays involving measurement of fluorescence excited in a fluid sample by an evanescent wave at a surface region of a totally internally reflecting substrate, the improvement comprising: exciting said fluorescence while flowing through an enclosure of fixed dimensions bounded in part by said region, a sufficient volume of said sample to maintain said enclosure filled with flowing sample.
2. The improvement according to claim 1 wherein said surface region is coated with a coating having a plurality of sites, each site being capable of having attached thereto a selected moiety of a chemical complex, said complex being capable of fluorescing when excited by said evanescent wave, including the step of maintaining the rate (v) of flow of said volume of sample at least at L/t;

where L is the length of said region, measured in the direction of the flow of said sample past said region; and

t is the time required for said coating to scavenge a static volume of said sample filling said enclosure.

3. The improvement as defined in claim 1 wherein at least the minimum cross-section dimension of said enclosure is of capillary dimensions with respect to said fluid sample.

4. In apparatus for assaying a fluid sample, said apparatus including a totally internally reflecting substrate transmissive to radiation capable of exciting fluorescence in fluorescent material disposed at least on a portion of the surface of said substrate, said substrate also being transmissive to said fluorescence, and means spaced from at least said portion of said surface of said substrate so as to define an enclosure bounded in part by said portion, the improvement comprising:

means for flowing substantially continuously said sample over at least said portion of said surface and in sufficient volume to fill said enclosure, while exciting said fluorescence.

5. In apparatus as defined in claim 4 wherein said substrate is an optical fiber.

6. In apparatus as defined in claim 5 wherein said enclosure is a tube coaxially spaced apart from and surrounding said fiber.

7. In apparatus as defined in claim 6 wherein the interspace between said tube and fiber is of capillary dimensions.

8. In apparatus as defined in claim 4 including a fluorimeter disposed for measuring fluorescence excited by said evanescent wave.

9. In apparatus as defined in claim 4 including means for controlling at least the rate of flow of said sample.

10. In apparatus as defined in claim 9 wherein said means for controlling said rate of flow comprises fluid pumping means.

11. In apparatus as defined in claim 9 wherein said portion is coated with a coating having a plurality of sites, each of said sites being capable of having attached