"Architextured" Fluid Management of Biological Liquids

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Novel "architextured" liquid-spreading and capillary flow systems are described as an adjunct to diagnostic procedures that range from the fully automated to techniques requiring no instrumentation. These systems were developed because of the observation that local features of macroscopic surfaces—roughness and chemical heterogeneity—influence both the spreading rate and equilibrium shape of liquid drops on solid surfaces. By use of organized patterns of surface structure complemented by surface chemical modification, this uncontrolled and undirected capillary flow is recast into flow behavior that is remarkably reproducible. Flow fronts are engineered to have prescribed shapes, and flow rates that are insensitive to the rheological properties of the biological liquids are obtained. To demonstrate the utility of fluid-management principles, we describe the implementation of architextured capillary configurations in microvolulme liquid dispensers and potentiometric analytical systems.

Additional Keyphrases: control of capillary flow, liquid spreading rheological properties of biofluids

The recent coupling of chemistry and biotechnology, and the extraordinary advances in immunochemistry, offer exciting diagnostic opportunities, including a cost-effective technical basis for movement toward alternative-site medicine; that is, from the hospital to the doctor's office to the home. For example, through clinical technology, a patient can monitor the concentration of certain therapeutic drugs in the body by using only one drop of whole blood, without any instrumentation, at home.

A common element in most clinical chemistry determinations is the requirement that the user successfully integrate the test liquid into the analytical element. This is a nontrivial and potentially constraining task, especially for devices designed to use small liquid volumes, which must be introduced with precision by users having various degrees of skill for such manipulations. Other complications include the need to protect the user from any potentially toxic reagents in the assay and to isolate the reagents and the test liquid from contamination in the environment. In this paper we will provide insight, by example, into the realities of our experiences at Eastman Kodak Co. in handling biological liquids. We will also propose methodologies that can turn seemingly random acts of nature into options and opportunities in the controlled surface spreading of liquids by capillary forces.

Consequences of Uncontrolled Flow

Dispenser Systems

Motivated by the introduction of dry reagent carriers such as multilayer thin-film elements (1), instrumentation innovators are developing for the clinical laboratory mechanized micro-techniques that can deliver precisely metered droplets of biological liquid onto appropriate substrates. A common method for dispensing liquid onto a test element for analysis is aspiration, followed by pressurized dispensing. That is, a disposable pipet tip is immersed into a sample liquid being held in a suitable container and the pressure in the pipet is reduced, drawing in the required amount of liquid. The pipet tip is then withdrawn from the liquid and moved to the station holding the test element. At that station, increased pressure is applied to the interior of the pipet sufficient to dispense the desired amount of sample liquid from the tip. If only a few microliters of liquid is required for the chemical assay, then the delivered volume amounts to a single drop on the tip of the pipet. To prevent cross-contamination between samples, the pipet tip is removable and disposable.

As the dispenser tip is withdrawn from the sample container, the liquid becomes entrained as a thin liquid film on the exterior surface of the tip. The presence of this residual liquid at the tip's orifice can result in significant error in dispensed volumes or might cause the dispensed liquid volume to move up the exterior surface of the pipet tip rather than to move onto the surface of a test element. Over time, the residual film will usually drain downward to the end of the tip, as shown in Figure 1, and ultimately will overwhelm the tip's ability to support both the accumulated film and the metered volume of liquid it is designed to deliver. The result is uncontrolled shedding of liquid, which contaminates the hardware and invalidates the assay.

It is tempting to think that this problem can be solved easily by fabricating the pipet tip out of a nonwettable material so that residual liquid will not cling to its surface. However, biological fluids are proteinaceous and, when aspirated, chemically modify even Teflon polymer-coated surfaces through protein adsorption, rendering these surfaces wettable.

Fig. 1. Failure in a micrometering device caused by drainage of entrained liquid on the external surface of a prewetted dispensing tip.
Liquid-Transport Devices

Various passive liquid-transport devices, that is, those free of moving parts, have been designed to deliver liquid over defined surface areas. For example, it is well known that liquids will flow spontaneously between two smooth, flat, polymer surfaces separated by a thin gap serving as a capillary flow channel, as long as the liquid wets the polymer surface. An elementary configuration is shown in Figure 2, in which liquid is introduced through a centrally located aperture in one of these plates and flows between the surfaces by the action of surface tension until either the liquid supply is exhausted or an obstruction in the channel is encountered. If no obstructions are present and the gap width is uniform, an orderly, symmetric radial flow pattern should be expected.

In reality, however, the flow is characteristically uncontrolled and undirected across these surfaces, reflecting the strong interaction of the liquid with the solid surfaces. The consequences of such flow include the possibility of forming trapped air pockets and thus incomplete wetting of the surface. Air pockets are particularly undesirable in automatic devices for microscopic examination of the liquid or the wetted surfaces because their existence is not easily detected and their interference with the analytical test cannot be accounted for.

This uncontrolled fluid flow situation becomes even worse if we try to incorporate chemicals relevant to the analytical test in a gelatin coating, placed on the inside surfaces of these polymer sheets. Introducing liquid into this capillary sandwich configuration swells the matrix of chemical and polymer, reducing capillary spacing and thus the rate of liquid transport. If the initial channel spacing is too small, capillary closure occurs, limiting flow extension and accentuating random, rapidly extending, irregular streaming, which limits flow quality (see Figure 3). Increasing the initial capillary gap to 5 mils (0.005 inch, 127 μm) improves the extent of flow but clearly does little to improve uniformity in liquid distribution. Part of the randomness in this flow profile is due to the rapid swelling of gelatin when it contacts water. But other problems include differential wettability of the inside surfaces of the channel caused by the chemical alteration of the coating as it interacts with the test liquid.

![Idealized Flow Profile](image)

Fig. 2. A generic capillary “sandwich” for distributing small volumes of liquid over large surface areas
The sandwich is constructed of two flat sheets of polymer separated by a uniformly thin gap. Fluid is introduced through a centrally located aperture in the top sheet.

![Actual Flow Profile](image)

![Graph](image)

Fig. 3. Highly irregular flow within a capillary sandwich when the inside surface of one of the polymer sheets is coated with a thin film of gelatin. Fluid penetration into the gap between the sheets is increased if the gap width is increased to 5 mils, but the irregularities in fluid distribution are not eliminated or improved (1 mil = 0.001 in. = 25.4 μm)

The frustrating feature of this example is that this sandwich configuration has great potential for distributing small quantities of viscous, nonideal fluids over large distances and surface areas in a protected environment. But the technique is semiquantitative, at best, if the movement of the liquid boundary is random and unpredictable. Thus, the challenge is to understand the causal mechanisms leading to random liquid distribution and then to learn how to exploit nature’s way of managing the flow of microvolumes of biological liquids.

Causal Mechanisms

The nature and rate of spreading of liquids over solid surfaces depend on two fundamental concepts: wettability and surface tension. Ideally, the wettability of a solid surface by a liquid can be characterized uniquely by the contact angle that the liquid surface (gas/liquid interface) makes with the solid surface (liquid/solid interface). A liquid placed on a smooth, clean, solid surface generally will remain in the form of a drop, having a definite angle of contact between the liquid and solid phases (2a). If this contact angle is <90°, such as with water on glass, the solid is considered to be wet by the liquid. If the contact angle is substantially >90°, e.g., water on Teflon polymer, the solid is not wet by the liquid. Cases where the contact angle is near 90°, such as water on human skin, are called transitional.

Unfortunately, in most attempts to measure the contact angle for a specific liquid/solid pair, one quickly discovers that the result depends critically on the cleanliness of the solid surface, its smoothness, and even the volume of the drop dispensed onto the surface. Real solid surfaces, such as those used in manufactured capillary systems, are not composed of ideal elements; that is, the surfaces are not perfectly smooth or chemically homogeneous and thus there is no single, unique value for the contact angle between the liquid and our solid surfaces (2b).

The degree of nonideality in a given situation is readily observed by placing a droplet of liquid on an inclined plane, as shown in Figure 4. Acted on by gravity, the stationary drop is distorted, creating two independent contact angles. The contact angle at the front portion of the drop (the advancing angle) is always larger than that at the rear of the drop (the receding angle). The difference between these two angles at the degree of inclination required to cause the drop to slide is a measure of the degree of nonideality in the system (3, 4). The phenomenon is termed "contact angle hysteresis." If the surface was ideal, with only one possible
contact angle, this static condition would never be achieved; rather, the drop would always slide off the surface, no matter how shallow the slope of the plate.

An important property that influences the spreading of a liquid drop on a solid is the surface-free energy of the solid. For example, the surface of Teflon polymer has a low surface energy and resists being covered by liquids such as water. However, treating this surface with a monomolecular film of protein creates an entirely different wetting behavior—one that is much more favorable towards wetting.

Another cause of contact angle variability, and the bane to surface chemists, is the rough nature of common surfaces. Although most surfaces appear smooth, they are actually randomly rough, with microscopic features such as hils, lines, or point defects randomly distributed in arbitrary orientations. Microscopic heterogeneities in the surface promote contact angle hysteresis (and make wettable surfaces appear more wettable) (5).

In summary, the chemical composition of the solid surface (only a few molecules thick) and the degree of microscopic roughness play a dominant role in determining the extent to which a given liquid will wet a solid. Whether or not a solid has been pre-wet by prior exposure to the liquid also can be a critical factor, because in the prior exposure a surfactant film may have been deposited, which can transform the surface chemistry. Finally, spatial variations in either surface composition or roughness can lead to gross irregularities and unpredictability in the flow of liquids over such surfaces, even though the surface nonuniformities are microscopic.

The other fundamental concept germane to capillary transport processes is surface tension. Surface tension is simply the force that acts at a tangent to the surface and perpendicular to any line drawn within the surface. The most common manifestation of surface tension is the spontaneous rise of liquid in a capillary tube, as illustrated in Figure 5. The speed with which a liquid rises in a capillary tube and the height of the liquid at equilibrium are determined by the magnitude of the capillary driving pressure, which is proportional to the product of surface tension γ and the cosine of the contact angle θ (2E):

\[ \text{capillary driving pressure} = 2 \gamma \cos \theta r \]

where r is the radius of the capillary tube. Thus, as the surface tension increases or the contact angle decreases, the capillary driving pressure increases. When conditions are less favorable for wetting, the magnitude of the capillary driving pressure is reduced, and can even become negative when the contact angle is >90°, such as with a tube constructed of Teflon polymer. That is, water will not rise in that tube. On the other hand, if the surface of the Teflon polymer tube is chemically modified with a protein film to make the surface wettable, the capillary driving pressure becomes strongly positive, and the rate of movement (uptake) of water is nearly equal to that in a glass capillary.

**Fluid Management**

The local rate of movement of a gas/liquid interface in a capillary-flow channel is determined by the surface tension of the interface and the local wettability of the solid surfaces. If construction materials are chosen arbitrarily, certain test liquids may enter the channel and flow freely, whereas other test liquids will not, being unable to wet the solid. Among those liquids that do enter the channel, the rate of flow may vary greatly because of difference in surface tension and contact angle. Furthermore, even if fluid flow occurs spontaneously, the flow most likely will be irregular because certain portions of the surface will be more wettable than other portions, and local physical imperfections will dominate the flow behavior. Even if the surfaces are perfectly smooth and chemically homogeneous, problems can arise from minor variations in the assembly process, leading to nonuniformities in (e.g.) the spacing between the solid surfaces. The local resistance to flow is greatest in the narrowest regions; thus, gap nonuniformities trigger flow irregularities.

The goal of fluid management is to defeat these inevitable factors that produce unacceptable flow patterns in capillary-flow systems. The philosophy is to impose macroscopic physical and chemical "defects" in the solid surfaces that will overwhelm the microscopic irregularities and force the liquid to flow by capillary action in a controlled fashion. Thus fluid management means the ability to control the transfer of microvolumes of biological fluids with respect to meniscus shape, flow front, velocity, and spatial extent in capillary-driven flow configurations. Our unique approach to fluid management in capillary-flow systems involves the use of organized patterns of surface structure complemented by surface chemical modification, a methodology we term "architecture."

For example, to minimize the impact of microscopic roughness and random physical imperfections on the spreadability of a liquid over a solid, we can emboss a pattern of parallel, linear grooves approximately 10-μm deep into a plastic sheet (see Figure 6). A droplet of liquid placed on this architextured surface will elongate dramatically along the axis of the grooves (Figure 7). The mechanism for this elongation appears to result from unhindered spreading along the grooves, which serve as capillary chan-
Microscopic Roughness vs. Macroscopic Roughness

Fig. 6. Macroscopic patterns of texture, in the form of parallel V-grooves embossed on the surface of polymer sheets, overwhelms the influence of microscopic surface imperfections and forces liquids to spread in a controlled fashion over the solid surface.

tannels and inhibit any spreading across grooves because of their sharp edges (6, 7). Both the magnitude and the form of this distortion of the droplet are predictable and can be controlled through selection of the macroscopic pattern of texture and the free energy of the surface of the materials.

Recognizing that preferential wetting in a predefined direction is achieved through the application of asymmetric patterns of macrotexture, we have expanded on this idea by creating a three-dimensional capillary device, as defined in Figure 8, having embossed patterns of texture on opposing faces, separated by a thin gap. Introducing fluid into this system produces flow patterns that are controlled by the synergistic interaction of macrotextured plates with the fluid. In particular, when the V-groove patterns are orthogonal, the resulting flow pattern is square-shaped with straight sides and remarkably sharp corners. The final dimensions of the free liquid boundary are determined by the volume of liquid delivered to the capillary sandwich.

This architextured flow-configuration provides, at all phases of the process, orderly patterns of fluid entry and transport. Further, the flow profile is independent of the liquid used—whether the liquid has a high or low surface tension, has a high or low viscosity, or is whole blood instead of serum. Therefore, the application of asymmetric, regularly rough, patterned surfaces becomes a logical transport configuration for achieving controllable and preferential fluid spreading (8).

Applications of Architextured Liquid Distribution Systems

Ion-Selective Electrode Fluid Bridge

Disposable test devices developed for potentiometric analysis of blood serum with use of two identical ion-selective electrodes are an ideal example of the applicability of this technology. The electrodes in such a device are overlaid with the fluid-distribution system, or bridge, that provides for ionic flow between a drop of test liquid and a drop of reference liquid. The bridge also has apertures that allow the test and reference liquids to contact their respective electrodes. An electrometer placed in contact with both electrodes detects a potential that is proportional to the difference in activity and, therefore, to the concentration of the ion being measured. The fluid bridge must be capable of both distributing viscous biological fluids in orderly patterns and controlling the rate of liquid advance to prevent short-circuiting an electrode with the wrong liquid. Further, the configuration must be compatible with the creation of a stable liquid/liquid interface to complete the electrical circuit.

The three-dimensional architextured capillary sandwich (Figure 8), configured as a liquid bridge (Figure 9), satisfies these functional requirements (9). The capillary sandwich is formatted in a rectangular shape with concentric holes cut at either end, centered over each electrode. Ultrasonic "welding" at predefined regions of this assembly produces a stable mechanical configuration. The flow patterns of the

Fig. 7. The parallel V-groove pattern of texture transforms a normally spherical sessile drop into an elongated, sausage-shaped structure aligned with the grooves.

Fig. 8. An architextured capillary sandwich, composed of two V-groove, embossed sheets oriented orthogonally to each other, and the impact of this architextured system on the resulting flow pattern.

Fig. 9. The application of the V-grooved, architextured capillary sandwich to a potentiometric, analytical device featuring ion-selective electrodes.
introduced sample liquids are definable and controllable. Each liquid first flows laterally to the edge of the bridge and completely wets the associated ion-selective electrode. Then, the liquids "march" toward each other with a square wavefront until they touch near the center of the bridge and complete the electrical circuit. Liquids in this system are contained only by the energy barriers created by the sharp, solid edges of the capillary sandwich.

Although this generic three-dimensional architextured bridge configuration is a particularly effective liquid-distribution system for both highly viscous and low-surface-tension fluids, it is costly to manufacture because each bridge unit must be constructed individually. An alternative configuration that can be manufactured in a continuous process is a laminated capillary channel, created by joining ribbons of embossed and planar polymer sheets. However, the reality of this configuration (Figure 10) is flow behavior that is not predictable because of its intrinsic sensitivity to fluid properties. Further, this type of construction strongly reflects, in flow quality, errors in manufacture such as capillary spacing, surface heterogeneities, and deformability of plastic components. If the high- and low-surface-tension fluids shown in Figure 10 were, respectively, the reference and test solutions, the poorly defined flow fronts would result in extensive mixing during the formation of the liquid junction, negating the test.

The question of how to couple the flow performance of a capillary sandwich with the manufacturability of the laminated capillary channel is answered through a configuration that captures the essence of the V-groove, an architextured surface capillary channel. In the composite, elemental embodiment shown in Figure 11, the polymeric capillary channel is composed of two laminated webs. One web is concave, featuring slotted, parallel ribs spaced at predetermined intervals. Typical construction includes a polystyrene embossed member and a poly(ethylene terephthalate) planar member. The fluid-management aspect of this configuration is demonstrated in the typical flow sequence by which a sample of human whole-blood advances one rib.

As Figure 11 illustrates, energy barriers, in the form of partially extending ribs containing a flowthrough slot, control liquid advance. The wetting process starts as a centrally directed meniscus extending into the unwetted flow chamber. Although there is ample space for liquid flow between the top of the ribs and the planar sheet, the liquid chooses, instead, to flow through the central aperture and then laterally outward to fill the gap between adjacent ribs. The flow from rib to rib is distinctly intermittent. Typically, 40% of the total time to advance one rib is spent in the quiescent mode (frames 1 and 2), with the meniscus noticeably curved but essentially stationary. As if by a predetermined signal, the meniscus then surges forward to fill the gap between ribs. As a result of the centrally weighted flow behavior, air is pushed from the center outward in front of the meniscus, which obviates air entrapment. By knowing the fluid pairs, the wetting process can be controlled accurately by adjusting the roughness parameters—rib height, spacing, and channel width—and by chemical modification through materials selection or surface coatings (10). Once one has a configuration capable of directing liquids to flow in predesignated regions at controlled velocities, it is simple to envision a single device that incorporates a number of potentiometric tests. One embodiment is illustrated in Figure 12, in which four independent pairs of ion-selective electrodes share a single but separate pair of reference and sample pools and a common liquid bridge (9).

Proteinaceously Prewetted Dispenser System

A second class of problems that can be solved with the aid of architextured surfaces is represented by the uncontrolled liquid metering process illustrated in Figure 1. As was mentioned earlier, precise metering of microvolumes of biological liquids is deceptively complicated, especially if it...
must be done by an automated pipetting system. The primary reason for this is that the aspiration step entrains liquid on the external surface of the pipet tip, which ultimately interferes with the precision of the metering process. Because of the sequence of events in the metering process—namely, aspiration followed by pressurized dispensing—entrainment of liquid is inevitable. Thus, the solution to imperfect metering in such systems is the removal of this residual liquid from the immediate vicinity of the dispensing orifice.

If the metering process were done manually, the operator no doubt would wipe off the tip. But to program a robotic metering device to wipe tips after each aspiration is highly undesirable. At worst, automated wiping represents a source of cross-contamination; at best, the additional automated mechanisms involved would lower the throughput rate and increase the expense of any such microdispensing system.

What is desired is an architextured pipet tip that passively withdraws the entrained liquid from the end of the tip, thereby isolating this liquid from that being dispensed from the orifice. There are two critical design elements to this tip (Figure 13): the barrel of the tip is in the form of a truncated cone, and the dispensing orifice is isolated from this conical section by a short, cylindrical tube. The cone is robust and acts as a capillary pumping station: at small radii the capillary pressure in the liquid film is high; whereas at large radii the capillary pressure is low, in accordance with the equation of Young and Laplace (2d):

\[ P_{r} - P_{a} = \gamma R \]

where \( P_{r} \) is the pressure in the film, \( P_{a} \) is the atmospheric pressure, \( \gamma \) is the surface tension of the liquid, and \( R \) is the radius of the inverted cone, which increases with height. This difference in film pressure between the narrowest and broadest end of the cone results in a net upward flow of liquid away from the metering orifice, against the action of gravity. The short cylindrical section at the orifice both supports the drop being metered and disconnects the film source from the drop-forming region (11). In contrast, conventional pipet tips are essentially cylindrical throughout; consequently, gravitational forces dominate over capillary forces in determining the film drainage process, and the entrained liquid flows downward along the external surface of the pipet and collects at the terminus of the tip.

Figure 14 photographically depicts in real time the performance of this self-cleaning architextured dispenser tip, immediately before and after wetting by a protein sample. The presence of a highly tapered conical section, its surface area ever increasing with height, acts as a focus of attraction for the liquid film, such that the contact line migrates away from the drop-forming region. As the liquid film "drains" upward, its thickness diminishes, because of the increased surface area; finally, the film coalesces into one or more major drops, eliminating gravity's dominance on film drainage. Attached by hysteresis and located well away from the critical drop-producing region, these drops are incapable of interfering with the nominal dispensing sequence, which now can produce precise microvolume drops, even when the sample is whole blood or some other highly viscous biological fluid.

In conclusion: To illustrate "architextured" fluid management as a means for coherently influencing liquid wetting behavior, we have detailed some specific design considerations embodied in devices constructed to control either liquid surface-spreading or capillary transport. In the latter example, fluid is distributed by means of capillary forces, induced within hollow vessels that include passive energy barriers in the form of patterns of macrotexture and chemical modifications through materials selection or surface

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**Fig. 12.** The extension of the concept of an architextured fluid bridge to a multiple-channel potentiometric analytical device

**Fig. 13.** Self-cleaning dispenser tip for metering precise microvolumes of biological fluids

Entrained liquid on the external surface of the tip is pumped by capillary action away from the terminus that holds the metered volume. \( R \), radius; \( P \), capillary pressure

**Fig. 14.** Actual performance of the self-cleaning tip, showing the upward movement and coalescence of the entrained liquid film over time

Total time span of this photographic sequence, \( \sim 10 \) s
treatment. These vessels, being hollow, are inherently unrestrictive, permitting fluids such as undiluted pathological sera or whole blood to be transported even at shear rates $<5$ s$^{-1}$. Although we necessarily have limited the number of examples described, a broad range of applicability is feasible.

References