ANALYTICAL BIOCHEMISTRY 31, 272–278 (1969)

Analytical Techniques for Cell Fractions
XVI. Preparation of Protein-Free Supernatants with a "Z"-Path Rotor[1]

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Received April 2, 1969

The General Medical Sciences-AEC or GeMSAEC fast analyzer system previously described (1–7) allows a number of reactions to be initiated over a very short period of time, with absorbancy measurements similarly limited to brief intervals. The strategy employed in these studies has been to describe and illustrate each of the several types of steps or procedures which might be employed as part of the GeMSAEC system. In subsequent papers these are incorporated into specific biochemical or clinical chemistry methods. The objective of this brief paper is to describe a general method for the rapid preparation of a number of supernatants simultaneously.

EXPERIMENTAL

The transfer disc previously described (2,3) incorporates depressions in which samples and reagents may be retained unmixed at rest, but which provides channels for fluid transport by centrifugal force into a ring of cuvettes. The same general principles have been employed in the device shown diagrammatically in Figure 1 and photographically in Figure 2. It is constructed of white Teflon and fits in the center of the G- IIB or C rotors or directly on the shaft of the IEC clinical centrifuge.

In operation a particulate suspension, or the solutions which

[1] Research conducted under the joint NIH-AEC Molecular Anatomy (MAN) Program supported by the National Institute of General Medical Sciences, the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission.

[2] Operated by Union Carbide Corporation Nuclear Division for the U.S. Atomic Energy Commission.

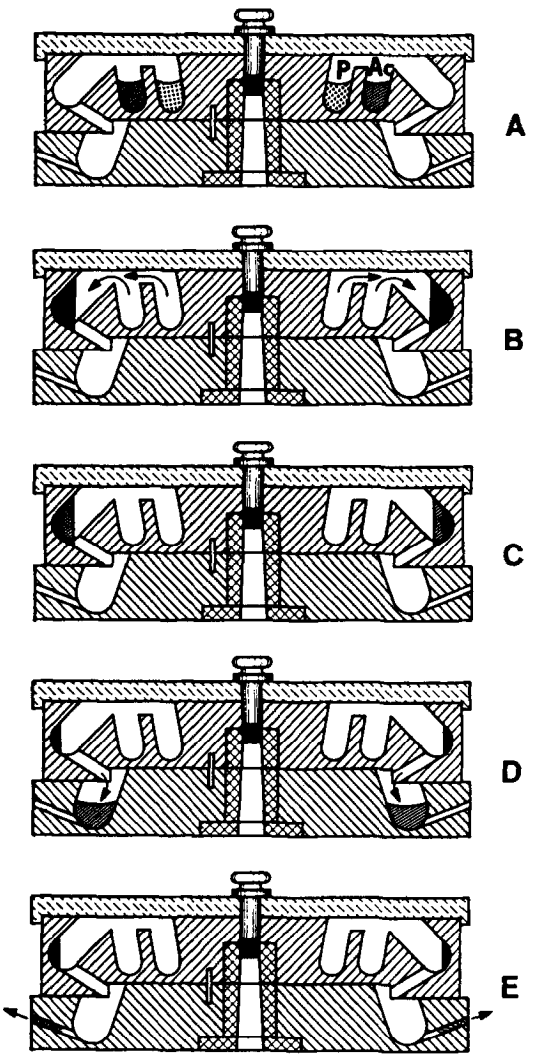


FIG. 1. (A) "Z"-path rotor at rest. Plasma (P) and precipitating acid solutions (Ac) are placed in appropriate chambers. (B) During centrifugation the two solutions pass to the sedimentation chamber and are mixed by rapid changes in rotor speed. (C) The precipitate is sedimented tightly against the sedimentation chamber wall. (D) After deceleration to rest, the clear supernatant is decanted into the lower supernatant holding chamber. (E) On reacceleration the supernatant is centrifugally transferred radially into a cuvet or a suitable measuring device. Samples may also be removed manually from the holding chamber. The maximum radius in the sedimentation chamber is 4.9 cm.

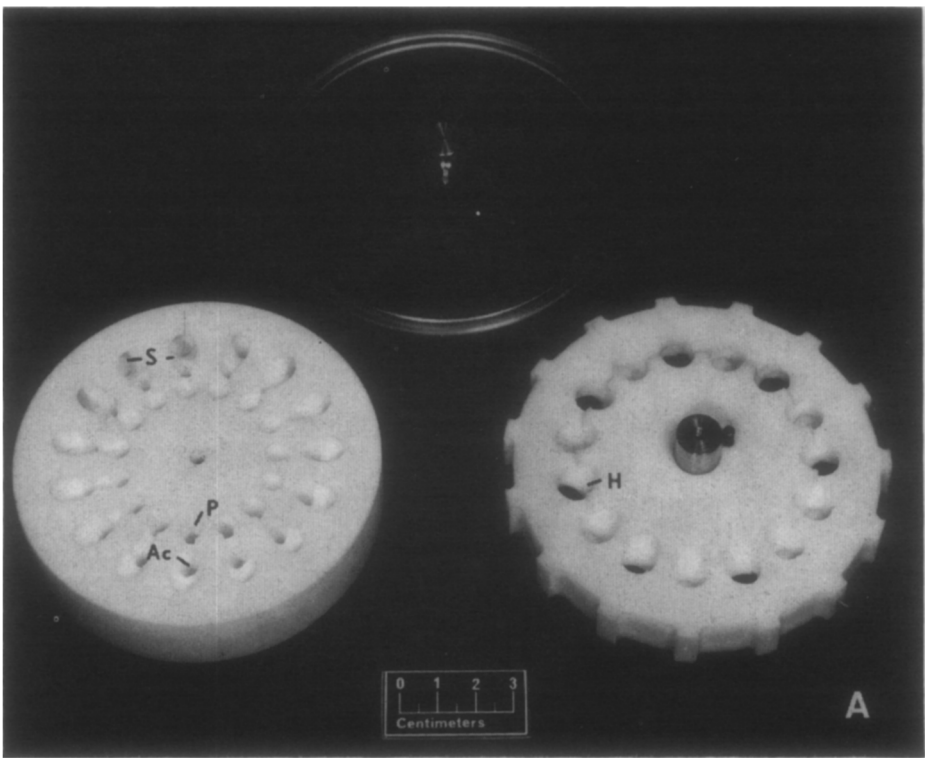
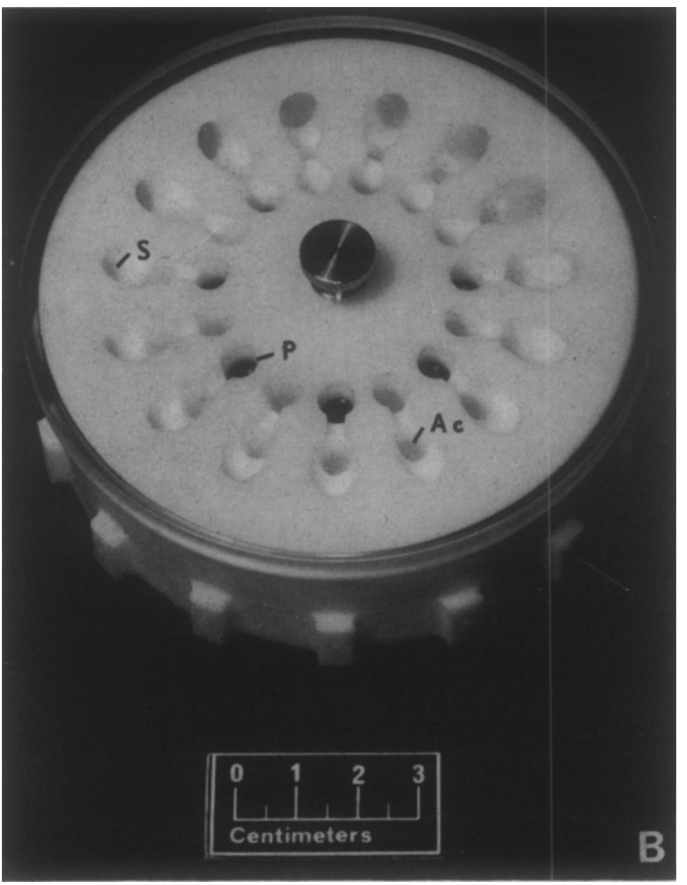


FIG. 2. (A) Disassembled "Z"-path rotor with colored solutions in sample and reagent chambers (P and Ac). These solutions are moved by centrifugal force into sedimentation chamber S. When the rotor is decelerated to rest, the supernatant drains into holding chambers H. (B) Completely assembled rotor showing transparent Lucite cover.

when mixed form a precipitate, are placed in the tilted receptacles (Fig. 1A). The rotor is accelerated causing the liquid or liquids to flow into the peripheral cavity indicated in Figure 1B. Centrifugal force maintains the suspensinon against the rotor wall as shown, and after acceleration to higher speed the suspended particles are sedimented to form a thin pellet (Fig. 1C). The rotor is now allowed to decelerate to rest, and the supernatant flows, with the assistance of very light air pressure if necessary, into the lower chambers (Fig. 1D). Reacceleration will move this particle-free supernatant out through the lower exit ports (Fig. 1E). Interior surfaces are designed so that the sample and reagent cavities do not unload until the centrifugal force is sufficient to hold a liquid in the sedimentation chamber. The three questions to be asked are: first, does fluid flow as proposed with no stray drainage or mixing; sec-



ond, is a uniform supernatant obtained; and third, is the recovery quantitative.

The fluid path is first horizontal in a generally centrifugal direction, then diagonally down after deceleration, and finally horizontal again, describing a pattern resembling the letter "Z." The rotor is therefore termed a "Z"-path centrifuge.

A Lucite cover is provided to minimize evaporation and prevent windage from deflecting liquids during centrifugal transfer.

Cross-leakage. An alkaline phenol red solution was used for initial studies. Alternate reservoir pairs were filled with 200μl of dye and 200 μl of distilled water, giving a total of 400 μl in each sedimentation chamber. The rotor was slowly accelerated to approximately 300 rpm and then rapidly to 3000 rpm, then allowed to coast to rest. The reservoir vessels were carefully examined for traces of

dye, which were rarely seen. The contents of the supernatant receptacles were also examined and no trace of cross-reaction found. 200 μl from each vessel was diluted with 5 ml distilled water and read at 556 nm using 1 cm cuvets in the Beckman DB spectrophotometer. No trace of dye could be discovered in the water series even though each was placed between dye samples, nor was dye found between the two discs. On a second acceleration to drain the lower receptacles, the fluid passed directly out the peripheral exit ports and did not spread between the discs.

Protein-free supernatants. Protein-free filtrates are required for a variety of purposes, including the estimation of acid-soluble nucleotides and of free amino acids, and for a number of clinical measurements including blood sugar. The precipitant employed and the ratio between sample and reagent may vary widely. A sensitive test for complete precipitation is the absorbance of the supernatant in the ultraviolet. Incompletely sedimented protein contributes both absorption and scatter. The principles of the "Z"-path centrifuge are illustrated by the preparation of protein-free supernatants from plasma as follows.

Ten reservoirs in the inner register are filled with 200 μl of partially hemolyzed human plasma containing sufficient hemoglobin to give a bright color to allow incomplete precipitation to be readily detected. All remaining reservoirs are filled with 200 μl volumes of 0.4 *M* $HClO_4$. The rotor is accelerated and decelerated rapidly to produce mixing as previously described (2), and then spun for 15 min at approximately 6850 rpm using an IEC clinical centrifuge. The rotor is gently tilted about 15° to assist drainage into the lower supernatant receptacles. From each, a 200 μl sample is withdrawn, diluted with 5 ml of distilled water, and read against distilled water at 260 nm in a 1 cm light path cell. In a series of 10 samples spun in the "Z"-path rotor, the absorbance observed was 0.186 with a standard deviation of 0.004 absorbance unit. This is a severe test of the system since the concentration of plasma in precipitating mixtures is usually much lower. The results were comparable to those obtained with similar volumes centrifuged at 2000 rpm in a PR–2 refrigerated centrifuge for 15 min.

In additional experiments, equal or superior reproducibility was obtained without speed variations to produce mixing (2), suggesting that in such small volumes (400 μl total) mixing readily occurs during fluid transfer and by diffusion and vibrational forces.

The recovery was not quantitative and varied between approximately 200 and 300 μl. Direct introduction of the sample from the

DISCUSSION

lower supernatant receptacles into a cuvet is therefore not feasible at present without the interposition of a suitable measuring device.

The "Z"-path rotor described allows a set of mixtures to be prepared simultaneously, centrifuged to remove a precipitate, the supernatant automatically decanted, and subsequently transferred to the cuvets of a cuvet rotor (1). The technique is applicable to colorimetric reactions involving the formation of a precipitate during color development, or to the preparation of supernatants for subsequent analysis. The simple device described is specifically adapted to precipitates which form firm adherent pellets. Where the pellets are not firmly packed, the sedimentation chamber may be modified to include a further depression of narrow diameter to contain the sediment.

With the device described, the supernatant is not quantitatively recovered. Additional measuring devices, such as those described in subsequent papers, are therefore required if the supernatant is to be reacted with additional reagents. The lower reservoir disc may serve as the sample disc for use with other conventional types of automatic analyzers. Recovery of the supernatant from the sedimentation chamber is markedly assisted by the addition of a small amount of detergent to the precipitating solution. While the device described will process 15 samples simultaneously, the basic principles are directly applicable to plastic or metal rotors handling larger volumes, larger numbers of samples, or both.

For higher speed operation, rotors reinforced with metal bands, or constructed entirely of stainless steel or titanium will be required. Provision for more than two reactants may be made, and the sedimentation chamber may slope upward as well as in the direction shown.

The "Z"-path principle is also useful in instances in which no precipitate is involved but two or more solutions are to be mixed together, incubated for a prolonged interval, and subsequently transferred to a cuvet rotor to be read. The principle may also be applied to the separation of plasma from the formed elements of blood.

SUMMARY

A new "Z"-path centrifuge rotor is described for the separation of pellets from supernatants in up to 15 samples simultaneously. The samples and precipitating solutions are stored in separate

chambers at rest, and are transferred to a sedimentation chamber by centrifugal force during rotation, where mixing occurs during rapid changes in speed, followed by sedimentation of the precipitate. The supernatant decants itself at rest into a third series of chambers, where it may be transferred centrifugally into radially disposed measuring devices, transferred with suitable pipettes, or used as a sample ring for conventional analyzers.

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