shaking at 0°, ~30% exchange was observed with chloroform and ~5% with ethylene dichloride. Ethylene dichloride could not be used in the experiments at the lower concentrations because the separation of the two phases became very slow with the large volumes used.

The rate constant calculated from experiment 1,[6] assuming the reaction to be bimolecular, is >1500 liter·mole^{-1}·sec.$^{-1}$ at ~0°.

The above results indicate that the exchange is extremely fast even under conditions of low concentrations and low temperature. Admittedly, we have no proof that exchange is not induced by the separation procedure, but considering the nature of the separation process, induced exchange seems unlikely.

(6) Friedlander and Kennedy, "Introduction to Radiochemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 287.

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ST. LOUIS 5, MO. RECEIVED NOVEMBER 18, 1950

Density Gradient Centrifugation: A New Separation Technique[1]

BY MYRON K. BRAKKE

Density gradient centrifugation is a technique for more efficient centrifugal separations of suspended particles. The author is unaware of any previous publication of the procedure outlined below.

A thin layer of the suspension to be fractionated is floated on a solution having a density gradient. If the suspended particles sediment ideally, *i.e.*, as discrete entities, during subsequent centrifugation, those of each sedimentation rate will travel down the tube as a separate zone. Centrifugation may be continued until the particles reach density equilibria, yielding a separation dependent on particle density only. If centrifugation is stopped earlier, a separation dependent on the sedimentation rates of the particles may be obtained. Theoretically, the latter variation should have the higher resolving power since for two particles to have the same sedimentation rates throughout a tube with a density gradient, their densities and ratios of mass to frictional constant must be identical. In addition to the density gradient, aqueous systems will usually have an osmotic pressure gradient. Then for two particles to sediment at the same rate throughout the tube they must not only fulfill the above criteria, but must also change hydration at the same rate with changing osmotic pressure.

This basic procedure can be modified for application to many different problems involving particles and large molecules of either biological or non-biological origin. Appropriate density gradients must be prepared for each situation. The method might be used as a separation procedure in either of the two ways noted above, as a criterion of purity, or as a technique for measuring densities of particles or large molecules. The use of density gradient tubes for density measurements of macroscopic droplets and particles was originated by Linderstrom-Lang.[2,3] In the present case the interpretation of the densities obtained would be complicated by the possible change in the solvation of the particle as it travels down the tube, since, in general, the liquids used for the density gradients in centrifugation are solvents for the particles concerned.

In the exploratory experiments in this Laboratory with density gradient centrifugation two types of non-ideal sedimentation have been recognized. The first type is due to aggregation of particles such as may be observed in any centrifugation. The second type is due to the sedimentation of droplets of solution containing particles. This phenomenon may be demonstrated by centrifuging a layer of a suspension floated on a denser liquid, which, however, has no density gradient. It probably occurs to a limited extent when the density gradient is not steep enough, causing a spreading of the zones.

That particles will sediment as discrete entities in a density gradient tube was shown by experiments with potato yellow-dwarf virus. Half a ml. of virus concentrate prepared by two cycles of differential centrifugation[4] was placed in a 15-ml. centrifuge tube on a density gradient prepared by layering 2 ml. each of sucrose solutions having densities 1.04, 1.08, 1.12, 1.16 and 1.20 in the tube and allowing diffusion to occur for a few days at 0°. After centrifuging at 3200 r.p.m. for 5 hr. in a horizontal head of an International 1-SB centrifuge at 10°, a zone, suspected of being the virus (S^{w}_{20} = 1150s),[4] was visible from 0.9 to 1.4 cm. below the meniscus. Samples were removed from the bottom of this and other desired zones by means of an inverted tip glass capillary and 0.1 *M* neutral phosphate buffer was added to give suitable equivalent dilutions. Their infectivity was compared by inoculating opposite half-leaves of *Nicotiana rustica* L. One-ml. samples which removed the zones 0.9 to 1.4, 1.9 to 2.4, 2.9 to 3.4, and 3.9 to 4.4 cm. from the original position of the meniscus gave a total on 3 half-leaves of 1871, 34, 6 and 1 local lesions, respectively. A second experiment gave similar results.

Even when density gradient centrifugation is performed in an angle head a definite virus zone may be obtained (Fig. 1). The stabilizing effect of sucrose density gradients on boundaries during angle centrifugation[5] may account for the relative sharpness of the zone obtained.

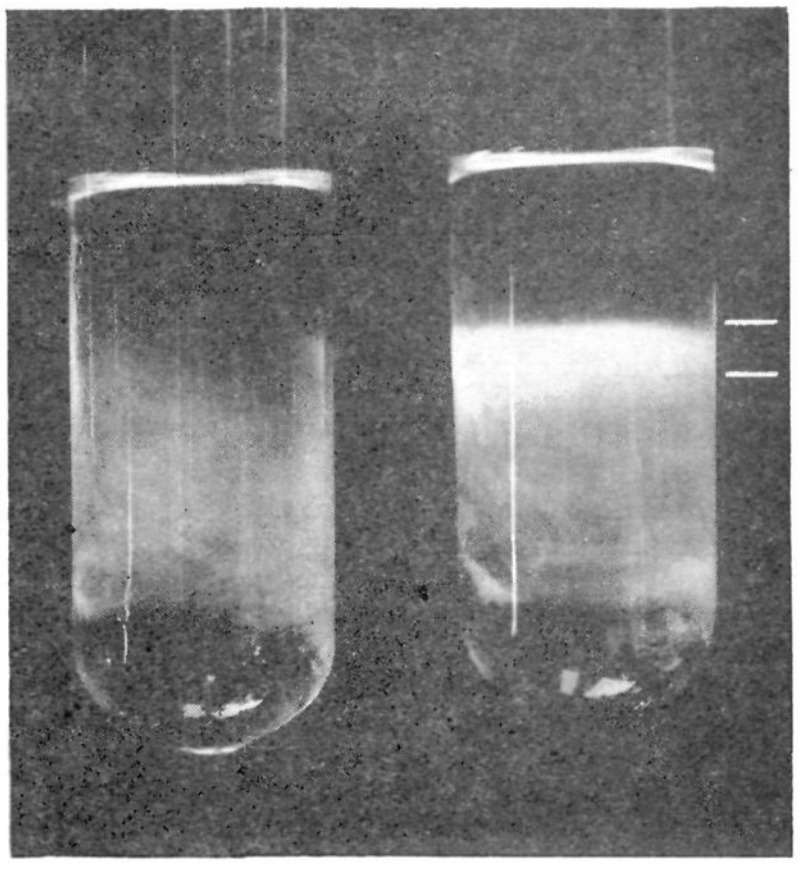

Fig. 1.—Density gradient centrifugation of potato yellow-dwarf virus. The tube on the right contains a concentrate prepared by two cycles of differential centrifugation and one density gradient centrifugation cycle from diseased *N. rustica* plants (virus zone indicated by lines). The tube on the left contains a similar concentrate from normal plants. Three ml. of the concentrate was centrifuged for 20 min. at 10,000 r.p.m. at 5° in a Servall SS-2 through a density gradient prepared in a 50-ml. tube from 7 ml. each of sucrose solutions of densities 1.04, 1.08, 1.12, and 1.16.

(1) This work was supported, in part, by a grant in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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In both these experiments, the separation obtained was dependent on sedimentation rates, *i.e.*, centrifugation was not continued long enough for the virus to reach a density equilibrium position.

Density gradient centrifugation is being applied in this Laboratory to the purification of plant viruses. With potato yellow-dwarf virus, whose zones are visible by scattered light, it has served as a rough but very convenient criterion of purity at the same time that it is being used as a separation procedure.

BROOKLYN BOTANIC GARDEN
BROOKLYN 25, N. Y. RECEIVED JANUARY 2, 1951

Isolation of *d*-3-Octanol from American Oil of Spearmint

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In the distillation of American spearmint oil (*Menthis spicati*), it was observed that certain portions of the monoterpene fractions possessed a heavy, fruity odor which could not be accounted for by the reported constituents of these fractions, *i.e.*, *l*-α-pinene, *l*-α-phellandrene, and, mainly, *l*-limonene.[1] The ceric nitrate alcohol test on these fractions was positive. Although the new compound could not be obtained in any degree of purity by fractional distillation, its allophanic ester (m.p. 182°) was readily precipitated when cyanuric acid gas was passed into the terpene fraction. Analytical data on the allophanate indicated that the new compound was an octanol. Saponification of the allophanate gave a dextrorotatory alcohol (b.p. 88–92° (33 mm.)) which was negative in the unsaturation test with bromine in carbon tetrachloride and in the iodoform test. Analysis of the alcohol and its α-naphthylurethan (m.p. 79–80°) supported the view that it was an octanol.

d-3-Octanol has been found in Japanese peppermint oil,[2] in Brazilian peppermint oil[3] and, both free and as the acetate, in European pennyroyal oil.[4] The α-naphthylurethan was reported[2,3] as melting at 81–82°. *l*-3-Octanol occurs in French lavender oil[5]; it forms an allophanate melting at 167° and an α-naphthylurethan melting at 81°. Racemic 3-octanol has been synthesized[2,6,7] (α-naphthylurethan,[7] m.p. 54°) and resolved.[6] The *d*- and *l*-forms were prepared also from *l*- and *d*-amylvinylcarbinol, respectively[8]; the α-naphthylurethan of the synthetic *l*-3-octanol was found to melt at 79–80°. In the work reported here *dl*-3-octanol was prepared by the reaction of *n*-amylmagnesium bromide and propionaldehyde. This compound was practically identical with the unknown alcohol in odor and physical properties.

The semicarbazone of 3-octanone has been reported[6] to melt at 112° (slow heating) and also[4] at 117–117.5°. Oxidation of the alcohol from spearmint oil with chromic acid gave an octanone (b.p. 160–165°) which formed a semicarbazone melting at 112°. The semicarbazone derived in the same manner from synthetic *dl*-3-octanol melted at 114°. A mixed melting point determination showed that the two compounds were identical.

On the basis of the weight of the allophanate obtained, *d*-3-octanol makes up about 1.1% of American oil of spearmint.

This work was done under the sponsorship of the Bristol-Myers Company, whose permission to publish is gratefully acknowledged.

Experimental[9]

Isolation of *d*-3-Octanol.—Oil of spearmint (A. M. Todd Company, Kalamazoo, Michigan) was distilled through a short Vigreux column under reduced pressure until the distillate amounted to about 25% of the original oil. This fraction was cooled in an ice-bath and treated with cyanic acid gas generated by heating cyanuric acid at 325–330° in a slow stream of carbon dioxide. When precipitation was complete, the allophanate was collected on a filter, washed with a small amount of petroleum ether, pressed out on porous paper, and allowed to dry. After recrystallization from ethanol, it melted at 182°. From 1460 g. of spearmint oil there was obtained 27 g. of crude allophanate.

Anal. Calcd. for $C_{10}H_{20}O_3N_2$: C, 55.53; H, 9.32; N, 12.96. Found: C, 55.74; H, 9.18; N, 13.21.

The allophanate was hydrolyzed by boiling under reflux for ten hours with aqueous sodium hydroxide solution (10%). The reaction mixture was extracted with ether and the extract was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the alcohol was distilled under reduced pressure; b.p. 88–92° (33 mm.), $n^{25}D$ 1.4259, $[\alpha]^{25}D$ +8.23° (abs. alc.).

Anal. Calcd. for $C_8H_{18}O$: C, 73.78; H, 13.93. Found: C, 73.95; H, 13.65.

Treatment of a small portion of the alcohol with α-naphthyl isocyanate in the usual way gave the α-naphthylurethan which, after recrystallization from Skellysolve B, melted at 78°.

Anal. Calcd. for $C_{19}H_{25}O_2N$: C, 76.22; H, 8.42. Found: C, 76.28; H, 8.38.

Oxidation of *d*-3-Octanol.—A portion of the alcohol (2 g.) was dissolved in acetic acid (15 ml.) and treated dropwise in the cold with a solution of chromic anhydride (2 g.) in a small amount of acetic acid. Then the reaction mixture was heated on the steam-bath for one hour, allowed to cool and neutralized with sodium hydroxide solution (10%). The product was removed from the reaction mixture by steam distillation and extracted from the distillate with benzene. After the solvent was removed, the residue was distilled from a small, modified Claisen flask. The material boiled at 160–165° and weighed 1.5 g. Further purification of this ketone was not attempted; instead it was converted to its semicarbazone which, after recrystallization from alcohol–water, melted at 112°.

Anal. Calcd. for $C_9H_{18}ON_3$: C, 58.66; H, 10.39. Found: C, 58.56; H, 10.08.

A sample (7.5 g.) of synthetic *dl*-3-octanol (b.p. 75° (20 mm.), $n^{25}D$ 1.4256), prepared by the reaction of *n*-amylmagnesium bromide and propionaldehyde, was oxidized with chromic anhydride in acetic acid in a manner identical with the oxidation of the octanol from spearmint oil. Five grams of 3-octanone was obtained; a portion of this ketone was converted to the semicarbazone, m.p. 114°. The melting point of a mixture of this semicarbazone and that derived from the octanol from spearmint oil was 113–114°.

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALA. RECEIVED NOVEMBER 28, 1950

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