Harvesting density gradients

The mode of harvesting depends very much on the type of tube used for the gradient, the distribution of particles in the gradient and the aim of the fractionation.

Using a syringe with flat-tipped metal cannula or Pasteur pipette

If the banded material is clearly observable and well resolved then the tip of a cannula or Pasteur pipette (preferably fashioned into an L-shape) can be placed in the band and the material withdrawn. This method can be used to harvest the entire gradient, if the tip of the L-shaped pipette or cannula is placed at the meniscus. It is however tedious, prone to error and difficult to obtain equal volume fractions if that is a requirement, but for a crude fractionation into four or five regions it is quite satisfactory.

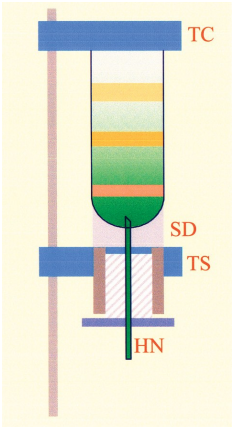
Using a peristaltic pump

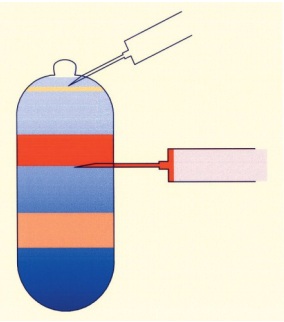
As a general rule the harvesting system should be devised so that the effluent from the tube should not have to pass through the pump but as long as the dead volume of the tubing is small compared to the volume of the gradient it is permissible to insert a narrow tube to the bottom of the tube and to aspirate the contents (dense-end first). Theoretically mixing will occur in the vertical section of the collection tubing as the decreasingly dense medium enters the bottom of the tube. In practice however this seems not to be a serious problem.

By tube puncture

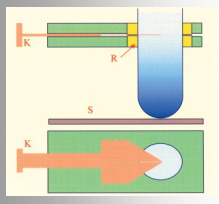
Practically this is best achieved by securing the tube vertically in some form of clamping device and to advance the needle through a rubber seal into the bottom of the tube by a screw or lever mechanism (see figure left below). The Beckman Fraction Recovery System incorporates such a device. If sealed tubes are used, then either the central plug should be removed (Optiseal™) or the top punctured with a syringe needle (Quick-Seal™) to allow air to displace the liquid which exits the tube under gravity.

The system is simple and the dead space of the collecting tube is very small and the gradient is collected almost ideally, the hemispherical section of the bottom of the tube directing banded material into the collecting needle. Thick-walled tubes however cannot be used and it is not a useful method if there is a large pellet which may obstruct the hollow needle.





It is also possible to collect a specific band within the gradient by puncturing the tube with a needle attached to a syringe just below the band and with the inlet to the needle uppermost, aspirating the band

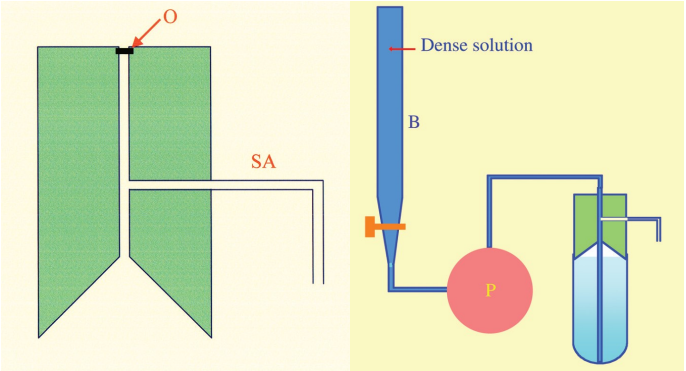


into the syringe (see figure right on the previous page). As with bottom puncture, air must be allowed to displace the column of liquid in the tube.

By upward displacement

A dense liquid introduced to the bottom of the tube can displace the entire gradient upwards and with a suitable device attached to the top of the tube, the gradient can be delivered into the collection tubes. To fit thin-walled flexible tubes the simple, cylindrical block of Perspex (Lucite or acrylic) shown in figure left below, should be slightly tapered towards the bottom (not shown in figure). The block contains a central channel, which leads to a hollowed-out cone, and a sidearm, which connects with the central channel. The dense unloading solution is introduced to the bottom via a long metal cannula inserted down the central channel and through the gradient. The gradient is displaced upwards by the incoming dense liquid into the cone and an 'O' ring around the cannula diverts the flow into the collection tubes via the side-arm. For non-flexible tubes the device requires sealing on to the tube with a gasket, under pressure.

By placing the dense unloading liquid in a burette and delivering it to the bottom of the centrifuge tube via a peristaltic pump (see figure right), the unloading process can be executed at a uniform flow rate and it is the only method which guarantees equal volume fractions. Collection of equal volume fractions by counting drops is inaccurate because of the variation in drop size due to the constantly changing density, viscosity and particle concentration in the effluent.



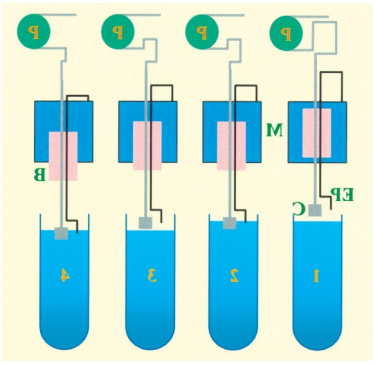
The best unloading medium is a low viscosity, dense, non-water-miscible, fluorocarbon such as perfluorodecalin (ρ = 1.9 g/ml), previously available from Axis-Shield as Maxidens™. Perfluorodecalin can currently be purchased from F2 Chemicals Ltd, Lea Lane, Lea Town, Preston PR4 0RZ, UK (tel: +44 (0)1772 775802, fax +44 (0)1772 775808).

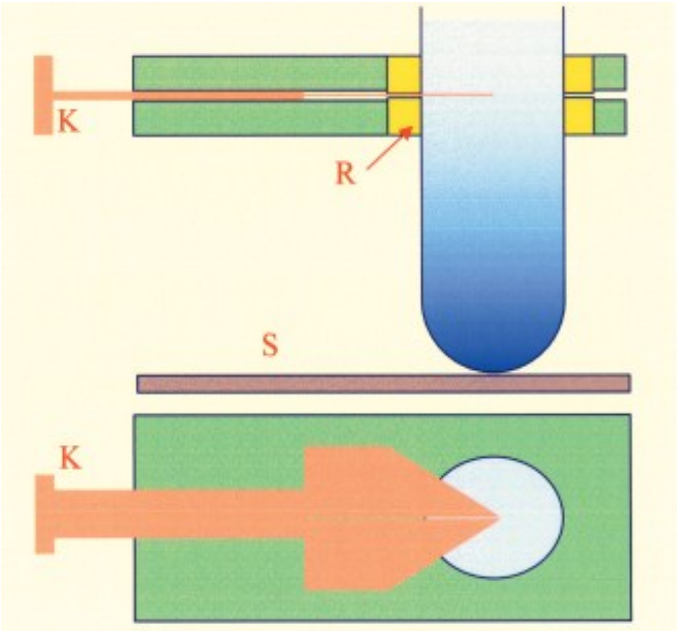
Automatic aspiration from the meniscus

The **Auto Densi-flow™** which is produced by the **Labconco Corporation** comprises a hollow metal tube which terminates in a small collection head (see pictures on next page); the upper end of the tube is connected to a peristaltic pump which aspirates the gradient. With the centrifuge tube clamped in place, a motor advances the collection head towards the gradient until the tip of an electronic probe, which is mounted at the side of the collection head, detects the meniscus of the gradient. As the motor is activated whenever the probe is not in contact with the meniscus, aspiration of the gradient and the consequent fall of the meniscus will causes the motor to advance the collection head continuously. In this way the entire gradient is collected in a smooth and continuous fashion. For further information regarding commercial availability contact either **Labconco Corporation**, Kansas City, Missouri (labconco@labconco.com) or **GRI**, Braintree, Essex, UK (gri@gri.co.uk). Note that the collection head of this device also provides an excellent means of depositing a continuous gradient, dense end first, from a two-chamber gradient maker.

Tube slicing

For the collection of specific zones or "cuts" of a gradient, a tube slicer may be a useful alternative (see figure below). It can only be used with thin walled tubes and its most common use is to remove material banded close to the top of a gradient (eg plasma lipoproteins floated on a density barrier). The tube is held vertically within the tube slicer by two silicone rubber rings and its position adjusted so that when the knife blade is advanced between the rings, the liquid above the knife blade is isolated from the rest of the gradient (the rings providing an effective seal at the cut). The medium above the blade can be recovered completely and the walls of the tube washed to remove any material adhering to them.





Influence of tube type on harvesting strategy

Open topped thin walled tubes (polyallomer, polycarbonate or Beckman Ultraclear™) for swinging-bucket or fixed-angle rotors can be unloaded by any of the above methods.

Thick-walled open-top tubes can be unloaded by any of the methods except tube puncture or slicing.

Thick-walled tubes with screw caps are best unloaded with the **Labconco Auto Densi-flow** or by aspiration from the bottom. It may be possible to use the **Beckman Fraction Recovery System** in the upward displacement by cannula mode (depending on the shape of the tube neck) but material may get trapped at the shoulder of the tube.

Quick-Seal™ tubes cannot be unloaded by any of the methods requiring the sealing of an unloading device on the top of the tube or by automatic aspiration, unless the tube is cut horizontally just below the shoulder. This is not easy and may cause disturbance to the gradient. Tube puncture is best suited to this type of tube.

Optiseal™ tubes may be unloaded by any of the methods. If upward displacement is chosen, the unloading solution must be introduced by tube puncture and a silicone tube attached to the neck should be used to collect the gradient.

Fraction Size

To be able to locate a particular fraction reproducibly in a gradient, an obvious requirement is that the harvesting procedure itself should be reproducible so that the material of interest always emerges in the same fraction number. Harvesting of each gradient should therefore provide:

• the same number of fractions, each of a reproducible volume

• satisfactory resolution of the particle(s) of interest

The volume of each fraction need not be the same, it is satisfactory that the volume of gradient in corresponding fractions from successive gradients is the same. The collection of equal-volume fractions can only be achieved in unloading systems in which a dense or light medium is delivered to the bottom or top (respectively) of the gradient from a burette. Drop counting is unsatisfactory because drop size changes with viscosity and particle concentration. Although the collection of fractions up to a graduation on the collecting tube can be used with simple tube puncture it is often difficult to achieve for volumes of less than 0.5 ml.

The maximum permissible volume of the fractions is obviously controlled by the linear separation of the particles of interest. Unless the separation of the particles of interest is so clear that they can be collected using a Pasteur pipette or syringe (see “Using a syringe …”), the first time that a fractionation is carried out it is a good idea to collect equal volume fractions of a number that can be readily analyzed. The volume of fraction will depend on the volume of gradient and on its resolving power; generally fractions of a volume equal approx 5% of the

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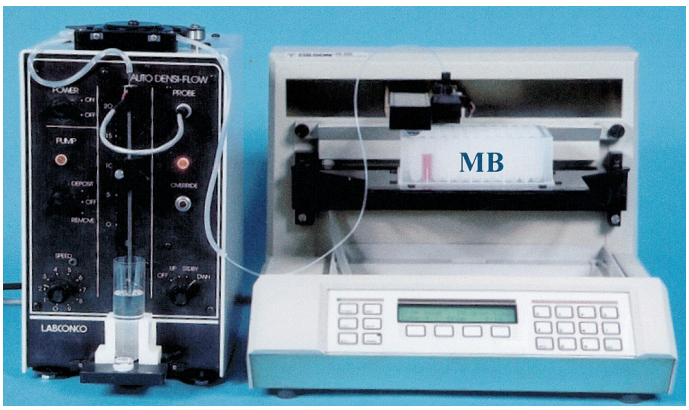
gradient volume are taken. This can then be modified in the light of experience.

An integrated gradient collection system

In the modern system illustrated in the picture on the right, the Labconco Auto Densi-flow is being used to harvest the gradient (from the meniscus) from a standard tube for a swinging-bucket rotor. The effluent from the peristaltic pump on top of the Auto Densi-flow is directed to the collection head of a Gilson FC205 fraction collector for dispensing into a 96-well polypropylene "Masterblock Deep-Well" plate (Greiner Bio-One Inc). These Masterblocks can easily accommodate volumes of up to 2.0 ml. The multi-well plate format for gradient collection allows simple gradient analysis, if the gradient fractions are subsequently sampled using a multiple channel automatic pipette (see below); it also provides an easy means of storage. The large-volume Masterblock can be replaced by a standard 96-well plate for the collection of smaller gradient volumes.



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