

KIT FOR ELECTROPHORESIS GEL MEDIUM

FIELD OF THE INVENTION

This invention relates to a medium or element for electrophoresis. More particularly, it relates to a kit and method of using the kit for conveniently and safely forming a polyacrylamide type polymeric gel medium suitable for electrophoretic separation of biopolymers such as proteins or for electrophoretic separation of fragments of DNA, RNA and their derivatives.

DESCRIPTION RELATIVE TO THE PRIOR ART

U.S. Pat. No. 4,704,198, which issued on Nov. 3, 1987, contains a comprehensive description of various aspects of electrophoresis. As described therein, and in numerous other publications, electrophoresis is based on the principle that charged molecules or substances will migrate when placed in an electric field. Since proteins and other biopolymers (e.g., DNA, RNA, enzymes and some carbohydrates) are charged, they migrate at pH values different from their isoelectric points. The rate of migration depends, among other things, upon the charge density of the protein or biopolymer and the restrictive properties of the electrophoretic matrix or medium. The higher the ratio of charge to mass, the faster the ion will migrate. The more restrictive the medium, the more slowly the ion will migrate.

In theory, separation of different proteins could be achieved readily in free solution provided that the molecules differed sufficiently in their charge densities. However, in practice, separations in free solution are difficult to achieve because the heat produced during electrophoresis can cause convection disturbances which distort the protein bands. Resolution of the individual proteins is compromised because the bands are broadened. Also, band broadening continues even after the electrophoresis has been stopped because of diffusion of dissolved solute. Therefore, electrophoresis in free solution is rarely performed. In practice, various supporting media are currently used to minimize convection and diffusion, and to effect separation both on the basis of size and of molecular charge.

Many support media for electrophoresis are in current use. The most popular are sheets of paper or cellulose acetate, agarose, starch, and polyacrylamide. Paper, cellulose acetate, and similar porous materials are relatively inert and serve mainly for support and to minimize convection. Separation of proteins using these materials is based largely upon the charge density of the proteins at the pH selected.

On the other hand, starch, agarose and polyacrylamide gels not only minimize convection and diffusion but also actively participate in the separation process. These materials provide a restrictive medium in which the average size of the polymeric network opening can be controlled to achieve a molecular fractionation in a desired molecular size range. In this way, molecular sieving occurs and provides separation on the basis of both charge density and molecular size.

The extent of molecular sieving is thought to depend on how much the gel network opening size is larger than the size of the migrating particles. The network opening size of agarose gels is so large that molecular sieving of most protein molecules is minimal and separation in that medium is based mainly on charge density. In contrast, polyacrylamide gels can have openings

whose sizes more closely approximate the size of protein molecules and so contribute to the molecular sieving effect. Polyacrylamide has the further advantage of being a synthetic polymer which can be prepared in highly purified form.

The ability to produce gels having a wide range of polymer concentrations, and, therefore, since the gel network opening is inversely proportional to polymer concentration, a wide range of controlled network opening sizes, as well as to form pore size gradients within the gels by virtue of polymer concentration gradients, are additional advantages of polyacrylamide as an electrophoresis gel medium. Control over opening size enables mixtures to be sieved on the basis of molecular size and enables molecular weight determinations to be performed. These determinations are especially accurate if the proteins are treated with a detergent, such as sodium dodecyl sulfate (SDS), which neutralizes the effects of molecular charge so that all SDS treated molecules, regardless of size, have approximately the same charge density values. This technique is referred to as SDS-PAGE electrophoresis.

Conventionally, polyacrylamide gel media have been prepared by free radical induced polymerization of a monomer such as acrylamide and a crosslinking agent such as N,N'-methylenebisacrylamide under oxygen-free conditions in the presence of water, a buffer, a polymerization initiator, and a polymerization catalyst. More particularly, since such polymerization can be inhibited by the presence of oxygen, polyacrylamide gel media for electrophoresis typically have been prepared by a process involving: introducing a previously deoxygenated aqueous solution containing acrylamide, a crosslinking (bis) monomer, a buffer, a free radical polymerization initiator and a polymerization catalyst into a cell formed between two glass plates with a selected clearance (e.g., 0.3-3 mm); and sealing the gel-forming solution from oxygen; whereupon the free radical polymerization proceeds so as to prepare the desired gel. Often this is done in situ by the scientist who is to conduct the electrophoresis.

The usual practice is for the manufacturer or the individual user to perform a free radical polymerization with acrylamide and a suitable bis monomer such as N,N'-methylenebisacrylamide (often simply referred to as "bis") in order to obtain a gel. Such gel formation is successfully done only as several precautions are taken, namely: (a) very high purity starting materials should be used; (b) the solution of monomers and buffer should be degassed to remove oxygen; (c) a free radical initiator and a catalyst must be quickly mixed into the degassed solution; (d) the solution should be quickly poured between two glass plates or down a glass tube, the lower end of which in either case is sealed to prevent leakage; and (e) the gelation should proceed with (i) oxygen largely excluded (i.e., to the extent feasible within the limitations of the equipment, oxygen is excluded) and (ii) adequate means for heat dissipation being present so that excess heat does not cause gel nonuniformities.

The cell employed for the preparation of the gel generally has a length of approximately 6 to 100 cm. Accordingly, the introduction of the gel-forming solution into such a long cell requires careful operation to prevent the solution from gelling before it is completely poured, thereby preventing the preparation of a uniform polyacrylamide gel medium of the desired length. Thus, the preparation of a polyacrylamide gel medium