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REVIEW

GEL ELECTROPHORESIS OF INTACT SUBCELLULAR PARTICLES

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1. INTRODUCTION

Electrophoresis* is the migration of charged particles in a conductive solution under the influence of an electric field. Pertinent conditions such as electric potentials between cell compartments or the interior and exterior of cells are frequent in nature and thus some kind of electrophoresis probably exists since the origin of life. Experimental electrophoresis according to Reiner [1] and Kolin [2] was initiated by Reuss [3] with the electrophoretic migration of colloidal clay particles. Classical electrophoresis continued to be carried out in solid support-free solution and was more recently improved by using apparatuses with special anticonvective devices [4–10]. In this fashion a separation of substances is achieved according to their surface net charge density, whereas differences in size and shape do not come into play [11,12]. Preparative cell electrophoresis is still exclusively performed by one of these techniques, the free-flow electrophoresis [13]. Another approach for overcoming convection was the introduction of anticonvective media (reviewed in ref. 14): paper [15–17], silica gel [18], glass wool and asbestos [19], cellulose fibers [20], sucrose density gradients [21–25], Metrizamide density gradients [26,27] and cellulose acetate [28] as well as gel matrices like gelatine [29], agar [30,31], starch [32,33], polyacrylamide [34–36], agarose [22] and mixed polymers [37–41].

Electrophoresis in gel media (gel electrophoresis) has become increasingly popular, since the retardation of electrophoretic particle migration by a gel (called sieving) allows for a separation according to size *in addition to* surface net charge

*The term Electrophoresis was created by Michaelis [239].

density. This increases the resolution of separation considerably as was first demonstrated for serum proteins [42]. Gel electrophoresis, therefore, is superior to electrophoresis in free or viscous solution in all cases, where the particles of the sample can enter the gel and do not chemically interact with each other or the gel matrix.

It is the purpose of this review to address the electrophoresis expert as well as the life scientist with little previous experience in electrophoresis and to make him familiar with the separation and characterization of subcellular particles by gel electrophoresis on the basis of their size, shape and charge differences. Detailed rationales and procedures will be provided which should suffice for equipping the reader with the know-how needed for the successful planning and execution of experiments.

2. THE SAMPLE

2.1. *Definition of subcellular particles*

Gel electrophoresis to date has primarily dealt with macromolecules. These comprise proteins in the size range of equivalent spheres of 1 to 10 nm radius, as well as nucleic acids containing up to several thousand basepairs. Comparably, applications in regard to subcellular particles have been less frequent. For the purpose of this review, subcellular particles are defined as bodies or vesicles formed by multiple aggregated and/or cross-linked macromolecules. These particles can be normal (in some cases symbiotic?) or parasitic cell constituents or synthetic products. Their size is usually smaller than the wavelength of visible light. Nucleic acids, consisting of only one or two macromolecular chains, are excluded. For reviews of nucleic acid electrophoresis see refs. 43–45.

2.2. *Sample preparation*

The samples need not be highly purified. On the contrary, it has been demonstrated that crude extracts of plants containing viruses are applicable (see Section 9.1.). The total amount of subcellular particles necessary depends on the sensitivity of the detection procedure (see Section 7) and on the amount of impurities which interfere with the detection of the particle under investigation. The detection range is usually from a few micrograms down to about 2 ng. The sample should be dissolved in the buffer which is used for electrophoresis. Minor impurities of inorganic ions are tolerable, otherwise the sample has to be dialyzed against electrophoresis buffer. Dialysis against concentrated solutions of sucrose (50–100%) or polyethylene glycol (in the case of osmotically sensitive particles) in buffer can be also used for sample concentration. For routine applications it may be more convenient to use commercially available kits for that purpose (e.g. ProDiCon, Pierce, Rockford, IL 61105, U.S.A.; Allington cell, ISCO, Lincoln, NE 68504, U.S.A.; Minicon or Centricon, Amicon, Danvers, MA 01923, U.S.A.). Another way of sample concentration is isopycnic centrifugation or the use of a discontinuous buffer system (see Section 3.2).

Samples that contain microscopically visible particles in substantial amounts need to be sedimented or (ultra)filtered in those cases, where the precipitated particles on top of the gel surface gradually dissolve and/or break down to cause vertical streaking. This phenomenon can be observed with particles like polystyrene size standards which have a tendency to aggregate. Disaggregation and a better particle solubilization may be achieved by the addition of detergents (see Section 3.3), urea (6–8 *M*) and/or an increase in ionic strength. Sometimes the additon of DNAse or RNAse to samples of totally encapsulated viruses is advisable in order to remove free nucleic acids. The stability of viruses can be improved by addition of bivalent ions like Mg^{2+} and Ca^{2+}. Finally, it should be mentioned that some particles, especially viruses with tails, have a tendency to stick to membranes, and adsorption onto gel matrices may also occur (see Section 4.2).

3. THE BUFFER

3.1. Gel electrophoresis in a continuous buffer

In most applications of electrophoresis to subcellular particles an identical buffer in the gel and electrode buffer reservoirs has been used. To maintain an evenly distributed and constant pH during the course of an electrophoretic experiment, relatively large electrolyte volumes may be used (0.5–3.0 l). Alternatively, electrode buffer reservoirs may be replenished continuously by pumping from a large volume; or, catholyte and anolyte may be continuously mixed [46].

The vast majority of particle separations by gel electrophoresis to date has been carried out in the physiological pH range 6–7.5. Within that range, buffers with high buffering capacity and low ionic mobility are to be preferred. Buffering capacity, of course, is best in the pH vicinity of the p*K* of the ionizable group in the pH range of interest. A suitable selection of buffers with amino groups of a p*K* close to pH 7 can be made from refs. 47–49 and a selection is presented in Table 1. These buffers have the further advantage of relatively low electrophoretic mobilities with the consequence of a low level of ohmic heating (Joule heating) during electrophoresis relative to conventionally used 0.05 *M* sodium phosphate buffer of pH 7.4 (with 1 m*M* magnesium chloride). The latter has successfully been applied to bacteriophages [50]. Buffer concentrations yielding an ionic strength of 0.03 *M* appear most suited to particle electrophoresis. Appendix 7 of ref. 51 lists a computer program in BASIC by D. Rodbard providing the concentrations required to achieve this ionic strength.

3.2. Multiphasic buffer systems

Electrophoresis in a discontinuous buffer [52] (also known as moving boundary electrophoresis*, MBE [53]) uses a multiphasic discontinuous buffer system (therefore called disc electrophoresis). The principle is illustrated and explained

*The term moving boundary electrophoresis in this review does not refer to electrophoresis in free solution in the Tiselius apparatus [240,241].

TABLE 1

BUFFERS WITH LOW ELECTROPHORETIC MOBILITY AND HIGH BUFFERING CAPACITY IN THE PHYSIOLOGICAL pH RANGE

It should be noted that some buffers containing primary, secondary and tertiary amines may react with monomeric acrylamide which causes a pH shift [238]. (Selection from a table of the Sigma Catalogue 1987, p. 306.)

Buffer	Abbreviation	pK_a (25°C)	Useful pH range
2-(N-Morpholino)ethanesulfonic acid	MES	6.1	5.5–6.7
Bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane; 2-bis(2-hydroxy-ethyl)amino-2-(hydroxymethyl)-1,3-propanediol	Bis-tris	6.5	5.8–7.2
N-(2-Acetamido)-2-iminodiacetic acid; N-(carbamoyl-methyl)iminodiacetic acid	ADA	6.6	6.0–7.2
Piperazine-N,N'-bis(2-ethanesulfonic acid); 1,4-piperazinediethanesulfonic acid	PIPES	6.8	6.1-7.5
2-[(2-Amino-2-oxoethyl)amino]ethanesulfonic acid; N-(2-acetamido)-2-aminoethanesulfonic acid	ACES	6.8	6.1–7.5
1,3-Bis[tris(hydroxymethyl)methylamino]propane	Bis-tris propane	6.8; 9.0	6.3–9.5
3-(N-Morpholino)-2-hydroxypropanesulfonic acid	MOPSO	6.9	6.2–7.6
N,N-Bis(2-Hydroxyethyl)-2-aminoethanesulfonic acid; 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid	BES	7.1	6.4–7.8
3-(N-Morpholino)propanesulfonic acid	MOPS	7.2	6.5–7.9
N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; 2-[(hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)ethanesulfonic acid	TES	7.5	6.8–8.2
N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid	HEPES	7.5	6.8–8.2
3-[N-Tris(hydroxymethyl)methylamino]-2-hydroxy-propanesulfonic acid	TAPSO	7.6	7.0–8.2
Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid)	POPSO	7.8	7.2–8.5
N-(2-Hydroxyethylpiperazine-N'-3-propanesulfonic acid	HEPPS; EPPS	8.0	7.3–8.7
Tris(hydroxymethyl)aminomethane; 2-amino-2-(hydroxymethyl)-1,3-propanediol	Tris; Trizma base	8.1	7.0–9.0
N-Tris(hydroxymethyl)methylglycine; N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine	Tricine	8.1	7.4–8.8
N,N-Bis(2-hydroxyethyl)glycine	Bicine	8.3	7.6–9.0

in Fig. 1. MBE is a powerful tool in the analysis of macromolecules [51], but has to date been applied only once to plant viruses and polystyrene size standards [54]. One stumbling block in its application to the separation of subcellular particles is that suitably fast migrating tracking dyes (marking the moving boundary) have not been found as yet. However, this technique can be used for the automatic electrophoretic concentration of samples prior to electrophoretic separation in a continuous buffer. For that purpose a concentrating (stacking) gel of low concentration (about 0.5% agarose) is formed. The three buffers, for the stacking gel and for the two buffer reservoirs (anolyte and catholyte), can be taken from published calculated buffer system compositions which generate the

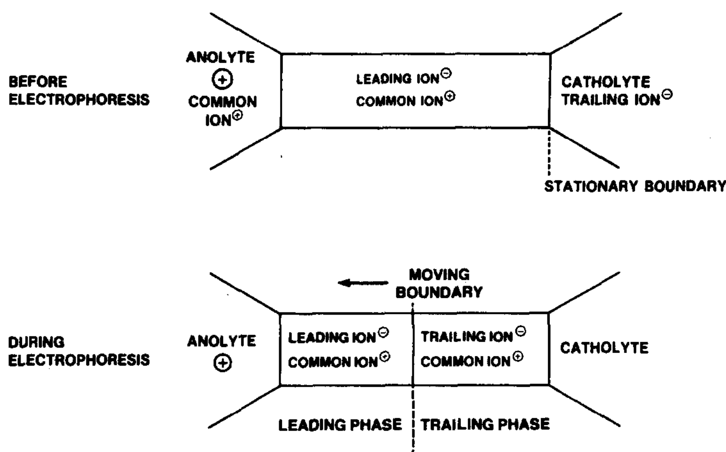

Fig. 1. Schematic diagram explaining moving boundary (discontinuous) electrophoresis. Anolyte and catholyte are the buffers of the electrode compartments, the rectangular field inbetween represents the gel. Three types of ions have to be distinguished in a discontinuous buffer system: (i) the leading ion (high net mobility), (ii) the trailing ion (low net mobility) and (iii) the counter ion of both, the common ion. The sample (not shown) is dissolved in catholyte and applied to the gel. In the electric field, leading ions and trailing ions form a moving boundary which is displaced towards the positive pole. A concentration of the sample is achieved by the fact that the leading ion mobility is selected to be larger and the trailing ion mobility to be smaller than the one of the sample which, therefore, is stacked between the two ionic species. The system shown is of negative polarity (leading and trailing ions are negatively charged) and represents the most popular variety of moving boundary electrophoresis. (Adapted from Chrambach [51].)

requisite moving boundaries (also designated as the stack). Formulations are available as a limited selection [55], as a computer output on microfiche [56] or from applications in the literature (Table 1 of Appendix 1 of ref. 51).

3.3. *Detergent and urea*

It has been possible to solubilize and electrophorese bacteriophages [57,58], plant viruses [59],vesicles [60] and cellular particles (semi-synthetic meningitis vaccines) [58] in the absence of detergent. Evidently, that is the preferable procedure in the interest of maintaining the native properties of the particle. In other applications, like those to polystyrene size standards on either agarose or polyacrylamide gels [54,61,62], detergent is required for solubilization. The requirement appears to progress with particle size. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) in 0.01–0.05 *M* concentrations has been able to solubilize particles up to 60 nm radius; 0.01% Triton X-100 has been capable of solubilizing particles as large as 150–200 nm radius [63,64]. Other detergents may be optimal in other applications [65,66].

Urea (6 *M*) has been added to the gel buffer in application of polyacrylamide gel electrophoresis (PAGE) to polystyrene sulfate particles [62]. Here, however, the urea served not to solubilize the particle, but rather to make the gel more transparent [67] for the visualization of the particle zones.

4. GEL MEDIA

Although starch gels can produce beautiful results in regard to protein separations, they are not applicable to electrophoresis of subcellular particles due to the narrow range of pore sizes which is not sufficiently large to allow for the entry of subcellular particles. Polyacrylamide gels have scarcely been used, e.g. in applications to tobacco rattle virus, cowpea yellow mosaic virus, bean pod mottle virus and broad bean mottle virus [68], bacteriophage ϕX174 [69], coliphage M13 and bacteriophage Qβ [70], M13 virions [71], bacteriophage T4D [72,73] and ribosomes [74,75]. By far the most popular gel matrix used for relatively large particles is agarose (reviewed in ref. 57).

4.1. Polyacrylamide

Polyacrylamide gels are synthetic polymers made by polymerization of acrylamide. Their porosity can be influenced by two parameters: the gel concentration (%T) [76,77] and the degree of cross-linking (%C) [78] achieved by copolymerization of bifunctional vinyl or alkyl compounds like cross-linking agents N,N′-methylenebisacrylamide (Bis) and N,N′-diallyltartardiamide (DATD)* and others. The gel concentration, %T, is defined as the total amount of monomer and cross-linking agent (in g) that is contained in 100 ml, %C is the quotient of cross-linking agent (in g) and %T multiplied by 100. The specification of 3%T and 20%C, for example, means that 2.4 g of monomer and 0.6 of cross-linking agent are contained in 100 ml. To achieve large pore sizes suitable for subcellular particles, %T has to be below 2.5 at 5%C [72]. These gels are almost liquid and are extremely difficult to handle. Another way of increasing the pore sizes of polyacrylamide would be to increase %C above 10 [68,78]. Orbán et al. [62] showed that within a very narrow concentration range one can obtain Ferguson plots of polystyrene sulfate particles with a radius from 22 to 60 nm using polyacrylamide of 30%C and gel concentrations ranging from 2 up to 3.2%T. The disadvantage of highly cross-linked gels is that they are fragile and have little wall adherance. Therefore, gels in tubes should be supported at the bottom by a gel plug (e.g. 10%T, 5%C) or by nylon mesh. The turbidity of these gels is reduced but still considerable at low concentrations that are applicable to subcellular particles. The addition of urea and polymerization at 50°C [67] further increases transparency.

Although polyacrylamide has been used for particle separations, it is inferior to agarose gels. Acrylamide and acrylamide-related cross-linkers have to be handled with care, since they are cumulative neurotoxins. Polymerized gels may contain 1–50% unreacted monomer (Fig. 10 of ref. 79), therefore, skin contacts should be avoided.

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*Gels cross-linked by DATD can be solubilized [242].

4.2. Agarose

Agarose is purified from agar [80–82], a polysaccharide which is isolated from red algae. Agar itself is quite unsuitable for many forms of electrophoresis due to its highly charged subcomponents (e.g. agaropectin) which cause electroendosmosis (see Section 8.2.3). Therefore, Hjertén [81] and Polson and Russel [22] replaced agar electrophoresis by electrophoresis in agarose. Later agarose electrophoresis [83] and quantitative agarose electrophoresis [84] was popularized by Serwer who has reviewed the application of agarose to electrophoresis [57,85]. Agarose electrophoresis and its pros and cons are also discussed by Chrambach [51,86].

The structure of agarose is described by Arnott et al. [87] and reviewed by Serwer [57] and Righetti [88]: two carbohydrate chains form a double helix with a radius of 1–2 nm. Several of these chains by hydrogen bonds aggregate to form a network of supercoil fibers (radius 20–30 nm). Structural models are pictured in refs. 57, 87 and 88. Agarose gels may be compared to a three-dimensional net made of rope: the rope represents the supercoil fibers, the strands of the rope correspond to the double-helical fibers. The holes in the net are equivalent to the water-filled pores of a gel. The net has to be considered quite irregular in regard to the diameter of the rope and the shape and size of the mesh (see Section 8.7); the sizes of the spacings are best conceived as asymmetric distributions [76].

Agarose is a natural product and, therefore, *the* agarose does not exist [51,86]. A lot of varieties, due to natural and/or chemical modification, are available and will be discussed briefly. Four classes can be distinguished (Table 2):

(i) In the category of relatively pure and underivatized agarose, three grades are available ranging from a high to low degree of electroendosmosis which depends on the amount of charged groups in the carbohydrate chain.

(ii) Another agarose family is specially prepared for isoelectric focusing and has the lowest, but still measurable endosmosis. This is achieved by blocking of the charged agarose groups by addition of clarified locust bean gum (IsoGel, FMC) [89,90] or by addition of quaternary positive groups to the gel matrix (IEF, Pharmacia; presumably HSIF, Litex) [86,91].

(iii) One group of derivatized agarose is designed for preparation of gels having a decreased melting point. This is of special advantage when remelting of the agarose for recovery of particles is considered. The melting point is lowered by hydroxyethylation of the agarose in regard to FMC and probably Litex products as well.

(iv) Recently, a new grade of agarose has also become available which on dissolving gives less viscous solutions. This agarose probably consists of relatively shorter carbohydrate chains and allows for the easier preparation of the gel concentration range above 3%. These gel concentrations have been prepared also with hydroxyethylated [92] and normal-grade [93,94] agarose, however, the procedure due to the high viscosity of the solutions was quite laborious. Low-viscosity agarose at high concentrations appears to be applicable to large macromolecules and thus is an alternative to polyacrylamide.

TABLE 2

COMPARISON OF AGAROSE SPECIES

Modified and expanded version of Table 1 in ref. 86.

Class	Manufacturer/agarose brand name	
	FMC*	Litex**
Pure and underivatized agarose		
Electroendosmosis is high	SeaKem HE & HEEO	HSA
Electroendosmosis is medium	SeaKem ME	HSB
Electroendosmosis is low	SeaKem LE	HSC
	SeaKem HGT	
	SeaKem HGT(P) (discontinued)	
Agarose for IEF		
Electroendosmosis is lowest	IsoGel	HSIF
Derivatized agarose		
Melting point is medium	SeaPlaque	EasyPlaque
		LS series
	(5.3% hydroxyethyl)	?
Melting point is low	SeaPrep	AgarSiéve
	(15.3% hydroxyethyl)	?
Low-viscosity agarose	NuSieve	

*FMC Bioproducts (Rockland, ME, U.S.A.).
**Litex (Glostrup, Denmark).

In general, agarose is by far the best gel matrix available in regard to separation of subcellular particles. It has the following advantages:

- Gels of low %T suitable for electrophoresis of subcellular particles are easier to prepare. Compared with polyacrylamide gels, they are mechanically stronger due to their double helix/supercoil fiber architecture [95].
- Compared with polyacrylamide, duration of staining and blotting procedures is shortened due to more rapid diffusion in the porous medium.
- Agarose is a non-toxic product.
- Agarose gels are not highly oxidative in contrast to polyacrylamide which is polymerized by a free radical-catalyzed reaction (p. 107 of ref. 51).
- Melting of the agarose (especially of hydroxyethylated species) potentially allows for recovery of thermostable particles.
- For an isolation of particles from agarose gels, it appears potentially important that agarose can be enzymatically degraded without any effect on protein structure [96].

Some disadvantages are:

- Gels are slightly turbid, unless gelling is performed by rapid cooling [93] or in the presence of urea or other hydrogen bond-breaking substances. These procedures, however, cause gels to be more restrictive.

- Some particles adsorb to the gel matrix. Often this problem can be avoided by choice of another agarose variety.
- Gels are sensitive to Joule heating.
- Adherence to glass walls is weak. However, this can be overcome by pre-coating the wall surface with a dry film of agarose [92,94].
- Agarose varies from batch to batch.

4.3. Gel casting

The preparation of polyacrylamide gels is described in refs. 51, 62 and 97–99. Agarose gels are best prepared by use of a microwave oven which shortens the procedure of agarose solubilization to a few minutes. In the case of heat-labile buffer constituents, agarose should be dissolved in distilled water of 75% of the final volume. The four times concentrated buffer solution is added afterwards. The agarose can be dissolved by either of two procedures. (i) The agarose solution is heated in an Erlenmeyer flask fitted with an air funnel made of plastic wrap (SaranWrap, Frappan, etc.). Water loss during the heating is determined by weighing the flask before and after heating and corrected for by addition of distilled water. (ii) Small agarose volumes can be heated for a *short* time in a tightly closed flask [61] without monitoring of water loss.

It should be stressed that heating in a tightly closed container can cause explosion, unless the heating time is very short or the container is pressure-proofed. Agarose solutions tend to have a boiling retardation. Heat-protective gloves should be worn and bottle openings have to be kept pointing away from the body in order to avoid severe burns.

The prepared agarose solution cooled down to about 60–70°C is poured into the horizontally aligned slab gel apparatus. Alternatively, agarose gel concentrations up to 2.3% can be filled by hot pipettes into hot agarose-pre-coated glass tubes the bottoms of which are closed by Parafilm [61]. Procedures suitable for higher agarose concentrations are described in refs. 92–94.

5. APPARATUS

Gel electrophoresis is performed in numerous varieties which are of two major types: tube gels and slab gels.

5.1. The tube apparatus

The first electrophoresis experiment reported was performed in tubes [3]. A modern tube apparatus applicable to "quantitative" gel electrophoresis of subcellular particles [54,60–62,98] was developed and described by Chrambach and co-workers [51,97] and is demonstrated in a schematic drawing in Fig. 2. This technique has the following advantages:

- Electrophoresis at different gel concentrations can be performed simultaneously in the same apparatus. This is of importance, since mobility data at different %T are essential for the Ferguson plot technique (see Section 8.2.1).

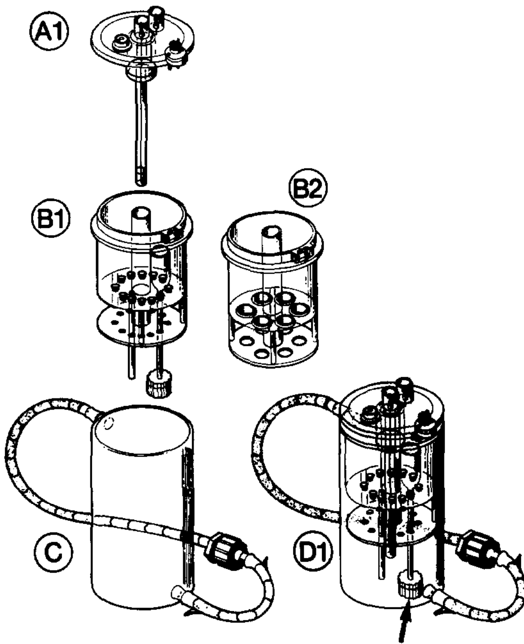

Fig. 2. Gel electrophoresis tube apparatus mostly made from pyrex glass. A1 = lid with electrode connections; B1 and B2 = upper buffer (catholyte) reservoir for 6- or 15-mm glass tubes (two 6-mm tubes with and without substance collecting cup, marked by arrow in D1, are shown); C = lower buffer (anolyte) reservoir with cooling jacket; D1 = the whole assembled apparatus. A similar model can be purchased from R&D Scientific Glass (Bethesda, MD 20892, U.S.A.). Other versions are distributed by Hoefer, Biorad, Miles and Buchler. (Adapted from Chrambach [51].)

- Gels can be examined separately during the run by removing the tubes and plugging the holes in the upper buffer reservoir (Fig. 2) with a rubber stopper. Electrophoresis in the other tubes is not perturbed.
- Electrophoresis can be performed in very fragile gels when the bottom of the tube is closed by a supportive gel plug [61,100].
- Easy application of larger sample volumes.
- No water condensation on the gels or loss of water due to evaporation.
- Constant cooling can be easily achieved.

The disavantages are:

- Visual comparison between samples at the same gel concentration is not as easy as on a slab gel.
- Unless naturally colored, fluorescent or pre-stained particles are used, the gels have to be removed from the tubes for staining. This may be difficult or impossible in the case of fragile gels, and even with stable gels involves the hazard of stretching the gel.

5.2. Slab gel apparatus

Electrophoresis in slab gels is the most popular. Horizontal slabs of agarose are preferred for the electrophoresis of relatively large subcellular particles due to the

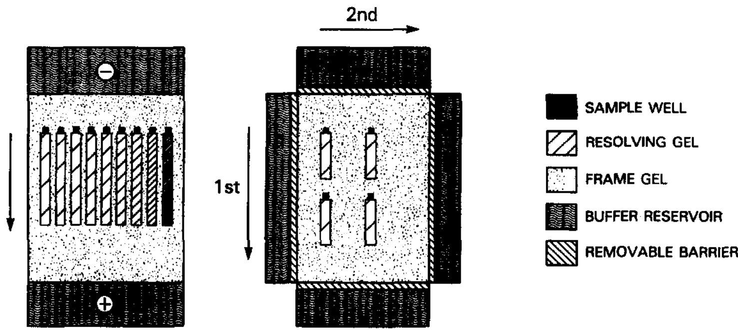

Fig. 3. Schematic view of the multi-channel submarine slab gel apparatus (left panel) described in ref. 104 and the two-dimensional submarine apparatus (right panel) published in ref. 107. The multi-channel type is commercially available from Aqueboque Machine Shop (P.O.B. 2055, Aqueboque, NY 11931, U.S.A.). For further details see Section 5.

fact that the necessary low concentrations of agarose give fragile gels which are best supported this way.

5.2.1. Uniform slabs

Slab gel electrophoresis at a single gel concentration is ideal when a large number of samples are to be compared, e.g. when fractions from gradient centrifugation [101] are screened or when processes like phage assembly [102,103] are investigaced. In the horizontal mode, gels can be run either uncovered by [83] or submerged in buffer.

5.2.2. Multi-channel slab gels

This type of gel and the submarine gel apparatus [104] is illustrated in Fig. 3 (left panel). Tracks within a pre-cast agarose frame (1.5–2%T) are filled with agarose gel of different %T (usually 0.1–1.5%). The technique allows the study of particles by applying the Ferguson plot technique [84] and different kinds of agarose can be tested side by side [57,105]. Further advantages of uniform as well as multi-channel slab gels are:

- Gel concentrations as low as 0.035%T can be handled [106]. This allows for a reasonably accurate determination of the free mobility, μ_0.
- Staining and subsequent photography is possible without moving the gels. This is essential for fragile gels.
- Gels can be easily accessed. Voltage or temperature measurements can be made at any location.
- Recycling buffer from electrode compartments and across the top of the gel surface gives a homogeneous buffer distribution even after a very long duration of electrophoresis, and at the same time ensures efficient cooling of the gel.

Some disadvantages are:

- Discontinuous gel electrophoresis cannot be performed in the submarine-type apparatus.
- In comparison with the tube apparatus cooling and temperature control is more elaborate.

5.3. *Two-dimensional agarose slab gel technique*

A recent development in agarose gel electrophoresis of subcellular particles is Serwer's introduction of a two-dimensional slab gel apparatus [107] (Fig. 3, right panel) which is a further development of the multi-channel-type of equipment. It is also made from Plexiglas, but has four (instead of two) buffer chambers and two sets of platinum wire electrodes. By switching cable connections and moving Plexiglas barriers it thus provides the possibility of switching the direction of the electric field by 90° after the run in the first direction is completed without touching of the fragile gel. The slab gel typically consists of an agarose frame of relatively high concentration (0.8–2%T) and four channels filled with a low gel concentration (0.1–0.2%T) as shown in Fig. 3 (left panel). Four samples per slab are applied in the sample wells and are then allowed to migrate electrophoretically along the length of the channel. After the field is switched the particles migrate in the second dimension into the frame gel perpendicular to the tracks. The principle of this technique is that in the low %T agarose particles are mainly separated according to charge, while in the high %T frame gel sieving according to particle size is more predominant. The technique and its advantages will be further described in Sections 8.5 and 8.6. Serwer's two-dimensional technique is not to be confused with the two-dimensional polyacrylamide electrophoresis according to O'Farrell [108] which achieves charge and size fractionation of proteins in a different procedure by isolelectric focusing (IEF, see Section 9.3) in the first and sodium dodecyl sulfate (SDS) electrophoresis in the second direction. O'Farrell's technique has not been applied to subcellular particles because SDS treatment* would degrade most of the particles into small subunits.

6. ELECTROPHORESIS

Prior to electrophoresis, the apparatus is aligned vertically (tube gels) or horizontally (slab gels). The particles are applied by a syringe or small pipette. Samples prepared as described in Section 2.2 can be used as such in the case of non-submerged horizontal gels. Otherwise, 5–20% of sucrose, glycerol, Metrizamide or $^{2}H_2O$ are added to increase the density of the sample solution. This allows for layering the sample beneath the buffer onto the gel surface when a tube or submarine-type slab apparatus is used. Furthermore, for a better monitoring of electrophoresis it is advisable to add a dye like Bromophenol Blue to the sample. To achieve a good separation, sample volumes should be small when a continuous buffer system is used. The multi-channel submarine apparatus is designed to hold a volume of about 30 μl in the sample well. The volume can be doubled by combining two sample well formers at the time of gel casting. In the case of larger samples, band sharpening can be achieved by applying the sample in gel electrophoresis buffer diluted by a factor between 2 and 5 [109].

*SDS, an ionic detergent [65,66], is applied to equalize the charge of investigated particle species. Thus, it is possible to achieve a separation according to size only which allows for the determination of relative molecular mass at one single gel concentration.

Sample volumes can be of the order of 1 ml and larger in discontinuous gel electrophoresis carried out in gel tubes [55,97,110–113]. At the present time it is recommended to use a multiphasic buffer system for the purpose of sample concentration only (see Section 3.2). The part of the gel containing the concentrated particle zone can be cut out and further analyzed by electrophoresis in a continuous buffer.

After a proper set up, the apparatus is closed and connected to the power supply which, ideally, is a precision power source covering the low voltage range (e.g. Hewlett-Packard No. 6115A). Relatively high currents of the order of 200 mA are required for the operation of the multi-channel submarine apparatus. Voltage (constant voltage setting) and temperature are monitored and have to be kept constant. The duration of the electrophoresis depends on the size and charge of the particle, the gel concentration, the field strength, the temperature and the pH, ionic strength and ion mobilities of the electrophoresis buffer. The time of electrophoresis is usually of the order of about 17–24 h both when the multi-channel apparatus (gel tracks of 12 cm length), phosphate buffer (Section 3) and gel concentrations from 0.1 to 1.5%T are used or when the tube apparatus is applied in the same concentration range (using a buffer with low electrophoretic mobility, see footnote on p. 336) at 20 or 0°C, respectively.

Although the applied voltages are relatively low, safety precautions such as safety interlocks and ground fault circuit interrupting devices are recommended. They are mandatory when power supplies with high current output are used.

7. DETECTION OF PARTICLES

Most of the particles are not colored and often they are applied at concentrations at which they cannot be detected by light scattering, especially when gels like agarose or highly cross-linked polyacrylamide are turbid. Some staining procedures and scanning devices will be reviewed briefly. An excellent review dealing with the problems related to particle detection has been published by Neuhoff et al. [114].

7.1. Staining of gels

Apart from immunological procedures for the specific detection of substances (see Section 9.5), methods for staining proteins by Coomassie Blue G-250 [115] or R-250 [46] are mostly applied. Neuhoff and co-workers [116,117] published a new Coomassie staining technique which is described as being more sensitive and more linear in the response; the same authors also investigated the influence of gel thickness and concentration on staining. Recently, a photothermal quantification of Coomassie Blue-stained proteins in polyacrylamide with a detection level of as low as 0.5 ng has been published [118]. Very sensitive, but far less linear, is silver staining with a detection level for proteins and nucleic acids in the low nanogram range. Techniques and their mode of reaction in special regard to polyacrylamide gels have been reviewed by Merril [119,120] and Heukeshoven and Dernick [121,122]. Procedures for silver staining in agarose gels are given

in refs. 123–126. A disadvantage of silver staining is that it is best applied to dried gels or very thin hydrated gels. Nucleic acids can be also very selectively detected by fluorescination with ethidium bromide which is almost as sensitive as silver stain. Ethidium bromide can be applied after electrophoresis in polyacrylamide [127] or agarose [128], it can be added to agarose gels before electrophoresis [61,129] or particles can be pre-stained with the dye without measurable alteration of their mobility values [61]. After fluorescination, particles can be detected under UV light of about 300 nm. Suitable light sources are, e.g., those manufactured by Fotodyne (New Berlin, WI 53151-0183, U.S.A.). The procedure including photography is described in detail in ref. 61. UV protection of eyes and skin is necessary. Since ethidium bromide is highly carcinogenic, contact with skin has to be strictly avoided.

7.2. Scanning of gels

Quite expensive and sophisticated computer-assisted scanners have been developed for evaluation of two-dimensional O'Farrell gels (see p. 317) which can contain thousands of spots. In regard to electrophoresis of subcellular particles revealing a relatively small number of spots or bands, simpler models linked to a personal computer (e.g. Biomed or LKB) in the price range of about US$ 20 000 may be useful, especially after upgrading the available software. Many scanners operate with laser beams the disadvantage of which is the scattering of coherent light; laser light photometry also does not obey Lambert-Beer's law [130]. A techique based on incoherent light, microscope optics and a software (written in FORTRAN) run on a VAX11/730 computer (Digital) which is especially tuned to quantitation problems has been published [114,131,132]. The scanner has become commercially available (ELSCRIPT 500 HP 2-D, Hirshmann, Unterhaching, F.R.G.). Neuhoff et al. [114] stress the fact that one-dimensional electrophoresis should be evaluated two-dimensionally and that a circular aperture is essential. A less sophisticated and less costly two-dimensional gel densitometer called GELSCAN (ca. US$ 700) has been recently described [133]. The report presents a do-it-yourself procedure for setting up the equipment operating with a Commodore (64 or 128) or Apple II personal computer system, a photocell and a projecting microscope as a light source. Software is described as well.

8. DATA ANALYSIS AND THEORY

8.1. Measurement of mobility values

The electrophoretic migration of a particle in a continuous buffer is recorded as absolute mobility ($cm^2\ V^{-1}\ s^{-1}$) which is the quotient of migration distance (cm/s) and field strength (V/cm). For convenience, values are usually multiplied by a factor of 10^4 or 10^5. Using a discontinuous buffer system, relative mobility values, R_F(disc.), are specified. R_F(disc.) is the migration distance of the particle in the resolving gel divided by the displacement of the front moving

boundary. The calculation of mobility values allows for the comparison of data sets derived from experiments performed at similar pH, ionic strength and temperature, but different time and field strength. Mobility, to be a meaningful characteristic of the particle, has to be independent of field strength. Thus, one must experimentally determine the maximal field strength to which mobility remains constant. This value, with polystyrene size standards, is about 1 V/cm [61]. Relative mobility values are less dependent on variation of temperature and field strength. Both types of mobility data are interconvertible (Sections 3.7 and 4.2 of ref. 153; calculation of mobility of front moving boundary, Table 2 of ref. 156 and p. 57 of ref. 51).

8.2. Independent determination of size and charge in "quantitative" gel electrophoresis

Electrophoresis in a gel matrix in addition to particle surface net charge density is dependent on the particle size which determines the resistance that a particle encounters when moving through a meshwork of fibers. The dependence on both particle size and charge qualifies gel electrophoresis as a unique separation method because other techniques are related either to size (e.g. gel chromatography, centrifugation) *or* charge (e.g. free-flow electrophoresis). In the following subsection it will be demonstrated how the parameters size and charge, although determining simultaneously the mobility of a particle, can be evaluated separately. In this connection the term *quantitative gel electrophoresis* has been introduced [51,97], where quantitative expresses a method based on mobility measurement in electrophoresis at different gel concentrations under controlled conditions (pH, ionic strength, temperature) and objective statistical evaluation.

8.2.1. Evaluation of the retardation coefficient (K_R) and free mobility (μ_0): explanation of basic principles in terms of linear Ferguson and R plots

Several approaches for the theoretical interpretation of electrophoretic mobility data have been published (e.g. refs. 134–139). In regard to gel electrophoresis of non-reptating particles (see Section 8.3.3) and attempting to explain mobility data in relation to gel structure, the Ogston theory [140] in its extended version [76] has the merit of making no assumptions concerning the nature of pore geometry. It is therefore preferred for the interpretation of mobility data.

The extended Ogston theory probabilistically describes the migration of a particle through a random and inert network of non-flexible fibers on the basis of the fractional volume that is available to the migrating particle. This volume is determined by the particle radius, R, as well as the fiber radius, r, and total fiber length, l'. It has been shown that the measured mobility, μ, and the free mobility (mobility in solution), μ_0, can be linked to these parameters by the following two equations [76, Fig. 15 of ref. 110]:

$$\log(\mu) = \log(\mu_0) - K_R \times T \tag{1}$$

$$K_R = \pi l' \times (r+R)^2 \tag{2}$$

The first equation is underlying the Ferguson plot which is the linear relation of log(mobility) versus gel concentration %T (see Fig. 4) or, in moving boundary electrophoresis, the plot of $\log(R_F)$ versus %T. This linear relation was first empirically found by Ferguson [141] in starch gel electrophoresis of proteins and afterwards popularized for polyacrylamide gels by Hedrick and Smith [142]. Chrambach and Rodbard [143] adopted for it the name of Ferguson plot. The Ferguson plot allows the determination of two parameters independently from each other (Fig. 4): the slope of the linear plot yields the retardation coefficient, K_R (a measure of particle size), and the antilog of the extrapolated value at 0%T is the free mobility, μ_0, which is related to particle surface net charge density*.

The retardation coefficient is also defined by eqn. 2. This relation valid for long-fiber gels becomes a linear expression after the square root is taken. The linear plot of $\sqrt{K_R}$ versus particle radius R (Fig. 5) is designated as the R plot. This plot has two applications. (i) After calibration (determining the K_R of particles of known size) the radii of unknown particles can be estimated according to their K_R. (ii) Gel fiber dimensions can be derived from it [76,94]: the negative intercept on the R-axis equals the negative value of the effective fiber radius (nm), $-r$, the intercept on the $\sqrt{K_R}$-axis equals the square root of the total fiber volume (of 1 g dry matrix material) divided by 10, and the curve slope represented by the factor $\sqrt{(\pi l')}$ is related to the total fiber length produced by 1 g of dry gel matrix material. The following calculations are given as an example: assuming that the full ranges of the $\sqrt{K_R}$-axis and R-axis of the plot shown in Fig. 5 are 0.5 and 6 nm, respectively, the radius of the unknown R is 1.9 nm, the fiber radius r is 1.1 nm, the intercept on the $\sqrt{K_R}$-axis is 0.097 and the curve slope c_1 is 0.090. According to the rationales published in ref. 94 the total fiber length l' is $0.090^2 \times 10^{16}/\pi$ or 2.6×10^{13} cm/g of dry gel matrix, the total fiber volume V_F is $0.097^2 \times 100$ or V_F is 0.95 ml/g of dry gel matrix.

8.2.2. *The influence of pH, ionic strength and temperature on K_R and μ_0*

Apart from gel concentration and field strength, electrophoretic mobility data of a particle are also dependent on pH, temperature and ionic strength. This is due to the relation of measured mobility, μ, to free mobility, μ_0 (eqn. 1), which strongly depends on the ionization of the particle. For the comparison of mobility values and for the calculation of the retardation coefficient, K_R, electrophoresis at constant and controlled conditions is therefore essential. The calculated K_R values are less dependent than the μ values as has been discussed previously [94], since they are not affected by changes in the free mobility (eqns. 1 and 2). The usually moderate effect of pH, temperature and ionic strength on K_R is due to their effect on the gel matrix by affecting the polymerization efficiency (polyacrylamide) or the degree of formation of hydrogen bonds (agarose). This

*The relation of μ_0 to net surface charge (valence) is discussed in ref. 134 (pp. 708–717). The valence of small spheres (protons/surface of a sphere) in the size range of proteins can be determined by program CHARGE of D. Rodbard (p. 57 of ref. 51; refs. 112, 156]. However, in regard to subcellular particles several assumptions underlying the calculations are violated and, therefore, the valence can be considered as a very rough estimate only [54].

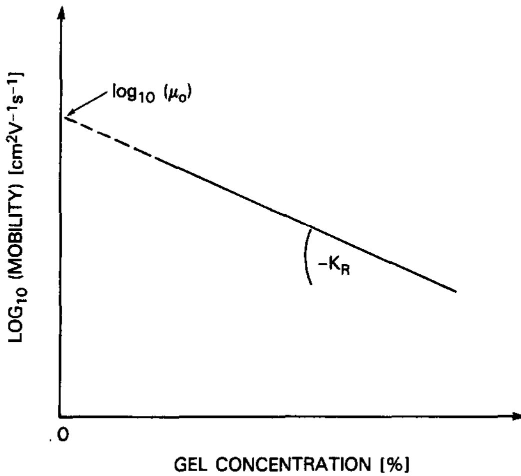

Fig. 4. Schematic illustration of a linear Ferguson plot which allows for determination of particle free mobility, μ_0 (related to surface net charge density), and the retardation coefficient, K_R. Explanations are given in Section 8.2.1.

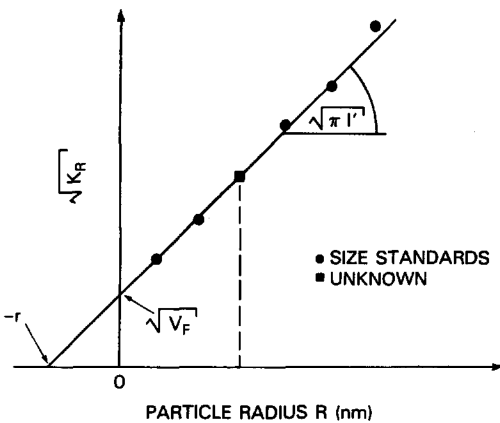

Fig. 5. Schematic illustration of the R plot used for determination of both particle size and gel fiber parameters. V_F = total fiber volume in ml produced by 1 g of agarose [94]. Further explanations are given in Section 8.2.1.

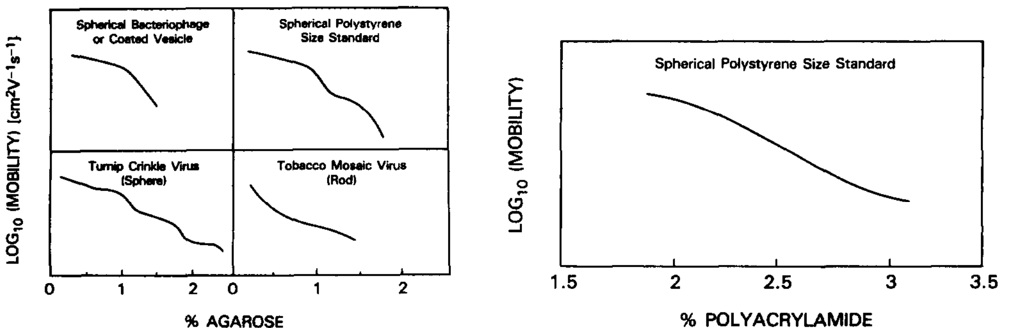

Fig. 6. Different types of non-linear Ferguson plots derived from agarose gel electrophoresis of the particles given in the boxes. The plot of tobacco mosaic virus is adapted from Serwer [155] and modified. For a discussion of these schematic plots see Section 8.3.

Fig. 7. Schematic non-linear Ferguson plot of a polystyrene size standard in polyacrylamide (Orbán

results in changes of gel fiber parameters r and l' to which K_R is related as shown in eqn. 2. It should be stressed, however, that the retardation coefficient changes considerably in regard to a chemical modification of the gel structure such as different degrees of cross-linking (polyacrylamide) [78] or gelling in borate buffer (agarose) [144].

8.2.3. *The effect of electroendosmosis on μ_0*

The theory of electroendosmosis (or electro-osmosis) is discussed by Morris and Morris (pp. 718–724 of ref. 134), and a special model in regard to agarose is presented by Waldmann-Meyer and Jacobsen [145]. Electroendosmosis always occurs when the gel matrix is bearing charged groups like the negatively charged sulfate, pyruvate and glucuronate residues that are covalently linked to the galactose molecules in the polymer chains of agarose [146–148]. In an electric field the hydrated positive (mobile) counter ions of the negatively charged gel matrix (stationary) migrate. This causes a waterflow towards the negative electrode which can be so intense that the gel is cracked by dehydration. In the case of a negatively charged particle migrating in agarose, electroendosmosis causes the measured mobility data and consequently the determined free mobility to be too small, since the particles move against the waterflow towards the positive pole and are retarded. Methods for the determination of electroendosmosis and the correction of μ_0 are reported by Ghosh and Moss [149] and Serwer and Hayes [57,150]. The degree of electroendosmosis in different types of agarose varies (see Section 4.2).

8.2.4. *Determination of particle mass*

According to the definition given above, subcellular particles consist of several molecules and may be of heterogeneous structure. The term molecular mass therefore does not apply. However, in cases of a comparison with macromolecules which are usually specified by their MW it may be of interest to specify the particle weight in kDa*. Applying rationales used for the determination of geometric mean radii from molecular weights (program RADIUS of D. Rodbard, Appendix 6 of ref. 51) and assuming that the spherical particle behaves like a single macromolecule, particle radius and weight (PW) are related by eqn. 3:

$$\mathrm{PW} = 4/3\,\pi R^3 N/V \qquad (3)$$

N (Avogadro's number) equals $6.023 \cdot 10^{23}$ and V usually equals 0.74 cm^3/g in the case of most proteins, but may vary due to the nature of a subcellular particle. In the case of viruses, e.g. which have a heterogeneous composition, an *average* V may be assumed to be that of proteins; polystyrene particles used in ref. 151 have $V = 0.952$. R is the geometric mean radius of a particle without hydration.

According to eqn. 3, the plot of $\sqrt{K_R}$ versus R (R plot, Fig. 5) can be replaced by the plot of $\sqrt{K_R}$ versus $\sqrt[3]{\mathrm{PW}}$ in the case when all particles under consideration are of almost the same density (factor V in eqn. 3).

*Note that the 'dalton' unit (Da) does not conform with the SI unit system. In spite of that it is used quite frequently.

8.3. *Non-linear Ferguson plots in the gel electrophoresis of subcellular particles*

Ferguson plots of macromolecules in polyacrylamide gels have been reported to be linear within the useful concentration range (see Section 8.2.1). By contrast, Serwer [84] first observed that Ferguson plots of bacteriophages in agarose of a concentration larger than approximately 0.8%T become non-linear. The curvature of plots obtained from agarose electrophoresis is expressed by different shapes (Fig. 6) and this seems to be related to the nature of the particle [151]. Recently, curved plots of polystyrene size standards (Fig. 7) in polyacrylamide have been reported [62].

The curvature of Ferguson plots needs not be of concern, if particle separation or isolation (see Section 9.2), isoelectric focusing (Section 9.3), titration curves (Section 9.4) or immunoelectrophoresis (Section 9.5) are required. However, the interpretation of curved plots is of importance (i) in regard to particle and gel fiber characterization resting on the evaluation of R plots (Section 8.2.1) and (ii) in regard to determination of the free mobility of a particle by gel electrophoresis and Ferguson plot technique (Section 8.2.1).

8.3.1. *Relatively rigid spheres: bacteriophages and vesicles*

Bacteriophages [57] and clathrin-coated vesicles [60] exhibit convex Ferguson plots (Fig. 6); these plots have a linear segment for a %T below about 0.8% and exhibit a disproportional decrease of mobility at higher gel concentrations (increase of curve slope between 1 and 1.6%). In an empirical-fitting [94] and later in a computer simulation study [152] based entirely on the extended Ogston theory [76] it has been demonstrated that the convex curvature can be accounted for by assuming that the agarose gel fibers become thinner and longer as the gel concentration is increased (see Section 8.4). This was hypothetically explained in terms of enhanced viscosity, steric hindrance and/or electrostatic repulsion between the sulfate groups of the polymer chains which may increasingly prevent agarose double-helical fibers to aggregate by hydrogen bonds and to form supercoil fibers [94].

8.3.2. *Elastic spheres: some plant viruses and polystyrene latex particles*

This category of particles in agarose gel electrophoresis is represented by a convex-type Ferguson plot with one or more segments where the reduction of particle mobility due to increase of gel concentration is less than expected (Fig. 6). The curve shape has been called convex–sigmoidal [61] (a sigmoidal curvature which is superimposed on a convex plot). Curve oscillations unlike the convex curvature do not appear to be gel-, but particle-specific, since they vary in number and they reproducibly occur at different gel concentrations depending on the sieved particle. A convex–sigmoidal Ferguson plot can be simulated by the model described in ref. 152 with the additional hypothetical assumption that the radius of a pliable particle is reduced upon sieving at higher gel concentrations (apparent compressibility, see Sections 8.4.2 and 9.4).

Gel electrophoresis in polyacrylamide also revealed a non-linear Ferguson plot of latex particles (Fig. 7) [62]. It is suggested that the slightly sigmoidal curve

shape can be explained as follows. The upper plateau of the sigmoid occurring below about 2.5%T possibly is due to the diminishing of the fiber network of the almost liquid gels leading to a transition from sieving by a fiber network to retardation by viscosity only. The cause of the curve flattening at higher %T is unknown, but it might be due to particle deformation. The medium %T range of the Ferguson plot reveals a linear relation. Curve oscillations became more pronounced and increased in number when larger particles were electrophoresed. None of the polystyrene particles (the same used in refs. 61 and 153, however, revealed a convex–sigmoidal Ferguson plot in polyacrylamide electrophoresis. It is, therefore, suggested [62] that the absence of a general convex curvature is an indicator of the fact that the polyacrylamide unlike the agarose fiber has constant fiber radius and total fiber length (l', per g of dry gel matrix) within the range of useful gel concentrations. This is in line with the linear Ferguson plots reported for spherical macromolecules [76,99]. The constancy of fiber dimensions can probably be attributed to the different modes of gel formation: aggregation of preexisting fibers (agarose) and polymerization (polyacrylamide) [152]. It should be noted that the polyacrylamide fiber presumably varies with different degrees of cross-linking [78,100,154].

8.3.3. Rigid rods exemplified by tobacco mosaic virus (TMV)

TMV particles with a radius of 4.5 nm and a length from 700 to more than 2000 nm can migrate in agarose gels and exhibit a concave Ferguson plot (a schematic drawing is shown in Fig. 6) which at higher gel concentrations becomes convex, as was demonstrated by Serwer [155]. This type of plot may be related to an orientation of TMV within the gel with its length parallel to the direction of migration designated as reptation in regard to electrophoresis of rigid DNA molecules [44,45]. A non-random orientation is presumably more pronounced at higher than at lower agarose concentrations with much larger spacings (see Table 3). There may be a continuous transition resulting in an increased effective size of the sieved particle at lower (0.1%) than at higher gel concentrations as is indicated by the decreasing steepness of the Ferguson plot below 1% agarose. Superimposed on this (above 1% agarose) seems to be the general convex curvature typical for wide-range Ferguson plots in agarose (see Section 8.3.1).

8.4. Computational evaluation of non-linear Ferguson plots

Ferguson plots derived from electrophoresis of subcellular particles in agarose are usually non-linear (Section 8.3), however, segments of the plot in the low agarose concentration range (below 0.4–0.8%T) can be linearized [61] and evaluated as described in Section 8.2.1 using linear regression analysis or the PAGE-PACK [112,156] programs of D. Rodbard which provide weighted and unweighted linear regressions, confidence limits as well as other statistical parameters. The evaluation of the non-linear Ferguson plot over the entire agarose concentration range which is of special interest for further characterization of the particle (Section 8.3) requires special techniques which will be discussed below.

8.4.1. Empirical approach in conjunction with the use of the extended Ogston theory

Convex Ferguson plots of rigid bacteriophages (Section 8.3.1) can be approximated by an empirical function of the type shown in eqn. 4 [94]. According to eqn. 1 $K_R \times T = a \times T^b$ and the retardation coefficient, K_R, is determined by eqn. 5.

$$\log(\mu) = \log(\mu_0) - a \times T^b \tag{4}$$

$$K_R = a \times T^{b-1} \tag{5}$$

Parameters a and b are variables and have to be determined by curve-fitting procedures [94]. Eqn. 5 demonstrates that K_R in the case of convex Ferguson plots ($b > 1$) varies with gel concentration. K_R at every %T is defined as the absolute value of the slope of the line that connects the curve point with the intercept on the log(mobility)-axis (obtained by curve extrapolation). This kind of mathematical approach and the evaluation of R plots for different %T gave the first indication that the convex curvature due to an increasing K_R can be explained by changes in the gel fiber structure: as %T increases the fiber radius of the agarose supercoil is reduced while the total fiber length, l', increases. A variation of agarose fiber dimensions with gel concentration has also been found by using freeze-fracture electron microscopy [157].

The evaluation by empirical functions has the advantage that relatively simple equations can be used and less time-consuming curve fitting is possible. It appears appropriate for rigid bacteriophages exhibiting convex Ferguson plots and it is the method of choice when a characterization of the gel fiber is of minor importance. The empirical curve-fitting technique cannot be applied in the case of more complex Ferguson plots mentioned in Sections 8.3.2 and 8.3.3.

8.4.2. Computer simulation using a model based on the extended Ogston theory

This approach uses equations derived from the extended Ogston theory [76] and is in principle a two-step procedure [153]. (i) Functions of the type given by eqn. 1 (containing eqn. 2 as a subfunction) are simultaneously fitted to electrophoretic data representing log(mobility) in relation to gel concentration obtained from differently sized particles with known radius R. This allows for the determination of fiber radius r, total fiber length l' and yields the free mobilities of the standard particles. (ii) Once the gel fiber parameters r and l' are standardized, the particle radii and free mobilities of unknown particles are determined by similar curve fitting.

Eqns. 1 and 2 as such are applicable, when Ferguson and R plots are linear. In the case of convex Ferguson plots (Section 8.3.1), the parameters r an l' have to be replaced by functions to account for the changes of gel fiber dimensions [152]: r diminishes while l' becomes larger as the gel concentration is increased. Furthermore, it was proposed that the size of the measuring probe (the migrating particle) determines whether effective fiber dimensions are more closely related to the agarose double helix or the agarose supercoil. While there was an indication for a particle size effect in regard to sieving of relatively small particles such as

proteins, it appeared that in the range of subcellular particles with larger radii only a dependence of r and l' on %T is detectable [152].

The convex–sigmoidal Ferguson plots (Section 8.3.2) can also be handled by the model described in ref. 152, when a decrease of particle size (particle compression) with increasing gel concentration is assumed [153]. Consequently, the particle radius R in eqn. 2 is replaced by a sigmoidal-type function which simulates a step-wise particle decrease. Apparent particle compressibility is especially observed with the sponge-like polystyrene particles (2.5% solids) and some relatively pliable plant viruses (see also Section 9.6).

A mathematical treatment of concave Ferguson plots exhibited by rod-shaped viruses appears feasible using two approaches. (i) The theory of Lumpkin and co-workers [138,139] describing the reptation of DNA molecules through randomly oriented tunnels within a gel may be applicable to rod-shaped viruses. (ii) The extended Ogston theory [76] is not strictly limited to spheres and is applicable to other particle shapes [77]. However, this theory in its present version would need to be changed to provide for particle reptation.

The simulation of electrophoretic mobility data allows for the most comprehensive interpretation of non-linear Ferguson plots available at present. It is an analytical tool for the investigation of the nature of the particle and the gel as well [158,159]. It should be stressed, however, that computer simulation can be used for evaluating the fit of a model to the results. However, a fit does not mean necessarily that the applied model represents reality. The simulation is also computer-time-consuming and thus expensive, since it involves program MLAB (DCRT, NIH) which can only be run on main-frame computer systems DEC-10 or DEC-20 (Digital). This fact also limits the availability of this simulation method, although program MLAB is exportable and used at hundreds of locations worldwide. A program in BASIC capable of handling moderately complex models on a personal computer is still outstanding.

8.5. Calculation of particle size using the two-dimensional technique

Two-dimensional electrophoresis performed by use of the apparatus described in Section 5.3 can be regarded as the special case of a two-data-point Ferguson plot obtained from an identical sample. Serwer et al. [46] have experimentally demonstrated that charge isomers with the same particle size are located along a diagonal line (the size line) and that the angle θ (between size line and gel track of the first dimension) can be used as a measure for particle radius. This fact can also be demonstrated (Tietz, unpublished results) by applying the mathematical model described in ref. 152 and Section 8.3.1 (Fig. 8). This approach is also used for predicting the lines of equal particle free mobility shown in Fig. 8.

8.6. Two-dimensional technique: pros and cons compared to the Ferguson plot method

The two-dimensional technique certainly is an elegant method for a number of applications [50]. In regard to subcellular particles it has the following advantages:

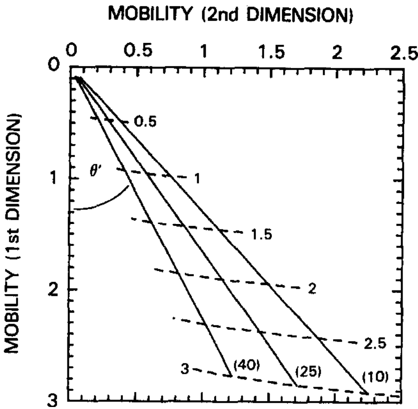

Fig. 8. Mathematical evaluation of two-dimensional gel electrophoresis of subcellular particles described in Section 8.5. The diagonal straight solid lines specify locations of equal size; number designations in parentheses give the corresponding particle radius in nm. Curved dashed lines mark the positions of particles with the same free mobility which are specified by numbers ($cm^2\ V^{-1}\ s^{-1}$, multiplied by 10^4, uncorrected for electroendosmosis). The angle θ' corresponds to θ that can be read off directly from the gel slab (published by Serwer et al. [46]) in those cases where field strength and duration of electrophoresis are the same in both directions. The calculation rests on the model derived from the extended Ogston theory and on the data base both described in ref. 152 (Dr. P. Serwer, University of Texas Health Science Center, San Antonio, TX, U.S.A., kindly provided published virus mobility data for ref. 152). First dimension = 0.1% agarose (SeaPlaque), second dimension = 1% agarose (SeaPlaque).

- Due to a higher resolution several substances including the standards can be compared simultaneously.
- Polydisperse particle populations which do not yield bands with a well defined maximum can be analyzed.
- Particle sizes can be easily determined from a photograph of the stained gel.
- Results can be obtained without moving of fragile gels.
- Irregular electrophoresis conditions such as retardation due to adsorption can be recognized [50]. For that purpose, the gel type and concentration of the first and second dimensions are identical. Under these conditions, all particles independently of their size will be located on a single size line (see Section 8.5) passing through the origin. The off-line position of a particle indicates a time-dependent mobility which may be related to adsorption or to denaturation.

Some limitations are:

- Free mobilities are difficult to determine, unless simulation tools are applied.
- Like a two-point Ferguson plot, the two-dimensional technique does not give any information about the nature of a particle. Abnormal migration due to a rod-shape or due to apparent compressibility may lead to incorrect size determinations, unless standard and unknown particle have the same order of irregularity.
- For a proper spot resolution and accurate size determination it may be necessary to experimentally determine the optimum %T required for the second dimension.

- Discontinuous buffer systems are not applicable.
- The originally designed apparatus [107] is not as yet commercially available.

Compared with this the Ferguson plot method has the following positive and negative aspects.

Advantages:

- Slab and tube apparatuses in several designs are commercially available at a relatively low cost.
- Ferguson plots allow for analytical studies in regard to gel structure and nature of the particle.
- The characteristic shape of a Ferguson plot may serve for particle identification.
- Free mobilities are easily obtained from agarose gel electrophoresis. This appears important for mutant analysis.

Disavantages:

- Several gel concentrations need to be prepared.
- Polydisperse particle populations cannot be examined.
- Evaluation of the entire non-linear Ferguson plots requires computational analysis.

The latter limitation to some degree can be overcome by restriction to linear plot segments (see above) or by use of the Zwaan method [160] and evaluation of a two-point Ferguson plot which also limits the effort of preparing different gel concentrations. The Zwaan method has been quite successfully applied, when polystyrene sulfate particles and some plant viruses of closely similar sizes were compared [61], both exhibiting convex–sigmoidal Ferguson plots. However, when detailed knowledge of both the gel fiber dimensions and particle characteristics are required, it is necessary to have more data points and to apply complex processing as used in other disciplines.

8.7. The pore size of a gel

In accordance with the Ogston model, a gel is considered a network of fibers which, in the ideal case, are non-flexible and inert and are randomly distributed. Hypothetically, such a fiber network in some respects can be compared to a heap of spaghetti representing the conditions in a gel magnified by a factor of 10^5–10^6. It is obvious that the spaces between the spaghetti vary considerably in shape and size and they cannot at all be compared with the uniform pores in a grid. Nevertheless, it is *statistically* possible to define an effective pore size. Rodbard [77] has reviewed different mathematical approaches. For long-fiber gels the eqns. 6 and 7 are presented [77] that can be derived either from the "random-spaghetti model" of Ogston [140] or rigid-grid model of Ornstein [36].

$$P_{0.5} = 1/\sqrt{(4 \times \pi \times l' \times 0.01 \times T)} \tag{6}$$

$$P_{M} = 1/\sqrt{(8 \times l' \times 0.01 \times T)} \tag{7}$$

Such calculated pore radii represent the radius of a "ball" that can be placed within the spacings of the three-dimensional fiber network. $P_{0.5}$ is the median pore radius in cm (most frequent pore size population), P_M is the mean pore

TABLE 3

COMPARISON OF EXPERIMENTALLY DETERMINED AND CALCULATED RADII OF AGAROSE GELS

P_M = mean pore radius; $P_{0.5}$ = median pore radius; further details are explained in Section 8.7.

%T	Pore radius (nm)							
	Experimental		Calculated by eqns. 6 and 7[a] (based on the extended Ogston theory)					
	IsoGel[b]	SeaKem LE[c]	SeaPlaque[d]		SeaKem HGT(P)[d]		IsoGel[e]	
			P_M	$P_{0.5}$	P_M	$P_{0.5}$	P_M	$P_{0.5}$
0.04		540[f] 345[g]	403	322			377	301
0.16	250		202	161			189	150
0.25		339[h]	161	128			151	120
0.4	130	233[h]	126	101			118	95
1	65	112[h]	72	58			46	36
1.6		96[i]	49	39			23	18
3		54[j]			29	23		
4		36[j]			22	18		

[a] According to Rodbard [77].
[b] Data of ref. 63.
[c] Data of ref. 64.
[d] Pore radii (nm) calculated on basis of the model in ref. 152 using eqn. 14 of ref. 152 for determination of the total fiber length, l'. A particle radius giving an R_F value of 0.7 (eqn. 15 of ref. 152) is used for gel concentrations (T) of 0.4% and larger.
[e] Calculated as above, except that the values of the gel fiber parameters r_{10}, r_{20}, M, BT and v_2 listed in Table 1 of ref. 152 are replaced by the values listed for $R10$, $R20$, MT, BT and V_2 in Fig. 3 of ref. 153.
[f] Polystyrene particle that does not enter the gel (Ferguson plot technique).
[g] Polystyrene particle that can enter the gel (Ferguson plot technique).
[h] Value obtained by eqn. 3 of ref. 63 (derived from both Ferguson plot and two-dimensional technique using viruses and polystyrene particles).
[i] Value obtained by eqn. 4 of ref. 63 (derived from two-dimensional technique using viruses).
[j] Determined by two-dimensional method using viruses.

radius (cm), T is the gel concentration in % (g per 100 cm^3) and l' is the total fiber length (cm) produced by 1 g gel matrix; $l' \times 0.01 \times T = \nu \times L$, where $\nu \times L$ is the fiber length per unit volume (cm^3) [77]. The fiber length, l', for linear Ferguson plots can be determined as outlined in Section 8.2.1. Calculation of pore sizes according to the example given in Section 8.2.1 for a gel concentration $T = 3\%$ would give $P_{0.5} = 3.2 \times 10^{-7}$ cm = 3.2 nm and $P_M = 4$ nm which indicates a very restrictive gel type. The treatment of non-linear Ferguson plots is discussed in refs. 94, 152 and 153 and also in Section 8.2. Table 3 contains calculated pore radii for agarose determined by electrophoresis of viruses and polystyrene size standards.

Righetti et al. [63] have published an empirical experimental method for determining pore sizes using polystyrene particles and very dilute agarose

(%T $\geqslant$ 0.16) or highly cross-linked (45–60%C) polyacrylamide gels. Linear plots of migration distance versus particle size at constant %T are presented. The pore size is estimated by linear extrapolation of the curve to 0 migration distance. The authors describe a maximum mean pore radius of about 250 nm for both gel types known to be very porous. This approach appears applicable as long as particles of almost equal charge (such as the polystyrene particles investigated) are sieved and the experimental conditions (%T, time, field strength, pH, temperature, ionic strength) are constant for the data to be compared. In other cases where the free mobility of the considered particle species varies, the author would like to suggest the following modifications: (i) Ferguson plots of the particles are obtained; (ii) the curves are shifted parallel (mathematically or graphically) in order to obtain for all of the plots an identical intercept on the log(mobility) axis; (iii) log(mobility) data for %T = constant are read from the transformed curves of (ii); (iv) the antilog of the mobility data obtained in (iii) is plotted versus particle radius.

Serwer and Hayes [64] recently published two strategies to obtain the pore size. The first method involves the Ferguson plot technique: mobility values at different %T are obtained and the gel concentration is determined where a particle just cannot enter the gel ($\mu = 0$); the pore size at this %T equals the radius of the excluded particle. The second procedure makes use of the two-dimensional technique (see Section 8.5): the particle radius R is plotted versus $\sqrt{\tan\theta}$ at a constant %T (θ is explained in Section 8.5); the gel pore radius is obtained by a linear extrapolation to $\sqrt{\tan\theta} = 0$. For example, Serwer and Hayes [64] find for SeaKem LE agarose (see Table 2) a pore size of 53 nm at 3%T (two-dimensional technique) and they describe that a latex sphere of 580 nm radius cannot enter gels with a %T as low as 0.04 while a sphere of 345 nm can enter but is excluded at a %T > 0.22–0.25 (Ferguson plot technique).

The results of Righetti et al. [63] and Serwer and Hayes [64] can be compared with the results obtained by calculating pore radii with eqns. 6 and 7 (Table 3) and using gel fiber parameters published in refs. 152 and 153 as well as the model described in ref. 152. Results calculated by eqns. 6 and 7 and experimental estimates [63,64] are of the same order. IsoGel appears to be more restrictive than the other gel varieties under comparison. Differences, at least in part, can be attributed to (i) a variation in buffer composition and the mode of agarose gelling [93], (ii) slight differences in the SeaKem, SeaPlaque and IsoGel agarose varieties and (iii) size distribution [63] of the commercially available polystyrene particles used in some of the experiments. It may be that agarose does not exactly exhibit the pore size distribution defined by the extended Ogston theory [76]. Moreover, particles larger than the mean gel pore size may still be able to migrate through the gel (i) by some kind of tortuous pathway [152], (ii) due to particle deformation (apparent particle compressibility, see Section 9.6) [61,151,153], and/or (iii) due to the fact that the dimensions of the pore's structure may change because of the thermal movement of the fibres. Such a fluctuation in the size of spacings may allow the passage of a particle that statistically should not happen (under the assumption that the size and shape of a local pore is constant). There-

fore, the pore size determined by particle exclusion [63,64] is probably always larger than the pore sizes calculated by eqns. 6 and 7.

9. APPLICATIONS

Gel electrophoresis has been applied successfully in regard to particle characterization and purification. However, its application faces strong competition from the traditional methods in this field such as electron microscopy, X-ray scattering, gel filtration, ultra-centrifugation or immunodetection which have proven to be reliable and to advance science in so many cases. During the last years, high-performance liquid chromatography has become available for the rapid size determination of subcellular particles [161,162]. It is not felt that electrophoresis is going to replace any of these techniques, however, it can supply additional tools:

- Unlike electron microscopy, electrophoresis does not allow determination of the detailed structure of a particle. But particle size and shape can be estimated and a very important advantage is that no dehydration or fixation (often causing artifacts [163]) is necessary.
- Unlike X-ray scattering, electrophoresis is not the method of choice for very precise particle size determinations. But electrophoresis can supply radii of particles available as impure preparations in the lower nanogram range as long as precisely determined standards are available.
- Unlike ultracentrifugation, electrophoresis is not suitable for determining particle density. But particles can be separated and isolated according to their size and – in addition – according to their charge.
- Unlike immunodetection, electrophoresis does not provide for a fast and highly specific particle recognition. But it allows for a particle characterization and to some degree particle recognition (under standardized conditions) in those cases, where an antibody is not available.

In the following subsections some more examples of successful applications of gel electrophoresis will be discussed.

9.1. Detection of nucleic acid-containing particles in crude extracts

Serwer et al. [27] demonstrated that due to the selectivity of electrophoresis and of staining for nucleic acid with ethidium bromide it is possible to detect minor amounts of viruses even in crude extracts of plants. Infected leaves of *St. Augustin* grass were mixed with 0.05 *M* sodium phosphate buffer (pH 7.4) and ground in a Waring blender for 5 min. The virus detection in unfractionated and partially fractionated extracts (centrifugation) is described and interference by cellular components was not detectable.

9.2. Isolation and purification of small organelles, particles and vesicles

Some preparative methods known from polyacrylamide gel electrophoresis [51,164,165] can be also applied to agarose electrophoresis of relatively large

particles. For example, particles can be isolated from tube gels* by electrophoresing them into sample collecting cups (Fig. 2) containing a dialysis membrane which allows passage of small ions whereas particles are retarded. Kedersha and Rome [166] recently reported on a quite simple method using a non-submerged agarose slab gel technique in combination with a continuous elution from a downstream well to purify and isolate several subcellular structures such as ribosomes, coated and smooth vesicles, filaments and particles of rectangular or ring-like shape. They stress the point that especially the separation due to charge allowed for purifications that otherwise are very difficult or not at all achievable. Purification of clathrin-coated vesicles is also reported in [167].

9.3. Isoelectric focusing

Isoelectric focusing (IEF) of small spherical plant viruses in 3% polyacrylamide [168] and of poliovirus as well as echovirus 1 in agarose [169] has been published. The principle is as follows. Application of an electric field causes the carrier ampholytes (a polymorphic mixture of amphoteric substances with different isoelectric point, p*I*) to move to different stationary positions within the gel at which they have zero net charge at the pH of the environment. This leads to the formation of a pH gradient. In a similar way, the viruses migrate within the gel until they have reached a pH that corresponds to the p*I* of the particle. A variety of this procedure is first to focus the carrier ampholytes and then to apply and to focus the sample. This is sometimes preferred to remove a part of unwanted chemicals resulting from the polymerization of polyacrylamide. In IEF, the ionic strength of the sample should be low in order to avoid a disruption of the pH gradient.

IEF allows for the separation of particles according to their p*I* and for measurements of the p*I* values. The pH gradient can be standardized by application of colored markers (e.g. Electran, BDH or IsoGel p*I* markers, FMC Bioproducts) or by contact electrode. Polyacrylamide is practically electroendosmosis-free and thus does not cause a considerable drift** of the pH gradient. Polyacrylamide gel, however, is rather restrictive and is only applicable to small viruses. Agarose*** gel (suitable for IEF, see Table 2), although it is not completely free of electroendosmosis, appears as the best choice for larger particles, unless focusing in a density gradient is preferred as has been described for poliovirus [170,171] and

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*This technique is applicable as long as tube gels (e.g. percentage agarose $\geqslant 0.3$) can be prepared without a supportive gel plug which prohibits the migration of the particles.

**A small drift, especially in the alkaline pH range, can be observed in all media [243]. The exact causes are unknown. Although the use of immobilized pH gradients in polyacrylamide can avoid the problem of a pH drift [244], this method presently appears not applicable to subcellular particles, since particles with increasing size (e.g. large proteins) have a tendency to adsorb to the matrix (J.S. Fawcett and A. Chrambach, NIH, personal communication).

***Agarose with a low content of charged groups was applicable to IEF after the addition of uncrosslinked polyacrylamide [245]. Agarose with a low surface charge can be obtained by treating solutions of normal agarose with anion exchangers (e.g. refs. 148, 245 and 246).

echoviruses [172]. Another alternative is IEF in a granulated gel (orthopox virus [173]).

Sometimes it is difficult to determine, whether particles have reached a focused position. It is, therefore, advisable to apply the same particle at the acidic and on the basic side of the gel and to determine p*I* values from the position of particles, once the pattern resulting from both applications becomes identical. This method can result in different focusing patterns [168,170,174] due to the following reasons (under the condition that samples are applied *before* focusing of the carrier ampholytes): (i) the sample becomes degraded; particles should not be applied at the extreme ends of the pH gradient. (ii) The particle may exist in pH-dependent interconvertible forms [174] which upon isolation and re-application (at the same position) would give identical patterns. If the appropriate focusing cannot be tested by sample application at different positions, the pH at the location of the sample has to be determined at different times of focusing. A sample has reached its p*I* when its pH position remains constant. Experimental p*I* values may be altered by complexing of the particle with carrier ampholytes [175]. Binding mechanisms in regard to nucleic acids, acid proteins and polyanionic substances in general are discussed in ref. 176. Complexing of a sample with carrier ampholytes can be ruled out by applying of the sample at both the acid and basic part of the gel *after* the carrier ampholytes are focused. In this way, each sample encounters different carrier ampholytes. An identical zone pattern independent of the locus of sample application would be proof that complexing with carrier ampholytes has not occurred.

9.4. Titration curves

This technique has previously been applied to proteins [177,178]. It is a two-step procedure: (i) a pH-gradient is formed by applying a strong electric field to a gel with admixed carrier ampholites (IEF); (ii) particles are applied in the sample well (see Fig. 9) and the substances are run perpendicular to the direction of the focusing field. This results in a sample-specific titration curve (Fig. 9)* that characterizes the migration rate of a particle within a pH range and also defines the isoelectric point, p*I*, at the point of the curve where particle mobility equals 0. This method has been recently applied for the charcterization of hapten-protein conjugates [179] and also to some plant viruses [180] (Fig. 9).

The advantages are:

- In addition to the isoelectric point (p*I*) this approach gives information regarding particle migration as a function of pH.
- The optimum pH for particle separation by gel electrophoresis can be easily selected and the p*I* gives information for the choice of suitable conditions for ion-exchange chromatography.

Disadvantages are:

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*Mobility-pH curves have been published for bean pod mottle virus [68,247] and carnation mottle virus [248]. These plots are similar to the titration curve reported here; they are, however, not obtained by using a single slab gel.

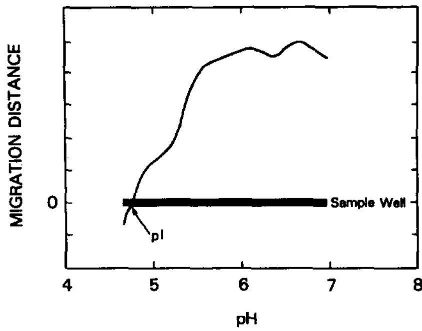

Fig. 9. Titration curve of turnip crinkle virus (see Section 9.4). Illustration according to Hurtt and Fawcett [180] using a computer program (Tietz, unpublished results) developed for a two-dimensional linearization of the pH gradient on the basis of pH measurements by contact electrode after the end of the experiment. The experimental set up is similar to procedures for protein titration curves described in brochures of FMC Bioproducts and LKB. Gel concentration, 0.8% IsoGel; focusing time of carrier ampholytes, 2 h at 350 V (about 10 W); gel electrophoresis of viruses, 2 h at 15 V/cm.

- Reduced band sharpness in comparison to isoelectric focusing. Therefore, particle populations with minor difference in p*I* cannot be distinguished and the determination of p*I* is less accurate.
- Migration distances are only of relative value, since the mobility values cannot be accurately evaluated due to variation of field and ionic strength and pH gradient within the gel.

9.5. *Immunoelectrophoresis*

Immunoelectrophoresis is frequently used in regard to proteins [181] and can be applied to viruses [182–185] to combine the resolving power of electrophoresis with the specificity of immunodetection. In the first step, gel electrophoresis is carried out in a horizontal non-submerged gel slab. Afterwards, two wells parallel to the direction of electrophoresis are cut into the gel on either side of the electrophoresed sample. One track is filled with a wide-spectrum antiserum solution, the other can contain a specific antibody. The antibodies diffuse into the gel and give precipitates with particles of the sample. The method has been applied for studying strains of tombus viruses [186,187]. Other specific applications are cited in ref. 182 and they are listed here accordingly: the characterization of plant viruses [188,189], the differentiation between virus strains of tomato bushy stunt virus [185,186], southern bean mosaic virus [190], red clover mottle virus [191] and peanut stunt virus [192], the differentiation between complete virions and their dissociated protein subunits [193–197] and the control of virion integrity following various treatments [198–200].

Experimental varieties are counter-immunoelectrophoresis (immuno-osmophoresis, electrosyneresis), rocket immunoelectrophoresis and crossed immunoelectrophoresis. The experimental conditions are discussed in detail in ref. 181 and the applicability to viruses is reported here as reviewed in ref. 182. Counter-

immunoelectrophoresis is based on the electrophoretic migration of negatively charged particles towards the positive electrode, while antibodies applied near the positive pole move into the opposite direction. The conditions are chosen in a way that the antibodies have almost zero net charge and they are moved within the gel by the electroendosmotic water flow (see Section 8.2.3). For example, glass slides coated with 1% agarose (high electroendosmosis, see Table 2) or agar can be used. Two sample wells, one at the anodic and one at the cathodic side, with a distance of about 2 cm are cut into the gel. The ends of the slides are connected by filter paper (moistened in buffer) with the electrode buffer compartments. Electrophoresis may be conducted at pH 6–7 and 12–15 V/cm. Precipitation lines are visible after 5–60 min and as little as 100 ng of virus can be detected. Special applications are given in refs. 201–206. Rocket immunoelectrophoresis allows for quantitative determinations of antigens [207,208]. For that purpose, the particles are applied in a series of lined-up small sample wells and are electrophoresed into a gel (e.g. 1% agarose, pH 8.6, 4–10 V/cm, 2–6 h) containing the antibody. The particles migrate towards the anode and form precipitation patterns of a rocket-like shape. Precipitation occurs when a certain equilibrium of antigen and antibody is reached which, depending on particle and antibody concentration, is achieved at different particle migration distances. This technique has been applied to cucumber mosaic and tobacco mosaic viruses [209–211]. Crossed immunoelectrophoresis is the combination of gel electrophoresis (first dimension) and rocket immunoelectrophoresis (second dimension) performed perpendicular to the first direction. This method is characterized as useful for analysis of very complex antigen–antibody systems [182] and was applied to *herpes simplex* virus [212,213].

9.6. Particle deformation

Computer simulation of electrophoretic mobility data on basis of the extended Ogston theory indicated an apparent compressibility of particles during electrophoresis* (see Sections 8.3.2 and 8.4.2) [151,153]. Compressibility might be caused by deformation of the particle due to shrinkage as well as by a change of shape (e.g. elongation). The simulation further revealed [61,153] that the absence of Ca^{2+} ions causes (i) a considerable swelling of turnip crinkle virus (12.8 ± 3.2 to 29.4 ± 2.6 nm radius) and a relatively minor expansion of pelargonium flower break (18.3 ± 0.7 to 22.1 ± 1.3 nm) and hibiscus chlorotic ringspot virus (20.3 ± 1.5 to 24.2 ± 2.1 nm) and (ii) has an effect on apparent compressibility patterns. A relation between size, bivalent ions (e.g. Ca^{2+}) and also pH (pH > 7) was detected by independent physical methods for tomato bushy stunt virus [214,215], brome-

*Conditions of electrophoresis [61]: 0.1–2.3% agarose (IsoGel); gel buffer, 0.24 *M* ACES (see Table 1), 0.0366 *M* HEM (Serva Fine Biochemicals), pH 6.5, 0°C, 0.03 *M* ionic strength; catholyte, 0.02 M ACES, 0.01 *M* potassium hydroxide; anolyte, 0.02 *M* HEM, 0.01 *M* hydrochloric acid, 0.01 *M* CHAPS (Calbiochem Biochemicals) was added as a detergent (see Section 3.3) to all buffers in regard to separation of polystyrene standards, however, it can be omitted in the case of viruses. Although different buffers are used, the electrophoresis corresponds to continuous buffer electrophoresis. No moving boundary (Section 3.2) is formed.

grass mosaic virus [216,217] and southern bean mosaic virus [218]. In the latter study it has been reported that removal of Ca^{2+} causes a swelling of the virus (pH 8.25) from 15 to 22 nm hydrodynamic radius which was detected by photon correlation spectroscopy. The mechanism of virus swelling has been studied on the molecular level by 0.8-nm resolution X-ray crystallography [214]. Structural comparisons of some small spherical plant viruses and the role of Ca^{2+} in particle swelling is reviewed in ref. 219. Swelling is caused by deprotonation of calcium binding sites (aspartate residues). The biological significance of particle expansion is unclear; it is speculated that this may be related with the different levels of calcium in the cytoplasm and extracellular fluid and its effect on virus entry and disassembly [214]. The swollen state in contrast to the compact one of plant viruses is very sensitive to enzymatic proteolysis [214].

Gel electrophoresis can be used to detect changes in particle size in solution and without any denaturing treatment. If particle deformation during gel electrophoresis at higher agarose concentrations can be confirmed by other methods, the computer-assisted gel electrophoresis seems likely to become a tool for characterization of particle texture under the influence of environmental conditions.

9.7. Detection of virus strains and mutants

Mutants may be expressed as differences in charge rather than in size [68]. There are several indications that virus strains can be distinguished due to charge as is described for tobacco mosaic virus [220], foot-and-mouth disease viruses [174] and tomato bushy stunt virus [221]. In the latter study it is suggested that differences in charge density are due to a different percentage of nucleic acids and a variability of protein composition. Furthermore, relations between virulence and particle charge [22,222] have been reported and the effect of aging on the mobility of saguaro virus has been briefly discussed [223]. The resolution of separation can be further increased by combining electrophoretic and immunological techniques (see Section 9.5). Thus, electrophoresis, IEF and/or immunoelectrophoresis appear as the methods of choice in all cases, where a characterization by size and shape alone is insufficient.

10. FUTURISTIC OUTLOOK

Presumably, gel electrophoresis will be more frequently used than at present for studying viral diseases and for the characterization and isolation of viruses and cellular organelles. Gel electrophoresis is a well established analytical technique in protein and DNA analysis and it appears that a number of techniques originating from those fields [51,224–228], such as preparative techniques [165], pulsed-alternating field electrophoresis [229,230], micro gels [231–234], ultrathin gels [235,236] or high-performance electrophoresis [237], can and will be applied to gel electrophoresis of subcellular particles. Another strategy might be the extension of the range of suitable particle sizes to larger subcellular particles and even small cells. This can probably be achieved by yet unknown gel matrices with larger fiber diameters that allow for the construction of a fiber network with

larger pores. This will involve an extended computational analysis to account for irregular particle shapes as well as particle deformation, the complexity of which increases with particle size [153]. Furthermore one could expect that particles of larger size cause deformations of the gel fiber – a phenomenon that has been neglected so far.

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12. SUMMARY

The review describes the application of gel electrophoresis to the characterization and separation of viruses, ribosomes, vesicles and other subcellular particles. The preparation of the sample, the choice of the buffer, the gel medium, the apparatus and the detection of the particle (staining and scanning) as well as the necessary theory are discussed. This includes the mathematical evaluation of experimental data on the basis of Ferguson plots using the extended Ogston theory. Simple methods and sophisticated computer simulation techniques are described and exemplified in application to the determination of particle size and charge, the pore size of the gel (unpublished data) and the two-dimensional agarose electrophoresis (unpublished). It is shown that the nature of the particle (e.g. spherical or rod-shaped, pliable or rigid texture) determines the shape of the non-linear Ferguson plot. In addition, the review gives a number of practical applications of gel electrophoresis, isoelectric focusing, titration curves and immuno-electrophoresis to subcellular particles. Pros and cons are evaluated. A comparison with other analytical procedures is made. The review is concluded by a futuristic outlook.

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