

Another series of gels was made by preparing a 1.5% solution of agarose in water and adding 1,3-dichloropropanol (1, 2, 3 and 4 mmol) and 10N NaOH (2.2, 4.4, 6.6 and 8.8 mmol) to 10 ml of the warm agarose solution. The gelling was for 4 days at 35° C. Electrophoresis of DNA fragments resulted in sharper bands in the gels with 2 and 3 than in the gel with 1 mmol of 1,3-dichloropropanol. It is noteworthy that the larger DNA fragments, above 500 bp, migrated further in the gel with 4 mmol than in the gel with 3 mmol of the cross-linker. Relative to ferritin, thyroglobulin migrated further in the gel with 4 mmol cross-linker. In addition, that gel was also softer than the gels with less cross-linker. Thus, there is an optimal cross-linker concentration and exceeding it may worsen not only resolution but also mechanical stability.

A series of gels containing 2% of agarose polymers and 0, 0.1, 0.2, 0.4, 0.6, 1.0, 2.0 and 4.0 mmol of 1,3-dichloropropanol, plus NaOH in 10% molar excess over chlorine, in 10 ml were prepared by incubation at 35° C. for 4 days. A portion of about 1.5 ml of each gel solution (in triplicate) was placed in polystyrene cuvettes, overlaid with paraffin oil and incubated under the same conditions. Electrophoresis of DNA fragments (7 V/cm, 1 h 50 min) showed improved resolution of DNA fragments below 1000 bp with increasing cross-linker concentration to about 1 mmol and remained good to 4.0 mmol. Transparency of the gels was determined by measuring the absorbance at 500 nm (Table 1) and recording a visible spectrum (400–800 nm) of the gels equilibrated at room temperature overnight. The spectrum of the agarose gel comprising no cross-linker is shown in FIG. 5. The absorbance values at all wavelengths decreased with increasing cross-linker concentrations and became essentially constant beyond 1 mmol. The visible spectrum of the gel prepared with 2 mmol of 1,3-dichloropropanol in 10 ml is shown in FIG. 6. The absorbance at 500 nm of this gel is over one order of magnitude lower than that of the standard agarose gel (Table 1). The UV spectrum, recorded after transferring a gel into a quartz cuvette, showed a low absorbance in the 300–400 nm region, a shoulder around 260 nm a steep rise below about 230 nm.

Two dimensional electrophoresis of lambda/Hind III fragments was carried out in a gel comprising 1.5% agarose polymer cross-linked with 1,3-dichloropropanol at 1.5 mmol per 10 ml. In the first dimension the gel was run at 2 V/cm for 16 h. After turning the gel by 90° the second dimension electrophoresis was carried out at 7 V/cm for 4 h. FIG. 9 shows that at 7 V/cm the 23 kbp fragment migrated further than the 9.4 kbp fragment. Since this result is the same as that shown in FIG. 2, it indicates that the mechanism of DNA migration in both gels is identical.

EXAMPLE 8

Gels comprising hydroxyethylated agarose and 1,3-dichloropropanol at different polymer/cross-linker ratios. A series of gels comprising 2% hydroxylated agarose polymer (SeaPlaque, FMC) and the cross-linker was prepared in the way described in Example 7. Electrophoresis of DNA fragments showed improved resolution, clearly visible already at the lowest cross-linker concentration, with respect to the standard gel. Measurement of absorbance at 500 nm demonstrated essentially no change at cross-linker concentration above 0.6 mmol in 10 ml. A visible spectrum of the standard SeaPlaque gel is shown in FIG. 7. FIG. 8 shows the spectrum of the gel comprising hydroxylated agarose polymer and 1.0 mmol of 1,3-dichloropropanol in 10 ml. The gel comprising hydroxylated agarose displays about one half of absorbance

of the underivatized agarose in the visible region (compare FIG. 5 and FIG. 7). On the other hand, the present gels which in addition to the same polymers comprise a cross-linker, showed less than one tenth of absorbance of the gels with no cross-linker. Actually, the absorbance of the present gels comprising hydroxylated agarose and higher amounts of the cross-linker is almost nonexistent in the visible region (FIG. 8).

EXAMPLE 9

Gels comprising agarose and 2,3-dibromopropanol prepared at different polymer/cross-linker ratios. A series of gels comprising 1% agarose polymer and 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mmol of 2,3-dibromopropanol in 10 ml was prepared. The gel solution included also sodium hydroxide in 10% molar excess over bromide. The gels were incubated at 35° C. for two days. Electrophoresis of DNA fragments showed improved resolution in the small molecular weight range, especially at 1–2 mmol of the cross-linker. The resolution was somewhat inferior to that achieved with gels cross-linked with 1,3-dichloropropanol. Occasionally, small bubbles could be noticed in the gels.

EXAMPLE 10

Gels comprising agarose and ethyleneglycol diglycidylether prepared at different polymer/cross-linker ratios. A series of gel comprising 1% agarose polymer in 0.1 M NaOH and 0.05, 0.1, 0.2, 0.4 and 0.6 ml of ethyleneglycol diglycidylether in 10 ml was prepared by incubation at room temperature for 4 days. Electrophoresis showed reduced migration distances with increasing cross-linker concentration. Resolution was inferior to that obtained with butanediol diglycidylether.

EXAMPLE 11

Gels comprising agarose and dimylsulfone prepared at different polymer/cross-linker ratios at various pH values for different time. Into a warm 1% agarose solution in 0.1M NaOH, 0.010 ml of divinylsulfone was added under rapid stirring. The solution was immediately transferred into a gel mode. The gel solidified within minutes. The solidification was faster at higher cross-linker concentrations which made it practically impossible to reproducibly prepare such gels. Electrophoresis showed a pronounced decrease in migration distances related to those in the standard gel.

Agarose solutions (1%) were prepared in 0.2M bicarbonate-carbonate buffers with pH 8, 9.5 and 11.0. To 20 ml of each of these solutions 0.1 ml of divinylsulfone was added and the gel solutions incubated at 35° C. for 2 days. DNA fragments migrated less and appeared as sharper bands in the gel formed at pH 8 than at pH 9.5. In the gel formed at pH 11 the fragments migrated less than in either of the two gels. The bands corresponding to small DNA fragments were the sharpest in this gel.

A series of gels was prepared which comprised 1% agarose in 10 mM NaOH and 0.020, 0.040, 0.060, 0.080 and 0.100 ml of divinylsulfone per 20 ml. After addition of the cross-linker each gel was immediately poured in the gel mold and left there for 15 min. The gel with 0.100 ml of the cross-linker was too weak for subsequent handling. The other gels were washed in water and equilibrated in the TAE buffer. Electrophoresis showed decreased migration distances with increasing cross-linker concentrations. The DNA bands in all four gels were more diffuse than in the above gel cross-linked at pH 11 for 2 days.