$$\log \mu = \log \mu_o - \eta l'(r+R)^2 T \times 10^{-16} \quad (1)$$

or

$$\log \mu = \log \mu_o - K_r T \quad (2)$$

where the retardation coefficient, K_r, is defined as

$$K_r = \eta l'(r+R)^2 \times 10^{-16} \quad (3)$$

Separation of two DNA fragments α and β can be written as $\triangle\mu = \mu_a - \mu_b = \mu_{oa} e^{-KraT} - \mu_{ob} e^{-KrbT}$. Since the free mobility μ_o of all DNA molecules is equal (Olivera et al., Biopolymers 2, (1964) 245–257) and maximal separation occurs when $d(\triangle\mu)/dT=0$, gel concentration T giving optimal resolution is:

$$T = \ln(Kra/Krb)/(Kra - Krb) \quad (4)$$

This equation corresponds to equation 25 of Rodbard and Chrambach (Proc. Natl. Acad. Sci. 65 (1970) 970–977). From that equation it follows that as Kra decreases, that is size of DNA fragment a becomes smaller, the gel concentration T necessary for resolution of fragments a and b increases. As noted above, however, when a gel of high polymer concentration is run in the submerged electrophoresis mode, separated bands are bent. Accordingly, from the prior art it seemed impossible to achieve optimal resolution by submerged gel electrophoresis without necessary adjustment of gel ionic composition in order to control the bending of separated bands.

The extended Ogston model teaches that very small ions are also retarded in a gel because even when radius R of an ion is zero, retardation coefficient is higher than zero (equation 1). However, from that equation it is not possible to predict at which minimal gel concentration, if at any, resistance to migration of buffer ions is so small that the difference in gel and buffer conductivity does not significantly affect the bending of bands. Experiments were designed in an attempt to find out whether there is such a gel comprising synthetic polymers, since resolution of macromolecules of small size in low percentage agarose gels is known to be inferior. In practice, the lowest workable concentration of a synthetic gel is determined by its mechanical stability which in turn depends mostly on polymerization efficiency of a starting monomer solution of a low concentration. This is true for acrylamide as well as many other hydrophilic and amphiphatic gels disclosed in (Kozulic and Heimgartner, U.S. patent application Ser. Nos. 293,840 and 696,696), which are incorporated herein by reference. The lowest practical gel concentration is in most cases around 4%. Since in 4% gels bending of bands was still noticeable (Kozulic, PCT/EP 92/00368), it appeared important to find a way for preparation of gels of even lower percentage. Most gels in prior art were polymerized in presence of a cross-linker having two vinyl double bonds but in some of them the cross-linker was a polymer with plurality of double bonds. That cross-linker was an agarose polymer substituted with allyl glycidyl ether (U.S. Pat. No. 4,504,641 to Nochumson), which is known as Acryl Aide™ (FMC Corporation). Since a mechanically more stable, low percentage gel was desirable, in an attempt to obtain such a gel the Acryl Aide™ agarose polymer was cross-linked through its hydroxyl groups prior to copolymerization. It was reasoned that a single large and branched cross-linked vinyl-agarose polymer will cross-link during polymerization many chains formed from the polymerizing monomer and thus improve mechanical stability of the matrix and resolution of small biomolecules. In an initial experiment, 6% poly(NAT) gels were polymerized with different amounts of the cross-linked vinyl-agarose. Transparency and mechanical strength of the resulting gels looked standard. During electrophoresis migration rate of the bromphenol blue tracking dye was normal. However, totally unexpectedly it was observed that resolution of DNA molecules was progressively lost as the amount of the cross-linked vinyl-agarose increased and at a certain cross-linker concentration there was no resolution at all. Surprisingly, the resolution was lost even when DNA fragments migrated essentially the same distance as in the control gel. At higher cross-linker concentrations the loss of resolution was accompanied by reduced migration rates. The loss of resolution occurred first in the low molecular weight range.

The above experimental data appeared to be of limited practical importance but they were a clue that lead to development of a new model for electrophoretic migration of macromolecules in gels. The model is described herein as follows. A gel is regarded as a block comprising randomly distributed polymer chains and water. The polymer chains have different motional freedom along their length. Regions of high motional freedom are separated by spots of low motional freedom, which correspond to cross-linking points in synthetic gels. There are no pores of any finite shape or size in transparent gels. Accordingly, there is no defined space or volume a macromolecule can enter before it begins to migrate in electric field. Once it starts migrating, the macromolecule pushes the polymer chains and thus creates the space it occupies. The macromolecules move along their path in discrete steps and in each step they pass through one gel layer. The gel layer is defined as a gel cross-section perpendicular to direction of migrating molecules. A gel layer is thicker than radius of the polymer chain but thinner than radius or length of the migrating macromolecule. Thus, the migrating molecule may encounter a second layer before it completely passes through the first one, however, resistance to its migration is given only by the second layer. Essential to this model is the notion that there are two ways a macromolecule can pass trough a layer. Thus, it will push aside polymer chains when on its path the macromolecule encounters a region in which polymers have a high motional freedom. In this way the macromolecule will open a "door" in the layer. When on its path the macromolecule encounters an area where polymers have a low motional freedom, it deforms the layer until an opening is formed. This opening is created at places where one or more polymer chains end or where the polymer chains are less cross-linked or entangled. The macromolecule thus creates a "corridor" in the layer. Creation of a "corridor" in one layer is accompanied by dislocation of some polymer chains in at least one layer above and below. If the migrating macromolecule encounters a similar place on the next layer, it will again open a "corridor". These two "corridors" may be connected into a single one and thus the same "corridor" can span several layers.

Whether a macromolecule will predominantly open "doors" or "corridors" on its way through the layers depends entirely on the balance of two forces. The first force acting on the layer is electrokinetic and it is exercised by all macroions moving in an electric field. This force is countered by resistance of polymer chains comprising the layer. The polymer chains resist to any change in their arrangement because during gel formation they have acquired energetically the most favorable position. When magnitude of the electrokinetic force in relation to resistance of polymer chains is high, "corridors" will be predominantly formed. When the two forces are of similar magnitude, a part of the openings will be "doors" and part "corridors". When