An Unexpected Mossotti: His Formula at the Basis of Dielectrophoresis in Modern Molecular Biology

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Abstract

The Clausius-Mossotti formula finds an unexpected application in modern molecular biology: it is in fact the foundation of dielectrophoresis, one of the most widely used chromatographic techniques for isolating biomolecules. In fact, dielectrophoresis overcomes some limitations of electrophoresis, also allowing the analysis and separation of neutral particles, such as individual cells. In this contribution, we briefly retrace some of the significant milestones in the history of dielectrophoresis, starting from its connection with Mossotti's studies.

Biomolecular separation techniques, also called "chromatography," have always been widely applied in the field of clinical analyses, of which they have always been one of the foundations, in order to isolate specific constituents within a heterogeneous sample. As the observation scale moves towards single cells, it is widely recognized how these techniques are increasingly important. A classic method of separating biological components is to exploit their different densities.

Another approach to develop separation techniques is based on the use of electric and magnetic fields. The evolution of these techniques has gone hand in hand with the evolution of knowledge of electrical and magnetic phenomena.

Electrophoresis was the first of these techniques. It is based on the application of a uniform electrostatic field to a sample to be analyzed. Biomolecules having a net charge – for example, due to ionizable groups – migrate within the sample. Based on the charge and mobility – that is, the coefficient that links the speed of a particle to the field to which it is subjected – the electrophoretic technique allows for separating the charged biomolecules. To improve performance, a gelatinous substance (agarose gel) is usually used as a sieve.

This approach is typically used to analyze and separate DNA nucleic acids, hence the great importance of the technique.

The electrophoresis phenomenon was first described in 1807 by Ferdinand Friedrich Reuss [1], who observed the migration of clay particles immersed in water in the presence of an external electric field. However, the first device to implement modern electrophoresis was made in 1937 by the Swedish biochemist Arne Wilhelm Kaurin Tiselius (Stockholm, Sweden, 1902 – Uppsala, Sweden, 1971) [2]. Thanks to his research on electrophoresis, Tiselius won the Nobel Prize in Chemistry in 1948, with the following citation: "for his research on electrophoresis and adsorption analysis, especially for his discoveries concerning the complex nature of the serum proteins."

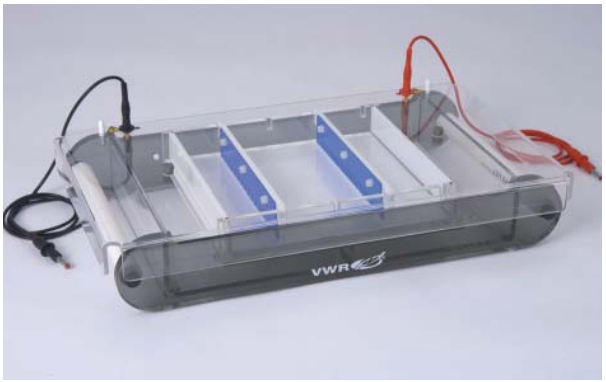

Figure 1. A commercial apparatus for electrophoresis (from [3]).

Although electrophoresis is a technique that has been known for two centuries and in use for more than a century [4], and is routinely employed in biomedical labs (Figure 1), it has a significant limitation: it cannot be applied to neutral particles, which are instead of increasing interest in modern molecular biology.

This is where the Mossotti formula comes into play. His formula, or rather the Clausius-Mossotti formula, was developed in 1846, but published 1850. If the particle of interest is neutral, but it is at least polarizable, there is still a possibility to exert on it a force similar to the electrophoretic force, ultimately allowing for separations of molecules as in the case of electrophoresis.

To that aim, let us consider a polarizable particle, describable as a uniform sphere of radius *r* and dielectric permittivity ε_p, immersed in a uniform medium of permittivity ε_m. If subjected to an electric field, $\mathbf{e}$, the particle is polarized. The dipole moment, **p**, envisaged by the Clausius-Mossotti formula is as follows:

$$\mathbf{p} = 4\pi\varepsilon_m r^3 K\mathbf{e}$$

$$= 4\pi\varepsilon_m r^3 \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}\mathbf{e},$$

where the term

$$K = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$$

is known as the Clausus-Mossotti factor. It is therefore sufficient that the electric field, **e**, is nonuniform so that the dipole moment induced on the particle can undergo a non-null force. In fact, an elementary dipole moment is sensitive to the gradient of the square of the electric field, $\nabla|\mathbf{e}|^2$. The force generated between the dipole moment and the nonuniform applied field is exerted on the polarized bioparticle of interest. This mechanism is the foundation of "dielectrophoresis," a phenomenon by which a neutral but polarizable particle is subjected to a force when immersed in a nonuniform electric field.

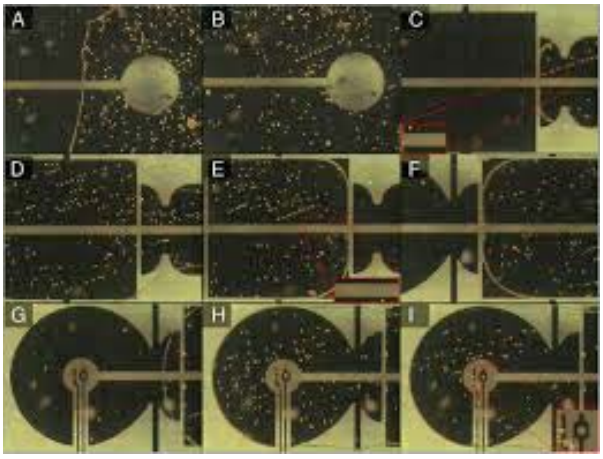

Figure 2. An example of an experimental dielectrophoretic device (from [9]).

The intensity of the dielectrophoretic force exerted on the biomolecule is therefore dependent on its dielectric properties and on those of the medium. This force also depends on the molecule's size and the configuration of the electric field, in particular, its gradient.

Strictly speaking, the history of dielectrophoresis begins as early as in the 1920s: Hatschek and Thorne had described something similar to the dielectrophoretic mechanism already in 1923 [5], and a patent filed in the United States in 1924 can actually be considered an electrophoretic technique [6].

However, it was Herbert Pohl, a professor at Princeton University and a pioneer of the dielectrophoretic technique in the 1960s, who gave to dielectrophoresis its current form. In 1966, Pohl and Hawk published the first demonstration of electrophoresis on yeast cells. That was the first case where dielectrophoresis was applied to living structures. The apparatus used was very simple. A signal generator was connected to a pair of electrodes in the "point and plane" configuration, that is, one of the two electrodes was needle-like whilst the other one was a classical planar plate. With their rudimentary apparatus, Pohl and Hawk showed that live cells were attracted by the electrodes, whilst dead, non-polarizable cells remained in solution. Pohl called this technique "the natural motion of neutral matter caused by polarization effects in a non-uniform electric field" [7].

However, the new technique was not immediately accepted, and indeed, many of Pohl's later works were refused for publication. Only in 1978, with the publication of his paper, "Dielectrophoresis: The Behavior of Neutral Matter in Nonuniform Electric Fields" [8], did the popularity

of the technique began to grow, although still remaining a niche topic.

Fortunately, a new generation of researchers appeared in the limelight later on, including Ronald Pethig, who was among the protagonists of a further refinement of the technique [9]. Pethig would later become one of the world's greatest experts on the subject [10].

It is interesting to note that in the current form developed by the generation following Pethig, many tricks have been introduced, such as the use of specific gels such agar-agar. Furthermore, various configurations/shapes of the electrodes (Figure 2) have been described [11].

One of the interesting features of the Clausius-Mossotti factor is the fact that it can change according to the actual combination of the particle's and medium's permittivities.

In recent times, the use of a wide range of frequencies has emerged more and more. In fact, depending on the frequency, the Clausius-Mossotti factor can significantly vary, and in particular, the magnitude and sign of the dielectrophoretic force depends on the difference between the polarizability of the particle and of the medium, as is apparent considering the term $\varepsilon_p - \varepsilon_m$ [12].

Using fields characterized by specific frequencies, it is therefore possible to manipulate particles of interest with high selectivity, obtaining very specific effects: cellular filtering with high specificity and without marking, and entrapment and identification of the electrical characteristics of unknown particles. Such results are difficult to obtain with traditional non-electrical chromatographic techniques, and are also even more interesting than the results of classical electrophoresis.

These properties are typically used to identify cancer cells camouflaged between healthy cells, allowing for rapid and minimally invasive diagnoses.

It is worth mentioning that there exists an effect similar to dieletrophoresis for magnetizable molecules, such as some blood constituents. The effect is known as "diamagnetophoresis," as the driving source is a magnetic field. The development of this recent approach allows for directly operating on red blood cells, which are sensitive to the magnetic fields thanks to the susceptibility of hemoglobin [13].

Finally, it is also worth noting that the two techniques can be used in combination. In this case, the technique is referred to as "dielectro-magnetophoresis." This strategy promises to be a harbinger of even more interesting developments in the field of molecular biology.

This is really an unexpected consequence of the work pioneered by Ottaviano Fabrizio Mossotti.

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