

# Technical Note

## Precision Magnetophoresis: A Novel Separation Technique For Magnetic Beads

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*In this note, we present an overview of a novel and fast magnetophoretic separation technique for superparamagnetic beads in solution. The technique employs uniform and well controlled magnetophoretic conditions in all the magnetic separator. This allows better quality control process and safer operation. The separation times are usually very fast, being the magnetic response of the beads and their concentration the major factors controlling the kinetics of the process. Separation times are shorter for beads capable of producing larger magnetic dipoles. Also, an increase of the concentration of the dispersion greatly reduces the separation times. This surprising effect is due to a cooperative process in which the magnetic fields generated by the beads themselves help to speed up the separation process. The separation process is reversible in the sense that when the magnetic field is suppressed, the beads can be easily redispersed again.*

### MAGNETOPHORESIS: THE CONCEPT

For many years, antibody-antigen interaction has been used. Recent advances in the synthesis and manufacture of magnetic particles have fueled a widespread interest for their applications. It is particularly relevant the case in which magnetic particles are tailored specifically to extract target solutes (proteins, soluble organic or metallic contaminants and many other examples). Due to their magnetic response, the motion of the particles can be controlled -in principle- using suitable magnetic fields. This fact opens the door to exciting new applications and possibilities such as manipulation and extraction by using magnetic fields.

The drift (directed motion) of magnetic particles due to the action of a magnetic field is called magnetophoresis. It has to be stressed that an uniform magnetic field does not produce any movement on the magnetic particles. The driving force for the magnetophoretic drift is the gradient of the magnetic field. A simple block magnet near an ordinary test tube generates a gradient of the order of 1 T/m, which suffices to easily observe the magnetophoretic drift of a dispersion of superparamagnetic beads. Of course, this crude magnetophoretic procedure can be greatly improved. In the last years, engineers have been working on the design of parameters such as the most appropriate geometries for magnetic separators and magnets (1) and developing numerical algorithms capable of predicting the separation power of the designed separators (2). These improvements, achieved in the last 10 years or so, have been based on the concept of high gradient magnetic separation (HGMS). In short, HGMS is

based on the observation that magnetophoretic velocities of magnetic beads are proportional to the magnetic gradient. Since typical magnetophoretic velocities are very low, the key is to develop systems with very high gradients (1,2). In order to generate the desired high magnetic gradients, the HGMS approach employs separator columns containing a packed bed of magnetically susceptible wires placed inside an electromagnet (1). When a magnetic field is applied across the column, the magnetic response of the wires produces very high field gradients around them. The suspension containing the magnetic beads is then circulated along the column and the beads passing near the wires are captured by the extremely high (but very inhomogeneous) gradients generated by them (2). A further step in magnetophoresis separation is provided by the so-called precision magnetophoresis (PMS) concept (3). In this approach, uniform and well controlled magnetophoretic conditions are employed in all the magnetic separator. This means that the same magnetic gradient is applied to all the magnetic separator and all the magnetic beads experience the same magnetic driving force. This allows better quality control process and safer operation. Another goal is to obtain very efficient separation: short separation times, high magnetic force and reduced magnetic bead loss even employing low gradients.

## SEPARATION TIMES IN PRECISION MAGNETOPHORETIC SEPARATION

In many cases, precision magnetophoretic separation (PMS) is very fast. As an illustration of the kinetics of this process, let us consider the specific example shown in figure 1. In this figure, we show the kinetics of magnetophoretic separation for a solution of different concentrations (from 0.01 g/L to 10 g/L) of M1-030/40 and M1-020/50 superparamagnetic microspheres supplied by Estapor® Microspheres (Merck Chimie SAS, France). The employed magnetophoretic setup is a SEPMAG LAB325 2042 apparatus, commercially available from SEPMAG Technologies (4). In the magnetophoresis experiment, a bottle of 3 cm diameter (25 mL) containing the dispersion was placed inside the SEPMAG apparatus. The initially brown dispersion (of high opacity) becomes transparent (negligible opacity) in a few minutes, with all particles at the wall of the bottle. When the field is removed or the particles are extracted from the magnetic separator, the beads can be easily redispersed. Note that the concentration is a very important factor in controlling the kinetics: as the concentration increases the magnetic separation speeds up. For example, in Figure 1(a) it can be seen that the M1-030/40 Estapor® Microspheres are separated after a time of about 180 s in the case of 0.01 g/L concentration. Increasing the concentration to 10 g/L reduces the separation time in a factor of around 6 (about 30 s).

At a first glance, this dependence with the concentration might appear unexpected. In fact, there is an interesting physical mechanism behind this observed behaviour. The PMS process is what it is called a cooperative process. In a way it is somewhat like the beads “talking” one with another and in some sense, “agreeing” in which direction they should move. In more technical terms, we may say that the magnetic beads move collectively, due to the magnetic interaction between the beads themselves. As a response to the external magnetic field (applied in the magnetic separator), the beads are strongly magnetized and generate their own, strongly inhomogeneous magnetic fields. When one bead approaches another bead of the dispersion they are strongly attracted, the final effect being that the beads move together collectively. This movement has been observed directly under a microscope (3). When the magnetic field is applied, the beads form long chains which move much faster than a single, isolated bead. It is essential to recall that these chains are reversible: when the magnetic field is removed, the chains rapidly disappear. This reversibility is due essentially to the superparamagnetic character of the magnetic core of the beads.

Apart from concentration, other factors which affect the kinetics of the separation process are the following:

- Maximum magnetic moment  $m$  of the beads: an increase in  $m$  significantly decreases separation times.
- Magnetic gradient: an increase of the gradient, decreases the separation time.
- Viscosity of the fluid: the higher the viscosity, the higher the separation time.
- The size of the system: higher volumes, larger separation times.

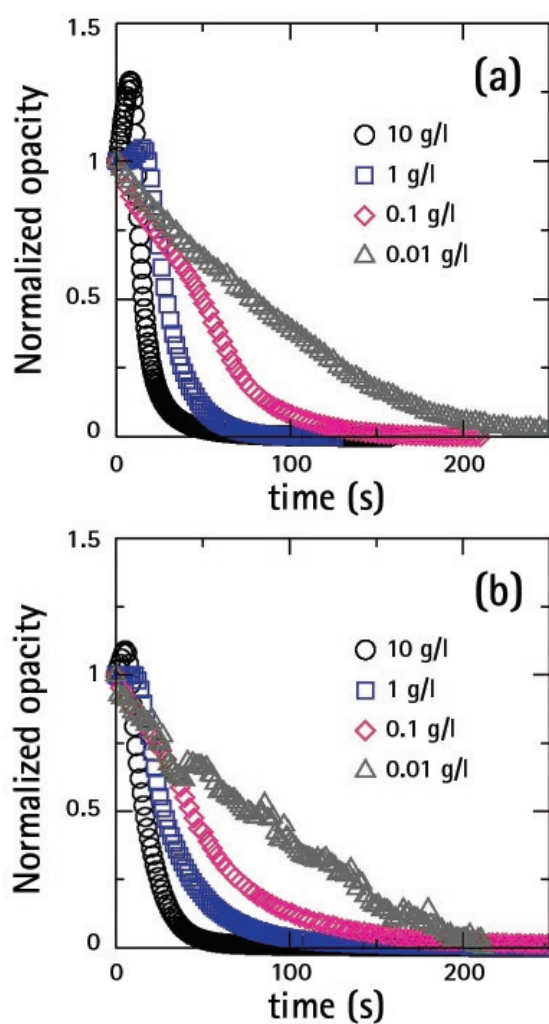


Figure 1: Separation times of dispersions of magnetic beads at concentrations from 0.01 g/l to 10 g/l using a SEPMAG LAB325 2042 magnetophoresis apparatus. The opacity of the dispersion (in arbitrary units) measures to which degree the beads are still dispersed, i.e. not separated or extracted from the fluid. As time goes on, the opacity of the dispersion decreases due to magnetophoretic separation of the dispersed beads. Panel (a) corresponds to Estapor® M1-030/40 beads and panel (b) corresponds to Estapor® M1-020/50. Figure reproduced with permission from reference (3). Copyright 2008 The American Chemical Society.

## MINIMUM PARTICLE SIZE FOR MAGNETIC SEPARATION

The magnetophoretic separation process is difficult for very small particles, for sizes in the nm range. In fact, it can be argued that magnetophoretic separation is only feasible for particles larger than a minimum size, which depends on the magnetic properties of the beads: the higher the magnetic response of the beads, the smaller the particles which can be separated. The investigation of the smallest particles which can be separated by magnetophoresis has been an intense topic of study (2,3,5). In the case of the PMS process, this minimum or critical diameter  $d_{\min}$  can be estimated from the following equation (3):

$$d_{\min}=(\mu_0 m^2/2k_B T)^{1/3}$$

where  $m$  is the magnetic dipole of the bead (in J/T) and  $T$  is the absolute temperature (the magnetic and Boltzmann constants are given by  $\mu_0=4\pi\times 10^{-7}$  N/A<sup>2</sup> and  $k_B=1.38\times 10^{-23}$  J/K respectively). At  $T=300$  K, the minimum diameter in nm is given by  $d_{\min}=6.72\times 10^{11}m^{2/3}$  where  $m$  is the magnetic dipole of the bead in emu. For example, in the case of M1-030/40 and M1-020/50 Estapor® Microspheres we obtain respectively  $d_{\min}$  of 20 nm and 40 nm, which implies that these magnetic beads will be easy to separate using precision magnetophoresis. Very small particles require a high magnetic content to be separable by using PMS. In order to give an idea of the power of the technique, let us recall that in the extreme limit of beads made only of pure magnetic material one can separate very small nanocrystals. For example, from the previous equation we can estimate  $d_{\min}\sim 7$  nm for pure magnetite nanocrystals.

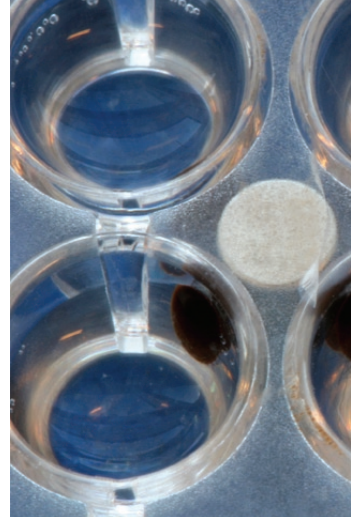
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