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Research review paper

Magnetic separations in biotechnology

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ABSTRACT

Magnetic separations are probably one of the most versatile separation processes in biotechnology as they are able to purify cells, viruses, proteins and nucleic acids directly from crude samples. The fast and gentle process in combination with its easy scale-up and automation provide unique advantages over other separation techniques. In the midst of this process are the magnetic adsorbents tailored for the envisioned target and whose complex synthesis spans over multiple fields of science. In this context, this article reviews both the synthesis and tailoring of magnetic adsorbents for bioseparations as well as their ultimate application.

Contents

1. Historical overview

Magnetism has long been employed in various scientific fields ranging from separation processes, imaging, analytical chemistry, drug delivery, data handling, acoustic reproduction, energy production and transportation. For an extended period of time the main application of magnetism was restricted to the mining and metal processing industries with the first patent for the separation of iron minerals being filed by William Fullarton in 1792 (Parker, 1977). Applications in the biotechnological field are far more recent. In the early 1940s, pure magnetic iron oxides were used in wastewater treatment to remove dissolved and colloidal biological substances (Pieters et al., 1994). The introduction of high gradient magnetic separation systems (HGMS) in the 1950s further heightened the use of magnetic particles in biotechnology but it was not until the 1970s that the use of selective magnetic adsorbents allowed the capture of valuable biomolecules (Dunnill and Lilly, 1974; Horisberger, 1976). In fact, at the time, greater interest was given to the possibility of using magnetic particles as enzyme immobilization matrixes (Adalsteinsson et al., 1979; Chaplin and Kennedy, 1976; Gellf

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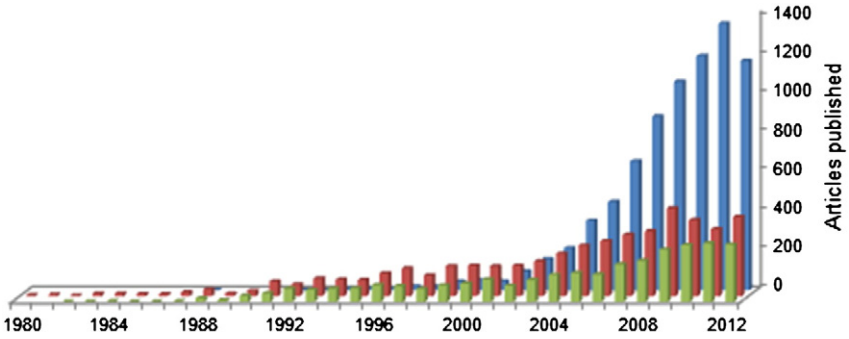

Fig. 1. Yearly breakthrough of the articles published covering all aspects of magnetic particles. The numbers were obtained from a Thomson Reuters' Web of Knowledge[SM] search (October 20th 2012) using the terms: ■ magnetic particles, ■ magnetic beads and ■ magnetic nanoparticles.

and Boudrant, 1974; Halling and Dunnill, 1979; Munro et al., 1977; Robinson et al., 1973; Van Leemputten and Horisberger, 1974). Nowadays, magnetic separations of cells, proteins and nucleic acids are routinely used in laboratories and available in automated stations for high throughput processes. However, the implementation of large scale magnetic separations in biotechnology has not yet been achieved. Nevertheless, in recent years, the preparative scale purification of a monoclonal antibody from an 100 l cell culture supernatant has shown the advantages of magnetic separations particularly in process time (Holschuh and Schwämmle, 2005).

It should be pointed out that magnetic particles are not solely used for separation purposes. From a literature search, without any application restrains, in the Thomson Reuters' Web of Knowledge[SM] using the terms "magnetic particles", "magnetic beads" and "magnetic nanoparticles" we can clearly see a significant increase in the number of publications in the last 20 years (Fig. 1). Among them, magnetic nanoparticles have received a thriving interest in the past 10 years reaching almost 1400 in 2011. Behind this driving force are many biomedical applications in drug and gene delivery, imaging, tissue engineering and magnetically induced hyperthermia that typically require very small particles (e.g. <100 nm). To the present day, several magnetic based biomedical applications have been subject to clinical trials (e.g. MagForce Nanotecnologies AG) and some products are already available in the market (e.g. clinically approved contrast agents Feraheme™, Lumirem®, Resovist®). To learn more about such applications the reader can resort to several recent review articles (Corchero and Villaverde, 2009; Kumar and Mohammad, 2011; Oh and Park, 2011).

2. Introduction

Magnetic separations offer a particularly unique propriety that allows unprecedented control of our target of interest. Given the non-magnetic behaviour of the vast majority of biological samples, the target can be separated in a rapid and highly selective fashion under very gentle conditions (low shearing forces). In fact, the purification of intact cells (Choesmel et al., 2004), organelles and cell compartments (Diettrich et al., 1998; Hornig-Do et al., 2009; Lawson et al., 2006; Lüers et al., 1998; Mura et al., 2002; Perrin-Cocon et al., 1999; Wittrup et al., 2010) and large protein complexes (Cristea et al., 2005, 2006; Gorchakov et al., 2008; Hofmann et al., 2002; Lange et al., 2000; Markillie et al., 2005; Raghavendra et al., 2010; Tchikov and Schütze, 2008) is possible.

In order to capture magnetic particles from solution, the magnetic force (F_m) exerted on them must be able to overcome particle diffusion as well as inertial, viscous, gravitational and buoyancy forces. The magnetic force exerted on a particle by the influence of a magnetic field is given by the following equation:

$$F_m = \frac{V_p \Delta\chi}{\mu_0}(B.\nabla)B \quad (1)$$

where μ_0 is the permeability of vacuum ($4\pi \times 10^{-7}$ T m A^{-1}), V_p is the volume of the particle (m^3), $\Delta\chi$ is the difference in magnetic susceptibilities between the particle and the surrounding medium (dimensionless) and B the applied magnetic field (T). For most applications, low magnetic field separators (<500 T/m) based in strong permanent magnets (e.g. neodymium iron boron, samarium cobalt) allow for the efficient recovery of the magnetic adsorbents at small bench top scales (up to 50 ml) and in fully automated robotic systems. However, their application is normally limited to batch operations and micrometric particles. When small or low magnetisable particles are used they have to be captured resorting to high gradient magnetic separators (HGMS). These systems typically consist of a canister filled with a magnetisable ferrous matrix (e.g. steel wool) to which a large external static field (~1 T), provided by an electromagnet or a strong permanent magnet, is applied. The resulting magnetic gradients are as high as 10^4 T/m generating forces large enough to capture even weakly magnetic particles in a flow stream (Ditsch et al., 2005b; Moeser et al., 2004). HGMS are the only suitable magnetic biopurification systems for large process volumes but up to date there is not a commercially available process scale system suitable for bioprocesses. Unlike the separators used in the mining industry these systems will have to ensure containment and be able to sustain harsh cleaning and sterilization procedures.

Separation processes can be either targeted to the removal of magnetic contaminants or for the recovery of an envisaged product. While the first simply requires a magnetic separator, the latter involves selective magnetic adsorbents. The selectivity of which can be tailored just like any chromatographic media. In fact, several companies already offer a vast portfolio of particles functionalized with affinity, ion exchange, hydrophobic and mixed-mode ligands. A few examples are shown in Table 1. To further increase the panoply of ligands possible, companies also offer pre-activated particles bearing amine, carboxyl, epoxide, thiol, aldehyde, bromoacetyl, cyanuric chloride, hydrazide, tosyl or isocyanate groups for functionalization. The application for which the magnetic particle is required ultimately defines its characteristics. Nevertheless, for biotechnological purposes some general guidelines may be defined.

2.1. Magnetic proprieties

For most applications, superparamagnetic particles with a high magnetic saturation are required. Superparamagnetism can be viewed as the combination of paramagnetic and ferrimagnetic behaviour (Fig. 2). Like paramagnets, superparamagnetic materials exhibit zero remanence, i.e. in the absence of an applied external magnetic field their average magnetization is zero. As a result, these particles show a better dispersibility in solution as they do not tend to magnetically interact with each other to form aggregates. However, their magnetic susceptibility is much higher than paramagnets and like ferrimagnets they reach magnetic saturation but without exhibiting a magnetic hysteresis loop. Highly magnetisable particles respond quicker as they generate greater

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