Analytica Chimica Acta 673 (2010) 101–108

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Flow-through immunomagnetic separation system for waterborne pathogen isolation and detection: Application to *Giardia* and *Cryptosporidium* cell isolation

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ARTICLE INFO

Article history:
Received 30 December 2009
Received in revised form 14 May 2010
Accepted 17 May 2010
Available online 25 May 2010

Keywords:
Continuous flow
Washing
Separation
Magnetic particles
Giardia
Cryptosporidium

ABSTRACT

Simultaneous sample washing and concentration of two waterborne pathogen samples were demonstrated using a rotational magnetic system under continuous flow conditions. The rotation of periodically arranged small permanent magnets close to a fluidic channel carrying magnetic particle suspension allows the trapping and release of particles along the fluidic channel in a periodic manner. Each trapping and release event resembles one washing cycle.

The performance of the magnetic separation system (MSS) was evaluated in order to test its functionality to isolate magnetic-labelled protozoan cells from filtered, concentrated tap water, secondary effluent water, and purified water. Experimental protocols described in US Environmental Protection Agency method 1623 which rely on the use of a magnetic particle concentrator, were applied to test and compare our continuous flow cell separation system to the standard magnetic bead-based isolation instruments. The recovery efficiencies for *Giardia* cysts using the magnetic tube holder and our magnetic separation system were 90.5% and 90.1%, respectively, from a tap water matrix and about 31% and 18.5%, respectively, from a spiked secondary effluent matrix. The recovery efficiencies for *Cryptosporidium* cells using the magnetic tube holder and our magnetic separation system were 90% and 83.3%, respectively, from a tap water matrix and about 38% and 36%, respectively, from a spiked secondary effluent matrix. Recoveries from all matrices with the continuous flow system were typically higher in glass tubing conduits than in molded plastic conduits.

1. Introduction

Protozoa, particularly, *Giardia* and *Cryptosporidium*, are among the most common reported waterborne pathogens. Isolation and detection of these pathogens from water samples presents a challenge to environmental laboratories. Existing methodologies for routine monitoring of *Giardia* and *Cryptosporidium* are only semi-quantitative. Most water samples contain few oocysts, and concentration techniques are necessary to obtain even a small number of oocysts to allow reliable detection. US Environmental Protection Agency (USEPA) has developed the standardized method 1623 for detection of *Giardia* and *Cryptosporidium* oocytes in water [1]. Method 1623 includes four major steps: filtration, immunomagnetic separation (IMS), fluorescent anti-body labeling, and optical microscopic cell counting. Among these major steps, IMS and cell counting pose significant operational challenges due to the long processing time and high risk of sample loss.

Immunomagnetic separation (IMS) is an established technique that involves application of anti-body-coated magnetic particles to separate pathogenic micro-organisms, biological cells, or chemical compounds from clinical, food, soil and environmental samples. Current standard IMS devices use 1–8 μm magnetic particles to test volumes of 1 mL [2,3]. In most cases, such a small sample volume contains low numbers of target organisms, which results in low sensitivity of detection. Attempts have been made to increase the sample size to 10 mL, which resulted in a 7-fold increase in sensitivity [4].

Several magnetic separation devices have demonstrated the separation of magnetic particles in microfluidic channels utilizing either external permanent magnets [5–7] integrated magnetic posts [8,9], or integrated micro-electromagnets [10,11]. However,

Abbreviations: USEPA, US Environmental Protection Agency; IMS, Immunomagnetic separation; MSS, Magnetic separation system; t_{s1}, The time required for the particle to travel to and be retained at the first trapping zone; T_0, The time required for a particle to travel through the entire fluidic channel and reach the separation chamber without any influence of magnetic force; T_m, The time required for a magnetic particle to travel through the entire fluidic channel and reach the separation chamber with the present of magnetic force effect from the magnet array; t_{Sn}, The time required for a magnetic particle to travel between two adjacent trapping zones; PC, Poly carbonate; PBS, Phosphate buffered saline; S1, Syringe 1; S2, Syringe 2; S3, Syringe 3; V1, Valve 1; V2, Valve 2; V3, Valve3; Pi1, Fluidic inlet 1; Pi2, Fluidic inlet 2; Pi3, Fluidic inlet 3; MA, Magnetic assembly (see supplementary materials); SM, Separation magnet (see supplementary materials).

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doi:10.1016/j.aca.2010.05.025

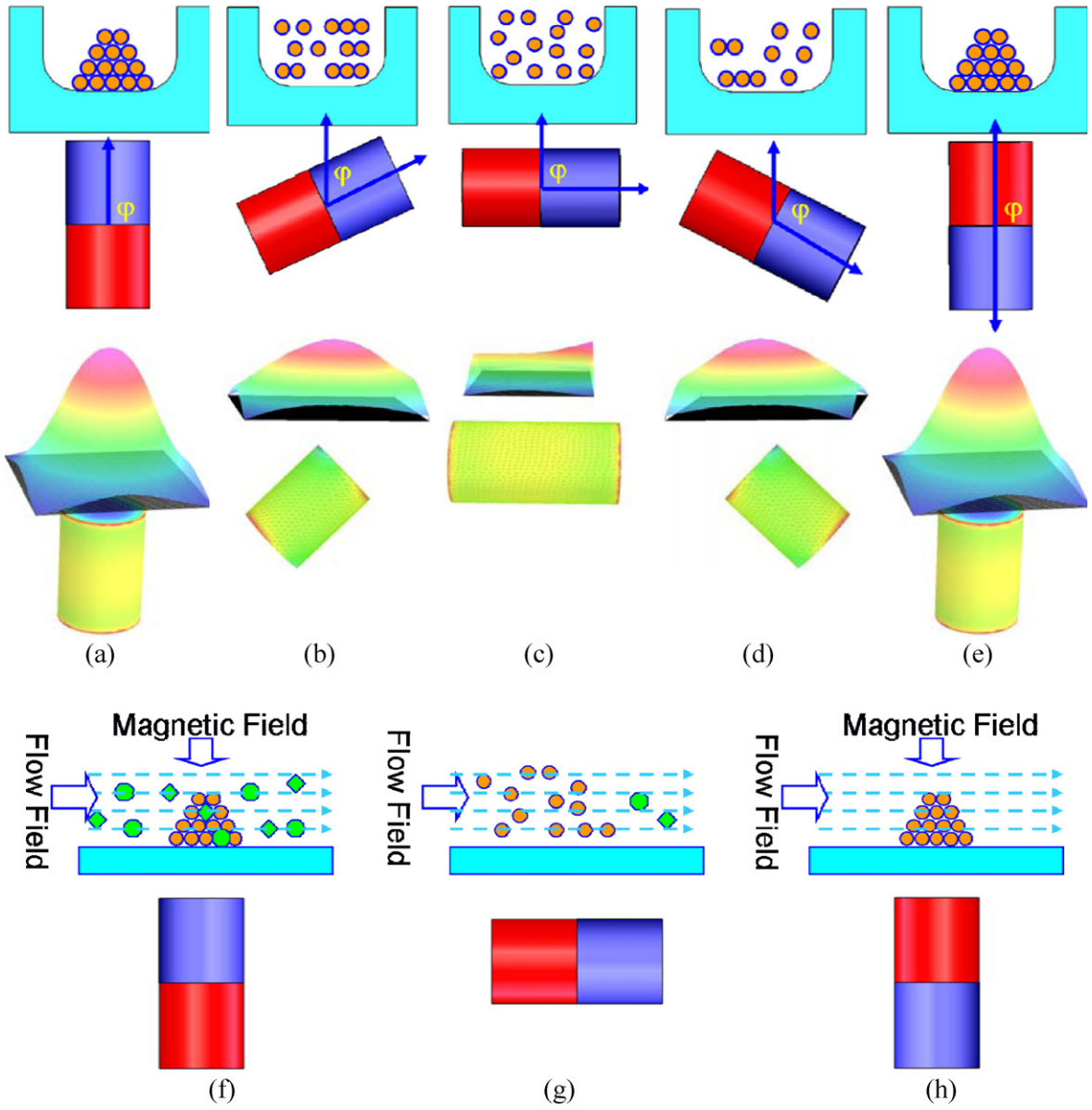

Fig. 1. Magnetic force modulation on magnetic particle suspension by magnet rotation. Magnetic force changes during the magnet rotation from maxima to minima then back to maxima, etc. Magnetic particles trapped in the channel when magnetic force maximum and released when the magnetic force minimum. (a) $T = t_1$, $\varphi = 0^\circ$, $B_{max} = 16$ mT (magnetic force is maxima); (b) $T = t_2$, $0^\circ < \varphi < 90^\circ$, $B_{max} = 4$ mT (magnetic force is moderate); (c) $T = t_3$, $\varphi = 90^\circ$, $B_{max} = 0.7$ mT; (d) $T = t_4$, $90^\circ < \varphi < 180^\circ$, $B_{max} = 4$ mT (magnetic force is moderate) (magnetic force is minima); (e) $T = t_5$, $\varphi = 180^\circ$, $B_{max} = 16$ mT (magnetic force is maxima); (f) both magnetic and flow fields are ON therefore, magnetic particles clustering while non-magnetic particles keep flowing downstream. But some non-magnetic particles can be physically trapped in the clusters; (g) magnetic field is OFF or weak therefore, magnetic particle clusters disperse in the fluid and flow downstream and non-magnetic particles also released and flow downstream with higher velocity than that of the magnetic ones; (h) purified magnetic particle cluster.

these devices and/or processes still have the drawback that the capture of the magnetic particles occurs only at the wall of channels/tubes in a static trapping mode. In practical applications, high magnetic particle concentrations are used and magnetic fields usually create relatively large aggregates during the magnetic separation process due to the significant dipole–dipole interactions [12]. Such a static aggregation process results in inhomogeneous aggregates. In other words, not only the magnetic particle-analyte get trapped, but other particulates (impurities) will also be physically trapped in the created aggregate and appear in different concentrations, depending on the original raw sample composition. For instance, method 1623 describes an experimental protocol based on IMS technology for the isolation, concentration and detection of *Giardia* and *Cryptosporidium* in water. During the magnetic separation process, a relatively large single aggregate is formed against the sample container (tube) and the carrier fluid is discarded. The separated sample is then suspended in a smaller volume with a new buffer and transferred afterward to downstream processes (detection). In such methods, the sample is washed by applying a washing buffer solution while the magnet is holding the magnetic particle-analyte aggregate. This washing step removes only impurities in the liquid surrounding the aggregate while other sandwiched impurities remain inside the aggregate. As such, it is necessary to carry out subsequent washing steps before the analyte can be analyzed further. However, in carrying out subsequent washing steps there is a risk that quantities of analyte may be lost during transfers between different washing containers, evaporation, and adsorption to the wall of the containers. In the case of a low concentration of analyte in the starting sample, as in the case of pathogen cells in drinking water, carrying out the extraction and washing of analyte