New optimised one micron magnetic bead platform for fast and efficient development of automated immunoassays

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Introduction

The use of magnetic beads as the solid phase in automated immunoassay instruments is well established and rapidly increasing. The critical and important criteria for the solid phase in immunoassays are:

- high capacity
- high signal to noise ratio
- easy development of assays
- efficient and reproducible immobilisation of antibodies (or other ligands)
- easy handling in manufacturing
- reproducible behaviour in automation without mixing requirements, and fast and efficient washing of beads

The objective of our work has been to develop new magnetic beads that fulfil the requirements for solid phases in automated immunoassays.

Materials and Methods

Dynal Biotech's well established proprietary technology is the production of monodisperse magnetic Dynabeads® with a renowned quality in the *in vitro* diagnostic immunoassay market. Based on this technology, we have developed a new one micron magnetic bead platform called Dynabeads® MyOne™.

To meet immunoassay requirements we have developed new magnetic one micron beads and optimised the following parameters:
- bead size
- surface area
- surface active groups
- surface properties like hydrophobicity and charge
- iron content
- magnetic properties.

Bead size and size distribution are determined by use of Coulter Counter measurements, whereas iron content is measured by total elemental analysis. Surface active groups like tosyl or epoxy groups where analysed by traditional wet chemistry methods. Magnetic properties were determined by magnetisation curves measuring the magnetisation with increasing and decreasing magnetic field strength.

Further we have characterised the physiochemical surface properties by contact angle measurements for hydrophobicity as well as zeta potential measurements for surface charge and isoelectric point. Both streptavidin and monoclonal antibodies have been used to determine protein immobilization efficiency. To demonstrate the feasibility of these new one micron magnetic Dynabeads® in immunoassays, the beads were coated with antibody or streptavidin, and introduced into existing immunoassay kits for Myoglobulin, D-Dimer and Intact PTH. The coated beads were simply replacing the existing capture solid phase with no further optimisation of assay conditions, and standard curves as well as samples analysed.

Results

Size and size distribution

Figure 1 shows the size distribution for Dynabeads® MyOne™ beads measured with Coulter Counter MS3. The size distribution for the main peak is 2 %. Amount of beads per gram dry weight was also determined by Coulter Counter measurements, and found to be approx. 10^{12} beads per gram dry weight.

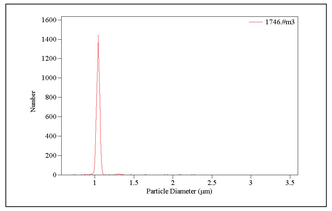
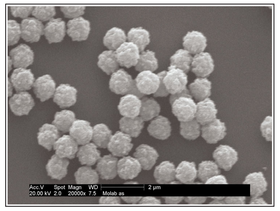


Figure 1. *Size distribution for Dynabeads® MyOne™ prototype 2 measured with Coulter Counter MS3.*



SEM picture of Dynabeads® MyOne™ Carboxylic Acid.

Magnetic properties

The iron content of Dynabeads® MyOn™e is 37 % Ferrite.

The magnetic material is evenly distributed throughout the beads, and shows no remanence (rest magnetism after removing the magnetic field). This is shown in the hysteresis curve in figure 2. This curve shows in addition the magnetic saturation level (at maximum field strength). The slope of the initial magnetisation at low magnetic field strength gives the magnetic mass susceptibility.

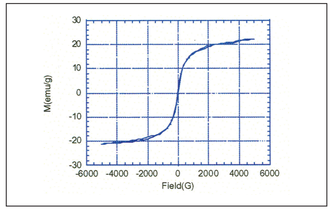


Figure 2. *Hysteresis loop for Dynabeads® MyOne™ Carboxylic Acid. Magnetisation as a function of magnetic field strength.*

Surface characteristics

Hydrophobicity of the beads is measured by Contact angle measurements (instrument Fibro Dat 1120)) on water droplets deposited on a dry bead layer. Hydrophobicity of the MyOne prototype beads can be compared to hydrophobicity of existing and well established Dynabeads® M-280 Tosylactivated. Dynabeads® MyOne™ Carboxylic Acid is in comparison very hydrophilic, due to a high negative net charge at neutral pH.

Zeta potential measurements were performed with a Malvern Instruments Zeta Sizer 2000-3000 HS. Results show again that the new Dynabeads® MyOne™ beads can be compared to Dynabeads® M-280 beads. Zeta potential measurements for beads coated with monoclonal mouse IgG1 antibody and streptavidin were included The results demonstrates that the protein immobilised may determine the Isoelectric point, as the negatively charged Dynabeads® MyOne™ Carboxylic Acid beads after coating with antibody have a similar pI as the more neutral Tosylactivated beads.

Results for hydrophobicity and Zeta potential measurements are given in Table 1.

Bead type	Contact angle °	Zeta potential Isoelectric point Uncoated beads	Zeta potential Isoelectric point Antibody coated beads	Zeta potential Isoelectric point Streptavidin coated beads
Dynabeads® M-280 Tosylactivated	120	7,0	4,9	5,1
Prototype 1 Dynabeads® MyOne™	120	7,2	5,1	5,4
Dynabeads® MyOne™ Carboxylic Acid	10	2,7	5,2	4,1

Table 1. *Contact angle and isoelectric point measured with Zeta potential.*

Sedimentation

Sedimentation was measured in a spectrophotometer measuring the absorbance at 450 nm over a period of time. Settling of beads gives reduced absorbance. Results for Dynabeads® MyOne™ Streptavidin were compared to Dynabeads M-270 Streptavidin and are shown in figure 3.

Titration of Dynabeads® MyOne™ bead amounts in Immunoassay

Geometrical surface area for 1,0 µm beads is approximately 2,5 times higher per weight compared to 2,8 µm beads, calculations based on bead surface area, number of beads and density of the beads. Hence the required amount of 1,0 µm beads was expected to be much less than 2,8 µm beads. Dynabeads® MyOne™ beads and Dynabeads® M-280 Tosylactivatedä were coated with antibody against D-Dimer and used in a sandwich assay for D-Dimer on an automated platform utilising chemiluminescence detection. Results are shown in figure 4 for 2,8 µm Dynabeads® M-280 and with 30, 40 and 50 % weight of Dynabeads® MyOne™ compared to Dynabeads® M-280.

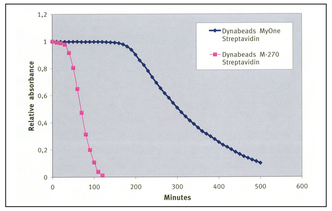


Figure 3. *Sedimentation of 1,0 µm and 2,8 µm Dynabeads® in aqueous solution measured as relative absorbance at 450 nm as a function of settling time (minutes).*

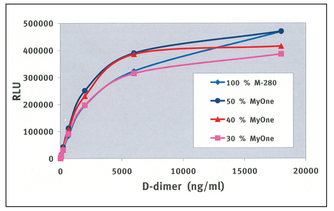


Figure 4. *Bead titration of different amounts of Dynabeads® MyOne™ beads in D-Dimer immunoassay compared to Dynabeads® M-280*

Antibody immobilisation

Antibody immobilization efficiency is always dependent on the antibody and conditions like antibody amount and antibody concentration, magnetic bead concentration and buffer composition during immobilization.
Typical result for antibody immobilization is **60-95 % binding of added antibody**, measured by the use of I^{125}-labelled tracer amounts of antibody.

Introduction of Dynabeads MyOne™ beads in immunoassays

Dynabeads® MyOne™ prototype beads and Dynabeads® M-280 Tosylactivated were coated with capture antibodies for D-Dimer and Myoglobulin and used in sandwich immunoassay kits developed on an automated platform respectively. Streptavidin coated beads were similarly prepared and used in sandwich immunoassay for intact PTH where biotinylated capture antibody was utilised. Results for standard curve measurements for the three different immunoassays are shown in figure 5, 6 and 7. Bead amount used was 40 % weight Dynabeads® MyOne™ compared to Dynabeads® M-280.

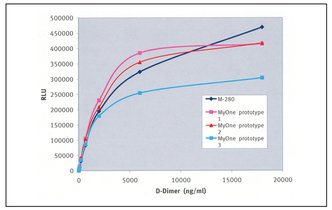


Figure 5. *Standard curve measurement for D-Dimer immunoassay with Dynabeads® M-280 (100 % weight), and three prototypes Dynabeads® MyOne™ (40 % weight).*

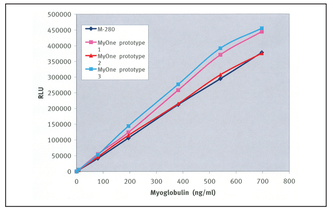


Figure 6. *Standard curve measurement for Myoglobulin immunoassay with Dynabeads® M-280 (100 % weight), and three prototypes Dynabeads® MyOne™ (40 % weight).*

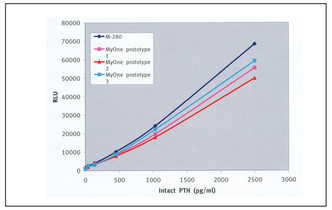


Figure 7. *Standard curve measurement for Intact PTH immunoassay with Dynabeads® M-280 (100 % weight), and three prototypes Dynabeads® MyOne™*

Measured RLU	Reference assay	Dynabeads® M-280 Tosyl	MyOne™ Prototype 1	MyOne™ Prototype 2	MyOne™ Prototype 3
Myoglobulin					
0 standard	273	199	261	300	203
1 standard (0,86 ng/ml)	356	611	730	794	819
Signal/noise	1,30	3,07	2,80	2,65	4,03
D-Dimer					
0 standard	331	297	350	371	188
1 Standard (25 ng/ml)	1350	3471	4692	4533	3891
Signal/noise	4,08	11,69	13,41	12,22	20,70
Intact PTH					
0 standard	n.a.	1575	1125	1096	842
1 Standard (38 ng/ml)		2468	2118	1907	2630
Signal/noise		1,57	1,88	1,74	3,12

Table 2. *Signal to noise ratio for Dynabeads® MyOne™ prototypes and Dynabeads® M-280 Tosylactivated in different immunoassays.*

Signal to noise ratio

Signal to noise ratios were determined for Dynabeads® M-280 and Dynabeads® MyOne™ prototypes as well as kit reference beads when available. Results are given in table 2. The results show excellent signal to noise ratios for all new prototypes.

Conclusion

The results show that new 1,0 µm Dynabeads MyOne, compared to the current Dynabeads M-280 Tosylactivated (2,8 µm), have an increased capacity of 250 %, thus enabling us to use 60 % less magnetic beads yielding the same results for dynamic range. The beads have a narrow size distribution and show very good dispersion properties and a slow sedimentation rate. The high Ferrite content (37 %) gives excellent magnetic properties with fast separation but still no magnetic remanence. The antibody immobilization efficiency is ranging from 60-95 % binding, depending on the antibody and conditions during coating.

Applying these beads in existing immunoassays, with a minimum of optimisation, gave an improvement in dynamic range and signal to noise ratios. Similar results are obtained using different surface compositions for immobilization of antibodies to the bead surface. Surface activated groups can be tailor made to suit the specific requirements of any immunoassay. From the results it can be concluded that these new beads fulfil the challenging criteria for magnetic solid phase in automated immunoassays.

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