Demonstration of improved light harvesting with novel silver plated paramagnetic particles in luminescent applications.

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INTRODUCTION

Due to the ease of manipulation and their large surface area, paramagnetic particles (MPs) are currently the solid phase of choice in automated clinical laboratory analyzers (1). Most automated clinical analyzers for immunoassays employ MPs as the solid binding phase. Also, light measurements, in the form of chemiluminescence, are the most common signaling mechanism employed today in automated clinical laboratory immunoalayzers (3)(4).

MPs of various sizes, magnetic moment, production method, residual magnetism, polymer capsule as well as surface functional groups are commercially available. Fundamentally, MPs are produced by either encapsulation of an existing magnetic core or by precipitation of magnetic salts in the presence of an existing polymer particle. Ideally, MPs should offer a large surface area (for maximum target binding), high magnetic moment (for fast collection), minimal residual magnetism (for easy re-dispersion to facilitate washing) and optimized colloidal status (to facilitate target binding) (2).

System and test developers on automated platforms optimize the performance of their immunoassay tests by improving the system design and by optimizing various components and steps of the immunoassay such as employing antibodies with improved characteristics, improved light emitting material, assay conditions and conjugation procedures tailored to the paramagnetic particles. The solid phase, in the form of MPs, represents a major component of immunoassay performance. The use of polymer-coated paramagnetic particles of ideal size with a high amount of surface functional groups/smaller parking area has been demonstrated to improve the test performance.

Higher light output from an immunoassay allows faster and more sensitive detection of very limited amounts of the analytes. Although the collected light signal from an immunoassay represents a small portion of the generated light, commercial sources of MPs employ black/brown magnetic pigment, which usually absorbs a large portion of the generated light. MPs represent the largest source of light loss in assays that employ them due to the light scattering effect on their dark surfaces. A new generation of light emitting detection systems requires a novel approach, not only with the instrumentation design and the test parameters, but also with the nature of solid support.

We have developed a proprietary process of silver plating a paramagnetic core material in order to convert color into much lighter color and in the meantime not to impede the magnetic moment to address the current problems. We reasoned that by increasing the efficiency of light harvesting over a silver-plated paramagnetic material, it would increase the collected light signal and therefore improve the performance of a binding assay that employ the silver-plated MPs.

MATERIAL, METHODS AND PROCEDURES

N-Cyclohexyl-*N'*-(β-[*N*-methylmorpholino]ethyl)carbodiimide *p*-toluenesulfonate salt (CMC), *N*-Hydroxysulfosuccinimide sodium salt (sulfo NHS), Rabbit Anti-Goat Alkaline Phosphatase (AP) conjugate (H and L specific), Rabbit Anti-Goat Horseradish Peroxidase (HRP) conjugate (H and L specific), Goat Anti-Mouse Horseradish Peroxidase (HRP) conjugate (H and L specific), SigmaFast OPD, Casein (vitamin free), were all purchased from Sigma Aldrich Canada (Oakville, ON).
Sulfo-SMCC Sulfosuccinimidyl 4-N-maleimidomethyl cyclohexane-1-carboxylate (Sulfo SMCC), 2-Iminothiolane•HCl (Traut's Reagent), Streptavidin Alkaline Phosphatase (AP) conjugate , BCA Protein Assay Kit , EZ-Link Sulfo-NHS-LC-LC-Biotin were purchased from Pierce (Rockford, IL).
Sephacryl S200 and PD 10 were purchased from Amersham Biosciences.
Lumigen APS-5 and Lumi-Phos 530 were purchased from Lumigen Inc.(Southfield, MI).
Goat IgG and Donkey Anti Goat (affinity purified) were purchased from Lampire Biologicals (Pipersville, PA).
Other reagents were of analytical reagent or laboratory grade.
Deionized, distilled water form Millipore system Direct-Q3 was used in all procedures.

1. Silver plating process

A proprietary electroless silver plating process was developed to coat MPs. MPs of various sizes were plated with a layer of silver, the thickness of which could be controlled by controlling the reduction parameters. Briefly, the MPs are degreased in acetone, then etched in sodium hydroxide solution and then activated by tin chloride. The activated washed and dried beads are plated by a process that employs silver nitrate and a reducing agent. The developed procedure was optimized to accommodate both the large surface area of the activated surface and the thickness of the plated silver layer. Also, the silver grain size was optimized for improved color conversion.

2. Polymer encapsulation process

The silver-plated MPs are polymer coated by proprietary polymer-encapsulation process that was developed. Briefly, the MPs are encapsulated by multiple-step process that combine both monomer and polymer assembly in order to deposit several layers of polymer that then functionalized. The process ensures silver surface isolation as well as minimizes any ion leakage that will be detrimental to biological samples.

3. Particle size determination

Digital microscopy was employed for size measurements (Carl Zeiss Primo Star, Carl Zeiss, AxioVision (version 4.6.1.0, Canon PowerShot A640)

4. Conjugation of goat IgG to Magnetic particles by two step procedure

A standard two-step conjugation procedure of the antibody to the carboxyl group on the MPs employing carbodiimide and NHS chemistry was employed. Magnetic beads were prepared with a first activation step followed by a conjugation step as follows:

- Magnetic particles (MPs) were adjusted to 200 mg/ml in volume of 1 ml.
- All samples were washed consequently with 0.01 M NaOH once, H_2O twice and 0.05 M KH_2PO_4 twice.
- MPs were separated from the washing solution by magnet.
- Washing was followed by activation step.
- MPs were activated by 0.0276 M sulfo-NHS and 0.0236 M CMC in 0.05 M KH_2PO_4 pH 6.0. for 1 hour incubation at room temperature (RT) with constant rotation.
- MPs were washed three times with 0.05 M KH_2PO_4 pH 6.0.
- 0.4 mg Goat IgG in 1 ml of 0.0375 M Borate, 0.05 M NaCl pH 8.5 was immediately introduced.
- After 2 hour incubation at RT, magnetic particles were washed with three volumes of 0.01 M PBS
- MPs were quenched for 30 min RT incubation with 0.01 M ethanolamine (0.01 M PBS, pH 7.4) with constant rotation.
- Blocking of the beads was completed by 1% Casein in 0.01 M PBS pH 7.4 for an hour RT rotation.

5. Preparation of Donkey Anti-Goat conjugates

- Obelin conjugate

  The bioluminescent protein Obelin was employed for light measurements. Conjugation procedure of IgG and Obelin was previously described (5). Briefly, SMCC-activated Donkey Anti Goat IgG was incubated with previously thiolated Obelin by Traut's reagent (SH-obelin) at a molar ratio of 1:10 for overnight at 4 C. The obtained conjugate was purified by gel filtration on Sephacryl S200 column (Amersham Biosciences) equilibrated with 0.2M NaCl, 0.005M EDTA, and 0.05 M MOPS, pH 7.1. Protein concentration was determined by BCA Protein Assay Kit (Pierce).
- Biotin conjugate

  Donkey anti-Goat Biotin conjugate was prepared using NHS activated long arm (LC-LC) biotin supplied by Pierce (Rokrord, IL). Biotinilation took place in 10 mM PBS pH 7.4 with 8 mol. eq. of NHS-LC-LC-Biotin for 30 min. The excess amount of biotin was removed by desalting. Protein concentration was determined by BCA Protein Assay Kit.

6. Bead Testing

Enzyme Linked Immunosorbent Assay (ELISA) "HALF SANDWICH"

Evaluation of MPs by an ELISA testing that employ either color generating substrate (OPD) or chemiluminescent material Lumigen APS-5 or by bioluminescence as follows:.

A - Evaluation by colorimetric measurements

- A 1 % MPs already conjugated with Goat IgG was prepared in 0.2 ml of 0.05M Tris, 0.15M NaCl, 0.1 % Tween 20, 0.01 M EDTA pH 7.4 (Washing buffer).
- Washing buffer was removed by magnet
- 0.2 ml in duplicates from 1:80k of Rabbit Anti-Goat HRP for in 0.05M Tris, 0.15M NaCl, 0.2 % Tween 20, 0.01M EDTA, 1 % Casein (Reaction buffer) were applied.
- Incubation for 30 min at RT with rotation.
- MPs were washed two times with washing buffer and transferred to white strips (Greiner Maximum Binding, flat bottom White*).
- One more washing step was applied in the wells before applying the substrate.
- 0.15 ml of from SIGMA FAST OPD was applied to each well, plate was incubated for 10min with constant shaking.
- 0.1 ml of already developed substrate was transferred on transparent plate, the reaction was stopped by 0.5 M H_2SO_4.
- The measurement was done with a plate reader at 450 nm (ThermoMax, Molecular Devices).
- Background of the MPs was evaluated by 1:80K Goat Anti-Mouse HRP (since the testing procedure is half sandwich type).

B - Evaluation by Chemiluminescent light measurements

- A 1 % MPs already conjugated with Goat IgG were prepared in 0.2 ml of 0.05M Tris, 0.15M NaCl, 0.1 % Tween 20, 0.01 M EDTA pH 7.4(Washing buffer).
- Washing buffer was removed by magnet.
- 0.2 ml in duplicates from serial dilutions 1:5k , 1:10k and 1:20K of Rabbit Anti-Goat AP conjugate in 0.05M Tris, 0.15M NaCl, 0.2 % Tween 20, 0.01M EDTA, 1 % Casein (Reaction buffer) were applied.
- Incubation for 30 min at RT with rotation.
- MPs were washed two times with washing buffer and transferred to white strips (Greiner Maximum Binding, flat bottom White*).
- One more washing step was applied in the wells before applying the substrate.
- 0.1 ml of Lumigen APS-5 was applied to each well, plate was incubated for 90 sec.
- The measurement took place with the particles on board in the white strips.
- The peak signal was collected for 1 sec by Luminoscan Ascent Thermoelectron.
- Background of the MPs was evaluated by 1:5 k Streptavidin-AP (since the testing procedure is half sandwich type).

C - Evaluation by Bioluminescent light measurements

An ELISA employing MPs conjugated to Goat IgG and DAG-Obelin conjugate was employed as follows*:*

- A 1 % MPs conjugated to Goat IgG was prepared in 0.2 ml of 0.0074M Na_2HPO_4, 0.0025M KH_2PO_4, 0.15M NaCl, 0.005 M EDTA, 0.005 M Mercaptoethanol, 0.1 % Tween 20, 0.02 M N-Acetyl Cystein, pH 7.4 (Washing buffer).
- Washing buffer was removed by magnet and two hundred microlitre (0.2 ml) of Donkey Anti-Goat-Obelin conjugate from serial dilutions of 5, 2.5 and 1.25 μg/ml (in duplicates) in 0.0074M Na_2HPO_4, 0.0025M KH_2PO_4, 0.15M NaCl, 0.050 M EDTA, 0.005 M Mercaptoethanol, 0.2 % Tween 20, 0.02 M N-Acetyl Cysteine, 1 % Casein (Reaction buffer) were applied.
- Incubation for 30 min at RT with constant rotation.
- MPs were washed two times with washing buffer and transferred on white strips (Greiner Maximum Binding, flat bottom White*).
- Two more washing steps were applied in the wells before measuring the signal, after final washing step final 0.05 ml of washing buffer was added to each well.
- Light emission was triggered by injecting 0.05 ml of 0.1 M $CaCl_2$, 0.05 M MES; pH6 in Luminoscan Ascent Thermoelectron.
- Signal was collected for 30 sec and the integration of kinetic curve was applied (area under the curve).
- Since the procedure for testing is half sandwich type, background of the MPs was evaluated by 2 μg/ml Mouse-Obelin.

***Evaluation of the amounts of MPs on the harvested light* (bioluminescence)**

- Light emitted from solid support (microtitre plate) coated with 5 μg/ml goat IgG, blocked with casein 1%, loaded with Donkey Anti-Goat-Obelin in Reaction buffer, defined in the previous section, was tested with serial dilution of MPs from 5 to 0.625 mg total amount of MPs. Light emission was triggered by 0.05 ml of 0.1M $CaCl_2$, 0.05M MES; pH 6 in Luminoscan Ascent Thermoelectron. Signal was collected as in previous section.
- Light emitted from solution phase 0.025 ml of Donkey Anti-Goat-Obelin with concentration 0.5 μg/ml in washing buffer, defined in the previous section, was applied on MPs with total amount from 5 to 0.625 mg in 0.075 ml washing buffer. Light emission was triggered by 0.05 ml of 0.1 M $CaCl_2$, 0.05 M MES; pH6 in Luminoscan Ascent Thermoelectron. Signal was collected as in previous section.

Enzyme Linked Immunosorbent Assay (ELISA) "FULL SANDWICH"

Evaluation of MPs by an "Full Sandwich" ELISA was prepared by using light emitting tests that employ the chemiluminescent material Lumi-Phos 530.

- A 1 % MPs conjugated with Goat IgG were prepared in 0.2 ml of 0.05M Tris, 0.15M NaCl, 0.1 % Tween 20, 0.01 M EDTA pH 7.4(Washing buffer).
- Washing buffer was removed by magnet.
- 0.2 ml of Donkey Anti-Goat-Biotin conjugate from serial dilutions of 5, 2.5 and 1.25 μg/ml (in duplicate) in 0.05M Tris, 0.15M NaCl, 0.1 % Tween 20, 0.01M EDTA, 1 % Casein (Reaction buffer) were applied.
- Incubation for 20 min at RT with rotation.
- MPs were washed two times with washing-buffer and transferred to white strips (Greiner Maximum Binding, flat bottom White*).
- 0.2 ml of Streptavidin-AP conjugate in the same reaction buffer, in duplicates, from 1:10k as above was applied
- MPs were washed two times with washing-buffer and transferred to white strips (Greiner Maximum Binding, flat bottom White*).
- One more washing step was applied in the wells before applying the substrate.
- 0.1 ml of from Lumi-Phos 530 was applied to each well, plate was incubated for 5 min.
- The measurement took place with the particles on board in the white strips.
- The peak signal was collected for 1 sec by Luminoscan Ascent Thermoelectron.

RESULTS AND DISSCUSIONS

RESULTS

A schematic depicting the total process of silver plating and then polymer encapsulation is shown in Fig 1

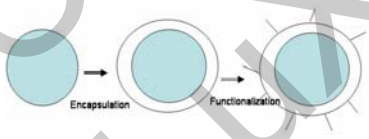

Fig 1.

Electroless surface silver plating is a well established process, although mostly done with flat surfaces. Silver plating of particles with large catalytic surface is a more delicate process, balancing the speed of salt reduction and the bath decomposing. As shown in Fig 2, our experimental results demonstrate the success in silver plating of MPs with a layer of silver.

Fig 2.

The thickness of the silver layer could be controlled to manipulate both chemical and physical characteristics of the particles. By controlling the concentration of reagents as well as their metered addition, the large catalytic surface of activated MPs of various sizes could be plated with a silver layer, without fouling the plating bath (Fig 3 and 4).

Fig 3.

Fig 4.

Although the metering of the reagents to the bath has resulted in well controlled color development, this did not impact the size of the silver grain as shown in Fig 5

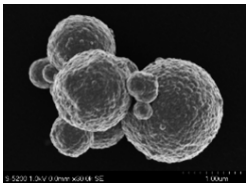

Fig 5.

The polymer coating process relied on both covalent and electrostatic binding of polymer chains to the silver surface to introduce polymer layers with large hydrophilic domains. As seen in Table 1 and Fig 6 and 7, the extended hydrophilic domains of the coated polymer have resulted in minimizing the non-specific adsorption of proteins as evidenced from the non-specific signal, using either light or color measurements.

Particle size (µm)	Black MPs		Silver MPs	
	Specific signal	Noise	Specific signal	Noise
8.5 µm	4.93E+07	6.83E+05	2.67E+08	2.23E+04
25 µm	7.64E+07	1.10E+03	2.05E+08	4.23E+04
35 µm	3.37E+07	1.27E+03	1.86E+08	6.35E+04
50 µm	2.70E+07	1.57E+03	9.40E+07	1.90E+04

Table 1

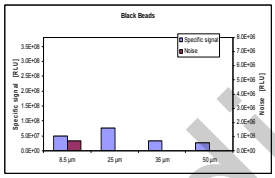

Fig 6.

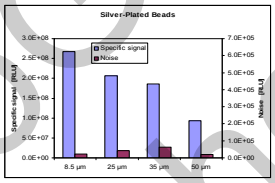

Fig 7.

As shown in Table 2 and 3 and Fig 8, 9, 10 and 11, introduction of surface carboxyl-group was successful as evidenced by the difference in active covalent coupling of the antibodies through carbodiimide chemistry in comparison to simple passive adsorption of the antibodies to the surface of both silver-plated (Table 2) or black MPs (Table 3).

Particle size(µm)	Bioluminescence			Colorimetric test		
	Active conj.	Passive adsorp.	Noise	Active conj.	Passive adsorp.	Noise
8.5 µm	2.67E+08	1.44E+08	2.23E+04	2.91E+00	1.61E+00	6.35E-02
35 µm	9.65E+07	7.34E+07	1.97E+04	1.77E+00	1.38E+00	4.65E-02
9 µm	1.70E+08	9.37E+07	4.32E+05	2.57E+00	1.85E+00	5.27E-01
5 µm	2.00E+08	1.41E+08	4.36E+05	2.72E+00	2.04E+00	8.25E-02

Table 2.

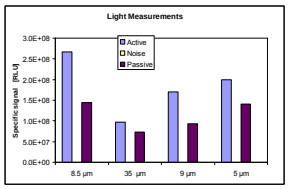

Fig 8.

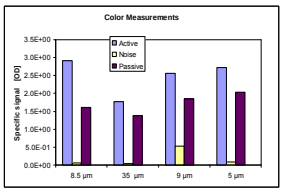

Fig 9.

Particle size(µm)	Bioluminescence			Colorimetric test		
	Active conj.	Passive adsorp.	Noise	Active conj.	Passive adsorp.	Noise
35 µm	2.18E+07	1.59E+07	6.59E+02	1.73E+00	1.49E+00	6.25E-02
6 µm	4.63E+07	1.29E+07	7.76E+05	2.73E+00	8.06E-01	1.54E-01
8.5 µm	4.93E+07	1.32E+07	6.83E+05	2.59E+00	8.68E-01	1.44E-01
9 µm	4.62E+07	1.62E+07	7.47E+05	2.90E+00	1.06E+00	5.61E-01

Table 3.

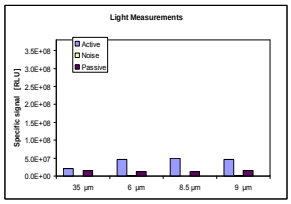

Fig 10.

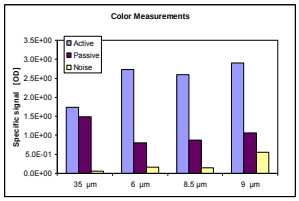

Fig 11

The density of the functional groups was not ideal. A balance between the amount of free radical added and the tolerated aggregation is required, as more free radical is needed to compensate for free radical consumption by the electro-active silver surface. Although we succeeded in polymer coating and functional group assembly, as evidenced by the immunoassay testing results using active conjugation, comparison to commercial beads demonstrates that the signal is lower than commercial beads by color measurements. However, as shown in Table 4 and Fig. 12 and 13, when light measurements are employed, the signal was higher with silver-plated MPs, in comparison to black MPs.

RAG-HRP	6 um Signal	Estapor 2.4 um Signal
1:160 k	3.68E+07	8.03E+06
1:320 k	2.05E+07	4.01E+06
1:640 k	1.11E+07	1.88E+06
0.0000	1.63E+04	4.90E+03

RAG-HRP	6 um Signal	Estapor 2.4 um Signal
1:40 k	9.64E-01	3.26E+00
1:80 k	8.61E-01	2.88E+00
1:160 k	5.36E-01	2.18E+00
0.0000	5.25E-02	1.42E-01

Table 4.

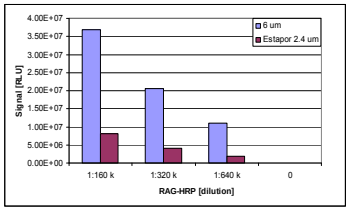

Fig 12.

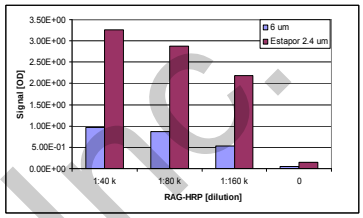

Fig 13.

The results of our immunoassay testing confirm the notion that a large portion of the light loss is due to the light scattering effect of the solid surface, regardless of the particle size. As shown in Table 5, Fig.14 and 15, immunoassay ELISA testing that employs color measurements, both the dark-colored and silver-plated MPs give equivalent signals, delineating the similarity of polymer coating characteristics and antibody binding capacity.

Particle size (µm)	Black MPs		Silver-plated MPs	
	Signal	Noise	Signal	Noise
25 µm	2.45E+00	4.50E-02	2.18E+00	4.25E-02
35 µm	1.77E+00	5.10E-02	1.76E+00	4.35E-02
50 µm	1.68E+00	3.90E-02	1.61E+00	4.50E-02
6 µm	2.73E+00	1.54E-01	2.35E+00	8.85E-02
9 µm	2.90E+00	5.61E-01	2.57E+00	5.27E-01
5 µm	2.85E+00	2.26E-01	2.72E+00	8.25E-02
3 µm	2.79E+00	1.29E-01	2.92E+00	7.55E-02

Table 5.

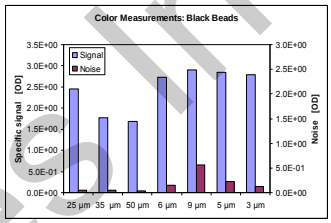

Fig 14.

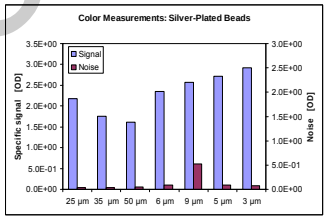

Fig 15.

In contrast, by employing light measurements, the silver plated MPs consistently give a higher light signal. As shown in Table 6 and Fig 16 and 17, the amount of harvested light is much higher with silver coated MPs in comparison to their black counterparts (between 2 and 7 folds). This was evidenced whether the light generation employed biomiluminescence (Table 6) or chemiluminescence (Table 7, Fig 18 and 19). Although no attempt was made to optimize the performance of the immunoassay testing, silver-plated MPs always give a higher light output. The amplitude of the light output depends on the size of the MPs, the level of generated light, and the amount of plated silver- all factors which impact the light scattering efficiency.

Particle size (µm)	Black MPs		Silver-plated MPs	
	Signal	Noise	Signal	Noise
25 µm	6.42E+07	1.57E+03	2.06E+08	4.23E+04
35 µm	3.37E+07	1.27E+03	1.86E+08	6.35E+04
50 µm	2.70E+07	1.57E+03	9.40E+07	1.90E+04
6 µm	4.62E+07	7.47E+05	1.70E+08	4.32E+05
9 µm	4.62E+07	7.47E+05	1.70E+08	4.32E+05
5 µm	6.07E+07	8.87E+05	2.00E+08	4.36E+05
3 µm	6.03E+07	1.10E+06	1.97E+08	3.15E+05

Table 6.

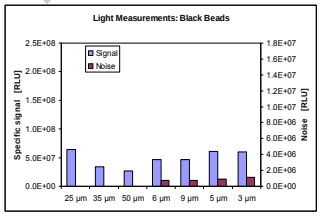

Fig 16.

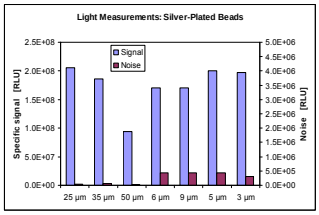

Fig 17.

Our ELISA testing results using both the full-sandwich and half-sandwich formats demonstrate the enhancement of the light harvesting effects of silver-plated MPs. As shown in Tables 7, 8 and Fig 18, 19 and 20, the light enhancing effect was evident, in both formats, with no attempt for any optimization for the employed ELISA.

Particle size	Black MPs		Silver-plated MPs	
(μm)	Signal	Noise	Signal	Noise
25 μm	**6.14E+05**	**3.11E+04**	**1.16E+06**	**6.12E+04**
35 μm	**4.14E+05**	**2.97E+04**	**1.28E+06**	**7.14E+04**
50 μm	**3.58E+05**	**3.15E+04**	**1.28E+06**	**6.76E+04**
6 μm	**8.48E+04**	**4.07E+02**	**2.07E+05**	**6.59E+02**
9 μm	**9.11E+04**	**3.77E+02**	**2.83E+05**	**6.30E+02**
5 μm	**1.20E+05**	**4.65E+02**	**2.17E+05**	**1.53E+03**
3 μm	**1.26E+05**	**6.46E+02**	**3.84E+05**	**1.33E+03**

Table 7.

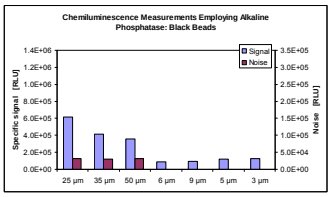

Fig 18.

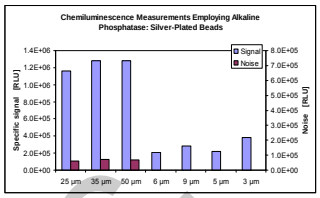

Fig 19.

DAG-Bio	6 μm		9 μm		5 μm		3 μm	
μg/ml	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs
5	**2.77E+05**	**1.43E+05**	**2.43E+05**	**1.12E+05**	**3.54E+05**	**1.53E+05**	**5.12E+05**	**1.31E+05**
2.5	**2.07E+05**	**8.48E+04**	**2.83E+05**	**9.11E+04**	**2.17E+05**	**1.20E+05**	**3.84E+05**	**1.26E+05**
1.25	**1.47E+05**	**7.01E+04**	**1.69E+05**	**7.09E+04**	**1.61E+05**	**8.95E+04**	**2.38E+05**	**9.31E+04**
0	**6.59E+02**	**4.07E+02**	**6.30E+02**	**3.77E+02**	**1.53E+03**	**4.65E+02**	**1.33E+03**	**6.46E+02**

Table 8.

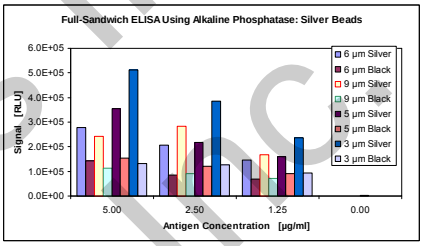

Fig 20.

In order to explore the reason(s) behind signal enhancement, various sets of experiments were carried out. Varying the amount of the MPs, the source of light generation and the amount of generated light were tested. As shown in Table 9, Fig 21, although the signal is always higher with silver-plated particles, the signal difference depends on the size of particles, the amount of emitted light as well as amount of particles employed. The notion of a major impact of light scattering effect was further confirmed by varying the amount of conjugate employed and therefore the amount of generated light in the presence of constant amount of beads (Table 10, Fig 22).

MPs (%)	25 mm		35 mm		50 mm	
	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs
2.5	**4.76E+07**	**2.16E+07**	**6.98E+07**	**1.17E+07**	**5.61E+07**	**1.54E+07**
1.25	**8.61E+07**	**2.74E+07**	**7.70E+07**	**1.13E+07**	**6.41E+07**	**1.61E+07**
0.625	**7.59E+07**	**2.51E+07**	**6.92E+07**	**1.14E+07**	**5.47E+07**	**1.82E+07**
0.3125	**7.72E+07**	**2.91E+07**	**6.62E+07**	**1.06E+07**	**4.51E+07**	**1.45E+07**

Table 9.

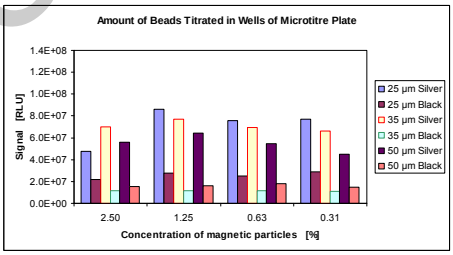

Fig 21.

Conjugate Conc.	6 μm		9 μm		5 μm		3 μm	
	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs
1:5k	**4.52E+06**	**1.88E+06**	**2.38E+06**	**1.49E+06**	**4.27E+06**	**2.73E+06**	**4.15E+06**	**2.29E+06**
1:10k	**2.69E+06**	**1.14E+06**	**1.87E+06**	**9.66E+05**	**2.99E+06**	**1.62E+06**	**2.96E+06**	**1.44E+06**
1:20k	**1.72E+06**	**6.36E+05**	**1.26E+06**	**6.16E+05**	**2.02E+06**	**9.04E+05**	**1.85E+06**	**9.25E+05**
noise	**3.09E+05**	**2.64E+05**	**1.34E+05**	**7.76E+04**	**2.65E+05**	**8.09E+04**	**2.51E+05**	**1.20E+05**

Table 10.

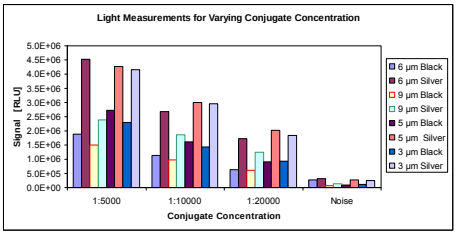

Fig 22.

To explore further the light scattering effect, the source of generated light was tested by the same immunoassay method except the light was generated with the light emitting conjugate bound to wells of microtitre plate in the presence of various amounts of MPs, only during light emission. As shown in Table 11, Fig 23, in comparison to control signal (no beads added to well), the light signal harvested from wells which contain black MPs was lower than those harvested from wells containing silver-plated MPs.

MPs(mg)	25 μm		35 μm		50 μm		8.5 μm	
	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs
5	**7.31E+07**	**5.18E+07**	**8.53E+07**	**8.10E+07**	**1.17E+08**	**7.23E+07**	**6.61E+07**	**2.98E+07**
2.5	**9.92E+07**	**8.01E+07**	**1.31E+08**	**1.19E+08**	**1.25E+08**	**1.32E+08**	**9.31E+07**	**6.99E+07**
1.25	**1.28E+08**	**1.09E+08**	**1.46E+08**	**1.21E+08**	**1.43E+08**	**1.36E+08**	**1.15E+08**	**1.05E+08**
0.625	**1.56E+08**	**1.43E+08**	**1.65E+08**	**1.44E+08**	**1.58E+08**	**1.68E+08**	**1.41E+08**	**1.36E+08**

Control signal 100%= 2.13E+08

Table 11.

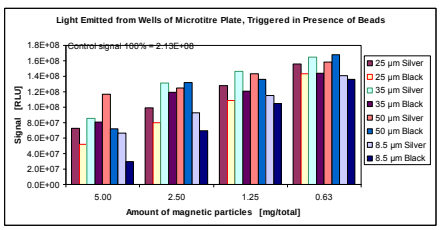

Fig 23.

Also, solution-phase light generation was tested. The presence of either black or silver-plated MPs during light generated from solution impacted the amount of harvested light. Wells containing silver-plated beads showed higher light signals than wells containing black beads, although the difference was lower than expected (Table 12, Fig 24).

MPs(mg)	25 µm		35 µm		50 µm		8.5 µm	
	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs	silver MPs	black MPs
5	4.86E+07	3.79E+07	5.21E+07	4.49E+07	5.65E+07	4.77E+07	4.92E+07	3.38E+07
2.5	5.58E+07	4.86E+07	6.37E+07	5.89E+07	5.77E+07	7.43E+07	5.96E+07	4.73E+07
1.25	7.22E+07	6.69E+07	8.32E+07	7.85E+07	7.93E+07	8.80E+07	7.22E+07	6.72E+07
0.625	8.38E+07	8.36E+07	9.44E+07	9.05E+07	9.37E+07	9.96E+07	9.13E+07	8.43E+07

Control signal 100% = 9.76E+07

Table 12.

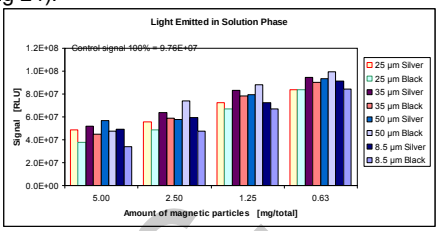

Fig 24.

As the process of silver plating is a process of core encapsulation, both silver-plated and black MPs employed have high density and are much less colloidal than comparable size commercial beads. The magnetic contents were intentionally kept high (85-90%) to facilitate particle collection. As such, the impact on signal generated elsewhere is lower than that if the light signal is generated from their surfaces, especially since the luminometer employed for measurements did not allow mixing during light generation. Although we employed plating and coating processes by which we could control the thickness of both the silver and/or the polymer shell, we did not succeed in developing totally colloidal particles.

The light harvesting enhancing effect of the developed silver-plated particles is evident and the amplified signal could be exploited in developing binding tests with higher sensitivities and/or extended measuring range.

CONCLUSIONS

Our results confirm the notion that a major portion of the lost light signal is due to the light scattering effect of the solid phase. In immunoassay bioluminescent measurements, silver coated MPs showed up to 7 fold improvement in the collected light signal, although the antibody coating efficiency of both was the same as the dark MPs. This improvement in the measured light signal was evident using color-converted paramagnetic particles of various sizes in comparison with the black counterparts. The differential effect was maintained with different amounts of generated light.

The developed particles show a promise in improving the testing sensitivity of binding assays when light measurements are employed (such as immunoassays as demonstrated here). However, the polymer coating process, which could control both particle-colloidal status and functional group density need much further optimization. This work is currently in progress.

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