The simultaneous detection of two analytes on the Agilis™ platform using silver and gold nanoparticles

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AgPlus diagnostics

CONTENTS

KEY POINTS

AgPlus expands capabilities to measure gold and silver NPs simultaneously on the Agilis™ system.

Successful dual detection of two analytes simultaneously.

Multiple applications in patient point of care for the dual detection of analytes.

Dual detection speeds up diagnosis

SILVER & GOLD NANOPARTICLES

Silver and gold nanoparticles are very widely used and studied due to their aqueous solubility, easy functionalisation and inert, non-toxic nature, whereby monodisperse particles with a tuneable diameter can be produced. Indeed, these particles have found their way into a multitude of assay systems ranging from lateral flow devices whereby the intense red colour of an AuNP gives rise to a qualitative signal, through to both electrochemical and photochemical systems whereby the intrinsic metallic particle is used as the detectable label due to the unique properties of the metallic particle, in many cases giving rise to a fully quantitative system.

The AgPlus system, for the detection of a single analyte, uses the plating of a silver nanoparticle onto a carbon electrode whereby the stripping of the metal from this electrode is measured and an assay signal recorded (Figure 1). The silver nano-particles form a charged aggregate due to the presence of ammonium thiocyanate. This is attracted to the electrode under a positive potential. The silver nano-particle at the electrode is converted to silver ions. The silver ions are measured electro-analytically giving rise to a measurable peak, the area of the peak is proportional to the concentration of molecule being measured.

Indeed, this fantastic technology is well understood, and we have used this for the detection of a wide range of biomarkers. Our system is a rapid, fully automated, quantitative, microfluidic system which is based on the afore mentioned electrochemical detection of the Ag+ ion which is to be used in a point of care setting and is currently under review from the FDA of both China and the US for the detection of various acute infections.

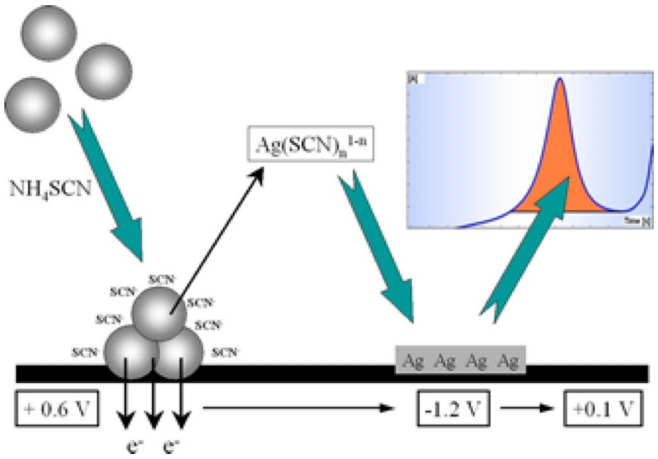

Figure 1. Schematic of the AgPlus assay chemistry measuring the concentration of silver ions.

The detection of multiple analytes simultaneously is an increasingly popular area of study. Whilst the Ag+ ion is where a sizable amount of our research and development is based, we also have the use of other metallic nanoparticles at our disposal. It is possible to use AuNPs in the same manner as described earlier for AgNPs on our system. Further to this, the Au+ ion is electrochemically stripped from the carbon electrode under a different potential to that of the Ag+ ion. With this in mind, we have shown the detection of two analytes simultaneously on our system with the use of both AgNPs and AuNPs in the same assay against different analytes. This method uses two sets of conjugates with two separate immunocomplexes being formed and measured at the same time (Figure 2) on our electrochemical system. This allows us to measure both analyte 1 and analyte 2 at the same time.

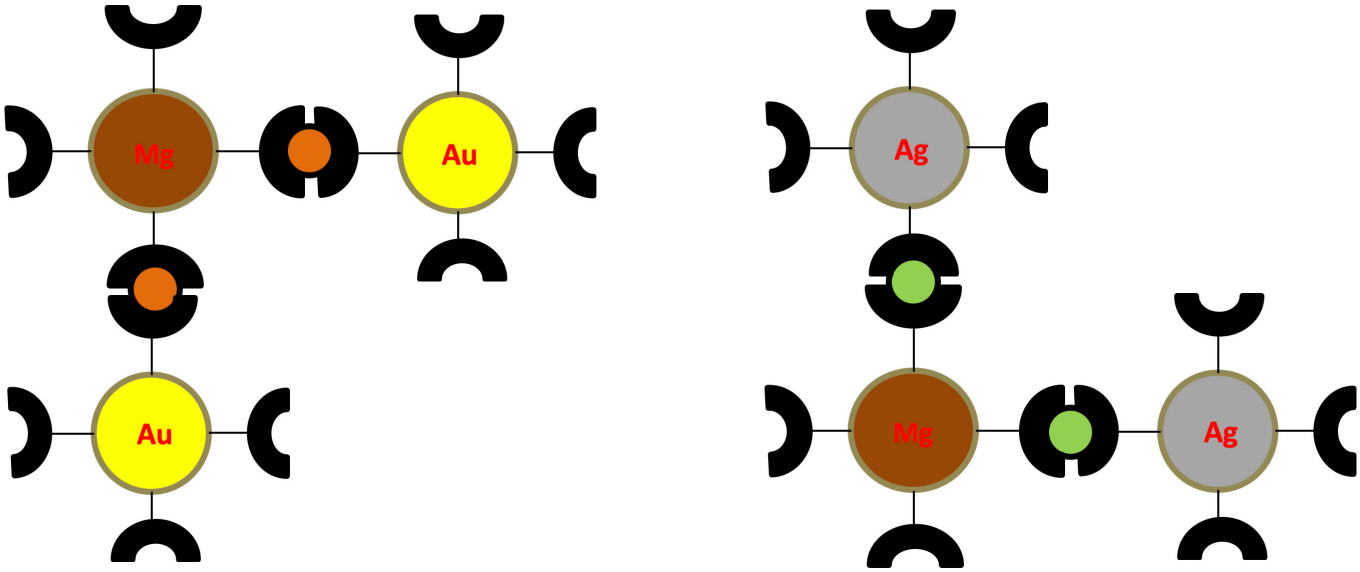

Figure 2. Cartoon representation of the simultaneous formation of two immunocomplexes using AgNPs for the green analyte (analyte 1) and AuNPs for the orange analyte (Analyte 2).

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With the idea that we can strip the Au and the Ag plated metals from the carbon electrode at different voltages, we can see via our stripping voltammetry that distinct Au and Ag peaks can be accomplished from a model buffer system (Figure 3).

Figure 3. Voltammogram gained from the dual detection of analytes on our system with using AgNPs as a marker for analyte 1 and AuNPs for analyte 2.

We can see that we are able to measure both the Ag and the Au signal independent of each other with good resolution of the two signals. We have shown that this can be taken forward whereby one analytes concentration are varied whilst the other analyte is kept constant which is reflected in both the voltammogram obtained and the subsequent instrument response from the integrated signals (Figures 4).

Figure 4. Top; Voltammograms obtained when the analyte 2 concentration is varied and the analyte 1 concentration is kept constant, Bottom; Subsequent integrated instrument responses.

The above data shows that as the analyte 2 concentration is increased, both the voltammograms and the instrument responses for the Au signal are increased whilst for the Ag signal, the voltammogram and the instrument response are kept constant.

This piece of work has shown that dual detection system for two different analytes can be accomplished on the AgPlus system using differing AuNP and AgNP simultaneously. This is particularly exciting as from at our instrument responses, we can see no interference between the two analyte signals. In our lab we are in the process of optimising this process and we are running development projects based around this type of expertise.

Stripping voltamagram for Au and Ag simulatneously formed immunocomplexes

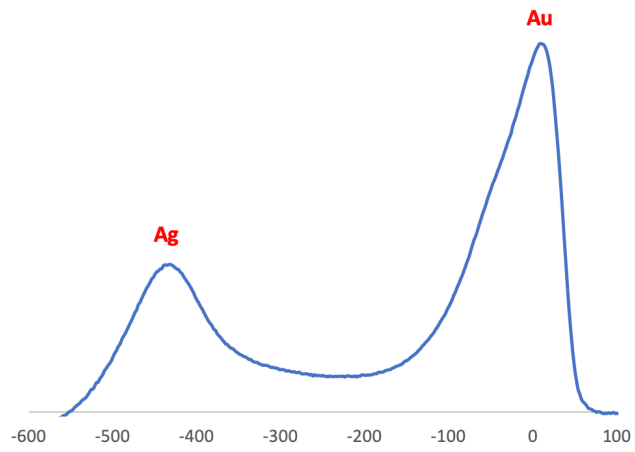

Figure 3. Voltammogram gained from the dual detection of analytes on our system with using AgNPs as a marker for analyte 1 and AuNPs for analyte 2.

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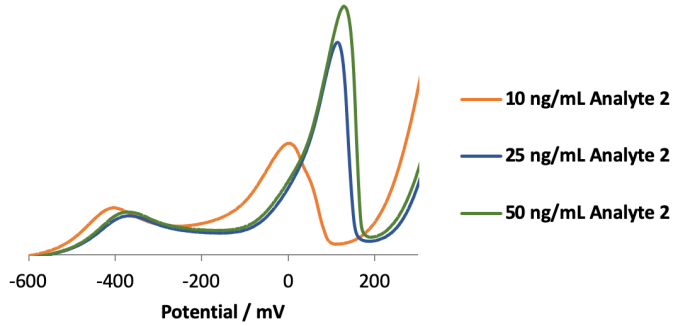
This technology has a huge potential and we expect to transfer all the benefits associated with our single analyte system on the Agilis™ reader of being rapid, fully automated, quantitative system onto a duplex system for the detection of multiple analytes at once. The Agilis™ system is a point of care system whereby results are available to a patient within 15 minutes, using a minimal amount of sample.

The diagnosis of several conditions is often not trivial and several different tests are required. For example in a clinical setting when a patient present with temperature which could be caused by a viral and a bacterial infection firstly being able with one test to distinguish this but then having the potential to determine the nature of the infection and indeed relative amounts and levels of different analytes become important, and in a point of care setting, we want results to be available rapidly.

This technology will cut down on the number of tests required as multiple analytes can be measured simultaneously, the downstream result being a faster diagnosis for the patient which in the case of many acute infections such as meningitis, sepsis and many others can be a life saving amount of time saved with this technology as the correct treatment can be administered to the patient.

Analyte 2 conentration is varied and analyte 1 concentration kept constant at 25 ng/mL



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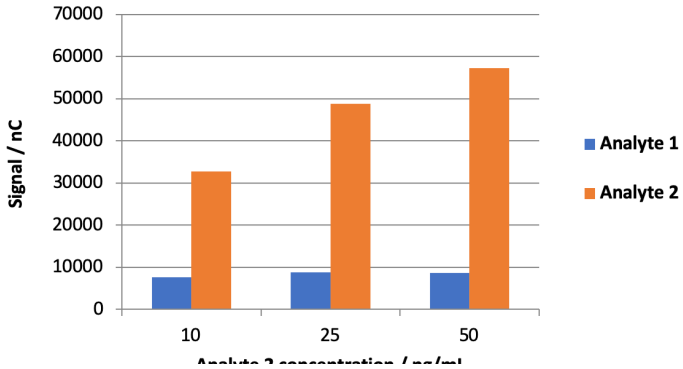

Figure 4. Top; Voltammograms obtained when the analyte 2 concentration is varied and the analyte 1 concentration is kept constant, Bottom; Subsequent integrated instrument responses.

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