Development of a Low-Cost Arduino Based Laser Nephelometric Instrumentation for High Sensitivity determination of the Inflammatory Marker C-Reactive Protein (CRP)

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Abstract— *A simple low-cost (<60 USD) Arduino based laser nephelometric instrument, which is suitable for high sensitive immunoprecipitation detection is presented. A measuring range of up to 49 mg/L of dog C-Reactive Protein (CRP) is reported. Furthermore, the limit of detection (LOD) is determined to be 2.1 mg/L. Only 24 µL of dog serum samples are required. Assay-time is 6 minutes. We successfully demonstrated the good performance of the device on two low level (0 and 10.2 mg/L CRP) dog serum samples. The coefficient of variation (CV) was 10% at the level of 10.2 mg/L.*

Keywords*— *nephelometry, immunoprecipitation, C-Reactive Protein, laser, Arduino.

I. INTRODUCTION

Immunoprecipitation reactions are used for the quantitative analysis of chemical substances in clinical chemistry. The operational principle is based on the formation of immuno-complexes through interactions between antiserum (antibody) and antigen (protein). These complexes can under certain premises aggregate and form precipitates which might scatter light. Both in clinical chemistry and under in vivo conditions the formation of immuno-complexes is well documented in scientific literature and books[1].

The immunoprecipitation reactions were for the first time described in the scientific literature already in 1897 by Rudolf Kraus, who published his work with antiserum against cholera and thypoid[2]. Immunoprecipitation is a technique involving precipitation of a dissolved antigen using antiserum that specifically bound to the antigen. The technique has become widely spread in biochemistry and is being used for isolation and separation of antigens[3], as well as, for analytical purposes[4] in clinical chemistry. Continuous improvement of diagnostic methods is of a vital importance for clinical chemistry to improve the diagnosis of diseases.

Antibodies (immunoglobulins) are proteins found in vertebrates with the specific purpose to defend the organism against infection[3]. The immunoglobulin mainly used in immunoassays is immunoglobulin G (IgG). Macromolecules, such as proteins, that are foreign to the organism can induce an immune response and are referred to as antigens. Antibodies (given as Ab) interact with antigens (given as Ag) to form immuno-complexes (given as AgAb) through weak interactions.

Each IgG-antibody contains two identical antigen-binding sites, and is therefore bivalent and thus able to cross link antigens (especially macromolecular antigens) to form large lattices. Once a lattice grows beyond a certain size, it precipitates out of solution (see Fig. 1).

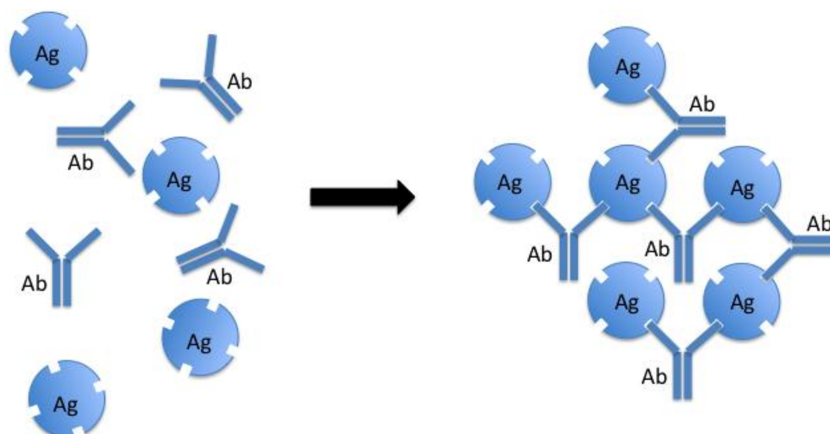

FIGURE 1: Figure illustrating the antibody (Ab) and antigen (Ag) binding and the formation of the insoluble immunoprecipitate complex (AgAb)

The binding reaction between the antibody and antigen is caused by weak interactions, and not by covalent bond formation[2]. This weak binding reaction resembles host-guest chemistry, in which the interaction involves 3-dimensional shapes, as well as, ionic bonds, London dispersion forces, dipole-dipole attractions, and hydrogen bonds[6]. Therefore, they are affected by conditions such as temperature, pH, polymers (e.g. PEG 6000, and Dextrans) and ionic strength. The reversible equilibrium reaction is given by reaction formula (1).

$$Ag_{aq} + Ab_{aq} \leftrightharpoons AgAb_{aq} \qquad (1)$$

$$K = \frac{[AgAb]}{[Ag][Ab]} \qquad (2)$$

The equilibrium constant of the reaction formula (1) can be calculated according to equation (2) and has experimentally found to have a value ranging[3] from 10^4 M^{-1} to 10^{12} M^{-1}. This means that the reaction nearly completely shifts towards the dissolved antibody-antigen complex. However, the dissolved antibody-antigen complex also reacts with free antibodies (that are in excess) and is further cross-linked into insoluble immunoprecipitate as illustrated in equation (3). However, it is also important to remember that when using polyclonal antisera directed against larger macromolecules like plasma proteins the antisera contains a spectrum of antibodies with varying affinity constants.

$$AgAb_{aq} + Ab_{aq} \longrightarrow AgAb_s \qquad (3)$$

Light entering a solution containing suspended immunocomplex particles is scattered. This scattering, caused by the particles, occurs in all directions due to reflection and refraction[7]. Therefore, immunoprecipitation can be measured with light scattering techniques such as turbidimetry and nephelometry. Turbidimetry measures the decrease of the transmitted light and nephelometry measures the scattered light of the sample. An ordinary spectrophotometer can be used for turbidimetry. For point-of-care applications, a standard spectrophotometer is costly, and turbidimetry does not perform as well as nephelometry in dilute solutions, which makes turbidimetry less sensitive. For nephelometry, specialized instrumentation is required, which is even more costly than standard spectrophotometers.

We describe in this paper the construction and the evaluation of a simple and low-cost Arduino based laser nephelometric instrument, which is suitable for rapid and high sensitive detection of immunoprecipitates. As a model application, we evaluated the device for the low-level determination of the inflammatory marker canine C-reactive protein (cCRP)[5]. The C-reactive protein measurements are used clinically for the detection and management of bacterial infections, ischemic necrosis of tissue, and active inflammatory conditions. Most commercially available point-of-care assays for CRP are only suitable for the measurements of CRP concentrations higher than 5-10 mg/L

II. MATERIALS AND METHODS

2.1 Reagents

The Immunoturbidimetric assay for canine CRP (art. No. CP2798) was obtained from Randox (Ireland). Two dog serum samples containing 0 mg/L and 10.2 mg/L CRP, respectively, were obtained from a local veterinary hospital.

2.2 Instrumentation

The Arduino based Nephelometer was built using the following components: Arduino Uno R3 (Arduino, Italy), Black Plastic Rod (Delrin®, D15mm * L40mm), Laser module 670 nm (Art. No.41002975, Elektrokit AB, Sweden), High-Sensitivity Light-to-Voltage Converter TSL257-LF (Art. No. 1226886, Farnell, UK), and an Electronic brick - 5V Relay (Art. No. 41010280, Elektrokit AB, Sweden). The Arduino based nephelometer construction is shown in Fig. 2. The nephelometer was controlled by a software, which is illustrated in Fig. 3. The instrument uses the inbuilt serial interface of the Arduino to transfer data to a computer or to a printer.

2.3 Methods

The method used for this experiment was copied from the instruction for use in the immunoturbidimetric assay for canine CRP. The dog serum sample was filtered through a sterile filter 0.2 µm (Sarstedt, Germany) and subsequently diluted 1:10 with H_2O. The measuring procedure consisted of a first step, where 450µl Reaction Buffer R1 and 24 µl sample were added in the glass vial (Waters, USA), which followed with a 1-minute incubation in the nephelometer, which resulted in signal N_1. Subsequently, 66 µl Antibody Reagent R2was added and the mixture allowed to incubate during 5 minutes in the nephelometer. The resulting signal N_2 was measured and finally the nephelometric signal was calculated as $\Delta N = N_2 - N_1$.

The glass vials (diameter 8.1mm – length 40mm) were used as cuvettes. However, the vials had to be marked so that the same orientation was always kept in the nephelometer, in order to avoid signal variations dependent on rotation of the vial. The standards used for the calibration curve contained the following concentrations of CRP: 0 mg/L, 10.4 mg/L, 22.6 mg/L, and 49.4 mg/L.

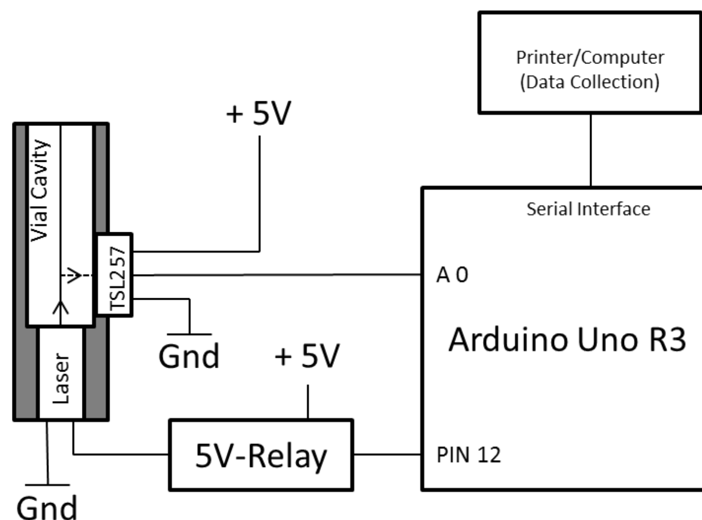

FIGURE 2: The construction of the simple low-cost Arduino based laser nephelometric instrument

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Laser Nephelometry Meter v2.0 Measures the nephelometry of a substance using a light detector and laser.
  modified 29 January 2018
  by D. H. Kriz

constint reactiontime1 =  60; //60 second pause before first measurement
constint reactiontime2 =  300; //300 second reaction time before second measurement
intbuttonState = 0;
intledPin = 0;
int A=0;
long B=0;
long sensorValue=0;
intnephelometry1=0;
intnephelometry2=0;
intnephelometry=0;

void setup() {
pinMode(2,INPUT);
pinMode(12, OUTPUT);
pinMode(13, OUTPUT);
Serial.begin(9600);
}

void loop() {

Step1:
buttonState = digitalRead(2);
  if (buttonState == LOW) {
goto Step1;
  }
digitalWrite(13, HIGH);
digitalWrite(12, LOW);
```

FIGURE 3: Arduino Software

III. RESULTS

We observed that the nephelometric signal (ΔN) caused by the immunoprecipitation reaction, increases with the CRP concentration in the range 0-49 mg/L (see Fig. 4). After an initial linear increase, the nephelometric signal saturates at higher CRP concentrations. We modelled a mathematical curve and found that the best-fit curve is y= -0.2546x2 + 24.02x + 127.41 (R2=0.9999).

A dog serum sample containing 0 mg/L CRP was measured 3 times and the following nephelometric signals were obtained: 111 AU, 146 AU, 119 AU, which gives an average of 125 ±15 AU. Using the best-fit curve equation above, the dog serum CRP concentration was determined to be -0.1 ± 0.6 mg/L.

Furthermore, a dog serum sample containing 10.2 mg/L CRP was measured 3 times and the following results were obtained: 332 AU, 291 AU, 303 AU, which gives an average of 309 ±17 AU. Using the equation above, the dog serum CRP concentration was determined to be 8.3±0.9 mg/L. The relative standard deviation (RSD) was found to be 10%.

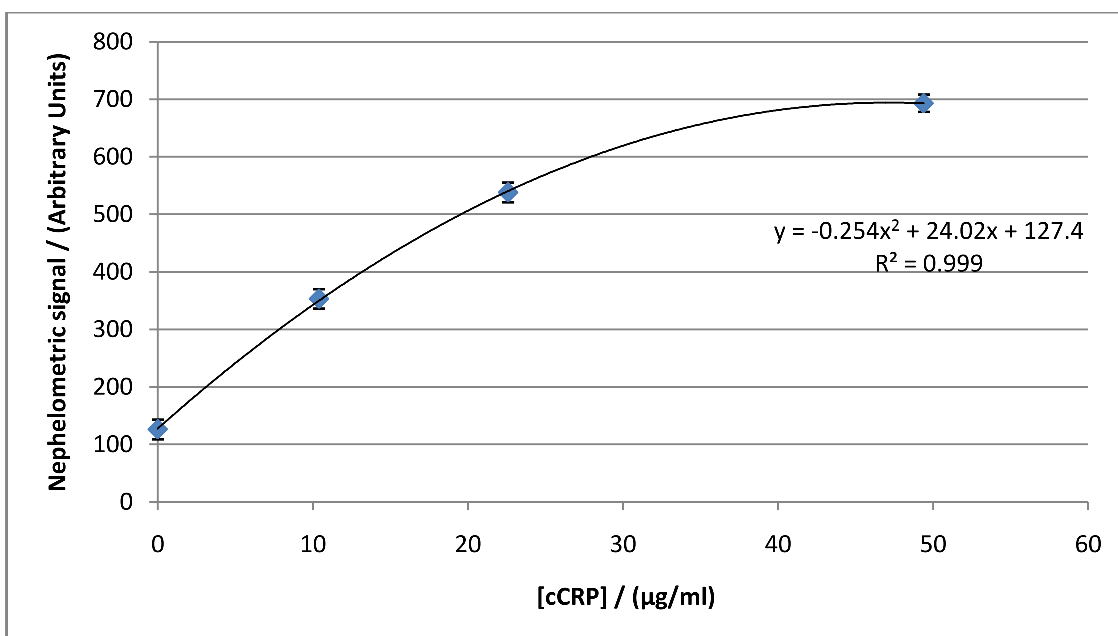

FIGURE 4: Signal observed with an Arduino based nephelometer for C-reactive protein concentrations in the range of 0-49 µg/ml in the sample.

IV. DISCUSSION

According to our results, we have demonstrated that the Arduino based nephelometer was capable of measuring on dog serum samples containing low levels of CRP (0 and 10.2 mg/L).

For the dog serum sample containing 10.2 mg/L CRP, we obtained 8.3 ± 0.9 mg/L, which is 19 % lower than anticipated. However, the RANDOX calibration solutions are based on human CRP, which could explain the slight deviation, as we are using antibodies towards canine CRP.

In order to estimate the limit of detection (LOD) of our device, we used the standard deviation value (SD=0.15 AU) obtained for the dog serum sample containing 0 mg/L CRP. The limit of detection is calculated by using Δy = 3*SD and the approximated linear calibration curve for the range 0-10 µg/ml (y=21.8x + 126). This results in a limit of detection (LOD) of 2.1 mg/L CRP.

The performance of the Arduino based nephelometer with relation to cost (<60 USD) was very satisfying. Of course, further optimization of the system is needed. This device would thus be of interest to use in many low-income countries if applied to human CRP.

V. CONCLUSION

In conclusion, we describe in this paper a simple low-cost (<60 USD) Arduino laser based nephelometric instrumentation, which is suitable for high sensitive immunoprecipitation detection. We successfully demonstrated the good performance of

the device on dog serum samples.

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