Digital-Based Image Detection System in Simple Silver Nanoparticles-based Cyanide Assays

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Abstract

Due to its high toxicity, qualitative and/or quantitative monitoring of cyanide in water is of great importance. The official existing methods for cyanide analysis, however, are not only expensive, but also require dedicated instruments and are unsuitable for field or on-site analyses. In the study herein, we demonstrate development of a new, simple, inexpensive and dedicated instrument free method for the determination of cyanide in water using silver nanoparticles (AgNPs) along with the digital- based image application as a detection system.

In this research, cyanide was quantified based on its ability to quench the colour of AgNPs which was captured by a digital camera and the pixel of the image was then used as the analytical response. The performance of this method was evaluated and compared to that of the spectrophotometric method. It was found that the AgNPs synthesized via reduction of $AgNO_3$*, by* $NaBH_4$ *with Sodium Dodecyl Sulphate (SDS) as a stabilizing agent, gave a maximum absorption at a wavelength of 400 nm, confirming the successful formation of AgNPs. This proposed method proved to be comparable and/or dependable in place of the previous methods (such as the spectrophotometric method) as it showed high precision, accuracy, recovery and very low limit of detection which were 0.96% (%RSD); 0.69% (%E); 97.79 ± 2.93% and 1.92 ppm respectively.*

Keywords: Portable Cyanide assays, Digital-Based Image, Silver Nanoparticles, On-site Analysis.

Introduction

Cyanide (CN^-) is one of the most hazardous environmental pollutants which at trace level is lethal to humans[1]. Due to its extreme toxicity, an admissible low limit of cyanide presence in water has been established by several international and national regulations.

As an example, the maximum permissible limit of cyanide in water set by the Indonesian government under the Health Minister Regulation is 70 $\mu g\ L^{-1}$ while the United States Environmental Protection Agency (US EPA) and World Health Organization (WHO) allow the maximum limit of cyanide in water to be 200 $\mu g\ L^{-1}$ and 50 $\mu g\ L^{-1}$ respectively[2]. Considering the extreme toxicity and the low standard requirement, routine on-site monitoring of cyanide in water is obviously necessary and not to mention, crucial to ensure safety among consumers. Therefore, this said monitoring process must be conducted using a simple and low cost yet highly-reliable analytical method. Among all of the official existing methods, ample attention has been given to the use of colorimetric method based on the use of UV-visible spectrophotometer. This was due to its rapidity, simplicity, ease of operation and lower limit of detection[3].

However, most of the reported colorimetric methods are based on the colour change due to the reaction of cyanide with dyes which can only be dissolved in organic solvents and therefore, cannot be effectively applied in the determination of cyanide in water[2]. Moreover, most of the dyes consist of complex structures which require laborious and time-consuming synthetic steps[3,4]. This, therefore, justifies that the interest in searching for a simple yet effective method for cyanide determination in water is of a continuous challenge.

Nanoparticles such as silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), unlike their bulk material counterparts, have been reported to be superior in that they have better water solubility and stronger absorption in the visible region. The latter characteristic was apparently due to their higher surface plasmon resonance band which strongly depends on a number of parameters such as size distribution, shape of the particles, and distance between particles[5]. Moreover, the above-mentioned characteristics of nanoparticles can be specifically tuned by using various chemical compounds adsorbed onto their surface.

Furthermore, the interaction with certain specific analyte can result in the colour change of the nanoparticles which is proportional to the concentrations of the analyte[6]. Thus, they are considered as future promising sensors for colorimetric analysis[7]. A number of research papers have reported the successful applications of nanoparticles for the simple determination of cyanide[8-11]. However, most of the reported nanoparticles applications for cyanide determination in water were based on the use of a spectrophotometer, which is not readily available in each laboratory especially those in the developing countries and therefore hampers their simplicity for the direct and on-site determination of cyanide in water[7-12].

In this study, we describe a simple and portable method for the determination of cyanide in water to overcome the above-mentioned problems associated with the existing official methods. This method was based on the colour change of AgNPs due to their aggregation induced by the interaction with cyanide ions. AgNPs were chosen as the colorimetric agent due to their ease of preparation and higher extinction coefficient in comparison to the AuNPs with the same size[6]. Digital image based (DBI) method which has been successfully utilized and reported elsewhere as an alternative method to the existing spectrophotometry was used to quantify the colour change to further simplify the determination of cyanide using this method without the need for a complicated instrument such as spectrophotometer[13-15].

Material and Methods

Materials: Silver nitrate, sodium borohydride, sodium dodecyl sulphate (SDS) and potassium cyanide were all in analytical grade and were used without any further purification. All the aqueous solutions in these experiments were prepared by using deionized water obtained from PT. Brataco (Indonesia).

Instrumentation: A digital camera with a characteristic and standard setting as described in table 1 was used to capture the pictures of standard and sample cyanide solutions for the digital-based image method. A UV-Vis spectrophotometer PG instruments T60 was used to characterize the formation of AgNPs and further used as a reference instrument for cyanide analysis using AgNPs. Image data processing was done by using free mobile phone software called colour shoot free, which was installed on a Lumia 625 LED Nokia. A simple customized box for the photographic system was designed by using a styrofoam container with the size of 25 x 30 x 25 cm which was fully covered from its outer side with a PVC black plastic. A 35-watt Philips LED bulb was placed in the top-centre inside the box to maintain the same environmental light condition during image recording.

Table 1
The characteristics of digital camera and the conditions of camera operation

Digital camera type Maximum resolution Camera sensor	Canon IXUS 155 20 mega pixels 1/2.3 type CCD
Photographic condition	
Exposure mode	Equivalent aperture
Focal length	35 mm
Metering mode	Centre-weighted average
Flash mode	Off
White balance	Auto
Image format	JPEG, (L)-5152 x 3864
Colour representation	RGB
Image processing hardware	Lumia 625 LED
Image processing software	Colour shot free

Procedure: The AgNPs were prepared according to the procedure applied in a previous study by Song et al[16] whereby the $AgNO_3$ which acted as the precursor metal salt was reduced by $NaBH_4$ in the presence of SDS as the stabilizer. Briefly, a mixture of 0.038-gram $NaBH_4$ and 0.085 gram SDS in 100 mL deionized water was added wisely by way of a dropper into an aqueous solution of 0.017 gram $AgNO_3$ under a continuous stirring for 1 hour. The formation of SDS-capped AgNPs was monitored by measuring the absorbance of the solutions using a UV-Vis spectrophotometer. The stability of the SDS-capped-AgNPs was also studied by measuring its absorbance using a UV-Vis spectrophotometer in the wavelength range of 350-500 nm at predetermined time intervals of 0, 1, 5, 10, 15, 20 and 24 hours.

The determination of cyanide in water samples using the digital-based image method was done by adding a 0.1 mL of either standard cyanide or sample solutions into 5 mL of the SDS-capped-AgNPs. The mixture was kept for 5 minutes to allow for complete reaction.

Image Capturing Procedure: A customized black box in a square shape with the size of 25 x 30 x 25 cm was used as a container to avoid the interference from ambient light (fig. 1). In order for the light inside the box to have the same contrast, the box was equipped with a 35 watt Philips lamp from the top-centre side. Before the sample was placed into the box, the sample was transferred into a glass vial. The picture was then captured through a "customised door" with a distance of 9 cm between the camera and the sample.

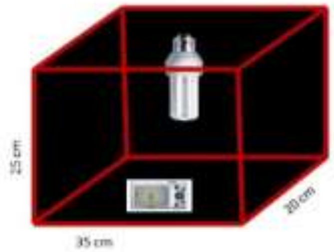


Figure 1: Customized Box for Image Acquisition

The image was then analysed using the Nokia Lumia mobile phone that was pre-installed with the software "colour shoot free" that can immediately convert the image into digital pixel intensity. The intensity of red (R), green (G), blue (B) of each sample solution was obtained directly from the software without the use of additional software. The RGB intensity for each solution was then manually recorded in a Microsoft excel file for further data analysis. The blue intensity was used as the analytical response as it showed a proportional response to the increase of cyanide concentration. The analysis of each sample was done in triplicate and the concentrations were determined based on the calibration curve.

A calibration curve of the CN^- standard concentration against the blue pixel intensity was constructed with the same procedure. The proposed digital-based image method was validated by determining its precision, accuracy, percentage of recovery and limit of detection. As a reference, the analysis was also conducted by the spectrophotometric method.

Results and Discussion

Preparation and characterization of AgNPs: This study utilized silver nanoparticles (AgNPs) as the colorimetric detection agents for cyanide determination in water. These AgNPs were prepared through the reduction of ionic Ag^+ by $NaBH_4$ in the presence of sodium dodecyl sulphate (SDS). It has been previously described that the purpose of SDS addition was to prevent the AgNPs from aggregation. This occurs through the steric hindrance mechanism[8,16]. It was found that the colour of the $AgNO_3$ solution immediately turned from colourless to yellow following the addition of $NaBH_4$, which clearly indicated the successful formation of AgNPs from Ag^+ ions.

Figure 2 indicates that the reduction of Ag^+ gave rise to a characteristic band at 400 nm which is due to the surface plasmon resonance of the AgNPs. This characteristic, as described by Caro et al[17], strongly depends on both the size of AgNPs and the environment where the AgNPs are dispersed. This can both be the medium and the compounds used to prevent the AgNPs from the aggregation. In some cases, the reaction between the analytes and the stabilizing agents can also result in the changes. This condition suggests that the SPR characteristic of AgNPs is tuneable and can therefore contribute to the applicability of SDS-capped AgNPs as sensing agents.

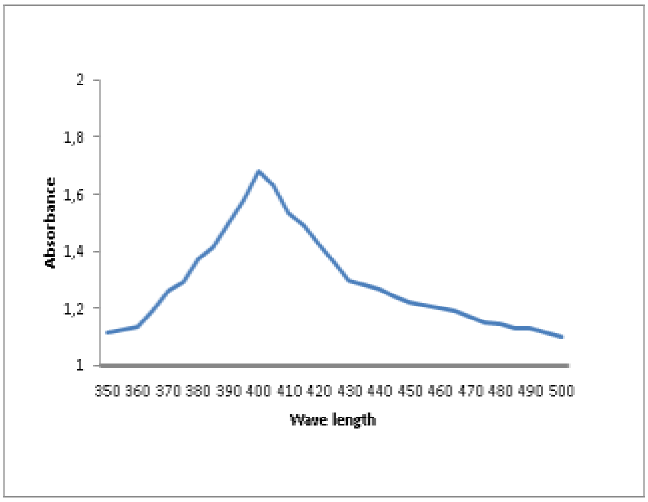


Figure 2: UV-Vis spectra of SDS-capped AgNPs

Stability Study of the AgNPs: The stability of the synthetized AgNPs was studied by continuously recording its absorbance (UV-Vis spectronic) and intensity (digital camera) for the duration of 24 hours. As depicted in figure 3, it was found that the AgNPs were stable for 24 hours period (1.660 ± 0.011) as they fluctuated to only about 0.66%. This constant trend could be attributed to the fact that the AgNPs were capped by the SDS on the surface, preventing them from aggregation. This trend is highly likely to remain for the longer duration. Similarly, the intensity of the AgNPs measured with digital camera showed highly stable characteristic (I = 42 ± 1 a.u.), whereby they fluctuated about 2.38% over 24 hours. The constant absorbance and intensity of AgNPs confirm their stability and therefore allow them to be used as colorimetric agent for cyanide determination in 24 hours.

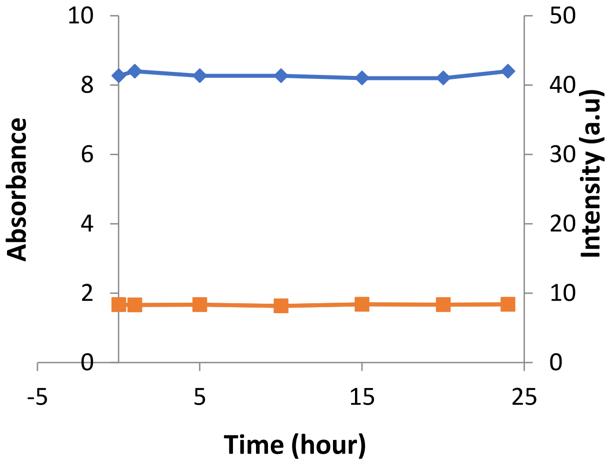


Figure 3: UV-Vis spectra of SDS-capped AgNPs

Effect of cyanide concentration on the AgNPs suspension colour intensity: The concentration (volume) of the cyanide added into the AgNPs suspension affected the colour intensity of the suspension. Figure 4 presents the individual intensity (Red, Green, Blue) change as the function of cyanide volume added into a 5 mL AgNPs. As the concentration increased, the blue intensity exhibited a linear dynamic trend while the red and green remained stable. Therefore, the blue intensity was then chosen and applied as the analytical response for further experiment.

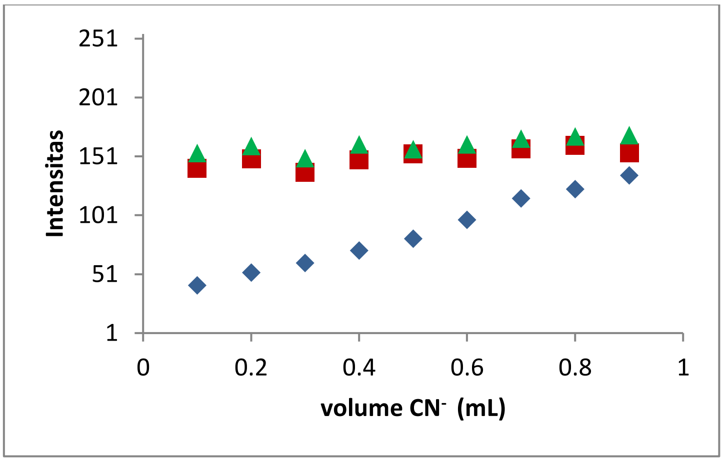


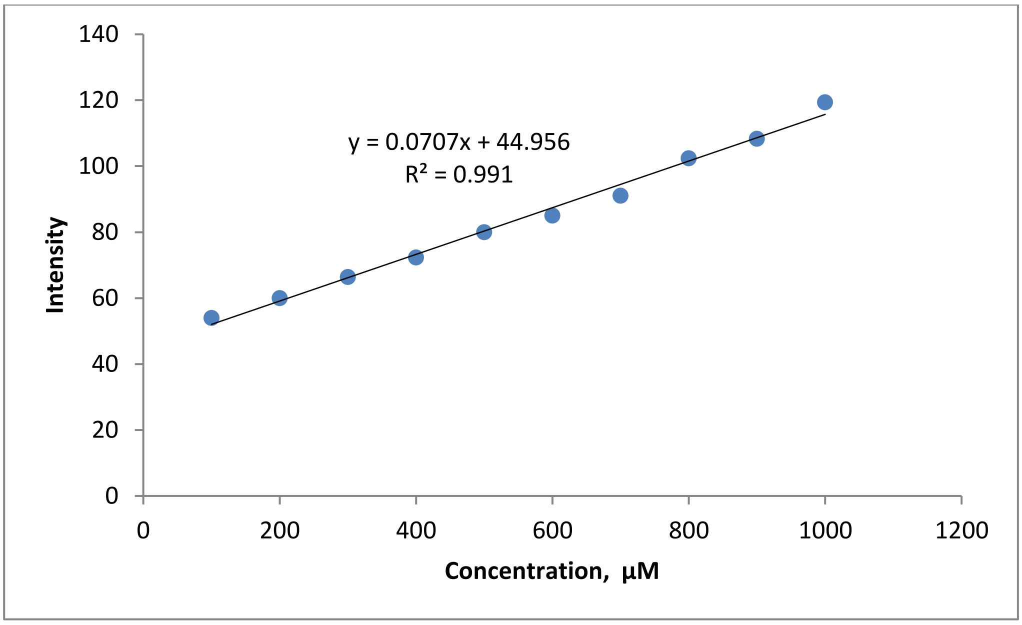
Figure 4: Profile of individual *Red, Green, Blue* (RGB) intensity in response to cyanide volume

Analytical Performance

Analytical figure merits: The analytical performance of the proposed AgNPs-based method was evaluated under optimal conditions. A series of solutions containing 100 µM; 200 µM; 300 µM; 400 µM; 500 µM; 600 µM; 700 µM; 800 µM; 900 µM and 1000 µM cyanide ion, were all measured with UV-Vis spectronic and a digital camera. The absorbance and intensities were then plotted with corresponding cyanide concentrations to construct linear curves in the range of 100-1000 µM (fig. 5). The analytical merits of this proposed method such as linear dynamic range, calibration curve equation, correlation coefficient, accuracy, precision and limit of detection were studied and compared to each other.

The obtained results were presented in the table 2. The linear relationship of both curves is expressed by coefficient correlations (R^2) of 0.991 and 0.996 respectively. This confirms that both methods complied with the Lambert-Beer law of an applicable method. The results also indicate the equivalent values of precision, accuracy and recovery of the proposed method in comparison to the spectrophotometric method. Our proposed method has however a higher limit of detection at 1.92 mg L^{-1} in comparison to the spectrophotometric methods which has a limit of detection at 0.204 mg L^{-1}. Nevertheless, the obtained limit of detection of the proposed method is reasonably acceptable with the compromise of other superior advantages of portability, simplicity, less time consumption and low analysis cost. This confirms the applicability of the proposed method for simple and direct measurement of cyanide in water.

Analysis of Synthetic Samples: The applicability of the proposed method to measure cyanide in water was examined by the measurement of several synthetic samples. The results were presented in table 2. These results illustrate the comparable concentration of cyanide in similar samples determined by using both the proposed method and the spectrophotometric method. It was important to note that in this experiment, the selectivity study was not conducted and the synthetic sample containing only cyanide in deionized water was used instead of a spiked environmental water sample. This was due to the highly selective reaction between the SDS-capped AgNPs and cyanide in the presence of other possibly interfering anions which had been previously demonstrated by Hajizadeh et al[8].



CN- (µM)	100	200	300	400	500	600	700	800	900	1000
Colour	●	●	●	●	●	●	●	●	●	●
Blue Intensity	54	59	65	73	81	84	91	108	103	108

Figure 5: Calibration curve

Table 2
Comparison of the analytical parameters of the proposed method and the spectrophotometric method

Parameters	**Methods**	
	Digital-based Image	**Spectrophotometric UV-Vis**
Precision (%RSD)	0.959	0.090
Accuracy (%Error)	0.694	0.931
Recovery (%R)	99.79 ± 2.93	99.275 ± 0.255
LOD (ppm)	1.92	0.204

Table 3
Determination of cyanide in several synthetic samples

C added µM	100	250	300	500	700	750	900
C measured by DBI (µM)	99.57	250.34	297.45	495.34	697.94	702.65	891.11
C measured by UV-Vis (µM)	99.50	248.84	298.37	495.74	695.360	743.38	891.22
Colour scale	●	●	●	●	●	●	●

Conclusion

In summary, a new, simple, portable and inexpensive method for cyanide determination in water based on the use of AgNPs has been developed as an alternative to the existing spectrophotometric method. It has been demonstrated through this study that the analytical performance of the proposed method was comparable to the existing spectrophotometric method with a reasonably low limit of detection. In contrary to the existing method, this said method requires no sophisticated instruments, thus, could be applied for direct and on-site determination of cyanide in water.

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