Real-time colorimetric hydration sensor for sport activities

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REAL-TIME COLORIMETRIC SENSOR OF DEHYDRATION AND OVER-HYDRATION FOR SPORT ACTIVITIES

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Abstract

Dehydration and over-hydration are a serious health concern in endurance sport activities, especially in tropical climates. Current methods for detection are inconvenient and not widely applied. Point-of-care hydration monitoring during a sport event is an ideal preventative therapy, as it allows personalize real-time feedback towards preventing hydration related injuries.

Gold nanoparticles allow colorimetric detection, whose color changes due to changes in colloidal stability. The red-shift in the plasmonic band induces a color change from red to purple upon aggregation that can be observed by the naked eye, which gives promising

27   applications in point-of-care preventative therapies. We developed a gold nanoparticle-based

28   colorimetric sensor for rapid detection of dehydration and over-hydration by monitoring

29   'proof-of-concept' artificial sweat ion concentrations. The colorimetric sensor was designed

30   to distinguish between dehydration and over-hydration through easily observed color changes.

31   In addition, the sensor exhibited a fast response time within minutes. The designed sensor is a

32   promising tool for point-of-care and real-time detection of dehydration and over-hydration.

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37   **1. Introduction**

38   Endurance sport activities are often associated with fatigue, muscle cramps, and hydration

39   imbalance. Among which dehydration is the most common due to the water loss in the form

40   of sweat to keep the body cool during intensive sport activities. The human sweat contains not

41   only water but also other mineral salts, lactic acid, glucose and urea [1]. Depending on the

42   rate of rehydration of the individuals, hydration imbalances will be developed leading to two

43   extreme conditions called dehydration and over-hydration [2]. Detection of hydration status

44   requires complicated procedures to analyze body fluids such as sweat, blood, urine and saliva

45   [3, 4] by analyzing body mass changes, urinary and hematological indices and salivary

46   parameters [5]. Therefore, a non-invasive and easy-to-use technique is still in need to give

47   real-time feedback during continuous strenuous exercise. Hydration status imbalance can be

48   represented by the variation of electrolyte concentration in the sweat. Electrolytes in the sweat

49   contain salts and dissolved ions such as sodium cations, which form the majority of the

50   available ions other than potassium and calcium [1]. Hence, sweat sodium cations were

51 chosen to determine the hydration status with normal physiological sweat concentration at 40

52 mM, over-hydration at 26.5 mM and dehydration at 47.9 mM [6, 7].

53 Gold nanoparticles (AuNPs) have been widely studied and applied in many sensing

54 application of inorganic ions [8] and biological analytes such as amino acids, peptides,

55 proteins, and DNA fragments [9-13]. The optical properties of AuNPs enable their use for

56 colorimetric transduction of molecular interactions simply by standard UV-Vis spectrometer

57 or even by the naked eye [14-15]. Indeed, the small size of these particles compared to that of

58 the visible wavelength gives rise to a strong extinction band in the visible wavelength regime

59 upon collective electron oscillations [16]. This band is the localized surface plasmon

60 resonance (LSPR) and its shape and position are closely dependent from local changes on

61 particle surfaces and from their aggregation/dispersion state. It was reported that the addition

62 of NaCl could induce the aggregation of the AuNPs, resulting in a color change because of

63 the alteration in LSPR [17]. We use this property here to monitor the hydration state in sweat

64 through the detection of standard values of salt concentration. The logic-gate based sensor

65 was developed by tailoring the formulation of AuNPs. The designed model is able to give

66 resolution in the concentration range of human body fluid. Figure 1 displays the working

67 principal of the sensor. This design is applied to detect 26.5, 40 and 47.9 mM of NaCl in

68 artificial sweat that correspond to over hydration, normal hydration and dehydration,

69 respectively. These values are based on the data of average population, but the designed

70 sensor can be customized for other ranges. The objective is displaying three distinguished

71 colors for these three concentration levels within few minutes, which can be discerned by the

72 naked eye. To the best of our knowledge, it is the first time applying AuNPs to sweat sensing

73 for hydration imbalance detection. Therefore, this user friendly and responsive sensor is

74 promising towards real time detection of ion concentrations in excreted body fluids.

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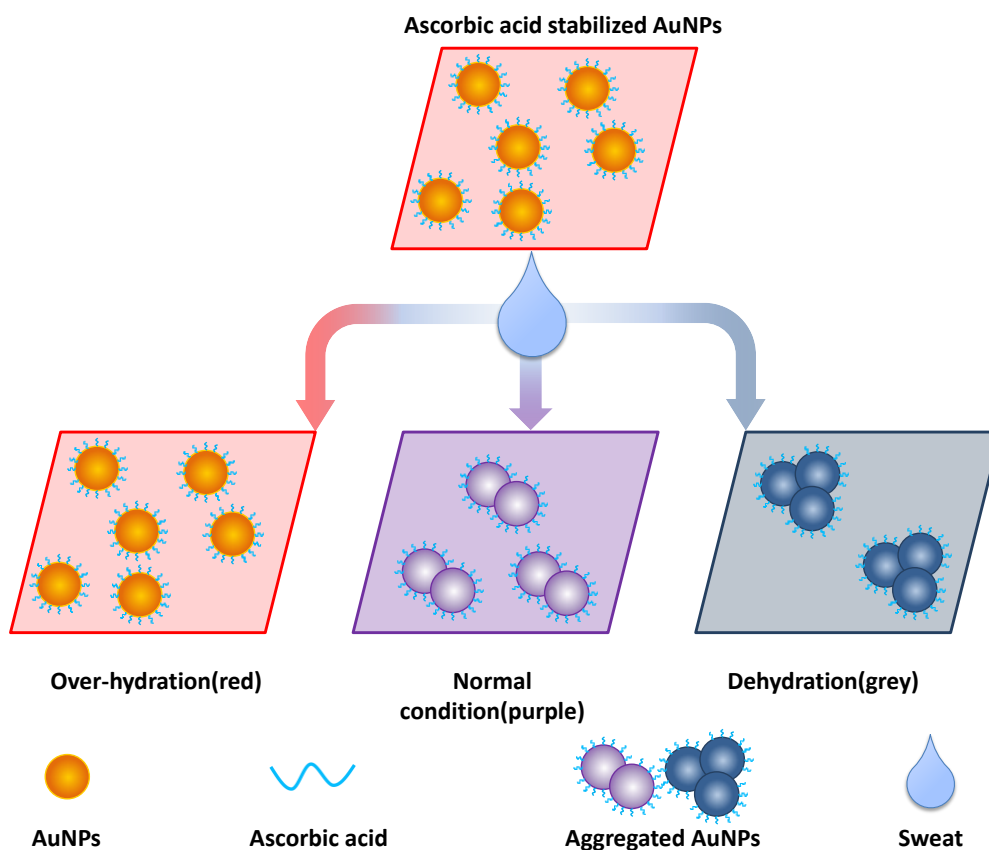
Figure 1. Schematic illustration of the colorimetric sensor.

2. Experimental Work

All chemicals were purchased from Sigma-Aldrich Singapore. The procedure to synthesize AuNPs by reduction of ascorbic acid was modified from the literature [18-20]. In all the reactions, gold nanoparticle solutions were used at 0.35 g L^{-1} and the volume ratio of NaCl solution or artificial sweat to AuNPs solution was 5:1 unless otherwise stated. Briefly, the synthesis was started with 100 uL of 100 g L^{-1} chloroauric acid (HAuCl$_4$ 3H$_2$O), mixed with 25 mL of deionized water and 1.5 mL of 21.2 g L^{-1} sodium carbonate solution. The reaction was started by adding 2 mL of 7 g L^{-1} ascorbic acid under vigorous stirring at 1000 rpm on the magnetic stirrer for 15 minutes. The solution mixed was chilled with ice at about 0-2 ˚C. The concentration of the obtained AuNPs solution is 0.35 g L^{-1}. Shimadzu UV-visible

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spectrophotometer was used to measure the absorption spectra of the AuNPs solutions whereas Malvern Zeta Sizer, described previously [21], was used to measure the size and zeta potential of the AuNPs. NaCl solutions in concentrations of 40, 26.5 and 47.9 mM were use as indicators of normal physiological condition, over-hydration and dehydration. The final optimized formulation was applied to artificial sweat of pH 6.7 according to EN1811 standard [22].

3. Results & Discussion

3.1 Colorimetric sensing by using Ascorbic Capped AuNPs: Solutions with different concentrations of NaCl (0, 26.5, 40 and 47.9 mM) were added to the red ascorbic-capped Au nanoparticles. The observed color changes at different incubation times (0 to 30 min) are shown in Figure 2.
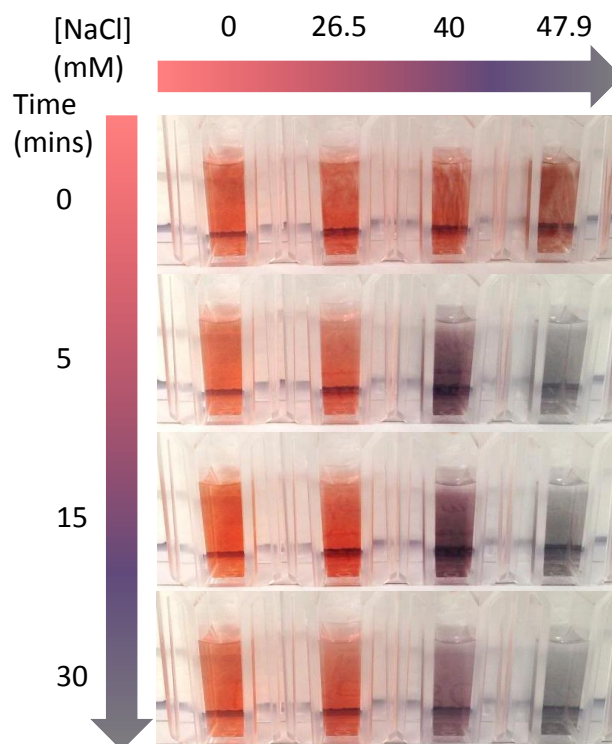
Figure 2. The different color of AuNPs solution in the presence of different concentrations of NaCl.

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107   Figure 2 shows that in absence of NaCl, the AuNPs solutions with deionized water (negative

108   control) undergo no color change during the experiment. The AuNPs solution with NaCl 26.5

109   mM also did not display any detectable color change. Starting from 40 mM NaCl, AuNPs

110   solutions changed color from red to purple within 5 minutes. Color was stable during the

111   following 30 min indicating that the detection is achieved at this early stage. The color of

112   AuNPs solution with NaCl 47.9 mM transformed rapidly red to grey color solution at 5

113   minutes, with no color change thereafter. The results show that different colors, easily

114   identified by the naked eye could be obtained rapidly at NaCl concentration of 26.5, 40 and

115   47.9 mM which indicates the sensor can distinguish the hydration imbalance situations.

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117   **3.2 UV-Vis measurement:** The color change of the AuNPs solutions was verified by

118   measuring the absorption spectra in the visible range by the UV-Vis spectrometer. Figure 3

119   shows the UV spectra of AuNPs in the presence of NaCl at a concentration of 26.5, 40 and

120   47.9 mM respectively as a function of time.

121   For 26.5 mM NaCl UV-Vis spectra weren't modified compared to the original AuNPs

122   solutions. Starting from 40 mM NaCl in the mixture, the intensity of the Plasmon band at 520

123   nm decreases while a broad band in the around 650 nm appears indicating the aggregation of

124   gold nanoparticles.
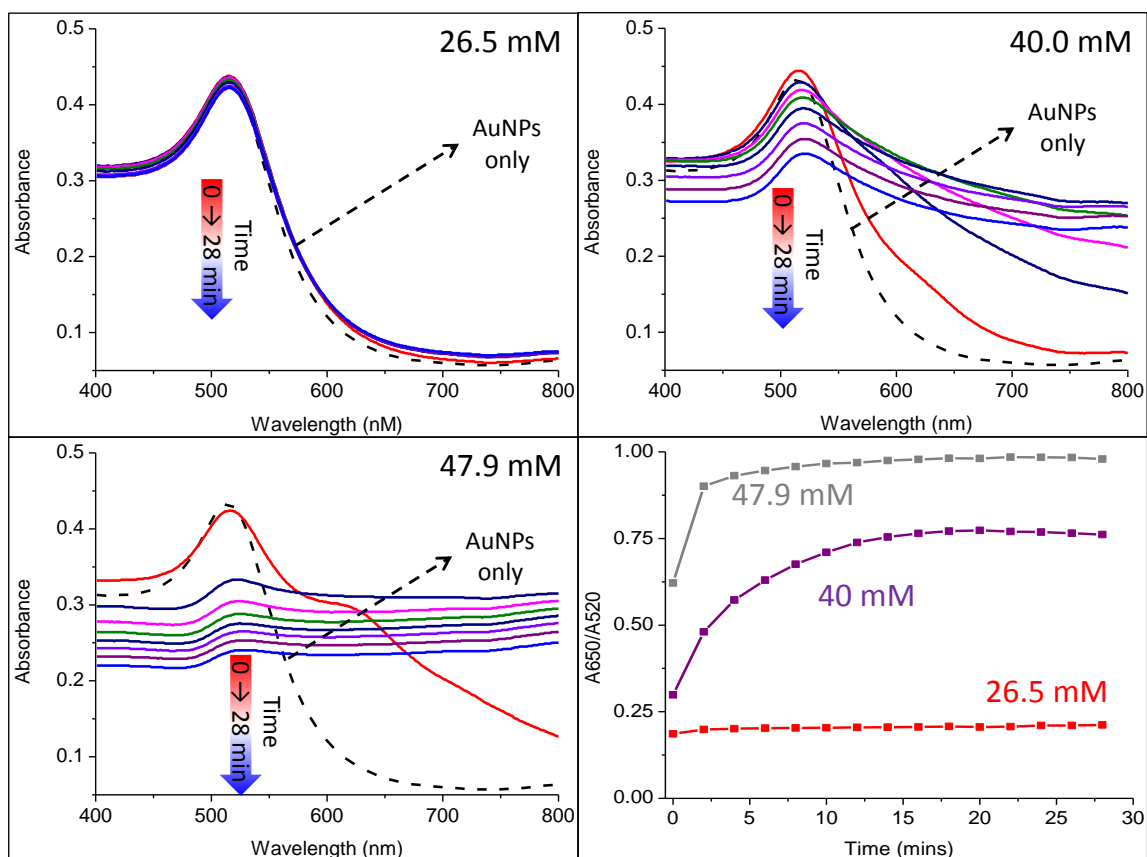
**Figure 3.** UV spectra of AuNPs in the presence of different concentration of NaCl as a function of time.

The ratio of absorbance at 650 to 520 nm (A650/A520) is a good indicator of the aggregation state of gold nanoparticle [23], this ratio in the presence of different concentrations of NaCl at different times is also analyzed in Figure 3. As the concentrations of NaCl increased from 26.5 to 40 and 47.9 mM, the A650/A520 ratio increased indicating the decrease of dispersed particles and the increase of AuNPs aggregates. This aggregation induces the color change of the mixture. In addition, A650/A520 ratio increases for NaCl concentration of 40 and 47.9 mM, indicating a higher aggregation state for high salt concentration, consistently with the observed color changes. Finally, the change in A650/A520 ratio takes place only during the first 5 to 10 minutes suggesting that the equilibrium is rapidly reached and the color change is stable during at least 30 min.

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3.3 Zeta Sizer Measurement: The sizes of AuNPs in the experiments were measured with

Malvern Zeta Sizer. Figure 4 represents the change in size and zeta potential of the AuNPs

solutions in the presence of NaCl solution as time proceeded. Upon the addition of the NaCl

solution, the zeta potential of the AuNPs solution was unusually modified. The kinetic

followed the previous observation by naked eye and UV-Vis, i.e. a rapid change and a

stabilization after 10 min.

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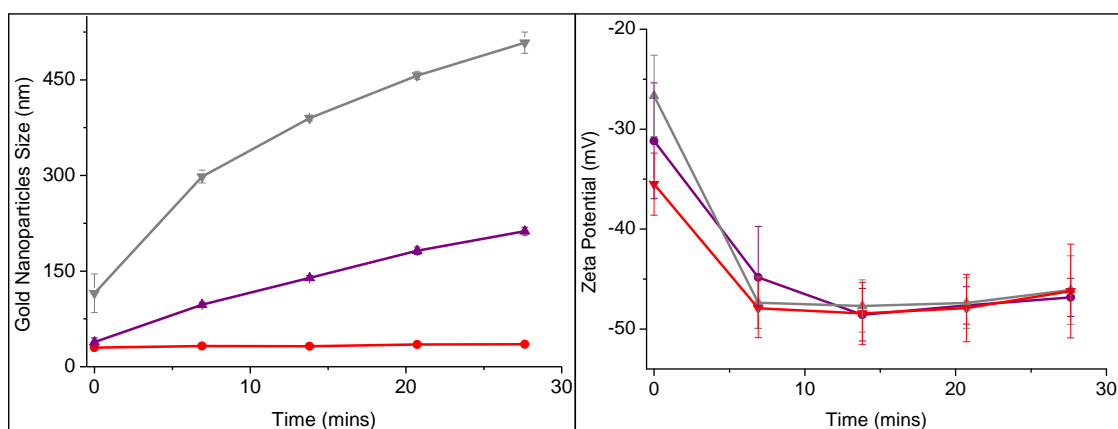
149 **Figure 4.** Size and Zeta Potential change of AuNPs as a function of time in the presence of different

150 concentrations of NaCl: 26.5 mM (red) 40 mM (purple), 47.9 mM (grey).

The hydrodynamic radius of the AuNPs prior to NaCl addition was less than 30 nm. For the

lowest NaCl concentration, 26.5 mM, this size wasn't modified during the measurement time,

consistently with previously evidenced absence of aggregation. For higher salt concentrations,

the measured radius increased without reaching a plateau. This discrepancy may simply result

from the difficulty to measure accurately aggregates size. Nevertheless, the observed changes

are in agreement with UV-Vis observation and evidence the dependence of colloids

aggregation step on the tested range of salt concentration.

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3.4 Detection of Artificial Sweat: The designed sensor was validated in artificial sweat samples. Artificial sweat was synthesized according to EN1811 standard. Figure 5 corresponds to the distinguished colors obtained by AuNPs solutions with the addition of EN1811 standard artificial sweat. The corresponding UV spectra are shown in the Supplementary Information (Figure S1) while the standard deviations (%) of the 26.5 mM, 40 mM and 47.9 mM samples are 1.8 %, 3.0 % and 1.0 %, respectively. Three distinct colors are obtained upon adding 26.5 mM, 40 mM and 47.9 mM of NaCl. We tested different concentrations of AuNPs and changed the ratio AuNP/NaCl. The optimal performances were obtained using 0.35 g L^{-1} AuNP solution with 5:1 volume ratio of artificial sweat to AuNPs solution. The results from visual experiment, UV-spectrum and Zeta-potential show the functionality of this colorimetric sensor with the advantages of simplicity and accuracy. The advantage of using ascorbic acid as the capping agent is that it is less stable compared to citrate and other capping and thus optimization of tuning the size of AuNPs with NaCl solution and artificial sweat is more sensitive to NaCl concentrations. Non-invasive methods such as epidermal tattoo potentiometric sodium sensors [24] or the tattoo lactate sensors are current available biosensors. Other sweat sensors such as wearable sweat rate sensors [25] are again dependent of the electronic circuits [26]. Nevertheless, to the best of our knowledge, there is still no colorimetric sensor that monitors sweat sodium concentration for sports activities that applies color change method which is very convenient for the user. We are currently conducting experiments on selection of substrates and deposition methods. Adsorbent pads made from cellulose extraction thimbles, polycaprolactone, cellulose membrane, hydrophilic filter paper and blotting pad will be tried while deposition methods such freeze-drying and vacuum drying will be applied to develop our sensor into solid form.
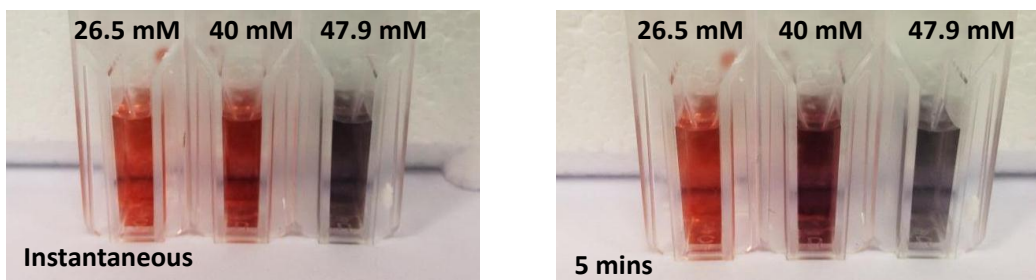
Figure 5. The different colors of AuNPs with artificial sweat.

4. Conclusion

In this study, a colorimetric sensor for the detection of hydration level in buffer and artificial sweat was designed and developed. Detection of sodium cations at concentrations indicating over-hydration, normal hydration and dehydration state was achieved using ascorbic acid capped AuNPs. These particles aggregation/dispersion state occurred in the desired range of salt. Colorimetric changes from red to purple to grey with increasing salt concentrations were easily observable with UV-vis spectrometer and naked eyes. This sensor responds according the actual human body conditions during over hydration and dehydration typical of endurance events. Unlike the common assessment methods for sweat, this design can provide real time feedback. It reduces all the intricate methods of monitoring hydration condition by a visual indication of color change. This novel sensor has been shown to be promising for detection of hydration levels with many desirable properties, including simple detection display, high stability, and fast response time. Finally, this model of sensor may be developed to other kinds of detections for small changes of biological substances in excreted fluids.

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211 **References**

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262   **Figure captions:**

263   **Figure 1.** Schematic illustration of the colorimetric sensor.

264   **Figure 2.** The different color of AuNPs solution in the presence of different concentrations of NaCl.

265   **Figure 3.** UV spectra of AuNPs in the presence of different concentration of NaCl as a function of

266   time.

267   **Figure 4.** Size and Zeta Potential change of AuNPs as a function of time in the presence of different

268   concentrations of NaCl: 26.5 mM (red) 40 mM (purple), 47.9 mM (grey).

269   **Figure 5.** The different colors of AuNPs with artificial sweat.
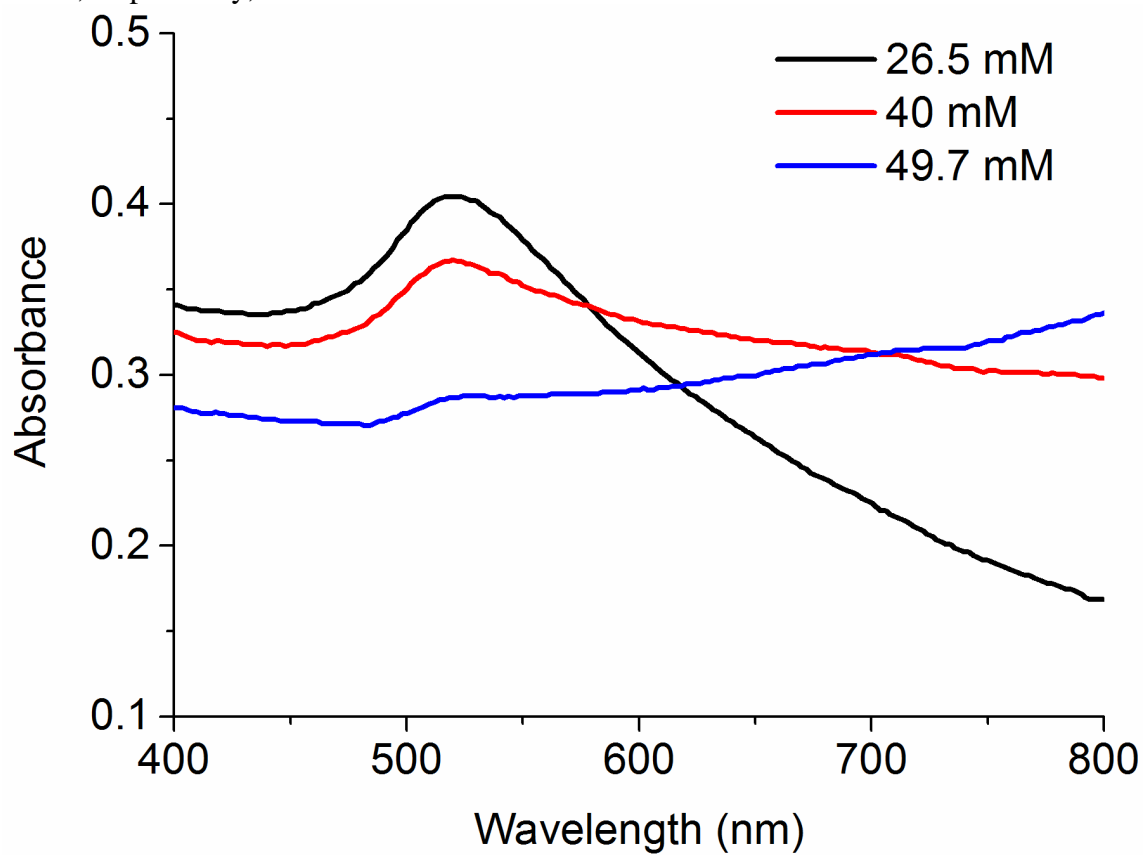
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274 **Supplementary Figure S1 UV spectra of AuNPs in the presence of artificial sweat.**
275 Standard deviations (%) of the 26.5 mM, 40 mM and 47.9 mM samples are 1.8 %, 3.0 % and
276 1.0 %, respectively, n=3.

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