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Silver Nanoparticle Formation-Based Colorimetric Determination of Reducing Sugars in Food Extracts via Tollens' Reagent

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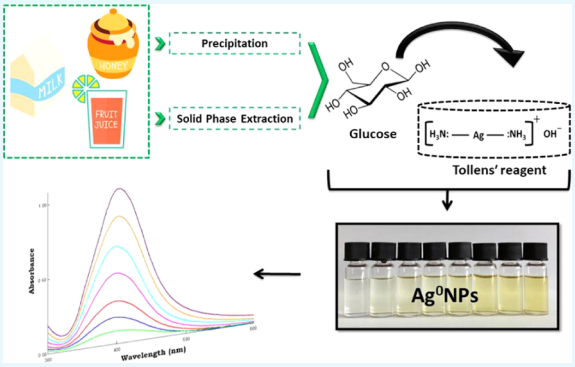
Supporting Information

ABSTRACT: A simple, sensitive, and nonenzymatic nanospectrophotometric method was developed for the determination of reducing sugars. The silver mirror reaction-assisted method is based on the in situ formation of silver nanoparticles in the presence of reducing sugars. All simple reducing sugars (glucose, galactose, fructose, mannose, maltose, and lactose) examined had perfectly linear regression equations. The detection limit for glucose was 40 nM. The proposed method could be selectively applied to various synthetic mixtures of reducing sugars with polyphenolic compounds, and to honey, milk, and commercial fruit juice as real samples using solid phase extraction as a clean-up process. The developed method was also statistically validated against conventional alkaline CUPRAC (cupric−neocuproine, Cu(II)−Nc) spectrophotometric method using Student's *t*- and *F*-tests.



INTRODUCTION

Carbohydrates are important macronutrients that serve as a basic energy source in human nutrition as well as specific functions in vital phenomena.[1] A few examples can be listed as ribose and deoxyribose in the structure of nucleic acids, galactose in some oils, and lactose in milk. Carbohydrates are polyhydroxy aldehydes and ketones and are classified under 4 headings as monosaccharides (simple sugars), disaccharides, oligosaccharides, and polysaccharides. Generally, free-form monosaccharides and disaccharides are called sugars and can be classified as reducing and nonreducing sugars according to their chemical reactions.[2] Sugars are indicators of certain food characteristics, such as taste, flavor, and naturalness.[3] Reducing sugars are not only related to food; it is an indispensable part of biological samples such as blood, serum, plasma, and tissue. The sugar content of certain foods and drinks is controlled by European legislation.[4] As sugars are known to play a vital role in the progression of major health problems (e.g., obesity) and diabetes disease, their total or single (especially glucose) identification is an analytical challenge. Sugar quantification is necessary in a variety of complex biological (e.g., blood and other biological fluids), food, and beverage matrices. Sugar-sweetened soft drinks, constituting the largest single source of calorie-rich U.S. diet and increasing the risk of obesity, require special investigation and monitoring.[5]

Reducing sugars (RSs) are usually determined by the Fehling method[6] requiring many analytical steps (including precipitation and titration), but different approaches for total sugar determination, based on capillary electrophoresis,[7] spectroscopic[8] and chromatographic[9] techniques, have been proposed. Several methods devised for the determination of sugars generally exploit the reducing properties of these carbohydrates, i.e., utilize the oxidation of reductive sugars with appropriate reagents. One of the oldest and most common of these is the 3,5-dinitrosalicylic acid method.[10]

The reducing properties of monosaccharides and disaccharides, as environmentally friendly materials, have also allowed the synthesis of nanoparticles, and there are numerous metal nanoparticle synthesis methods in the literature related to this advantage. The most commonly used of these is silver nanoparticles (AgNPs) synthesized with the aid of reducing sugars. In particular, glucose has been used for many years as a reducing agent for electroless deposition of silver mirrors in the Tollens' process.[11,12] One of them is the preparation of a stable aqueous dispersion of silver nanoparticles during the Tollens' process developed by Yin et al.[13] The Tollens' process for the preparation of AgNPs with a relatively narrow size distribution may potentially be such an advantageous process for Ag(I)-reductive assays carried out in alkaline media (such as the proposed sugar assay) that the as-synthesized aqueous dispersions of AgNPs remained stable for at least 1 year[13] without requiring any extra stabilizing agent. Thus, different synthetic procedures aiming at the production of silver nanoparticles of various shapes and sizes were developed using reducing sugars.[11,14] Tollens' process has not only been used for the synthesis of silver nanoparticles but also applied to

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the development of certain colorimetric assays of different analytes utilizing the formation of AgNPs. For gallic acid (GA) determination, Wang et al. proposed an assay based on in situ formation of AgNPs using a modified Tollens' process.[15] Li et al. developed a new colorimetric assay for the determination of *o*-phenylenediamine (OPD) using Tollens' reagent.[16] These assays (for GA and OPD) relied on the reduction of Ag^+ by the related analyte in the presence of NaOH and aqueous NH_3, resulting in a color change from colorless to yellow, which could be detected by localized surface plasmon resonance (LSPR) absorption with the use of an UV−vis spectrophotometer or even naked eye. Recently, Chaiendoo et al. have proposed a colorimetric sensor based on Tollens' reagent modified-silver nanoclusters (AgNCs) templated by poly(methacrylic acid) for the determination of formaldehyde.[17]

A colorimetric method based on metal nanoparticle formation for the determination of sugar was proposed by Palazzo et al.,[18] in which gold nanoparticle synthesis was performed in changing glucose concentrations. The authors claimed that their colorimetric method could determine all reducing sugars (including sucrose), where the limit of detection was 10 μm for glucose,[18] but the useful analytical range of this "pink assay" was rather narrow with a nonlinear response. Particle aggregation could only be prevented with the addition of the CTAB surfactant, and the whole assay was rather lengthy (90 min). On the other hand, silver has the highest plasmon excitation efficiency, and at the same time, it offers a distinct advantage for colorimetric sensors as the only material capable of producing SPR absorption in the entire visible spectrum (400−1000 nm).[19] Owing to these advantages, AgNPs have been synthesized in different shapes and sizes under varying conditions using different reducing agents (citrate, borohydride, ascorbic acid, plant extracts, etc.) and are still used as colorimetric sensors for various analytes. Another option for the successful production of AgNPs is the use of saccharide reductants which are cheap and favorable aldehydes. Thus, it was the aim of this work to combine the economic and environmentally friendly nature of AgNP synthesis using saccharides with the determination of reducing sugars in a simple and practical nanocalorimetric assay. As reducing sugars could be best oxidized with Ag(I) in the alkaline region, we thought that the best way to accomplish this aim was to use the Tollens' procedure (i.e., reducing Ag^+ from a diamminesilver(I) complex) so as to control the nanoparticle size such that analytical wavelength selection could be easily made without red-shifts of LSPR maxima. We were able to reduce $Ag(NH_3)_2^+$ with reducing sugars (i.e., glucose, fructose, galactose, mannose, maltose, and lactose) to zero-valent silver nanoparticles (Ag^0NPs) under special conditions, and thus devise a direct spectrophotometric method for reducing sugar quantification in food extracts by measuring the 410 nm absorbance pertaining to the LSPR band of nanoparticle solution having a yellow color. This method was both very sensitive (at nM levels) and exhibited excellent linearity of absorbance versus sugar concentration.

RESULTS AND DISCUSSION

Optimization Parameters of the Developed Method.

The developed method was optimized considering several important parameters such as the amount of NaOH, reaction time, temperature, and molar ratio of $AgNO_3$ and NH_3. Glucose was chosen as the representative reducing sugar for optimization studies.

First, experiments were performed with 0.1 M NaOH in the volume range of 0.1−0.5 mL for choosing the optimal amount of NaOH. Although low amounts (0.3 and 0.2 mL) of NaOH were ideal for high concentrations of glucose, this amount was not sufficient for low concentrations of glucose solutions. Likewise, in a similar study, Balavandy et al. had observed that the increased ratio of NaOH-to-alginate led to a blue shift of the LSPR peak of nanoparticles to a lower wavelength in parallel to a decrease in the particle size of AgNPs.[20] Therefore, the appropriate amount of NaOH was determined as 0.4 mL for low and high concentrations of glucose. The optimal amount of NaOH was determined as 8.0×10^{-3} M (in final conc.), obtained using 0.4 mL of 0.1 M NaOH solution in a final volume of 5 mL.

For choosing the optimal reaction temperature, experiments were performed in the presence of 0.1 M NaOH at 25, 30, 45, 60, 65, and 70 °C. No results were obtained at low temperatures, and the ideal results (maximum absorbance) were obtained at temperatures over 60 °C. Both the low (2.0×10^{-6} M) and high (1.0×10^{-5} M as initial conc.) concentrations of glucose were studied, and the optimal reaction temperature was chosen as 70 °C for both concentration values.

For choosing the optimal reaction time, experiments were performed between 0 and 15 min. The developed method was applied to glucose samples at both low (2.0×10^{-6} M) and high (1.0×10^{-5} M) concentrations, and the obtained results were compared. The optimal reaction time for both the concentration values was obtained as 6 min, beyond which the results did not change.

Experiments were carried out in the presence of 0.1 M NaOH for choosing the molar ratio of $AgNO_3$-to-NH_3. The final volume was 5 mL, the amount of 2.0×10^{-3} M $AgNO_3$ was reduced when the amount of 1.0×10^{-2} M NH_3 was increased. Both low (2.0×10^{-6} M) and high (1.0×10^{-5} M) concentrations of glucose were studied. Optimal amounts of 2.0×10^{-3} M $AgNO_3$ and 1.0×10^{-2} M NH_3 were selected to be 0.6 and 2 mL, respectively.

Initial concentrations of $AgNO_3$ and NH_3 were determined according to the amount of free ammonia present in the medium, considering the Ag(I)−ammine complex formation (i.e., species distribution) diagram (Figure 1).

Ag^+ ion combines with NH_3 in a two-step reaction. It first picks up one NH_3 molecule to form a one-coordinate complex (eq 1).

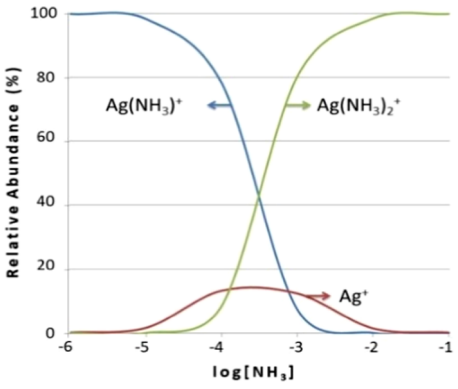

Figure 1. Formation curve of Ag(I)−ammine complexes (the species distribution curve drawn by one of the authors "Selen Durmazel" using the corresponding stability constants).

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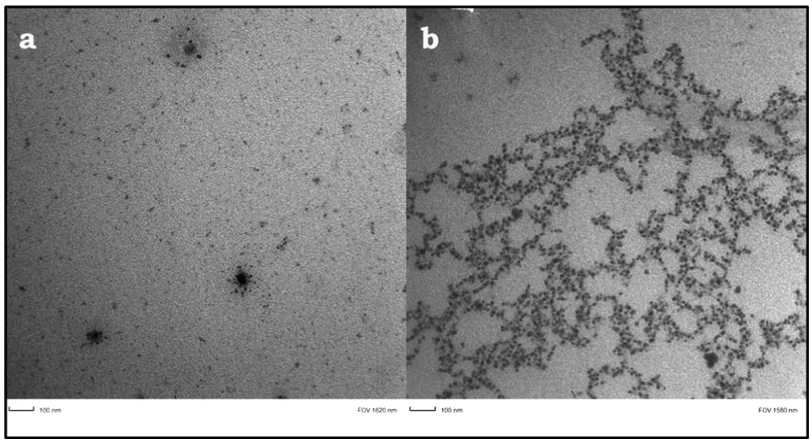

Figure 2. TEM images of the AgNPs formed from the redox reaction between 7.2×10^{-6} M (final conc.) glucose and $Ag(NH_3)_2^+$ in the absence (a) and presence (b) of NH_3 under special conditions (in alkaline medium and 70 °C water bath for 6 min).

$$Ag^+_{(aq)} + NH_{3(aq)} \rightleftharpoons Ag(NH_3)^+_{(aq)} \quad (1)$$

This intermediate complex then picks up a second NH_3 molecule in a separate step.

$$Ag(NH_3)^+_{(aq)} + NH_{3(aq)} \rightleftharpoons Ag(NH_3)_2{}^+_{(aq)} \quad (2)$$

Essentially, all of the silver is present as the Ag^+ ion at very low concentrations of NH_3. As the NH_3 concentration increases, the dominant species soon becomes the two-coordinate $Ag(NH_3)_2^+$ ion, with only a minor contribution of the 1:1 complex within the whole range (eq 2). Even at NH_3 concentrations as small as 10^{-3} M, most of the silver is present as the $Ag(NH_3)_2^+$ ion (Figure 1), because the logarithm of the cumulative stability constant of the 1:2 silver−ammine complex is $\log \beta_2 = 7.0$ at 15 °C.[21] Under our experimental conditions of the excess NH_3 ligand, it was clear that essentially all the silver(I) was bound to the two-coordinate $Ag(NH_3)_2^+$ complex ion. This would reduce the standard redox potential of the Ag^+/Ag couple by $(RT/nF) \log \beta_2 = 0.0592 \times 7 = 0.42$ V, enabling the formation of controlled-size silver nanoparticles.

During the preparation of the Tollens' reagent, excess ammonia should be avoided rendering the test much less sensitive. Otherwise, the formation of addition products between ammonia and aldehydes would reduce the amount of free aldehyde available for the reaction with ammoniacal silver nitrate. A similar reduction might also take place if a too high concentration of alkali was used. When alkalinity and oxidative conditions were excessive, Yin et al.[13] observed that the LSPR peaks red-shifted (with a significant reduction in the corresponding band intensities) possibly resulting from the formation of an ultrathin layer of silver oxide (Ag_2O) on the surface of preformed AgNPs. Under strongly alkaline conditions, aldehydes may also undergo the side reactions of aldol condensation and Cannizzaro reaction. To minimize these undesired side reactions, Tollens' reagent should be prepared with care, ensuring that ammonia was not in great excess and pH was not excessively high.[22]

Possible Mechanism of the Proposed Method. Although Tollens' test, carried out in a Ag^+−NH_3 system, has been used for over hundred years for detecting aldehydes by the silver mirror reaction, the test becomes more sensitive by initially adding a few drops of NaOH.[22] The sensitivity of the test was reported to increase with increasing pH and temperature, where free alkali (OH^-) was supposed to act as an accelerator at pH > 10.

$$RCHO + H_2O \rightarrow RCH(OH)_2 \quad (3)$$

$$RCH(OH)_2 + OH^- \rightarrow R(CHOH)O^- + H_2O \quad (4)$$

Oxidation of an aldehyde to carboxylic acid involves the transfer of two electrons, and requires two silver(I) ions through a free radical intermediate, with the following mechanism

$$R(CHOH)O^- + Ag^+ \rightarrow R(CHOH)OAg \quad (5)$$

$$R(CHOH)OAg \rightarrow R(HCOH)O^\bullet + Ag^0 \quad (6)$$

$$R(CHOH)O^\bullet + Ag(NH_3)_2{}^+ \rightarrow RCO_2H + NH_4{}^+ + NH_3 + Ag^0 \quad (7)$$

$$RCO_2H + OH^- \rightarrow RCO_2{}^- + H_2O \quad (8)$$

The Ag^+ ion as a reactant of eq 5 may be in the form of an aqua-ion or an amine-complex.[22] When Tollens' reaction is used in the reduction of silver(I) salts by reducing sugars to metallic Ag, three reaction steps should be taken into account:[11]

(1) Precipitation of silver oxide (Ag_2O):

$$2AgNO_{3(aq)} + 2NaOH \rightleftharpoons Ag_2O_{(s)} + 2NaNO_3 + H_2O \quad (9)$$

(2) Dissolution of Ag_2O by forming diamminesilver(I) complex:

$$Ag_2O_{(s)} + 4NH_3 + 2NaNO_3 + H_2O \rightleftharpoons 2[Ag(NH_3)_2]NO_3 + 2NaOH \quad (10)$$

(3) Reduction of diamminesilver(I) complex by RS to Ag^0:

$$2[Ag(NH_3)_2]^+ + R - CHO + H_2O \rightleftharpoons 2Ag^0_{(s)} + 4NH_3 + R - COOH + 2H^+ \quad (11)$$

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A blue-shift (to shorter wavelengths) is usually observed in the surface plasmon resonance (SPR) absorption maxima of spherical AgNPs accompanying a decrease in particle size, due to repulsive effects on nanoparticles.[23] Dubas and Pimpan made a green synthesis of AgNPs that they used for NH_3 sensing, and observed a blue-shift in the localized SPR wavelength of nanoparticles accompanying a color change from purple to yellow; the authors attributed this blue-shift (in NH_3-containing solution) to the repulsive effect of $Ag(NH_3)_2^+$ ions adsorbed on nanoparticles as well as to the increase in water content of the nanoparticle environment, resulting in more hydrophilic and isolated particles with a shorter wavelength-shifted spectrum.[24] Likewise, Amirjani and Fatmehsari were able to reduce the number of free AgNPs by the polyol method of nanoparticle synthesis and used them for ammonia sensing; the localized SPR band of homogeneous AgNPs in NH_3-containing solution was distinctively blue-shifted at around 400 nm.[25] In our case, since AgNPs were generated in an ammonia solution, the stable $Ag(NH_3)_2^+$ cationic complex adsorbed on AgNPs is responsible from this repulsive effect on nanoparticles, causing the blue-shift to 410 nm.[24] The AgNPs formed as a result of the redox reaction between RS (i.e., representative compound: glucose) and $Ag(NH_3)_2^+$ were characterized by transmission electron microscopy (TEM); the TEM images of AgNPs obtained from {Ag(I) + glucose} in the absence and presence of aqueous NH_3 are shown in Figure 2a,b, respectively, where silver nanoparticles did not exhibit a uniform particle size distribution in the absence of ammonia, whereas with NH_3, approximately 10 nm-sized spherical nanoparticles were produced.

Another important factor in the formation of uniform AgNPs is the rather small potential difference constituting the major driving force of $Ag(NH_3)_2^+$ oxidation of glucose. The presence of ammonia entraps Ag^+ ions in a relatively stable complex of $Ag(NH_3)_2^+$, and as a result, the Ag^+/Ag standard redox potential decreases from +0.80 V pertaining to uncomplexed silver ion to +0.38 V characteristic of $Ag(NH_3)_2^+$. On the other hand, the gluconate/glucose redox potential in acid solution is 0.056 V vs standard hydrogen electrode,[26] but decreases with increasing pH in ammonia solution. If silver(I) ion is employed without ammonia, the silver ion is reduced so rapidly that oversaturation would lead to the formation of colloidal silver in a black and cloudy suspension.[27] Silver(I)−ammonia complexation reduces the redox potential gap between the Ag(I) oxidant and sugar reductant (i.e., about −0.15 V at pH 7, depending on the type of sugar) also giving rise to a slower reaction[28] thereby preventing oversaturation (which would otherwise generate a large number of nuclei growing to highly variable particle sizes) and enabling uniform-sized AgNP deposition at a definite redox potential controlled by stable complex formation.

Analytical Figures of Merit. Analytical performance parameters of the developed and reference methods for glucose determination are summarized in Table 1.

The absorbance signals recorded by the proposed method against blank solution were linearly dependent upon the concentrations of the certain reducing sugars. The visible spectra of AgNPs (formed from the reduction of $Ag(NH_3)_2^+$ by RS in alkaline medium), using varying concentrations of glucose in the μM range, are shown in Figure 3. The absence of a shift in LSPR band maxima as a function of glucose concentration was indicative of the absence of oversaturation,

Table 1. Comparison of Analytical Performance Parameters of the Developed and Reference (Alkaline CUPRAC) Methods for Glucose Standards

parameter	proposed method	reference method
linear range[a]	9.6×10^{-7} to 7.2×10^{-6}	6.0×10^{-6} to 3.0×10^{-5}
LOD[b]	4.0×10^{-8}	6.0×10^{-7}
LOQ[c]	1.3×10^{-7}	6.0×10^{-6}
linear regression equation[d]	$A = 1.5 \times 10^5 C - 0.0665$	$A = 3.6 \times 10^4 C + 0.0063$
correlation coefficient (r)	0.9999	0.9999
RSD (%) (n = 7)	0.9	0.6

[a]In mol L^{-1} units at final concentrations (i.e., in the cuvette for spectrophotometric measurement). [b]Limit of detection in mol L^{-1} units at final concentrations (LOD = 3 σ_{bl}/m, σ_{bl} denoting the standard deviation of a blank and m showing the slope of the calibration line). [c]Limit of quantification in mol L^{-1} units at final concentrations (LOQ = 10 σ_{bl}/m, σ_{bl} denoting the standard deviation of a blank and m showing the slope of the calibration line). [d]A stands for absorbance, C molar concentration. All correlation coefficients (r) were found using absorbances which were repetitively measured three times (N = 3).

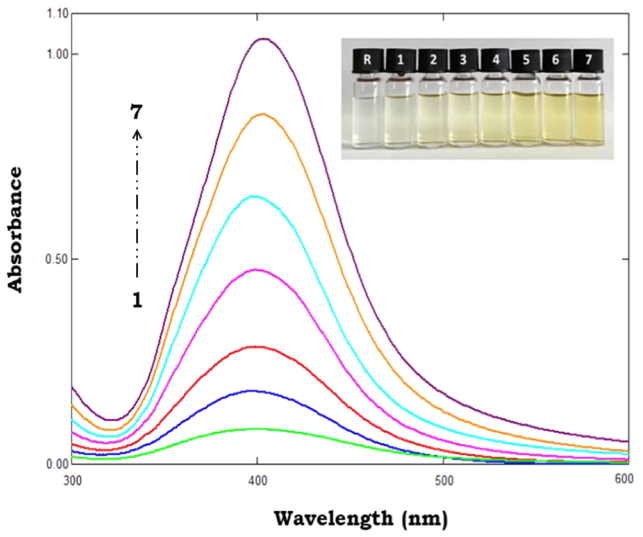

Figure 3. Visible spectra of AgNPs (obtained from the reduction of $Ag(NH_3)_2^+$ by RS in alkaline medium) at (1) 9.6×10^{-7} mol L^{-1}, (2) 1.6×10^{-6} mol L^{-1}, (3) 2.4×10^{-6} mol L^{-1}, (4) 3.6×10^{-6} mol L^{-1}, (5) 4.8×10^{-6} mol L^{-1}, (6) 6.0×10^{-6} mol L^{-1}, and (7) 7.2×10^{-6} mol L^{-1} final concentration of glucose, and the color images of these samples are shown in the inset figure (R: blank). (Inset photograph was taken by one of the authors Selen Durmazel).

yielding uniform-sized AgNPs, as controlled by careful adjustment of the ammonia concentration.

The equations emerging from linear regression analysis, with the corresponding correlation coefficients, were found by applying the proposed and reference methods to select reducing sugar samples, as tabulated in Table 2. Evaluating the data in Table 2, it was obvious that the developed method yielded about one order-of-magnitude higher slopes for the regression equations (i.e., indicating higher sensitivity of determination) than the alkaline CUPRAC assay for tested sugars.

Taking advantage of the enhanced sensitivity of localized SPR absorption of silver nanoparticles produced from the

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Table 2. Linear Regression Equations and Correlation Coefficients for the Relevant Reducing Sugars Obtained by the Proposed and the Reference Methods

reducing sugar	linear regression equations[a]	
	proposed method[b]	reference method[c]
D-(+)-glucose	$A = 1.5 \times 10^5 C - 0.0665$	$A = 3.6 \times 10^4 C + 0.0063$
	$r = 0.9999$	$r = 0.9999$
D-(+)-galactose	$A = 2.5 \times 10^5 C - 0.0874$	$A = 3.2 \times 10^4 C - 0.0045$
	$r = 0.9996$	$r = 0.9997$
D-(−)-fructose	$A = 0.9 \times 10^5 C - 0.0196$	$A = 3.5 \times 10^4 C - 0.0107$
	$r = 0.9996$	$r = 0.9995$
D-(+)-mannose	$A = 1.7 \times 10^5 C - 0.1413$	$A = 2.5 \times 10^4 C - 0.0196$
	$r = 0.9996$	$r = 0.9996$
D-(+)-maltose monohydrate	$A = 2.9 \times 10^5 C - 0.0973$	$A = 4.1 \times 10^4 C - 0.0408$
	$r = 0.9996$	$r = 0.9994$
D-(+)-lactose monohydrate	$A = 3.3 \times 10^5 C - 0.1413$	$A = 4.2 \times 10^4 C - 0.0157$
	$r = 0.9999$	$r = 0.9994$

[a]A stands for absorbance, C for molar concentration, and r for linear correlation coefficient. All correlation coefficients (r) were found using absorbances that were repetitively measured three times ($N = 3$). [b]Linear ranges (in final conc.) were 9.6×10^{-7} to 7.2×10^{-6} mol L^{-1} for glucose, 6.0×10^{-7} to 4.8×10^{-6} mol L^{-1} for galactose, 9.6×10^{-7} to 9.6×10^{-6} mol L^{-1} for fructose, 1.2×10^{-6} to 9.6×10^{-6} mol L^{-1} for mannose, 1.2×10^{-6} to 6.0×10^{-6} mol L^{-1} for maltose and lactose. [c]Linear ranges (in final conc.) were 6.0×10^{-6} to 3.0×10^{-5} mol L^{-1} for all sugar standards.

Tollens reagent by glucose, our detection limit was 40 nM with a fixed analytical wavelength and linear calibration, whereas glucose oxidase (GlOx)-based enzymatic assays of glucose via H_2O_2 detection remained within the mM−μM range. A few examples of GlOx-based assays for glucose detection are the CdSe/ZnS quantum dots fluorescence method of Gill et al.,[29] the carbon nanotube voltammetric system of Wooten et al.,[30] and the AgNP dissolution colorimetric method by H_2O_2 of Gao et al.,[31] which all had milli-to-micromolar sensitivities for glucose.

Statistical comparison between the proposed and the reference methods applied to glucose standards and milk samples was made on 7 and 5 repetitive analyses, respectively. Sugar contents of these samples were found with the aid of calibration curves for glucose. The results of Student's t-test and F-test are shown in Table S-1, basically exhibiting no significant difference (at 95% confidence level) between the accuracies and precisions of the two methods.

Hydrolysis of Sucrose. Various saccharides were used to prepare silver nanoparticles.[32,33] The most widely used of these compounds were monosaccharides, among which glucose was preferred, although substantially all monosaccharides had reducing properties. Some studies were also published regarding the use of disaccharides such as maltose (formed by two glucose molecules) and lactose (formed by a glucose and galactose molecule) as reducing agents.[28,34]

In the tested real samples, the sugars present were basically glucose, fructose, and sucrose for fruit juices,[35] lactose for milk, and sucrose, glucose, and fructose for honey.[36] Lactose, containing a hemiacetal group, is already a reducing sugar, thereby not requiring any preliminary hydrolysis.[37]

The disaccharide saccharose (or sucrose) is a nonreducing sugar which can be hydrolyzed with acids or enzymes. Since hydrolysis of each molecule of sucrose yields one molecule glucose and one molecule fructose,[8] the indirect determination of sucrose becomes possible. According to literature reports, acidic hydrolysis of sucrose is generally carried out with hydrochloric acid. However, in the presence of Cl^- ion, the precipitation equilibrium of $AgCl_{(s)}$ becomes dominant and the formation of $AgCl_{(s)}$ colloids was observed. Therefore, sucrose hydrolysis was carried out with dilute sulfuric acid.[38] The solubility product of AgCl is at the order of 10^{-10}, whereas that of Ag_2SO_4 at 10^{-5}. Therefore, the possibility of Ag_2SO_4 formation does not pose a risk. Besides, in the ammonia-containing medium of AgNP formation, $\log \beta_2$ for the $Ag(NH_3)_2^+$ complex is 7.0, obviously suppressing Ag_2SO_4 precipitation. The outcome of acidic hydrolysis was investigated with the application of the proposed and reference methods to the hydrolysate together with the possible hydrolysis products of (glucose + fructose) in a mixture. Sugar contents (expressed as glucose equivalents) of sucrose hydrolysate and of a synthetic mixture consisting of (glucose + fructose) are given in Table 3. These findings confirm that the developed method can be accurately applied to total reducing sugar determination in complex samples having sucrose hydrolysate or equivalent components (Table 3).

Table 3. Glucose Equivalent Sugar Contents of Sucrose Hydrolysates and of Synthetic Mixtures Comprising Glucose and Fructose (1:1), as Determined by the Proposed and Reference Methods

sample	proposed method (mg glucose equiv L^{-1})	reference method (mg glucose equiv L^{-1})
sucrose[a] hydrolysate	179.3	166.3
(glucose + fructose)[b]	186.5	173.8
sucrose[a] hydrolysate	379.8	346.1
(glucose + fructose)[b]	364.9	372.1

[a]Two different samples of sucrose were hydrolyzed, having initial concentrations of 0.5 and 1.0 mM, corresponding to 180.2 and 360.3 mg glucose equivalent per liter, respectively ($N = 3$). [b]Synthetic mixtures were prepared as theoretically equivalent to the hydrolysis products of sucrose.

Analysis of Synthetic Mixtures and Real Samples. To evaluate the interference effects arising from polyphenolic compounds expected to be present in real samples (fruit juices, honey etc.), suitable synthetic mixtures were prepared and solid phase extraction (SPE) was applied to the polyphenolic-containing samples, at the end of which recovery values were calculated. SPE is an increasingly useful sample preparation technique, the products of which are widely used for sample extraction, concentration, and clean-up.[39] SPE with a C18

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stationary phase has been demonstrated to be appropriate for the separation of sugars from phenolic compounds in complex samples.[40] In this regard, the developed method was applied two times to the synthetic mixtures before and after SPE separation (the steps of which are depicted in Figure S-1) to see the reagent color stemming from (sugars + phenolic interferents) versus sugars alone. The expected versus found results (reported as milligram glucose equivalent per liter of sample) and errors of recoveries are depicted in Table S-2. The synthetic mixtures (1−3) would produce positive errors before SPE separation due to the interfering effect of polyphenols to the proposed method. However, the recoveries recorded after SPE (i.e., ranging between 95.2 and 104.2%) demonstrated that reducing sugars could be totally separated from common phenolics and precisely estimated in synthetic samples (Table S-2). Experimental results (unreported) also demonstrated that polyphenolics could be efficiently separated from sugars and be determined following elution from the two SPE cartridges using 80% aqueous methanol as the eluting solvent.

In choosing real samples, polyphenol-rich samples, were preferred to see their possible interference to reducing sugar assays. With sequential use of two distinct SPE cartridges (C18 and polyamide), phenolic interference was successfully eliminated. The results (as grams glucose equivalent per 100 mL or 100 g) versus declared amounts are depicted in Table S-3. In honey samples, the findings of both methods were lower than the declared amounts. In spite of the additional hydrolysis procedure applied, a significant change in the reported sugar content of honey was not noticeable. Data in Table S-3 show 7−14% variations between the declared and found contents of certain real samples such as milk, apricot-apple juice, and honey; however, it should be considered that the actual sugar contents of these samples were not declared on their packing labels, bringing some uncertainty to the sugar content of commercial samples.

CONCLUSIONS

Since the concentration of reducing sugars is controlled by European legislation for certain foods and beverages, it is important to develop simple, inexpensive, and sensitive methods for the determination of these sugars in food samples. In the literature, there are reducing sugar determination methods based on metal nanoparticle formation, but these methods either do not provide a linear calibration or do not yield sufficient sensitivity. For example, methods based on gold nanoparticle formation provided such a large redox potential gap between the oxidant and reductant that it was extremely difficult to control the resulting nanoparticle size.[18] Therefore, this study aims to develop a spectrophotometric method based on the controlled formation of nanoparticles with reducing sugars. The principle of the method relies on the formation of silver nanoparticles by the reduction of the Ag^+ ion in the presence of aqueous ammonia and sodium hydroxide to zero-valent silver (Ag^0) by reducing sugars. Although there are a number of nanoparticle-based determination methods for reducing sugars in the literature, it is not possible to adjust the particle size and distribution in alkaline solution necessarily used for sugar oxidation, which could only be made feasible by the Tollens reaction incorporating ammonia for alkalinity. Thus, a single analytical wavelength with excellent linearity of response was achieved. The limit of detection of the developed method was at the nM level, and both the linear correlation coefficient (r = 0.9999) of the calibration study and the molar absorption coefficient (ε = 1.5 × 10^5 L mol^{-1} cm^{-1}) were satisfactory for glucose as the representative compound. The method has also been validated against the alkaline CUPRAC method as the reference method of reducing sugar determination established in the literature. The recommended method is simple and cost-effective, which can conveniently be applied even in traditional laboratories because of the easy accessibility and low cost of the reagents used. It is believed that this work may open the way to innovative studies employing nanotechnology in sensitive and linear-response sensing of reducing sugars. The LOD values for commercial test strips and electrochemical micropaper-based analytical devices range around 15 and 26 mg dL^{-1} (about 1 mM), respectively, whereas the medically relevant glucose concentrations lie between 70 and 120 mg dL^{-1}.[41] Hamedi et al. fabricated a microfluidics paper device to measure glucose between 2.7 and 22.2 mM.[42] Compared to the accuracy level (≥100 mg dL^{-1}, approx. 5.5 mM) of commercial blood sugar detectors used as medical devices, the developed method is much superior in terms of sensitivity by allowing the measurement of glucose in the nM range. Designing novel approaches to estimate low concentrations of glucose continues to attract attention, because it has potential use in several areas, such as food industry, clinical chemistry (such as tear fluid),[43] and diabetic diet monitoring. The quantification of glucose at very low concentrations in intra- and extra-cellular fluid may be of potential interest in bacteriology as a part of improving fermentation technology after extended periods of incubation.[44]

EXPERIMENTAL SECTION

Materials and Chemicals. Silver nitrate ($AgNO_3$), anhydrous 2,9-dimethyl-1,10-phenanthroline (neocuproine: Nc), sucrose, D-(+)-glucose, D-(−)-fructose, D-(+)-galactose, D-(+)-mannose, D-(+)-maltose monohydrate, D-(+)-lactose monohydrate, (+)-catechin hydrate, (−)-epicatechin, chlorogenic acid, gallic acid monohydrate, L-(+)-ascorbic acid, and potassium sodium tartrate tetrahydrate, Discovery DPA-6S (250 mg, 3 mL), and Discovery DSC-18 (1 g, 6 mL) SPE cartridges were purchased from Sigma-Aldrich (Steinheim, Germany). Copper(II) chloride dihydrate, ammonia solution (NH_3), sodium carbonate and sodium hydroxide (NaOH), potassium hexacyanoferrate(II) trihydrate, and zinc sulfate heptahydrate were obtained from Merck (Darmstadt, Germany). Sulfuric acid (H_2SO_4) was obtained from Carlo Erba (Italy).

Instruments. The spectra and absorption measurements were recorded in matched Hellma Suprasil black quartz cuvettes (optical thickness: 10 mm) using a Shimadzu UV-1800 ultraviolet−visible spectrophotometer (Kyoto, Japan). Incubation of the reducing sugar-containing standard and/or samples was performed using the Wisd WiseBath water bath for both the proposed method and reference alkaline CUPRAC method. Agilent Vac Elut 12 position manifold (California) and Isolab vacuum pump (Istanbul, Turkey) were used for solid phase extraction (SPE) processes. Transmission electron microscopy (TEM) measurements of nanoparticles were performed using a QImaging Retiga 4000R.

Preparation of the Solutions. All glassware and plastic materials used throughout the work were cleaned with freshly prepared aqua regia (HCl/HNO_3, 3:1, v/v), rinsed with distilled water, and dried in air prior to use. All concentrations

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given under experimental conditions were the initial values unless otherwise stated.

The proposed method solutions: all solutions used throughout were prepared daily, except for NaOH. A Stock solution of $AgNO_3$, aqueous NH_3, and NaOH were prepared at 2.0×10^{-3}, 1.0×10^{-2}, and 0.1 M concentrations, respectively, in ultrapure water.

Alkaline CUPRAC method solutions: $CuCl_2$ solution (1.0×10^{-2} M), alkaline solution as 0.5 M NaOH containing 2% (w/v) Na_2CO_3, and 0.1 M sodium potassium tartrate were prepared in ultrapure water. Neocuproine (Nc) solution (1.5×10^{-2} M) was prepared daily in absolute ethanol.

Stock solutions of the related reducing sugars and phenolic compounds (for investigation of interference effects) were prepared at 1.0×10^{-3} M concentration in ultrapure water, and the working solutions of these analytes were prepared by diluting from their stock solutions with ultrapure water. The concentration ranges of the working solutions of related RS were as follows; 2.4×10^{-6} to 1.8×10^{-5} M for glucose, 2.4×10^{-6} to 2.4×10^{-5} M for fructose, 1.5×10^{-6} to 1.2×10^{-5} M for galactose, 3.0×10^{-6} to 2.4×10^{-5} M for mannose, 3.0×10^{-6} to 1.5×10^{-5} for maltose, and 3.0×10^{-6} to 1.5×10^{-5} M for lactose.

Recommended Procedure for RS Determination. To obtain the reaction mixture, 0.6 mL of 2.0×10^{-3} M $AgNO_3$ solution, 0.4 mL of 0.1 M NaOH (wait for approx. 30 s for Ag_2O formation), and 2 mL of 1.0×10^{-2} M aqueous NH_3 were added to a test tube, respectively. Then, 2 mL of (x) M RS standard or sample solution was introduced into the test tube, and the reaction mixture was incubated for 6 min in a thermostated water bath at 70 °C. At the end of this time, the test tube was cooled in an ice bath, and pale yellow sol formation, the absorbance of which was directly proportional to the increase in concentration, was observed. The absorbance at 410 nm was recorded against a blank solution excluding RS using a UV/vis spectrophotometer (Figure 4).

Reference Alkaline CUPRAC Spectrophotometric Method. The total reducing sugar content was evaluated by the conventional alkaline Cu(II)−Nc spectrophotometric method as described by Başkan et al.[8] One milliliter of 1.0×10^{-2} M $CuCl_2$, 1 mL of 1.5×10^{-2} M Nc, x mL of sample, (1 − x) mL of distilled water, 1 mL of 0.5 M NaOH containing 2% (w/v) $NaCO_3$, and 1 mL of 0.1 M sodium potassium tartrate solution were added to a test tube in this order. The mixture in the stoppered tube was incubated for 20 min in a thermostated water bath at 60 °C. The tubes were cooled to room temperature, and their absorbance values at 450 nm were measured against a blank solution excluding RS using a UV/vis spectrophotometer.

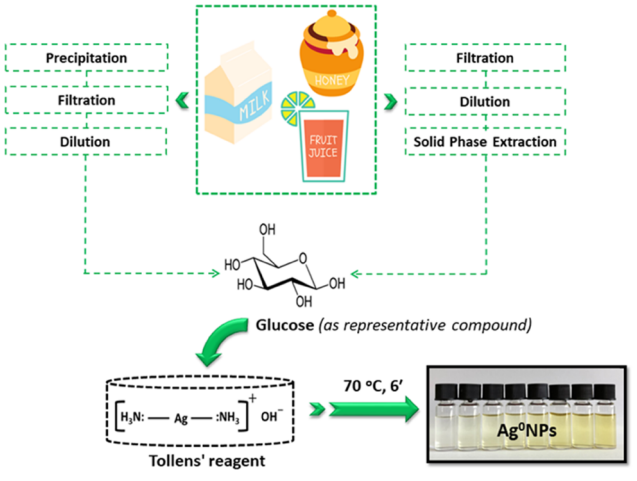

Figure 4. Schematic presentation of the proposed method for the determination of reducing sugars in food extracts. (Inset photograph was taken by one of the authors Selen Durmazel).

Preparation of Real Samples and Synthetic Mixtures. The commercial fruit juice, honey, and UHT whole milk samples were supplied from a local market of Istanbul. The honey sample was prepared by weighing 1.0 g of honey, made homogeneous by a certain amount of distilled water and diluted to 100 mL with H_2O. The milk sample extraction procedure was carried out as described by Kumar et al.[45] Briefly, 5 mL of milk sample was taken in a 14 mL centrifuge tube. 0.5 mL of potassium hexacyanoferrate(II) trihydrate (3.6% aq.) solution was introduced into the sample and vortexed for 1 min. Then, 0.75 mL of zinc sulfate heptahydrate (7.2% aq.) solution was added and vortexed for 1 min. The mixture was centrifuged at 10 000 rpm for 5 min.[45] Two milliliters of the supernatant was filtered through the GF/PET microfilter, adjusted to pH 7 with 0.1 M NaOH and diluted to 250 mL with ultrapure water. The commercial fruit juice sample was prepared by filtration through the glass fiber/poly(ethylene terephthalate) (GF/PET) microfilter and 1 mL of the filtrate was diluted to 50 or 100 mL with distilled water.

Standard mixtures (1−3) were prepared of with the use of standard solutions of reducing sugars and certain phenolics, the selection of which was performed according to their occurrence in natural samples such as fruit juice and milk. Besides, these sugar mixtures were prepared in the absence of phenolics, enabling the comparison of their alkaline CUPRAC recoveries. The final concentrations of synthetic mixture constituents are indicated below:

(1) 1.50×10^{-4} M glucose, catechin, and chlorogenic acid, separately.
(2) 1.67×10^{-5} M glucose, fructose, galactose, maltose, lactose, catechin, and chlorogenic acid, separately.
(3) 1.50×10^{-5} M glucose, fructose, galactose, maltose, lactose, ascorbic acid, gallic acid, catechin, epicatechin, and chlorogenic acid, separately.

Hydrolysis of Sucrose. The hydrolysis process was carried out using dilute sulfuric acid as described by Bower et al.[38] Twenty-five milliliters of sucrose stock solutions (0.5×10^{-3} and 1.0×10^{-3} M) containing 0.1% (w/w) H_2SO_4 were introduced into a 100 mL-conical flask. The mixture was stirred vigorously in a silicon oil bath at 160 °C for 3 min. After 3 min, the flask was removed from the bath and the solution was allowed to cool at room temperature. Then, the hydrolysate was neutralized with 0.1 M NaOH and diluted with ultrapure water before applying the proposed and reference methods.

Solid Phase Extraction (SPE) for Sample Clean-Up. C18 and polyamide SPE cartridges were used to eliminate phenolic compounds and other possible interferents of fruit juices and honey samples to clean up sugars. The stages of the clean-up process were described in the Supporting Information (SI) (Figure S-1).

Statistical Analysis. All assays were carried out in triplicate for each sample and standard. Descriptive statistical analyses were performed using Excel software (Microsoft

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Office 2013) for calculating the mean and the standard error of the mean. The precision and accuracy of two methods (the developed and reference method) were compared by the *F*-test and Student's *t*-test.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00761.

Solid phase extraction (SPE) for sample clean-up (Figure S-1); statistical comparison of the proposed method with the reference alkaline CUPRAC method for glucose and milk sample determination (Table S-1); total reducing sugar content of synthetic mixtures with respect to the proposed method (Table S-2); and total reducing sugar content of studied samples, including the declared and found values by proposed and reference methods (Table S-3) (PDF)

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Gropper, S. S.; Smith, J. L. *Advanced Nutrition and Human Metabolism*; Wadsworth Cengage Learning: CA, 2012.

(2) Cummings, J. H.; Stephen, A. M. Carbohydrate terminology and classification. *Eur. J. Clin. Nutr.* **2007**, *61*, 5−18.

(3) Gomis, D. B.; Tamayo, D. M.; Alonso, J. M. Determination of monosaccharides in cider by reversed-phase liquid chromatography. *Anal. Chim. Acta* **2001**, *436*, 173−180.

(4) Neeley, W. E. Simple Automated Determination of Serum or Plasma Glucose by a Hexokinase/Glucose-6-Phosphate Dehydrogenase Method. *Clin. Chem.* **1972**, *18*, 509−515.

(5) Apovian, C. M. Sugar-sweetened soft drinks, obesity, and type 2 diabetes. *JAMA* **2004**, *292*, 978−979.

(6) AOAC*Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, 1995.

(7) Toutounji, M. R.; Van Leeuwen, M. P.; Oliver, J. D.; Shrestha, A. K.; Castignollesa, P.; Gaborieau, M. Quantification of sugars in breakfast cereals using capillary electrophoresis. *Carbohydr. Res.* **2015**, *408*, 134−141.

(8) Başkan, K. S.; Tütem, E.; Akyüz, E.; Özen, S.; Apak, R. Spectrophotometric total reducing sugars assay based on cupric reduction. *Talanta* **2016**, *147*, 162−168.

(9) Zhang, S.; Li, C.; Zhou, G.; Che, G.; You, J.; Suo, Y. Determination of the carbohydrates from *Notopterygium forbesii* Boiss by HPLC with fluorescence detection. *Carbohydr. Polym.* **2013**, *97*, 794−799.

(10) Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426−428.

(11) Michalcová, A.; Machado, L.; Marek, I.; Martinec, M.; Sluková, M.; Vojtěch, D. Properties of Ag nanoparticles prepared by modified Tollens' process with the use of different saccharide types. *J. Phys. Chem. Solids* **2018**, *113*, 125−133.

(12) Sangsuk, S. Preparation of high surface area silver powder via Tollens process under sonication. *Mater. Lett.* **2010**, *64*, 775−777.

(13) Yin, Y.; Li, Z.-Y.; Zhong, Z.; Gates, B.; Xia, Y.; Venkateswaran, S. Synthesis and characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process. *J. Mater. Chem.* **2002**, *12*, 522−527.

(14) Wan, Y.; Guo, Z.; Jiang, X.; Fang, K.; Lu, X.; Zhang, Y.; Gu, N. Quasi-spherical silver nanoparticles: Aqueous synthesis and size control by the seed-mediated Lee−Meisel method. *J. Colloid Interface Sci.* **2013**, *394*, 263−268.

(15) Wang, H.; Chen, D.; Wei, Y.; Chang, Y.; Zhao, J. A simple and sensitive assay of gallic acid based on localized surface plasmon resonance light scattering of silver nanoparticles through modified Tollens process. *Anal. Sci.* **2011**, *27*, 937−941.

(16) Li, N.; Gu, Y.; Gao, M.; Wang, Z.; Xiao, D.; Li, Y.; Lin, R.; He, H. Colorimetric determination of o-phenylenediamine in water samples based on the formation of silver nanoparticles as a colorimetric probe. *Spectrochim. Acta, Part A* **2015**, *140*, 328−333.

(17) Chaiendoo, K.; Sooksin, S.; Kulchat, S.; Promarak, V.; Tuntulani, T.; Ngeontae, W. A new formaldehyde sensor from silver nanoclusters modified Tollens' reagent. *Food Chem.* **2018**, *255*, 41−48.

(18) Palazzo, G.; Facchini, L.; Mallardi, A. Colorimetric detection of sugars based on gold nanoparticle formation. *Sens. Actuators, B* **2012**, *161*, 366−371.

(19) Evanoff, D. D., Jr.; Chumanov, G. Synthesis and optical properties of silver nanoparticles and arrays. *ChemPhysChem* **2005**, *6*, 1221−1231.

(20) Balavandy, S. K.; Shameli, K.; Abidin, Z. Z. Rapid and green synthesis of silver nanoparticles via sodium alginate media. *Int. J. Electrochem. Sci.* **2015**, *10*, 486−497.

(21) Seth, R. L.; Dey, A. K. Calculation of the composition and stability of silver ammine complexes from solubility data. *J. Inorg. Nucl. Chem.* **1961**, *17*, 312−316.

(22) Benet, W. E.; Lewis, G. S.; Yang, L. Z.; Hughes, D. E. P. The mechanism of the reaction of the Tollens reagent. *J. Chem. Res.* **2011**, *35*, 675−677.

(23) Smitha, S. L.; Nissamudeen, K. M.; Philip, D.; Gopchandran, K. G. Studies on surface plasmon resonance and photoluminescence of silver nanoparticles. *Spectrochim. Acta, Part A* **2008**, *71*, 186−190.

(24) Dubas, S. T.; Pimpan, V. Green synthesis of silver nanoparticles for ammonia sensing. *Talanta* **2008**, *76*, 29−33.

(25) Amirjani, A.; Fatmehsari, D. H. Colorimetric detection of ammonia using smartphones based on localized surface plasmon resonance of silver nanoparticles. *Talanta* **2018**, *176*, 242−246.

(26) Bolzan, A. E.; Iwasita, T.; Vielstich, W. On the electrochemical oxidation of glucose: identification of volatile products by on-line mass spectroscopy. *J. Electrochem. Soc.* **1987**, *134*, 3052−3058.

(27) Hussain, J. I.; Talib, A.; Kumar, S.; AL-Thabaiti, S. A.; Hashmi, A. A.; Khan, Z. Time dependence of nucleation and growth of silver nanoparticles. *Colloids Surf., A* **2011**, *381*, 23−30.

(28) Kvítek, L.; Prucek, R.; Panáček, A.; Novotný, R.; Hrbáč, J.; Zbořil, R. The influence of complexing agent concentration on particle size in the process of SERS active silver colloid synthesis. *J. Mater. Chem.* **2005**, *15*, 1099−1105.

(29) Gill, R.; Bahshi, L.; Freeman, R.; Willner, I. Optical detection of glucose and acetylcholine esterase inhibitors by H_2O_2-sensitive CdSe/ZnS quantum dots. *Angew. Chem., Int. Ed.* **2008**, *47*, 1676−1679.

(30) Wooten, M.; Karra, S.; Zhang, M.; Gorski, W. On the direct electron transfer, sensing, and enzyme activity in the glucose oxidase/carbon nanotubes system. *Anal. Chem.* **2014**, *86*, 752−757.

(31) Gao, Y.; Wu, Y.; Di, J. Colorimetric detection of glucose based on gold nanoparticles coupled with silver nanoparticles. *Spectrochim. Acta, Part A* **2017**, *173*, 207−212.

(32) Mehta, S. K.; Chaudhary, S.; Gradzielski, M. Time dependence of nucleation and growth of silver nanoparticles generated by sugar reduction in micellar media. *J. Colloid Interface Sci.* **2010**, *343*, 447−453.

DOI: 10.1021/acsomega.9b00761
ACS Omega 2019, 4, 7596−7604

(33) Filippo, E.; Serra, A.; Buccolieri, A.; Manno, D. Green synthesis of silver nanoparticles with sucrose and maltose: morphological and structural characterization. *J. Non-Cryst. Solids* **2010**, *356*, 344−350.
(34) Panáček, A.; Kvítek, L.; Prucek, R.; Kolář, M.; Večeřová, R.; Pizurová, N.; Sharma, V. K.; Nevěčná, T.; Zbořil, R. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J. Phys. Chem. B* **2006**, *110*, 16248−16253.
(35) Stój, A.; Targoński, Z. Use of sugar content analysis for the estimation of berry juice authenticity. *Pol. J. Food Nutr. Sci.* **2005**, *14*, 403−409.
(36) Kamal, M. A.; Klein, P. Determination of sugars in honey by liquid chromatography. *Saudi J. Biol. Sci.* **2011**, *18*, 17−21.
(37) Seager, S. L.; Slabaugh, M. R. *Carbohydrates, Organic and Biochemistry for Today*; Brooks/Cole: New York, 2000.
(38) Bower, S.; Wickramasinghe, R.; Nagle, N. J.; Schell, D. J. Modeling sucrose hydrolysis in dilute sulfuric acid solutions at pretreatment conditions for lignocellulosic biomass. *Bioresour. Technol.* **2008**, *99*, 7354−7362.
(39) Cocchi, M.; Durante, C.; Grandi, M.; Lambertini, P.; Manzini, D.; Marchetti, A. Simultaneous determination of sugars and organic acids in aged vinegars and chemometric data analysis. *Talanta* **2006**, *69*, 1166−1175.
(40) Kylli, P. Berry Phenolics: Isolation, Analysis, Identification, and Antioxidant Properties. Academic Dissertation, EKT-series 1502; University of Helsinki, Department of Food and Environmental Sciences: 2011; pp 38−39.
(41) Nie, Z.; Deiss, F.; Liu, X.; Akbulut, O.; Whitesides, G. M. Integration of paper-based microfluidic devices with commercial electrochemical readers. *Lab Chip* **2010**, *10*, 3163−3169.
(42) Hamedi, M. M.; Ainla, A.; Guder, F.; Christodouleas, D. C.; Fernández-Abedul, M. T.; Whitesides, G. M. Integrating electronics and microfluidics on paper. *Adv. Mater.* **2016**, *28*, 5054−5063.
(43) Yao, H.; Shum, A. J.; Cowan, M.; Lähdesmäki, I.; Parviz, B. A. A contact lens with embedded sensor for monitoring tear glucose level. *Biosens. Bioelectron.* **2011**, *26*, 3290−3296.
(44) Salinas, E.; Morcillo, P.; Sanz, M. I.; Raba, J. Continuous-flow/stopped-flow system incorporating a rotating bioreactor based on a double redox catalytic cycle and electron transfer mediated by osmium complexes. Application in the determination of extremely low levels of glucose. *Instrum. Sci. Technol.* **2002**, *30*, 281−293.
(45) Kumar, N.; Kumar, H.; Mann, B.; Seth, R. Colorimetric determination of melamine in milk using unmodified silver nanoparticles. *Spectrochim. Acta, Part A* **2016**, *156*, 89−97.

DOI: 10.1021/acsomega.9b00761
ACS Omega 2019, 4, 7596−7604