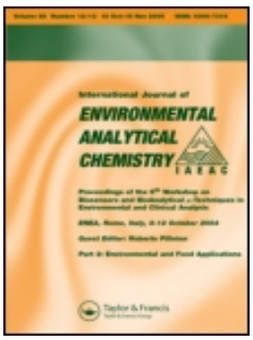

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Carbon nanoparticles based non-enzymatic glucose sensor

Indra Neel Pulidindi and Aharon Gedanken*

Department of Chemistry and Kanbar Laboratory for Nanomaterials at the Bar-Ilan University Center for Advanced Materials and Nanotechnology, Bar-Ilan University, Ramat-Gan 52900, Israel

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The analytical method reported herein relates to the detection of glucose in the range of 0.04–5.2 mM, with the aid of *in situ*-generated carbon nanoparticles (NPs) as a sensor. Hydrophilic carbon NPs with specific absorbance value at 275 nm were obtained in a reaction between glucose and urea at 120ºC for 20 minutes in an autoclave. The afore-mentioned chemical reaction was developed into a chemical sensing process for glucose detection. A good linearity over a wide range of glucose concentration (0.04–5.2 mM) with a correlation coefficient of 0.9948 and a detection limit of 0.04 mM is observed. The current assay for glucose sensing based on UV light absorbing carbon nanoparticles is simple, reproducible and fast. The process is devoid of either electrochemical – enzymatic (glucose oxidase) or non-enzymatic assays reported thus far.

Keywords: glucose sensor; carbon nanoparticles; urea; spectrophotometry

1. Introduction

Moses burnt the golden calf in the fire, ground it to powder, strewed it upon the water and made the children of Israel drink it. Thus, knowledge of the synthesis of small particles is not something new. However, the intelligence required to view, handle and use such small particles is definitely new.

Glucose sensing is inevitable in health care as well as food and beverage preparation processes [1]. Recently, quantitative estimation of glucose has become more important with the growing interest in the production of fuels and chemicals generated from biomass through glucose [2–5]. Metal, metal oxide and alloy nanoparticles have been extensively studied for glucose sensing application [6–12]. Non-enzymatic glucose sensors are being developed [13–16]. Enzyme-free glucose sensors have an edge over the enzymatic counter parts in terms of stability, simplicity and reproducibility [17].

The research reported on herein is a tale of serendipity. It all happened when a glucose–urea solution was accidentally subjected to autoclave treatment, resulting in the product solution turning yellow owing to the formation of small carbon particles.

Such carbon nanoparticles, with peculiar properties, and generated *in situ,* were used as a sensor to detect the mM level concentrations of glucose in the analyte.

Spectrophotometric (colourimetric) methods permit the rapid and accurate determination of small quantities of analytes. Several methods have been developed for the colourimetric determination of glucose. Timell *et al*. [18] developed a colourimetric method for determining the sugar in the concentration range of 20–500 μg mL^{-1}. This method is based on the characteristic colour developed as a result of a reaction between *o*-amino diphenyl, and reducing the sugar in acetic acid when heating the reaction mixture in a boiling water bath. Miller [19]

*Corresponding author. Email: gedanken@mail.biu.ac.il

has proposed a modified dinitro salicylic acid (DNS) method for the determination of reducing sugars. This method is based on the measurement of the intensity of the colour produced by the reduction of 3, 5 dinitro salicylic acid to 3-amino, 5-nitro salicylic acid and the corresponding oxidation of the aldehyde group of the sugar to the carboxylic group. Belal *et al*. [20] have developed a spectrophotometric method for the determination of monosaccharides (glucose and fructose) in the concentration range of 1–10 μg mL^{-1}. This method is based on the formation of osazone by the reaction between the monosaccharide and phenyl hydrazine hydrochloride and the subsequent reaction between the osazone and the tetra cyano ethylene, generating a red colouration with an absorption maximum at 455 nm .

Toghill and Compton [21], in their voluminous review on glucose sensors, foresaw and predicted the possibility of carbon nanomaterial-based technology superseding the current enzyme-based glucose sensing technology. Wilson and Zhang [22] have provided a comprehensive account of the recent developments in the *in vivo* sensing of glucose. Thus, the objective of the current investigation is to exploit the *in situ*-generated carbon nanoparticles, with an excellent hydrophilic property, as a tool for the spectrophotometric determination of the concentration of glucose in a given analyte at mM levels. We describe an absorption measurement of a carbon nanoparticle solution formed from the reaction between urea and glucose, and suggest this technique as a sensor for determining glucose concentrations down to 0.04 mM.

2. Experimental

Glucose (product no. G8270), galactose (product no. G0750) and urea (product no. U5128) were purchased from Sigma and used as received. Stock solutions were prepared afresh in deionized water. Just prior to their use, the stock solutions were adjusted to the desired concentrations. The glucose–urea solutions were subjected to autoclave treatment in a Tuttnauer cat 2007 autoclave. The autoclave treatment of the glucose–urea samples was carried out at 120ºC for 20 minutes. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM) images were recorded on Tecnai G2 FEI and JEM 2100 JEOL, respectively. A glucose–carbon nanoparticle composite was obtained from glucose–urea solutions after autoclave treatment followed by lyophilization using a Christ LOC-1m Alpha 2–4 lyophilizer. The X-ray diffraction pattern of the glucose–carbon nanoparticle composite was recorded on a Bruker AXS D Advance powder X-ray diffractometer (using monochromator CuKα radiation, $\lambda = 1.5418$ Å). The UV-visible spectra of the glucose–urea samples after autoclave treatment were recorded on a Cary 100 scan Varian UV/vis spectrophotometer. ^{13}C NMR spectra were recorded on Bruker Advance DPX 300 using D_2O as a solvent.

Generation of carbon nano particles from glucose: Typical method of generation of carbon nano particles involved heating a solution containing glucose (2 wt.%) and urea (0.2 wt.%) in an autoclave at 120ºC for 20 min. The colourless glucose–urea solution turns yellow indicating the formation carbon nano particles. Varying amounts of carbon nanoparticles were generated by systematically varying the initial amount of glucose (Table 1).

Table 1. Analytes with varying amounts (0.04–5.2 mM) of glucose.

Sample code	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Glucose (0.1%), mL	0	0.1	0.2	0.4	0.6	0.8	1.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0
Glucose, mM	0	0.04	0.07	0.15	0.22	0.30	0.37	0.74	1.48	2.22	2.96	3.7	4.44	5.18
Urea (10%), mL	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Water, mL	14.0	13.9	13.8	13.6	13.4	13.2	13.0	12.0	10.0	8.0	6.0	4.0	2.0	0
Total volume, mL	15	15	15	15	15	15	15	15	15	15	15	15	15	15

3. Results and discussion

When subjected to autoclave treatment at a temperature of 120ºC, a solution of glucose (2%) and urea (0.2%) resulted in the formation of a yellow colouration, as shown in Figure 1(A). The UV-vis spectra of the glucose (2%) – urea (0.2%) solutions before and after the autoclave treatment are shown in Figure 1(B).

The colourless glucose (2%) – urea (0.2%) solution before autoclave treatment showed no absorption. In contrast, the yellow colour obtained from the glucose (2%) – urea (0.2%) reaction under autoclave treatment exhibited a characteristic absorption band between 250–400 nm with an absorption maximum at 275 nm and a shoulder at 330 nm. Such an absorption band is typical of an aromatic pi system originating from π–π* transition of -C = C- chromophore. Haipeng Liu *et al*. [23], have also observed the presence of such terminal and internal 'C = C' bonds in the carbon nanoparticles (< 2 nm) derived from candle soot. Haitao Li *et al*. [24], made similar observations regarding carbon nanoparticles generated from glucose–NaOH and glucose–HCl solutions with the aid of ultrasonic treatment.

The transformation of a colourless glucose–urea solution to yellow is attributable to the formation of carbon nanoparticles generated *in situ*. Moreover, the carbon nanoparticles are hydrophilic. Use of urea facilitates the *in situ* generation of ammonia yielding suitable pH condition required for the formation of carbon nanoparticles from glucose under the reaction conditions.

Under the hydrothermal conditions employed currently, urea decomposes to yield ammonia, which is transformed to ammonium hydroxide as shown below [25]:

$$CO(NH_2)_2 \Leftrightarrow 2NH_3 + CO_2$$
$$NH_3 + H_2O \Leftrightarrow NH_4^+ + OH^-$$

The basic (alkaline) conditions thus generated facilitate, the polymerization by dehydration and the subsequent carbonization of glucose to carbon nanoparticles as shown below:

$$C_6H_{12}O_6 \Leftrightarrow 6C + 6H_2O$$

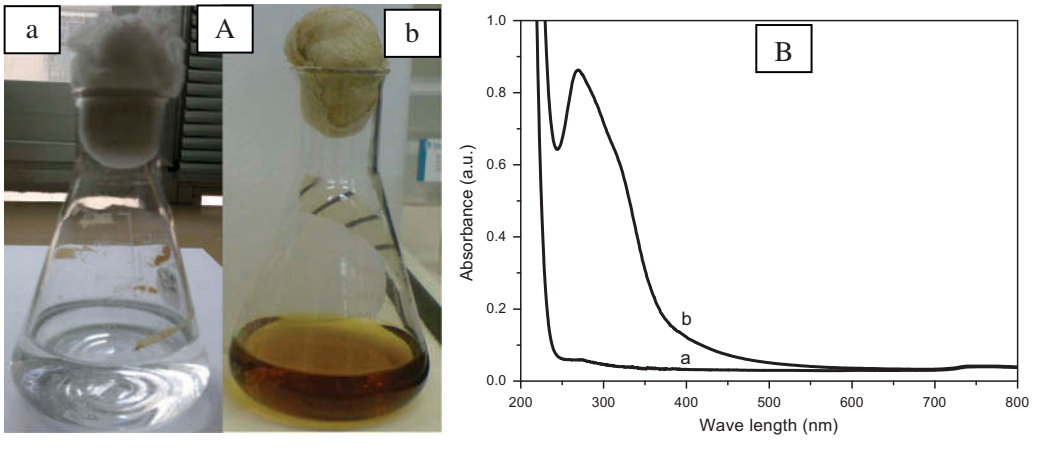

Figure 1. (A). Colour of Glucose (2%) – urea (0.2%) solution before (a) and after (b) autoclave treatment. (B). UV absorption spectra of glucose (2%) – urea (0.2%) solutions before (a) and after (b) autoclave treatment.

Haitao Li *et al.* [24], could bring about such conversion of glucose to carbon nanoparticles under ultrasonic irradiation in the presence of NaOH. The role of urea in the current methodology is exactly the same as that of NaOH employed by Haitao Li *et al.* [24], i.e. to generate alkaline conditions for the polymerization of glucose and its subsequent aromatization. Sun and Li [26] have provided a mechanistic view of the conversion of glucose to aromatic carbon particles under hydrothermal conditions. Ryu *et al*. [27], have identified HMF as the dehydration product from fructose which acts as a precursor to the formation of soluble polymer via inter molecular dehydration in the presence of phloroglucinol. The soluble polymer subsequently undergoes aromatization via intra molecular dehydration leading to the formation of *C* = C.

The transmission electron micrographs of the carbon nanoparticles generated *in situ* in the glucose (2%) – urea (0.2%) solution upon autoclave treatment are shown in Figure 2. Spherical carbon nanoparticles with a mean diameter of 17 nm are observed in the samples obtained soon after the autoclave treatment (Figure 2a), and also after a long standing (3 months) (Figure 2b) of the autoclave treated solution (carbon nanoparticles are seen on the edges of the lacy TEM grids). This indicates that the colloidal solutions containing carbon nanoparticles are indeed stable over a long period, and the carbon nanoparticles remained soluble.

Zhu *et al*. [28] also observed the formation of such a yellow coloured solution when subjecting a glucose–poly(ethylene glycol) solution to microwave treatment for 5 and 10 minutes. The transformation of colourless glucose–poly(ethylene glycol) to yellow and subsequently to dark brown was attributed to the formation of carbon nanoparticles (2–4 nm) with fluorescent and electro chemiluminescence properties. Very recently, Haitao Li *et al*. [24] made similar observations by exposing glucose–NaOH and glucose–HCl solutions to ultrasonic irradiation for a period of 4 hours. The change in the colour of the glucose–alkali or glucose–acid solution from colourless to brown was attributed to the formation of spherical and small carbon particles (less than 5 nm). In addition, the aforementioned carbon nanoparticles showed excellent photoluminescent properties.

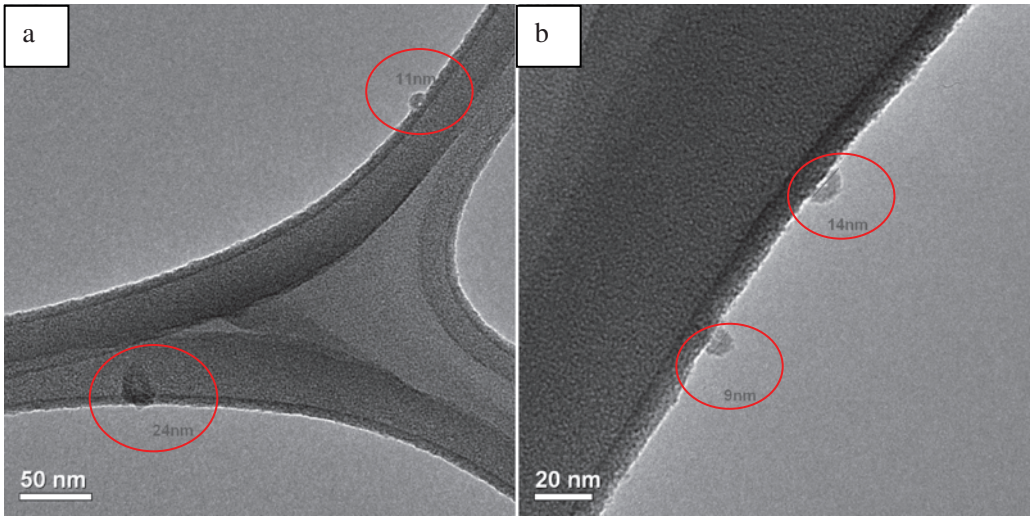

Figure 2. TEM images of carbon nanoparticles generated *in situ* in the glucose (2%) – urea (0.2%) solution after autoclave treatment: fresh (a) and after 3 months (b).

The glucose–carbon nanoparticle composite was isolated from the glucose (2%) – urea (0.2%) solutions subjected to autoclave treatment by lyophilization. The glucose–carbon nanoparticle composite was analyzed by X-ray diffraction studies. A broad diffraction peak between 5–28º (2θ) was observed, typical of amorphous carbon nanoparticles (Figure S1). Zhu *et al*. [28], also observed a broad X-ray diffraction peak between 10–30º (2θ) when preparing fluorescing carbon nanoparticles from glucose using microwave irradiation.

3.1 *Glucose assay*

The assay for glucose is based on the characteristic absorption of the *in situ*-generated carbon nanoparticles with an absorption maximum at 275 nm. Solutions with varying concentrations of glucose (0.04–5.2 mM) have been prepared in 20 mL glass scintillation vials, as shown in Table 1.

The TEM image of one of the products obtained from the working solutions (S13) with 5.2 mM of glucose upon autoclave treatment is depicted in Figure 3. Spherical carbon particles with a mean diameter value of 14 nm (Figure 3 and Figure S2 b) are generated *in situ* in the glucose (5.2 mM) – urea solution upon autoclave treatment.

The statistical (mean) diameter of the carbon nanoparticles generated from the solutions with two different initial concentrations of glucose namely 0.111 M (glucose (2 %) – urea (0.2%) solution after autoclave treatment) and 5.2 mM (glucose (0.1%) –urea (10%) solution after autoclave treatment). The mean diameter values, as deduced from the above TEM images were respectively 17 and 14 nm for the initial glucose of 2% and 0.1% glucose solutions (Figure S2). This indicate that the size of the carbon nano particles generated remained almost unchanged in the whole range of glucose concentrations.

The samples (glucose–urea solutions) with varying amounts (0.04–5.2 mM) of glucose and a constant amount of urea placed in glass scintillation vials were subjected to autoclave treatment for 20 minutes. The absorption spectra of the solutions described in Table 1 after autoclave treatment were recorded and are shown in Figure 4.

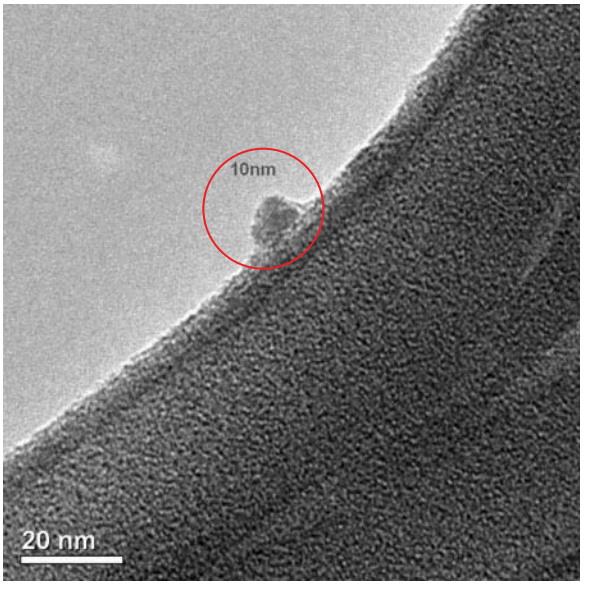

Figure 3. TEM image of carbon nanoparticles generated *in situ* in the glucose (933.3 ppm) – urea (S13) solution after autoclave treatment.

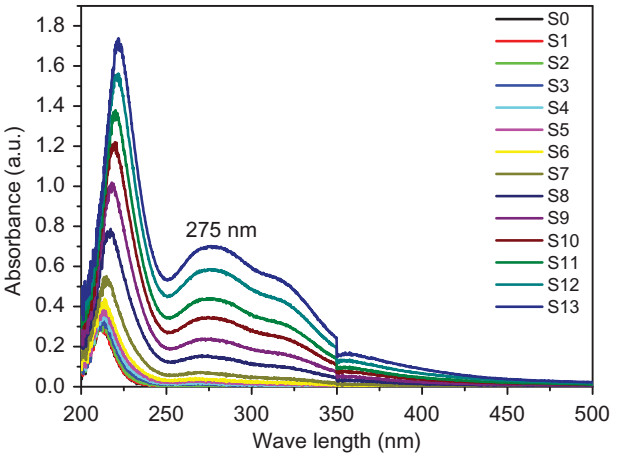

Figure 4. UV-Vis spectra of glucose (0.2%) – urea (10%) solutions with varying amounts of glucose (from 0–933 ppm).

When the amount of glucose increases from 0.04 to 5.2 mM (or 7–933 μg mL^{-1}), a steady increase in the intensity of the absorption band at 275 nm is observed. The variation in the absorption value at 275 nm as a function of glucose amount is depicted in the calibration plot shown in Figure 5. A good linearity over a wide range of glucose concentration (0.04–5.2 mM) with a correlation coefficient of 0.9948 and a detection limit of 0.04 mM (S/N = 1.5) is observed. A comparison of the performance of the current glucose sensor with other sensors based on carbon nanomaterials reported in literature has been made and summarized in Table 2. In addition, to being sensitive, the sensor need to be selective. So as to evaluate the selective nature of the sensor glucose dectection (0.04–4.4 mM) is tested in the presence of known amount of galactose (0.3663 mM). A comparison of the absorbance values of the two sets of

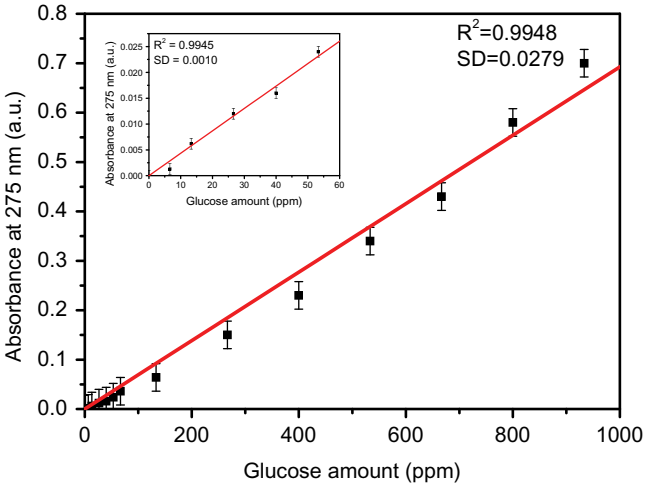

Figure 5. Calibration plot for the ppm level detection of glucose (Inset: the variation of absorbance at 275 nm as a function of glucose concentration (< 60 ppm)).

Table 2. Comparison of the performance of the current sensor with other carbon based sensors reported in literature.

S. No.	Carbon material	Linear range (glucose conc. in mM)	Limit of detection (μM)	References
1	Carbon nanoparticles	0.04–5	40	Current study
2	Ordered mesoporous carbon	2.5–5	20	23
3	SWNT films	0.01–2.16	10	24
4	MWCNT	$2 \times 10^{-3-11}$	1	25

samples containing glucose alone (Table S1) and containing a known amount of galactose along with varying amounts of glucose (Table S2) showed no effect of the presence of galactose on glucose detection over a wide range of glucose concentrations (7–933 μg mL^{-1}) (Figure S3). In addition, to the effect of presence of galactose, the effect of maltose on glucose selectivity has also been tested. Also, the addition of maltose had no effect on glucose detection. Thus the sensor is selective towards glucose detection.

The applicability of the glucose sensing methodology described herein has been tested for monitoring the kinetics of the fermentation of glucose to ethanol (Table S3). The electronic spectra of the aliquots of samples from the fermentation broth collected as regular intervals of time and subjected to glucose sensing assay were depicted in Figure S4. The wt.% conversion of glucose to ethanol as a function of time could be evaluated from the absorbance values. For comparison, the samples from the fermentation broth have also been analyzed by ^{13}C NMR so as to know the wt.% conversion of glucose as a function of time during the fermentation reaction (Figure S5) [29]. A good correlation was noticed between the glucose conversion values deduced from the UV-Vis method and ^{13}C NMR spectroscopies indicating the authenticity of the current glucose sensing process (Table S4).

Thus mM levels of glucose have been analyzed. Based on the analytical performance (LOD and linear range) as well as applicability the current glucose sensor is superior to most of the existing electrochemical sensors [21, 30]. Superior features of the current sensor involve: (i) absence of an enzyme, (ii) detection of glucose via reduction, not oxidation process and (iii) absence of noble metals.

4. Conclusion

Water soluble carbon nanoparticles generated *in situ* in the glucose–urea solution have been effectively exploited as a sensor for the determination of mM levels of glucose in the analyte. The potential analytical features that make the current chemical sensor for glucose a practically applicable device involve: (i) the linear range for the glucose detection from 0.04–5 mM which is well with in the realm of blood glucose concentration (2–10 mM), (ii) the limit of detection (LOD) of 40 μM (S/N = 1.5), (iii) independent of the use of enzymes which are pH sensitive and expensive, (iv) use of simple tools involved in the sensing technique, namely the spectrophotometer and the autoclave, (v) selectivity towards the exclusive detection of glucose and (vi) application, for instance, to monitor the progress of the fermentation reaction used in bioethanol industry.

Supplementary material

Supplementary Figures S1–S5 and Tables S1–S4 are available online at http://informahealthcare.com/doi/suppl/10.1080/03067319.2013.782488.

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