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Short communication

Naked eye detection of infertility using fructose blue–A novel gold nanoparticle based fructose sensor

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ABSTRACT

A simple and low cost colorimetric method, requiring no instrumentation, is presented for the detection of fructose in human semen, a marker of seminal vesicle function. In this study we have synthesized a novel gold nanoparticle (AuNP) based sensor, named as fructose blue, by co-functionalizing AuNPs with 3-aminophenyl boronic acid (APB) and L-glutamic acid-(2,2,2)-trichloroethyl ester (GTE). The red-shift in the plasmon absorption spectra of fructose blue with different fructose concentrations accompanied by colour change of the solution from red to blue is the principle applied here for the estimation of fructose. The novel co-functionalized nanoparticles (NPs) have better colour change response for fructose than that of the earlier reported fructose sensors based on AuNPs functionalized by the APB moiety alone. The proposed method showed linearity in the range of 0.5–6 mg/mL with a detection limit of 0.3 mg/mL, and exhibits excellent selectivity for fructose over a collection of sugars. The method was successfully applied for detection of fructose in real samples of semen and agrees well with values obtained from conventional methods. The method depicted here for the detection of semen fructose is indeed superior to the existing methods in the sense that it can be performed at home as a preliminary self-screening test by patients suspecting infertility for warranting further medical attention and provides privacy also. Moreover the method is important, particularly in third world countries where high-tech diagnostic aids are inaccessible to the bulk of the population.

1. Introduction

Infertility affects 13–18% of couples and male factors accounts for up to half of all cases (Iammarrone et al., 2003). More than 90% of the male infertility causes are due to low sperm counts, poor sperm quality, or both (Gonzales and Villena., 2001; Buckett and Lewis-Jones., 2002; Artifeksov., 1991). A large number of substances have been found in sperm plasma and among them, fructose occupies the most important place in biochemical investigation (Ndovi et al., 2006). In the routine of semen analysis, determination of fructose concentration in the seminal plasma has been recommended as a marker of secretory activity of the seminal vesicle. Any change in biochemical composition of semen, such as reduced fructose levels are positively correlated with low seminal volume, low sperm motility and high sperm chromatin stability (Rajalakshmi et al., 1989; Videla et al., 1981). Hence this sugar has been studied extensively, because it is considered as a marker of seminal vesicle function. The desired semen fructose for an individual is 1.5–6 mg/mL; however levels less than 1.5 mg/mL indicate a low testosterone level or seminal vesicle insufficiency.

Several techniques exist for the detection of fructose, including gold and silver NP (Kadir et al., 2004) based sensors and amperometric (Biscay et al., 2012), electrochemical (Hajar et al., 2013), and fluorescence methods. Although some of these methods can be used to detect fructose with excellent selectivity and sensitivity, they require expensive materials or sophisticated instrumentation that is cumbersome to operate and produce a reliable read-out. Hence a visual detection method is highly attractive because it can be easily interpreted by the naked eye or low cost, portable instruments.

Metallic NP based sensing has emerged as an important colorimetric tool for the detection of biomolecules linked to the onset of diseases to aid in early diagnosis. Among them, AuNPs are extensively used for molecular sensing due to the wide opportunity it offers in the design of easy to perform methods (Aslan and Prez-Luna., 2002). Optical sensing based on surface plasmon resonance (SPR) absorption, exhibited by NP, has been used in shaping analytical tools in clinical diagnosis (Perez-Luna et al., 2004; Daniel and Astruc, 2004; Durne, 2009; Link and Sayed, 2003). A small change in the size, shape or local environment leads to tunable opto-electronic properties which in fact are used in

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sensing applications (Aslan et al., 2004). On aggregation of the NPs the absorption maxima shift to longer wavelength, resulting in the colour change of the colloid from wine red to blue due to mutually induced dipoles that depends on interparticle distance and aggregate sizes (Aslan et al., 2004).

There has been an ever increasing demand for the development of simple, cost effective methodologies in an easy to read out format for the detection of clinically relevant molecules to aid in clinical diagnosis. Simple procedures which could be performed at home without the need of sophisticated instruments possibly bring radical changes in rural health care management. Visual detection of disease by specific marker molecules in biological fluids such as urine, saliva, or blood is an attractive approach to address these issues. A colour change observable by naked eye in response to the concentration of the analyte can be an indication of the disease condition warranting further medical attention. In the present study, we describe a new method for the estimation of semen fructose, a marker of seminal vesicle function based on AuNPs cofunctionalized with L-glutamic acid-(2,2,2)-trichloroethyl ester and 3-aminophenyl boronic acid ligands by carbodiimide chemistry. The proposed sensor shows good response to fructose and hence provides a simple detection by naked eye eliminating the use of sophisticated instruments.

2. Experimental section

The list of used reagents can be found in the Supplementary information (S11).

2.1. Apparatus and equipment

The list of used apparatus and equipments can be found in the Supplementary information (S12).

2.2. Synthesis and functionalization of AuNPs

Detailed procedure for the synthesis of AuNP, AuNP2, AuNP3, fructose blue, fructose sensing using fructose blue and the selectivity of the method are available in the Supplementary information (S13–S19).

3. Results and discussion

3.1. Functionalization of AuNPs

AuNPs synthesized yielded spherical particles with an average diameter of 16 ± 2 nm (Fig. S1A) and SPR absorption at 519 nm (Ai et al., 2009; Liu and Lu, 2004). After adding Tween 20, the SPR maxima of AuNPs shifts to 522 ± 1 nm due to the physical adsorption of surfactant and is consistent with the reported shifts of the band upon formation of dielectric layers around colloidal metals (Perez-Luna et al., 2004). The SPR maxima further shifted to 524 ± 2 nm upon chemisorptions of 16-MHDA, indicating the formation of a thicker monolayer around the AuNPs (Fig. S2). On conjugating the –COOH group of 16-MHDA with the amino group of GTE, the SPR further red-shifted to 528 ± 1 nm (Fig. S2). The spectral shift is not accompanied by any broadening, confirming non-aggregation of particles at this stage of modification. Further modification with APB resulted in red-shift in the SPR to 533 ± 1 nm (Fig. S2).

As shown in Table 1S, the zeta potential of AuNP was reduced from -35.3 ± 0.41 mV to -32.1 ± 0.21 mV, on conjugating with 16-MHDA. The size also increased (20 ± 3 nm, Fig. S1B), further reflecting the presence of 16-MHDA on the particle surface. Even after the modification the net negative charge on the NP surface stabilized them against aggregation in water. Further modification with GTE resulted in an increase in the particle size to 24 ± 2 nm (Fig. S1C), with a decrease in the zeta potential to -8.43 ± 0.34 mV (Table 1S). The net negative charge is further decreased (-3.21 ± 0.50 mV, Table 1S) in the formation of FB, confirming the conjugation process with an increase in the particle size to 28 ± 3 nm (Fig. S1D).

3.2. Fourier transform infrared spectroscopy (FTIR)

We recorded FTIR spectra of the AuNPs to get further insight on the surface modification (Fig. S3). Citrate AuNPs showed a peak around 1509 and 1399 cm^{-1}, characteristic of citrate ions (spectra not shown). AuNP3 showed intense peak around 1629 cm^{-1}characteristic of amide I (−C=O), a strong –C−O stretching band at 1096 cm^{-1} and on conjugation with APB, the peak at 1629 cm^{-1} reduced and an additional peak at 1644 cm^{-1} which is the characteristic peak of amide I, confirms the conjugation of APB moieties onto the activated −COOH group of AuNP3.

3.3. Effect of fructose on the SPR absorption maxima of FB

On adding varied amounts of fructose (0.5–6 mg/mL) to FB, the SPR absorption band was found to red-shift from 533 to 557 nm, reflecting the formation of aggregates having enhanced sizes. The corresponding spectra are shown in Fig. 1. A red-shift of the SPR with concomitant broadening of the spectrum occurred in a concentration dependent manner. Fructose causes a change in the absorbance since it induces aggregation of AuNPs through specific molecular interactions with FB as depicted in Fig. S2.

If only a small number of fructose molecules are available in the system, aggregation will not proceed to a significant extent because of the limited availability of the molecule that bridges NP together. The degree of aggregation is concentration dependent, and hence when more fructose molecules bind to FB more aggregation occurs. When the concentration of fructose is large enough, specific binding sites become unavailable for fructose mediated aggregation. The magnitude of wavelength shift was concomitantly increased with concentration of fructose and beyond 6 mg/mL it leveled off, indicating the saturation of available binding sites for fructose (Fig. S4). The variation in absorbance correlated linearly with concentration, and the method exhibits a linear range for fructose between 0.5 - 6 mg/mL with a detection limit of 0.3 mg/mL. The lowest amount of fructose that produced a shift in the SPR was 0.3 mg/mL, which was taken as the detection limit of the method.

FB contained a boronic acid motif, which is a well known sugar binding group (Wang et al., 2002; Striegler, 2003). Although several boronic acid based sensors for saccharides have been reported (Liu et al., 2011; Gao et al., 2003; Jiang et al., 2006),

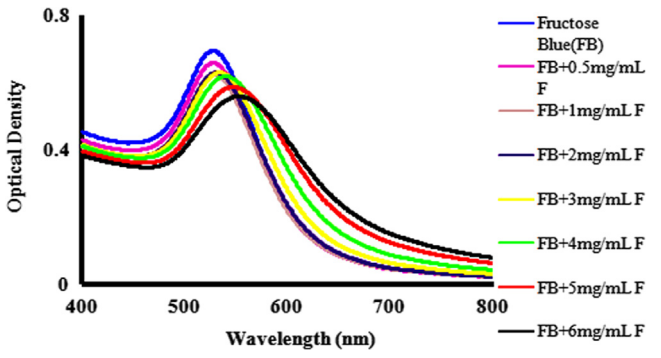

Fig. 1. UV–visible absorption spectra of FB showing red-shift on reacting with different quantities of fructose standard.