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Smart platform for the time since death determination from vitreous humor cystine

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ABSTRACT

In this work, we report the smart application of AgNPs based sensors for determination of time since death (TSD) via recognition and quantification of vitreous humor (VH) cystine as well as provide the portability for on spot determination of TSD. The lower detection limit was found to be 7.0 ng/ml with prominent selectivity. It was found that there is a linear correlation between the VH cystine concentration and TSD as the concentration of cystine increases up to 96 h ± 3.9 h. Further for the first time TSD determination is given a smart approach and it proves to have a great utility up to 24 h ± 2.6 h. The linear regression equation between TSD (the dependent variable), RGB intensity of cystine concentration till 24 h (the independent variable) was found to be TSD = 26.69 + −0.05*x. The proposed method gives the smart detection, portability, rapidity, sensitivity, selectivity as well as cost effectiveness for determination of time since death.

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1. Introduction

Estimation of time since death (TSD) is a crucial part of medico-legal investigations. An exact determination of TSD is of prime importance to the forensic medico legal expert in any investigation because it may narrow down the field of suspects and may help in recognition of the departed. In spite of its great importance it is very difficult to determine the exact TSD within the limit of probability. The longer the time interval between death and body examination the wider will be the limits of probability (Rentoul and Smith, 1973). Merely on the basis of autopsy findings it is seldom to estimate exact time of death, an appropriate range of TSD can be inferred from the changes which take place after death. The traditional methods applied to determine the TSD such as cooling of body, changes in eye, postmortem staining, rigor mortis, decomposition changes, contents of stomach and bowels, contents of urinary bladder and circumstantial evidence, only provide an approximation (Vishal et al., 2004; Mathur and Agrawal, 2011; Palmiere and Mangin, 2012). Biochemical analysis of different body fluids has proven to be a useful supplementary procedure in relation to their death interval as compared to the other traditional methods (Tumram et al., 2014). Body fluids like blood, pericardial fluid, synovial fluid, spinal fluid, aqueous humor and vitreous humor show biochemical changes immediately or shortly after death, which can help to ascertain TSD. All these changes possess its own time factor or rate and progress in a well methodical fashion until the body undergo putrefaction. Thus determination of these chemical changes can evidence to be beneficial to establish time since death more precisely as these body fluids are positioned in a well-protected closed sections making the chances of contamination to a lesser amount (Vishal et al., 2004; Arroyo et al., 2005; Aggarwal et al., 1983).

Out of these body fluids, vitreous humor (VH) is unique and possesses many advantages for bio-chemical analysis as compared to other fluids, due to its accessibility (Swann et al., 2010; Sturner, 1963), ease of sampling (James et al., 1997), less susceptibility than blood to rapid chemical changes (Coe, 1969) and relative independence from environmental influences (Henry and Smith, 1980). VH is preserved despite of serious trauma to head and is much less subject to contamination or putrefactive change than either blood or CSF (Sachdeva et al., 2011). VH is a fluid relatively well protected from post-mortem degradation and contamination and due to its post-mortem immovability, vitreous humor is highly utilized in forensic pathology as compared to blood. Various biochemical constituent of vitreous such as potassium (Adelson et al., 1963; Madea et al., 1990), sodium (Wilkie and Bellamy, 1982), calcium (Wilkie and Bellamy, 1982), urea (Leahy and Farber, 1967; Mulla, 2005) etc. have been studied frequently in relation to the TSD. Very few authors namely Patrick and Logan (1988) and Girela et al. (2008) studied amino acid in relation to TSD. According to Eloy Girela et al. taurine, glutamate and ascorbate showed

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significant increase with increasing TSD. However till now no study has been reported on cystine relation with TSD.

Cysteine has gained interest due to the presence of highly reactive thiol group. Cysteine is a non-essential amino acid and forms a dominant part of most of the peptides and proteins which is quite common in nature and plays an important role in many biological systems. When exposed to atmospheric effects it gets oxidized to L-cystine which is a dimer of two molecules of L-cysteine containing a weak disulphidic bond, which plays a crucial role in protein structure and folding. The analytical determination of this amino acid is not only important for TSD but any change in its concentration can cause diseases like HIV, cancer, sepsis, ulcerative colitis and many others (Droge and Holm, 1997) however it is also important to clinical diagnosis purpose. Methods such as spectrofluorimetry (Ensafi et al., 2009), colorimetry (Morioka and Kobayashi, 1997; Grote, 1931; Shinohara, 1935), iodimetry (Virtue and Lewis, 1934), liquid chromatography (Wang et al., 2007), electrochemistry (Mikus et al., 2003; Li et al., 2010; Damle et al., 2010) and spectrophotometry (Chrastil, 1989; Chrastil, 1990) are developed for the detection of cystine. Colorimetric methods are considered to be more appropriate, attractive and simple as compared to the sophisticated instruments applied till date. On the basis of the aforementioned facts there is a keen need to develop methods which can be applied to different biological/non-biological samples in a very trace amount and are rapid, sensitive and selective, for that nanotechnology can help. In the field of nanotechnology, metal and metal oxide nanoparticles are more profoundly used due to their excellent unique properties and wide range of application in various fields. From them, silver nanoparticles are considered to be one of the commercialized nanoparticle as it has found its applications in catalysis, biosensors, electronics, pharmaceuticals, therapeutics, optics and other areas due to their unique size-dependent optical and electrical properties, good stability, low toxicity, good biocompatibility etc (Desireddy et al., 2013). Due to these properties of silver nanoparticles they are frequently applied for the detection of thiol containing amino acids. Till now nanoparticles are not applied for TSD determination from any biological fluids.

Now a days various authors have attracted towards smartphone, as smartphone based colorimetric sensor prove to be an inventive technology (Guan-Hua et al., 2014; Sumriddetchkajorn et al., 2014; Moonrungsee et al., 2015). Smartphone provide a simple colorimetric detection (Shen et al., 2012). The colorimetric data acquired from smartphone as a digital image is further converted to analytes concentrations. This advancement provides a new direction in colorimetric detection as it can be a good substitute for spectrophotometers for “on-spot” detection.

Here, inspired by our previous studies (Lodha et al., 2014, 2013; Ansari et al., 2015) and literature reports, we used green approach to synthesize AgNPs for determination of cystine and studied its correlation with time since death, as one of the realistic approaches in forensic science as well as in clinical diagnosis. At the same time we utilized the smartphone as a smart approach for determination of cystine, as smartphones equipped with a high-resolution digital camera have become almost omnipresent in society which can be used to produce an image very conveniently. RGB values calculation, with the Adobe Photoshop CS6 software, of these images can be used for the quantitative analysis. To the best of our knowledge, this is the first time that silver nanoparticles have been applied with smart approach for the determination of TSD via cystine from VH. Thus, in this work, we have made an attempt to give smart, rapid, sensitive, cost-effective and lab on chip method that provides easy determination of cystine.

2. Materials and methods

2.1. Silver nanoparticles synthesis

Silver nanoparticles were synthesized by following reported method (Zhao et al., 2009) with some modifications (See Supplementary data) and it was synthesized in CEM Discover Benchmate microwave using single mode and continuous power at 2.45 GHz.

2.2. Colorimetric detection of cystine using synthesized silver nanoparticles

For colorimetric detection of cystine, 1 ml standard solution of cystine (0.1 μg/ml) was added to the 2 ml of freshly prepared 87 μM AgNPs after adjusting the pH to 7.0 by using PBS buffer (2 ml). The mixture was allowed to react for 10 s at room temperature (25 °C). The 87 μM AgNPs solution immediately turns pinkish red from yellow which was visible to the naked eye. These colorimetric change was recorded with UV–vis spectrophotometer using cells with 1 cm path length against the reagent blank. Further to determine its hydrodynamic diameter and characteristic vibrational frequencies in the infrared region, Dynamic light scattering (DLS) and FTIR analysis were carried out.

2.3. Photographic system

To photograph the coloured product of 87 μM AgNPs-Cys complex, a simple photographic safety cabinet system as described earlier (Ansari et al., 2015) was set up. This safety cabinet had white interior to produce the same lighting and environment condition to all samples. The color product was transferred into 2 ml Eppendorf tube and photographed in cabinet with the help of built in digital camera of the iPhone 5S in flash off mode with the high dynamic range (HDR). In iPhone 5S backside illumination (BSI) CMOS image sensor was used which facilitate more incident light to reach the light sensing silicon which provide better sensitivity to detect small differences in photon emission from darker products as compared to any DSLR camera. The images were then transferred to computer and were processed by using Adobe Photoshop CS6. The intensity of red, green and blue color were measured, the whole procedure was repeated five times and the average of intensity of each was recorded in Excel (Version 2013) spread sheet for data analysis.

3. Results and discussion

3.1. UV–vis spectra

The synthesized 87 μM ascorbate-AgNPs gives bathochromic shift in the presence of cystine and optimum pH for the reaction was found to be 7 (see, Supplementary data). The interaction between 87 μM ascorbate-AgNPs and cystine was studied by different techniques like UV–vis spectroscopy, FTIR and DLS. The molecular-linker-based aggregation mechanism between AgNPs and cystine was monitored by UV–vis spectrophotometer. The surface plasmon resonance (SPR) absorption of ascorbate-AgNPs solution showed absorption maxima at 410 nm which remained same even after months. With the addition of cystine into clear solution of AgNPs, the yellow color of AgNPs immediately changes to pinkish red with the shift in the surface plasmon resonance absorption maxima to 486 nm. With the addition of cystine, the absorbance at 410 nm decreases and a new band upturns at 486 nm as shown in Fig. 1, which confirms the binding of cystine with AgNPs. From the series of experiments, it was concluded that as the aggregation increases the size of the particles also increases which result in

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