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Poly (9-(2-diallylaminoethyl)adenine HCl-co-sulfur dioxide) deposited on silica nanoparticles constructs hierarchically ordered nanocapsules: Curcumin conjugated nanocapsules as a novel strategy to amplify guanine selectivity among nucleobases

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ABSTRACT

Poly (9-(2-diallylaminoethyl)adenine HCl-co-sulfur dioxide) (Poly A) deposited on silica nanoparticles self-assembles to form hierarchically ordered nanocapsules. These nanocapsules can be conjugated with curcumin. The curcumin-conjugated nanocapsules are found to be spherical in size and their size ranges between 200 and 600 nm. We found that curcumin conjugated with silica nanoparticles marginally shows a selectivity (~20%) for guanine over adenine, cytosine, thymine and uracil, but this selectivity is extraordinarily amplified to more than 500% in curcumin-conjugated nanocapsules prepared from the above procedure. FT-IR spectra along with lifetime measurements suggest that specific interaction between adenine moieties of Poly A nanocapsules and thymine/uracil does not affect the fluorescence of poly A nanocapsules. Thus, the sensitivity and selectivity for guanine estimation is due to hydrophobic interactions, which are assisted by the low water solubility of guanine as compared to the other nucleobases. The present method illustrates a wider linear dynamic range in the higher concentration range as compared to the reported methods. Finally, the degradation study proves that stability of curcumin is improved dramatically in such nanocapsules demonstrating that nanotechnology could be a viable method to improve selectivity of specific analyte and robustness of probe molecule during fluorescence based bio-sensing.

1. Introduction

Nanotechnologies and nanomaterials are getting widely utilized in sensor designing since past 10 years (Zhang et al., 2009). During this period this field has witnessed fast development and advancement. The importance of nanomaterials is not only referred to their geometric size but also due to the fact that a change from macro-/micro- to nano-scale qualitatively changes the physicochemical characteristics such as magnetism, optical refraction, electric conductivity, thermal stability and strength, light absorption and emission. It also produces new materials of the same chemical nature, but having properties and reactivity that are lacking in macro- and micro-scopic materials, thus opening new possibilities for sensor designing. All types of nanoparticles can be incorporated into different inorganic and organic materials to develop nanoscale sensors. Such nanosensors can be applied in both gas and liquid media analysis (Shtykov and Rusanova, 2008). Recently, there have been tremendous demands for utilizing nanomaterials as biosensing probes. Based on the hybridization between a target and its complementary probe, various electrochemical and optical methods have been successfully used (Sassolas et al., 2008).

The introduction of nucleic acids' fluorescent labeling method has opened up the ability of utilizing such systems for further research and development. The recent years have witnessed rapid growth of fluorescent probes as well as wide development of homogenous fluorescence assays, including those based on fluorescence resonance energy transfer (FRET) or quenching mechanisms for nucleic acid detection (Didenko, 2006). These probes like molecular beacons are labeled with both a quencher dye and a fluorescent reporter, where the reporter fluoresces when the two dyes are physically separated after the hybridization (Wang et al., 2011). At the same time determination and separation of nucleobases is an important and challenging task due to the significance of these compounds in numerous biological processes. Adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U) are the building blocks of both RNA and DNA that play an important role in the storage of genetic information and protein biosynthesis

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(Brown, 1987). Thus, the identification and estimation of individual nucleobases are crucial to understand the sequence of nucleic acids (Blazej et al., 2005). DNA sequencing has been of great interest for human genomics, forensic sciences, genetic engineering and medicinal applications. Selective estimation of individual nucleobases has tremendous potential application during DNA sequencing (Adams et al., 2007). Besides being one of the building blocks of DNA/RNA (to store genetic information and for protein biosynthesis) (Wang et al., 2008), guanine concentration is used to measure degree of oxidative damage to DNA (Li et al., 2012), because guanine is easily oxidized by different types of oxidants and free radicals (Li et al., 2012; Abbaspour and Noori, 2008). The widespread effect of guanine on cerebral circulation and coronary, control of blood flow, prevention of cardiac arrhythmias, modulation of adenylate cyclase activity, inhibition of neutrotransmitter release, etc. Zhu et al. (2013) have made guanine estimation a challenging and crucial task in clinical medicine, biological science and analytical chemistry.

Available methods for guanine estimation based on electrochemical assays, chromatography and capillary electrophoresis suffer due to labor-intensive electrode procedures, poor stability of electrodes or complicated and expensive equipment. The simple, fast and selective fluorescence method has attracted for selective determination of nucleobase. Further fluorescence markers are widely used in electrophoresis where a fluorescent marker specific to particular nucleobase may ease the analytical procedure during nucleic acid sequencing (Mahmodian et al., 2008). The interaction of gold nanoparticles and nucleobases has been reported, where experiments showed that A, G, C and T specifically interact in a sequence based manner with the surfaces of gold (Sharma et al., 2007). Moreover, the interaction of organic nanoparticles with nucleobases has been investigated showing different fluorescence responses for the four nucleobases (Xu et al., 2008).

In the present work, we have applied the method of self assembly to synthesize nanocapaulses based on Poly (9-(2-diallylaminoethyl)adenine HCl-co-sulfur dioxide) (Poly A). Poly A as prepared in our lab is a polymer of adenine along with a poly amine backbone. In this nanocapsule formation method, Poly A is deposited on silica nanoparticles (NPs) to which curcumin is incorporated subsequently as a fluorescence probe for the detection of nucleobases, in specific for the estimation of guanine. The nanocapsules are of various sizes ranging from 200 to 600 nm. The detection mode is based on the change in the fluorescence intensity of curcumin. The advantages of such technique are: (i) synthetic procedure is easy, (ii) it does not require long hours of nanocapsules preparation, (iii) materials are stable and robust, (iv) it is highly selective for guanine among nucleobases and, (v) it has a wider linear dynamic range.

2. Materials and methods

2.1. Materials

Curcumin was obtained from Acros Organics. Poly A was synthesized as shown in Fig. 1A using a procedure explained earlier (Bouhadir et al., 2012). The nucleobases including adenine, guanine, cytosine, thymine and uracil (structure shown in Fig. 1B) were obtained from Sigma-Aldrich and their stock solutions were prepared in de-ionized water. Silica LUDOX® HS-40 Colloidal Silica was also obtained from Sigma-Aldrich.

2.2. Preparation of Poly A nanocapsule

Encapsulation of curcumin inside the nanocapsules was made using Poly A as the structure directing agent. In this process 1.3 mL

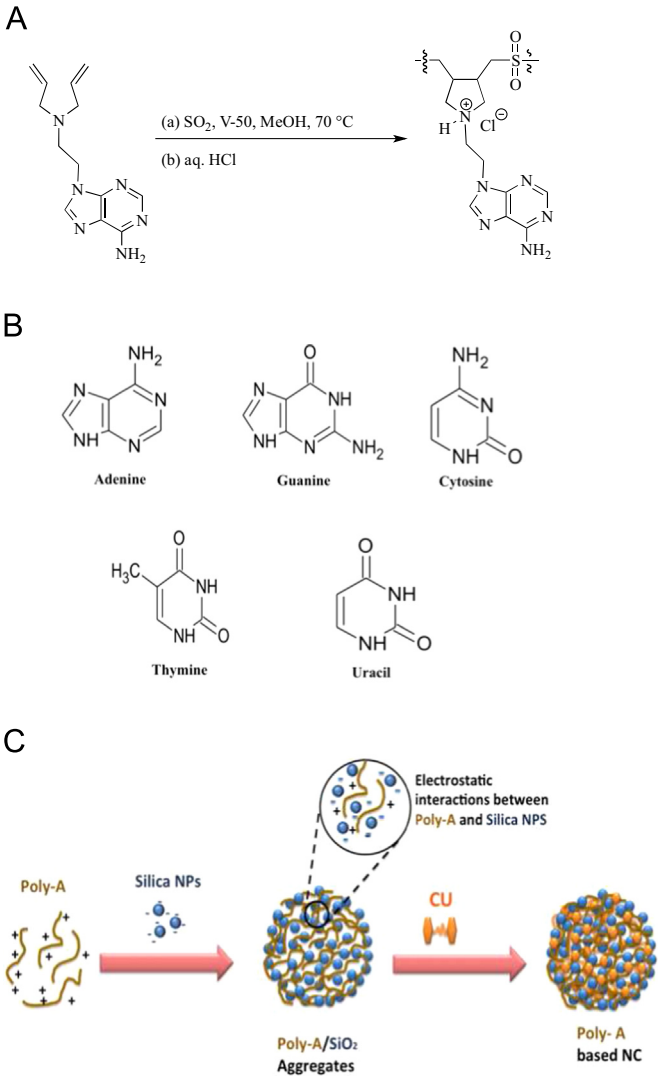

Fig. 1. (A) Chemical structure of Poly (9-(2-diallylaminoethyl)adenine HCl-co-sulfur dioxide) (Poly A). (B) The nucleobases used and their corresponding chemical structure. (C) Illustration of Poly A interacting with SiO_2 NPs, and curcumin to form nanocapsules.

of Poly A (2 mg mL^{-1}) was gently vortex mixed for 20 s with 7.8 mL of negatively charged silica nanoparticles. The obtained cloudy suspension was aged for 2 h; then it was centrifuged for 20 min at a speed of 4450 rpm. Poly A and SiO_2 aggregates were collected. To the latter precipitate 0.5 ml of curcumin (1 mg mL^{-1} prepared in 10% acetone/de-ionized water solution) and 2.5 ml of de-ionized water were added. The mixture was allowed to age for 30 min, and again centrifuged for 20 min at a speed of 4450 rpm. The precipitate of nanocapsules formed was washed with de-ionized water for 3 times to remove the excess of curcumin. The preparation method is depicted in Fig. 1C. Finally it was dispersed in 3 mL of de-ionized water for further characterization and investigation.

2.3. Morphological characterization

Scanning electron microscopy (SEM) analysis was done using a Tescan, Vega 3 LMU with Oxford Edx detector (Inca XmaW20) SEM, where 3 mg of the nanocapules was dissolved in 5 ml of de-ionized water, and a few drops of the nanocapsule suspension were mounted on an aluminum stub, coated with carbon adhesive. After being dried the sample was ready for the SEM analyses. The