Layer by layer assembly on inorganic microparticles for enzyme encapsulation

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Introduction

Health care needs of patients are deciphered by diagnostics and therapeutics, both of which are dependent upon the detection of physiological and pathological biochemicals present in the body. Development of technologically advanced systems for analysis of biochemicals has caught pace since the last decade. The developments aid in the decentralization of the facilities, capital and skilled labor which previously were concentrated in the laboratory and employed the use of analytical sophistication. Analytically sophisticated methods although accurate, are time consuming and suffer with the disadvantage of the inability to monitor concentrations in real time situations and where the instantaneous results are required. The development of biosensors has played a vital role in instant analysis of biochemicals under real time conditions using invasive and non invasive techniques, which offer a low cost, rapid and simple to operate analytical tools. The concept of self monitoring using such systems will revolutionize the clinical practice. Such biosensors would provide capability of detection of analytes in a sensitive and specific manner with other advantages like rapid response, inexpensiveness and ease of use.
The main objective of the work is to develop a novel matrix suitable for enzyme encapsulation so that the system can be used for developing an enzyme based biosensors. Enzyme encapsulation is the most critical factor because it is subjected to inactivation in presence of several matrices. An attempt to load enzymes in microporous inorganic matrices coated with layer by layer (LBL) assembled polyelectrolytes was made (G. Sukhorukov, 1998). Development of nanoengineered inorganic particles containing polymers has amplified the prospects of enzyme encapsulation for *in vivo* implantation. These would provide the necessary specificity and sensitivity for the detection of biochemical analytes maintaining the stability of the enzymes.

Materials and Methods

Materials

Calcium chloride was purchased from Merck Ltd, Mumbai, sodium carbonate was purchased from Lobachemie Pvt. Ltd. Mumbai, sodium poly (styrene sulfonate) (PSS, MW~70,000), poly (allylamine hydrochloride) (PAH, MW~70,000), Fluorescien isothiocyanate-dextran (FITC-dextran) (Mol. wt. 70 KDa and Mol wt. 150 KDa), Tetramethylrhodamine isothiocyanate (TRITC), Peroxidase (HRP)(type VI, obtained from *Horseradish*, 298 purpurogallin units/mg) and glucose oxidase (GOx) (type VII, obtained from *Aspergillus niger*, 221000 units/gm) have been purchased from Sigma-Aldrich. All chemicals were reagent grade and used as received.

Methods

Preparation of calcium carbonate microparticles

Calcium carbonate microparticles were prepared by precipitation, due to the reaction between calcium chloride (1 M) and sodium carbonate (1 M) in presence of PSS (1%) to form PSS doped calcium carbonate (H. Zhu, 2005 and A. Antipov, 2003). FITC-dextran of two molecular weights was loaded as a model compound having high molecular weight on to the microparticles at a concentration of 0.2 mg/ml. HRP and GOx loading on the calcium carbonate microparticles was done by mixing (2 mg/ml) solution during precipitation.

Layer by Layer assembly on inorganic particles

The PSS doped microparticles of $CaCO_3$ were thoroughly washed with deionized water before coating with nanofilms via the LbL technique (G. Decher, 1992; R. Srivastava, 2005). The PSS doped Calcium carbonate particles were coated with a layer of PAH which followed the LBL to lead $CaCO_3$ (PSS)-PAH-PSS (Q. Zhao, 2006). Two bilayers were coated over the $CaCO_3$ core.

Characterization

Microparticles were characterized for particle size and zeta potential using optical microscope (Zeiss) and dynamic light scattering (Brookhaven Instruments, USA). Zeta plus (Brookhaven Instruments, USA) was used for the determination of zeta potential (ζ) in order to determine the electrophoretic mobility of the particles. This was carried out to know the charge of the adsorbed layer in relation to the medium in which it is suspended.

Encapsulation of FITC-dextran

A calibration curve for the fluorescence emission was prepared using concentrations in the range (0-0.2 mg/ml). FITC-dextran encapsulation in the microparticles was studied using fluorescence spectrophotometer by analyzing the supernatant after loading and centrifugation. FITC-dextran loaded and TRITC loaded $CaCO_3$ microparticles were also visualized using confocal imaging.

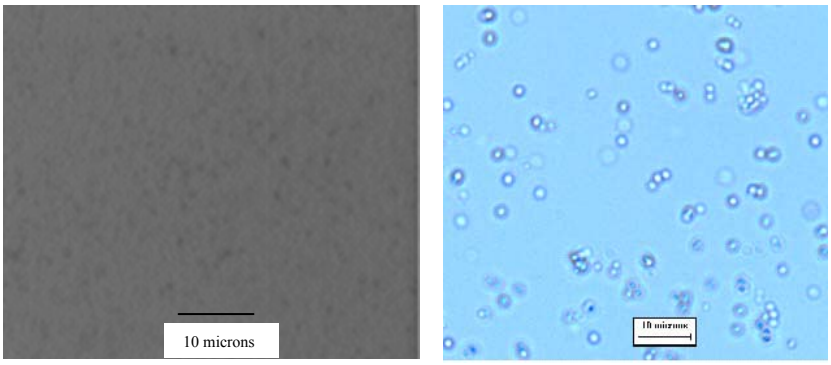
Enzyme encapsulation study

HRP and GOx in (1.5 mg/ml) were loaded on $CaCO_3$ microparticles and analyzed using fluorescence spectrophotometry and UV spectroscopy.

Results

Particle size analysis

Particle size measurement of microparticles formed after precipitation was carried out by optical microscopy and dynamic light scattering. Optical microscopic images of microparticles indicate microparticles of uniform size 2-3 μm.



(a) (b)

Fig. 1: Optical images of uncoated microparticles (a) 10 X and (b) 60 X

DLS analysis of the same was also attempted which showed the effective diameter of the particles to be 5 μm with polydispersity of 0.12. Since the DLS measurement cannot be performed on higher particles due to Rayleigh scattering, it showed a higher effective diameter and a higher polydispersity value.

Zeta potential measurement

Zeta potential measurements of uncoated and coated microparticles were performed in order to confirm the LBL assembly over the microparticles.

Zeta potential of bare calcium carbonate microparticles was found to be -7 mv. PSS doped microparticles showed a zeta potential value of -18.48 mv which showed increased stability in suspension form. This provided as template for attachment of subsequent PAH layer. Subsequent attachment of different layers showed corresponding changes in the zeta potential as shown in Fig. 2.

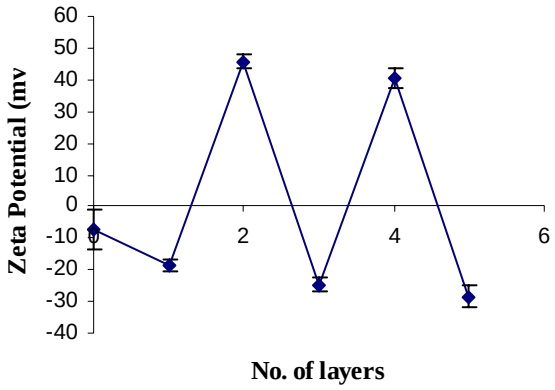


Fig. 2: Zeta potential measurement of LBL assembled particles with PSS and PAH

Encapsulation of FITC-dextran

Fluorescence spectrophotometric study for $CaCO_3$ microparticles showed encapsulation of FITC-dextran indicated by decrease in fluorescence intensity in supernatant solution in comparison to blank (Fig. 4). Spectra indicates that encapsulation of 70 KDa FITC-dextran occurs to an extent of (n=3) 68.6 % (5.41). On the other hand, encapsulation of 150 KDa FITC-dextran occurs to an extent of (n=3) 33.1% (1.21) on loading with 0.2 mg/ml solution of FITC-dextran as calculated from the calibration curves (Fig. 3).

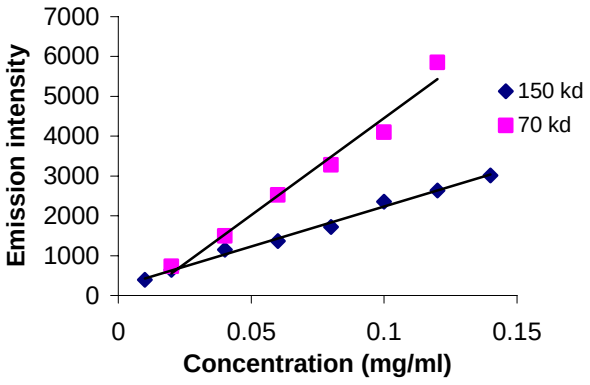


Fig. 3: Calibration curves of fluorescence emission intensity of FITC-dextran

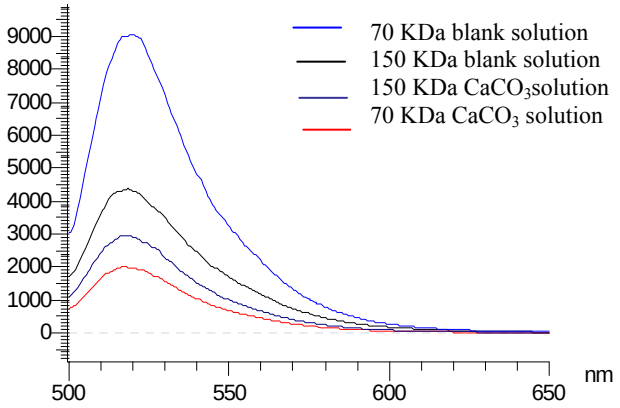


Fig. 4: Fluorescence emission spectra of FITC-dextran loaded on $CaCO_3$

Confocal microscopy

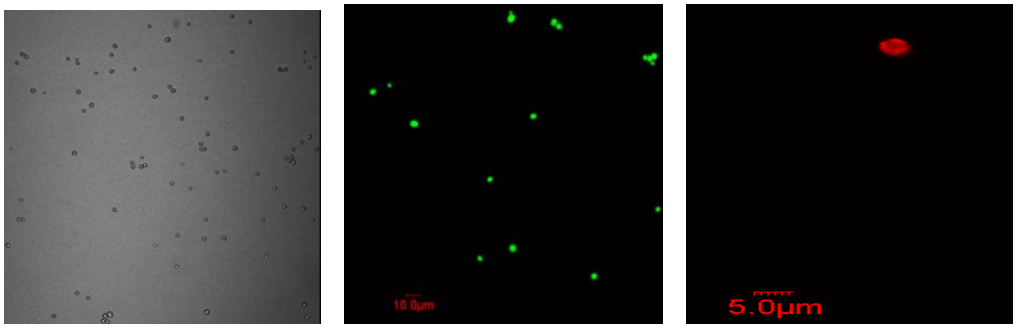


Fig. 5. Confocal images of (a) blank $CaCO_3$ microparticles (b) FITC-dextran (70 KDa) loaded on $CaCO_3$ microparticles, (c) TRITC loaded on $CaCO_3$ microparticles.

Encapsulation of FITC dextran was confirmed using confocal microscopy, Fig. 5(b) indicates the fluorescent confocal images of loaded $CaCO_3$ microparticles with FITC-dextran and Fig. 5 (c) indicates TRITC loaded $CaCO_3$ microparticles in comparison blank microparticles

Enzyme encapsulation study

Fluorescence emission scans show a decrease in fluorescence intensity for GOx and HRP in supernatant solution in comparison to blank enzyme solutions indicating encapsulation in microparticles (Fig. 6).

Sample	Absorbance	Absorbance
GOx solution	0.293 (244 nm)	-
GOx supernat.	0.282 (244 nm)	-
HRP solution	0.290 (242 nm)	0.107 (213 nm)
HRP supernat.	0.251 (242 nm)	0.056 (213 nm)

Table 1.UV absorbance of enzyme solutions loaded on $CaCO_3$ microparticles

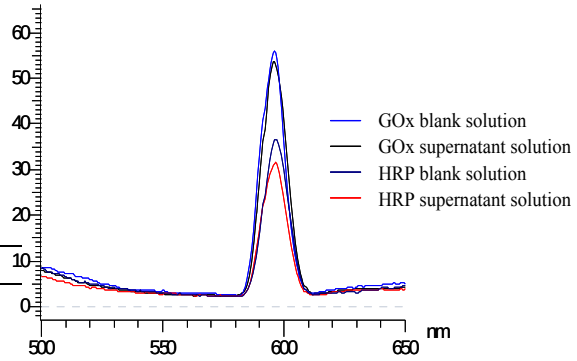


Fig. 6. Fluorescence emission spectra of GOx and HRP loaded on $CaCO_3$ microparticles obtained at excitation wavelength of 295 nm.

UV spectrophotmetric analysis also showed a decrease in the intensity in supernatant solution in comparison to blank (Table 1). Both scans show that the encapsulation of HRP occurs to a greater extent in comparison to GOx due to a lower molecular weight.

Conclusions

Precipitation of calcium carbonate from calcium chloride and sodium carbonate in presence of PSS leads to formation of uniform sized inorganic microparticles of size 2-3 μm. Further, LBL assembly was confirmed by measuring the electrophoretic mobility using zeta potential. The alternating zeta potential values confirmed the successful coating of PSS and PAH. The microparticles being microporous can be loaded with high molecular weight compounds like enzymes. FITC-dextran loading was analyzed for studying high molecular weight compounds. FITC-dextran loading showed encapsulation of 68 and 33 % of 70 KDa and 150 KDa, respectively. Qualitative analysis for enzyme encapsulation using fluorescence spectroscopy and UV spectroscopy showed that HRP undergoes a greater degree of encapsulation in comparison to GOx in the microporous $CaCO_3$ microparticles.

References

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