

Chemiluminescent peroxyoxalate nanoparticles for hydrogen peroxide analysis

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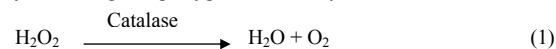
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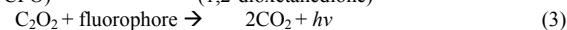
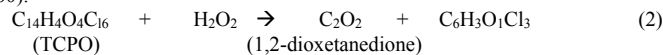


INTRODUCTION

In recent times several matrices have been developed for applications relating to encapsulation of drugs, enzymes and dyes for biosensor preparation. The development of newer polymeric matrices has opened new avenues in basic, applied scientific research and industrial applications. Chemiluminescence is a powerful and versatile analytical tool for sensitive and selective detection of a wide variety of chemical species (Hu Y 2007) (Tahirovi A 2007). Numerous reports have been published on the usefulness of chemiluminescence in analytical chemistry and these have been of increasing interest in food, pharmaceutical research, quality control because they are simple and relatively inexpensive. Hydrogen peroxide (H₂O₂) forms an important catalytic intermediate of many physiological, biochemical, atmospheric and pathological reactions. A number of methods have been used for the determination of hydrogen peroxide, spectrophotometry, fluorimetry, amperometry, and chemiluminescence. H₂O₂ produced in any reaction can be quantitatively measured either directly or by converting it to oxygen and water using following reaction (Equation 1) and then subsequently estimating using oxygen sensitive dyes.



Conventionally H₂O₂ has been estimated using luminol, 2, 7 dichloro fluorescein, rhodamine derivatives, pentacene, europium based compounds, boronates and peroxyoxalate esters. These have been tried for a long period for intracellular imaging and estimation of H₂O₂ *in vivo* due to their bio and chemiluminescent properties. Peroxyoxalate esters have recently gained importance in imaging and determining the production of H₂O₂. They are known to be converted to dioxetanedione intermediate which is a high energy compound (Equation 2 and 3), with capability to excite fluorophores. Lee *et al* have demonstrated their usage for *in vivo* imaging of H₂O₂. In presence of H₂O₂ peroxyoxalate moiety are converted to dioxetanedione, which subsequently excites pentacene giving an emission at a near infra red wavelength (Lee D 2007) (Arnous A 2002). The mechanism of the excitation of fluorophore by the dioxetane dione intermediate was explained by Stevani *et al* (Stevani CV 2000).



The main objective of the work is to develop a polymeric matrix suitable for encapsulation of oxygen sensitive fluorophore, so that the system can be used for biosensor preparation. Development of polymeric nanoparticles encapsulated with fluorophores and enzymes which can amplify the prospects of biosensor with *in vivo* implantation. These would provide the necessary specificity and sensitivity for the detection of biochemical analytes maintaining the stability of the enzymes. Platinum porphyrin was used as a oxygen sensitive fluorophore, which could be excited on formation of 1, 2- dioxetanedione intermediate.

MATERIAL AND METHODS

4-hydroxy benzyl alcohol, 1, 8- octane-diol and oxalyl chloride were purchased from Sigma-Aldrich, India. Polyvinyl alcohols (PVA), triethyl amine, tetrahydrofuran, sodium chloride, and dichloromethane were purchased from Merck Ltd, India. All chemicals were reagent grade and were used as received.

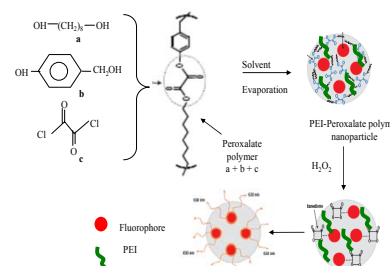


Figure 1: Schematic diagram of mechanism of chemiluminescent analysis of peroxide

Synthesis of peroxyoxalate polymer

The peroxyoxalate polymer was synthesized according to a modified method developed by Lee *et al*. Briefly, 4-Hydroxybenzyl alcohol (16 mmol) and 1, 8-octanediol (2.4 mmol) were dissolved in dry tetrahydrofuran (10 ml), under nitrogen, to which triethylamine (40 mmol) was added dropwise at 0 °C. This mixture was added to oxalyl chloride (18.3 mmol) in dry tetrahydrofuran (20 ml) at 0 °C. The reaction was kept at room temperature overnight (Lee D 2007).

Purification of polymer

The reaction mixture was purified by quenching with a saturated brine solution, and extracted with ethylacetate. The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under vacuum. The polymer was isolated by precipitating in dichloromethane/hexane (1:1).

Preparation of polymeric nanoparticles

Polymer (35 mg) was dissolved in 8 ml of dichloromethane, to which PEI solution in acetone was mixed; The mixture was added to 8 ml of a polyvinyl alcohol solution (5.0% in phosphate buffer pH 7.4) and to form a fine oil/water emulsion. A nanoparticle suspension was prepared by rotary evaporation of dichloromethane for 30 min. This led to formation of PEI-peroxyoxalate polymer nanoparticles loaded with fluorophore.

Encapsulation of fluorophore

A fluorescent dye (1 mg) in 200 µl of dichloromethane was mixed with peroxyoxalate polymer and PEI solutions and poured in PVA (1 %) solution.

Characterization

Synthesized polymer was characterized by Nuclear Magnetic Resonance (NMR) for completion of reaction and formation of peroxyoxalate polymer. The polymeric nanoparticles were characterized using

scanning electron microscopy (SEM), transmission electron microscopy (TEM), and fluorescence spectrophotometry.

RESULTS AND DISCUSSION

NMR

4-hydroxy benzyl alcohol shows NMR signals at 1.57 (protons attached to α carbon), 4.61 (aliphatic alcohol), 4.81, (aromatic alcohol), 6.81- 7.24 (Phenyl protons) ppm; 1, 8 – Octanediol on the other hand shows signals at 1.33-1.4 (δ carbon), 1.36-1.38 (γ carbon), 1.50, 1.54-1.59 (β carbon), 3.61-3.66 (α carbon) and 7.27 (aliphatic alcohol) ppm.

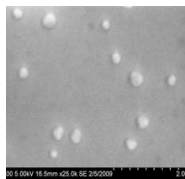


Figure 2: SEM image of PEI peroxalate nanoparticles

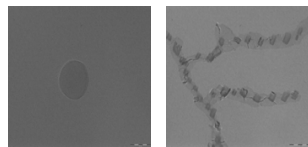


Figure 3: TEM image of (a) peroxalate polymer nanoparticles and (b) Dye loaded nanoparticles of peroxalate polymer.

The synthesized peroxalate polymer shows NMR signals at 1.1-1.59 (protons attached to α , β , γ and δ carbons of 1, 8 octane diol), 1.93-1.94 (α carbon attached to phenyl ring), 5.27 (aromatic alcohol), 7.19-7.26 (phenyl protons). The NMR signals of the polymer were characteristic for the proposed synthesized polymer as shown in the figure. An attempt to calculate the molecular weight using NMR method showed that the molecular weight of polymer was found to be approximately 13, 000 Dalton.

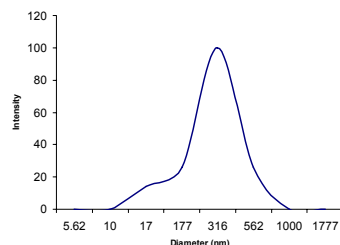


Figure 4: Particle size distribution analysis of polymeric peroxalate- PEI nanoparticles

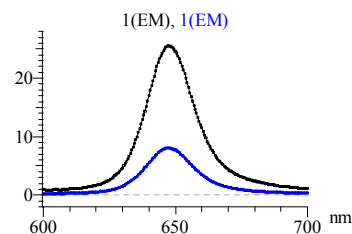


Figure 5: Fluorescent emission spectrum of encapsulated Pt porphyrin (blue) and Pt porphyrin in dichloromethane (black)

Particle size and zeta potential analysis

Particle size as studied from SEM showed that the nanoparticle size lied in the range of 250-300 nm (Figure 2). TEM analysis showed that spherical polymeric nanoparticles were formed having 250 nm (Figure 3). Size distribution analysis using dynamic light scattering showed a distribution curve having mean hydrodynamic diameter of 300 ± 150 nm with polydispersity index of 0.31 (Figure 4). TEM analysis of dye loaded shows precipitated dye molecules in surfactant coating, which clearly indicates that fluorophore has been encapsulated inside the nanoparticle matrix. Zeta potential analysis showed that PEI-peroxalate nanoparticles showed a zeta potential of -20 mV (± 5 mV) indicating stability, and lower aggregation potential of nanoparticles.

Fluorescence spectrophotometry

Encapsulation of Pt-porphyrin was characterized using fluorescence spectrophotometry. Pt porphyrin showed an emission peak at 650 nm when excited at 550 nm (Figure 5). The fluorescence intensity encapsulated fluorophore in nanoparticle showed a reduced intensity in comparison to fluorophore solution made in dichloromethane.

CONCLUSIONS

A modified polymer synthesis method was used to prepare nanoparticles of PEI-peroxalate polymer. The polymer synthesis was confirmed using NMR. The size distribution analysis showed that the nanoparticle size was 300 nm which was confirmed by SEM and TEM. The successful encapsulation of Pt-Porphyrin indicated that these nanoparticles can be used for dye loading. It showed an emission maximum of 650 nm when excited at 550 nm.

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