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MORPHOMETRIC AND ELEMENTAL ANALYSIS OF A POLYMER BASED NANOPARTICLE- FLAVONOID ENCAPSULE

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ABSTRACT In this study a polymer based nanocarrier platform suitable for encapsulation of a flavonoid Apigenin (DIONC – Apigenin) applicable for cancer therapy was developed and a morphometric and elemental analysis of the nanoparticle assembly was done by SEM – EDS. The SEM reports showed that a net with tiny particle hanging on the polymer dextran coated nanocomposite, which reveals the embedding and incorporation of Fe_3O_4 nanoparticle in dextran polymer film, The iron oxide nanoparticles increases the electrostatic surface area of the polymer dextran by increasing its folds and changing it into a net and providing a favorable environment for trapping drugs inside it. In the elememtal maps C,O and Fe was deduced. The DIONC – Apigenin capsule were examined for particle size and zeta potential analysis. The average particle diameter of the DIONC – apigenin capsule was 208.2nm and the zeta potential of dextran nanoparticle composite and flavonoid was found to be -18.7mV.

KEYWORDS : Iron Oxide Nano particle, Dextran, Apigenin, SEM, EDX

INTRODUCTION:

A nanocarrier platform encapsulated with a number of drugs for various applications has become a challenging endeavor. Among the various polymers present, dextran of microbial origin is promising for drug encapsulation in applications like cancer.

Nanoparticle of metallic origin have proved successful. The size range especially of 50-200nm have found to be apt to promote the nano carrier to tumour site. The smaller size of nano particle is an added benefit for their applications (1). Circulation half life of nanoparticle within the body could be prolonged by surface grafting of some polymers to the respective nano particles. Metallic nano particles have strong capability to overcome challenges and thus proved to be efficient drug delivery system. For cancer therapy amidst the available types of nanoparticles supermagnetic iron oxide nano particles proved better. Iron oxide nano particles are prepared by various methods including co-precipitation, micro emulsion methods, hydrothermal precipitation and thermal decomposition.(4,5,6,7). Among which the co precipitation is more commonly applied. In this method Fe(II) and Fe (III) salts are co precipitated in aqueous solution in presence of NH_4OH or NaOH(8).

Polymer assembled nano particles act as very powerful drug delivery vectors as they achieve high drug loading capacity along with controlled drug release and aimed targets. Since the polysaccharide dextran has multiple reactive sites it is suitable for being developed as a co polymer system with magnetic metal nano particles. Metallic nano particles are protected against oxidation by the coating provided by dextran (9). Hence dextran proves to be a most suitable drug carrier with added advantages like low toxicity, high stability, low pharmacological activity and protects conjugated drugs from degradation (10). An added advantage is the presence of numerous reactive hydroxyl groups in this polymer dextran (11)

Apigenin chemically known as 4′, 5, 7,-trihydroxyflameavone, with molecular formula $C_{15}H_{10}O_5$ is one of the flavonoid molecule that has gained interest as beneficial agent for human health with cancer preventive and/or therapeutic properties. This plant flavone, Apigenin is abundantly present in common fruits such as grapefruit, plant-derived beverages and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts and in some seasonings. The most common sources of apigenin is the herbal tea Chamomile prepared from dried flowers of *Matricaria chamomilla* [12].

The present study was carried out by incorporating an anti cancer agent i.e the plant based flavonoid apigenin into a polymer based nano-composite platform. To better understand and correlate the role of dextran as a carrier system for anti cancer agents was emphasized.

SUBJECT AND METHODS:

Materials

$FeCl_3$ (anhydrous), $FeCl_2.4H_2O$, NaOH (Merck), Dextran extracted from a bacterial isolate (12), Apigenin (4,5,7-trihydroflavone (Alpha Brand) was procured from Jayam Scientific suppliers, Coimbatore. Deionized water was used throughout the experiment.

Synthesis of Iron Oxide Nano particles
Alkaline Co- precipitation method

Magnetic iron oxide was prepared by alkaline co-precipitation [11] of Ferrous and Ferric salts in aqueous solution. Briefly, $FeCl_3$ and $FeCl_2$•$4H_2O$ in 100 ml deionized water were mixed in the 2:1 molar ratio and magnetically stirred at a temperature of 80◦C. The alkaline mixture was slowly precipitated with 1 M NaOH at a pH of 12.0. Black precipitate resulted indicating the formation of iron-oxide nanoparticles and the system was allowed to continue under the same reaction condition for 2 hrs. to ensure complete precipitation. The precipitate residue was washed in distilled water after complete settling. The iron-oxide nanoparticles formed were dried overnight and stored.

Magnetically Stirred

$2FeCl_3 + FeCl_2.4H2O + 8NaOH \longrightarrow Fe_3O_4 + 4H_2O + 8NaCl$

Iron(III) Chloride Iron(II) Chloride pH 12.0 at 80°C Iron Oxide (Black precipitate)

Coating of iron oxide nano particles with dextran

The dried iron-oxide nanoparticles (1mg) were dispersed in 3% dextran [12] and stirred for 6 hrs at 80◦C. The suspension was washed with deionized water. And the particles were dialyzed against deionized water.

Characterization of the nanocomposite
Morphometric and elemental analysis of DIONC under SEM-EDX

The size, morphology and characterization of homogeneity of the coatings of the dextran with iron oxide nano particles (DIONC) were examined by high resolution Scanning electron microscopy (SEM) (JEOL, JSM6390, Japan). A little amount of dried dextran was applied to the SEM stub by means of an adhesive tape and coated with 10nm Au in a sputter coater. The surface of the sample was visualized in SEM The objective of performing Energy dispersive X- ray analysis (EDX) analysis on dextran coated iron oxide nanoparticles is to investigate the elemental presence.

Elemental composition was analyzed using FEI Quanta FEG microscope equipped with Oxford Link EDX microanalysis hardware.

Development of polymer based nanoparticle – apigenin flavanoid encapsulation

Dextran (3%) with 1mg nanoparticle was used as a delivery platform for the flavanoid apigenin (1µm). The mixture was kept for stirring at 80 ° C for 6 hours. Then the suspension was centrifuged (10,000 rpm) and supernatant removed and the pellet was twice washed with distilled water and re-suspended in 1mL distilled water and dialyzed against distilled water for three days with consequent change of water in between. Following which the pellet was kept for drying in oven at 28°C O/N.

Morphometric and elemental analysis of DIONC – Apigenin encapsule by SEM - EDS

The morphology and characterization of homogeneity of the coatings of the dextran with iron oxide nano particles and flavanoid apigenin encapsulation were examined by high resolution Scanning electron microscopy (SEM) (JEOL, JSM6390, Japan). A little amount of dried dextran was applied to the SEM stub by means of an adhesive tape and coated with 10nm Au in a sputter coater. The surface of the sample was visualized in SEM

Elemental composition was analyzed using FEI Quanta FEG microscope equipped with Oxford Link EDX microanalysis hardware
Particle Size and Zeta potential determination

The dextran-nanoparticle composite and falvanoid apigenin (DIONC – Apigenin) mixture were dispersed into 10mL of distilled water and sonicated for 5 minutes before measurement. The obtained homogenous suspension was examined for particle size and zeta potential, size analyzer (Malvern zetasizer Version 6.32, MAL 1037088).

RESULTS AND DISCUSSION

Iron Oxide Nanoparticles synthesis and Coating with dextran (DIONC)

The super paramagnetic iron oxide nano particles, are prepared by co-precipitating a stoichiometric mixture of ferrous and ferric salts in an aqueous medium. The thermodynamics of the reaction require a ratio of 2:1 for Fe_2+/ Fe_3+, and a pH between 8.0 and 14.0. The precipitated magnetite is black in colour. The precipitate residue of iron oxide nanoparticle after complete settling and washing with distilled water was dried O/N to obtain an amorphous dry black powdery material ready for surface coating with a suitable polymer. (Plate No.1). Dextran was coated to the iron oxide nanoparticles by reacting a mixture of ferrous chloride and ferric chloride , under alkaline conditions.

Plate 1. Black amorphous powdery Iron Oxide Nps
Morphometric and elemental analysis of DIONC under SEM-EDX

The micro structure and composite homogeneity of the composite were investigated using SEM/EDX. The surface homogeneity and morphology of dextran coated iron oxide nano composite (DIONC) was observed. Numerous pores were observed in the reticular surface of dextran (Figure 1). A net with tiny particle hanging on it is noticed from dextran coated nanocomposite, which reveals the embedding and incorporation of Fe304 nanoparticle in dextran polymer film. The net shape may be attributed to interaction between the dextran and surface charged Fe_3O_4 nanoparticle. Providing of proper surface coating and development of some effective protective strategies to keep the stability of magnetic iron oxide NP was done by grafting or coating with dextran.

The elemental local analysis was performed using an energy dispersive spectroscopy detector, the EDX spectrum or elemental maps of C, O and Fe for sample prepared by coating of dextran with iron oxide nanocomposite was obtained (Figure 2). Apart from these elements no other impurities were detected. It is known from scientific literature that intensities of any spectral lines in EDX analysis are proportional to elemental abundance.

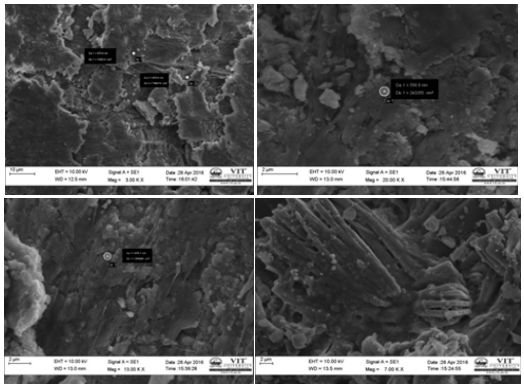

FIG 1 SEM images of NP coated with Dextran at variousmagnific ations

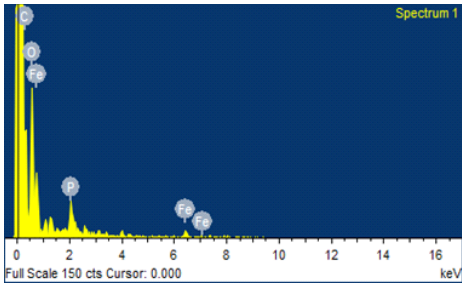

FIG 2 EDAX spectra of DIO-NPs (Dextran coated Iron Oxide Nano particles)

3.3 Morphometric and elemental analysis of DIONC – Apigenin encapsule by SEM – EDS

The most desirable magnetic nano particles are composed of the iron oxide core surrounded by a biocompatible surface coating which shows stabilization under physiological environment

The Fe_3O_4 nanoparticles increase the electrostatic surface area of dextran by increasing its folds and changing it into a net and providing a favorable environment for trapping drugs inside it. The formation of a polymer-nanoparticle conjugate resulted in a bifunctional platform that can be used for guided drug delivery via exploitation of the inherent magnetism possessed by the iron oxide core. Here the SEM micro structure reveals the porous structure and its holding capacity to accommodate the iron oxide nano particle thus providing a favourable environment to trap the anti cancer flavanoid – Apigenin (Figure 3).

The EDX spectra shows that the plant flavanoid apigenin was adsorbed with high percentage after using DX/Fe_3O_4 nanocomposite, by expressing intensity in spectral lines which is proportional to the additional elemental abundance. The elemental composition is confirmed to be C, Fe and O, with a broader spectral lines of O, confirming the presence of the flavanoid, and further with no other impurities (Figure 4).

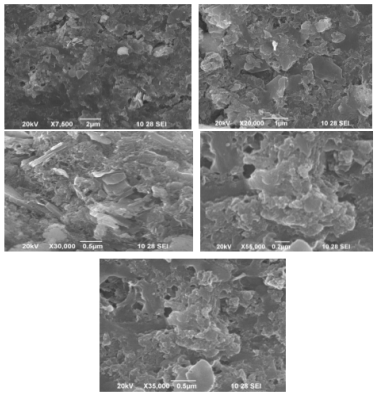

FIG 3 SEM Images of DIO-NPs – Apigenin composite at various magnifications

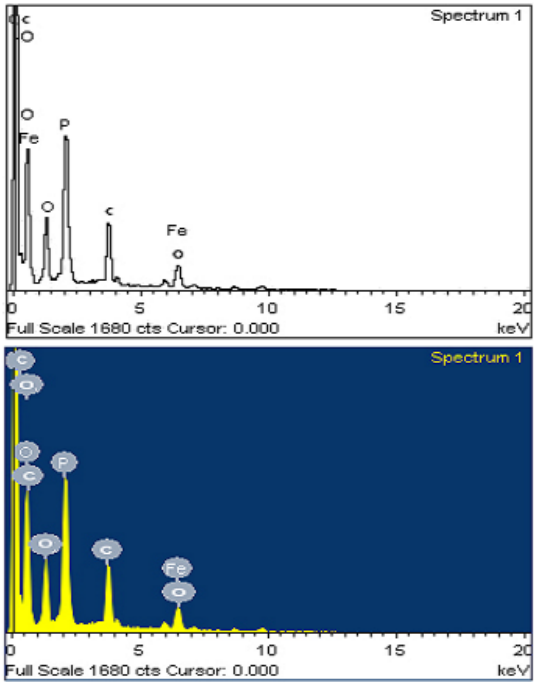

FIG 4. EDAX spectra of DIONC - Apigenin (Dextran coated Iron Oxide Nano particles encapsulated Apigenin)

Particle Size and Zeta potential determination

The average particle diameter of the DIONC – apigenin capsule was 208.2nm (Figure 5) and the zeta potential of dextran nanoparticle composite and flavonoid was found to be -18.7mV (Figure 6).

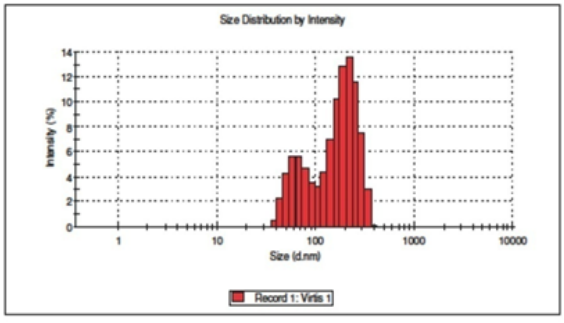

FIGURE 5. Particle Size of DIONC- Apigenin

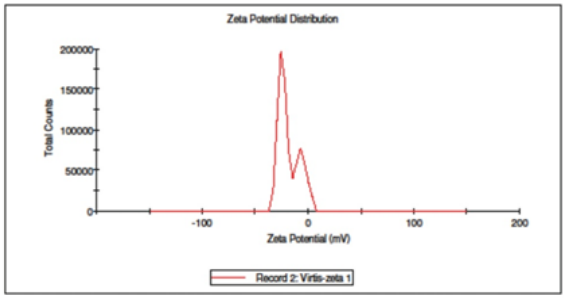

FIG 6. Zeta potential determination of DIONC- Apigenin

CONCLUSION

To conclude we report that the present study revealed that dextran based nanocarrier platform is a promising tool for drug encapsulation In order to investigate enhanced delivery of anticancer agent in further studies the composite was subjected to morphometric ,elemental analysis and particle size determination.

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