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Nanoemulsion drug delivery by ketene based polyester synthesized using electron rich carbon/silica composite surface

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ABSTRACT

A new carrier matrix for nanoemulsion drug delivery was synthesized from glycine as the raw material, using mesoporous/microporous electron rich carbon–silica composite surface (MAC_{800}). MAC_{800} was prepared from rice husk in two-stage carbonization. The surface area, pore volume, and pore size distribution of MAC_{800} were measured, using nitrogen adsorption isotherms at 77 K. The unpaired electron density of MAC_{800} was measured in electron spin resonance spectroscopy (ESR), using TEMPOL (4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl) as the reference spin probe. Glycine was converted into ketene at the surface of MAC_{800}, which further underwent radical polymerization to form a low molecular weight ketene polymer (LMKP) of ester structure. The structure and the properties of LMKP were confirmed through ^{13}C, ^{1}H and DEPT nuclear magnetic resonance (NMR) spectroscopy, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and size exclusion chromatography (SEC). The two hydrophilic drugs namely ciprofloxacin hydrochloride (CPH) and gentamicin sulphate (GS) were chosen for the nanoemulsion preparation and characterization. They were characterized for morphology, interaction of drugs with the polymer and their crystallinity, using HR-TEM, DSC and XRD, respectively. The encapsulation efficiency of the LMKP towards the drugs ciprofloxacin hydrochloride and gentamicin sulphate were 26% and 12%, respectively. The dissolution studies of the nanoemulsion were carried out for the pH 6.5, 7.4 and 8.0. The cytocompatibility studies were done for LMKP as well as nanoemulsion using Hep2 epithelial cells.

1. Introduction

The ciprofloxacin hydrochloride (CPH) and gentamicin sulphate (GS) can be used against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a low potential for toxicity [1]. The excess of the oral intake of antibiotics in significantly greater concentration would deposit in the urine rather than the serum. Consequently, such agents would have sufficient cytotoxicity to activate bladder cancer cells [2].

A controlled drug delivery system will transport and deliver a homogeneous and constant release of an appropriate antibiotic dose, while not disrupting the drug's effectiveness [3]. Among the drug delivery systems, nanoparticle systems are considered as a profound technology.

Nanoparticles are defined as solid, submicron-sized drug carriers that may or may not be biodegradable [4,5]. Nanoencapsulation of drugs involve the formation of drug-loaded particles with diameter ranging from 1 to 1000 nm [6]. Nanoparticles are receiving considerable attention for the delivery of therapeutic drugs. The submicron size of the nanoparticles offers a number of distinct advantages over microparticles, including relatively higher intercellular uptake.

Nanoemulsions, a type of nanoparticle drug delivery system are a group of dispersed particles used for pharmaceutical and biomedical aids that show great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies. They exist in a wide variety of forms that are dictated by the particle components. Developments in nanoemulsion technology are likely to lead to a much greater use of this medium in future pharmaceuticals.

An ideal drug carrier should satisfy a number of important requirements like favorable interaction with the drug, high delivery capacity, suitable drug release profile, preservation of drug activity during delivery to the target site, and no undesirable effects on the bioenvironment [7].

The most desirable polymeric matrix for drug delivery is one that is hydrophobic, stable, strong, flexible, soluble in organic solutions, has a low melting point and degrades linearly with the passage of time in an aqueous environment [8]. The nanoemulsions prepared from the low molecular weight polymers are promising carrier

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systems for drug delivery due to the safety of the material and fast degradation (hours, days) of the polymer [9].

Heterogeneous catalyst was considered as the best to synthesis the low molecular weight polymers. Polymer synthesis using heterogeneous catalyst remains weakly developed till now [10,11]. Heterogeneous catalysis is an attractive approach since it allows simple isolation of the desired compound, easy elimination of by-products and excess reagents, facile separation and recovery of the material [12]. Therefore, modern industrial polymerization processes use heterogeneous catalysts to circumvent reactor fouling (polymer adhesion at reactor walls) and to enable the direct morphology control of the polymer products [13]. Surface-initiated polymerization (SIP), which is a type of heterogeneous polymerization, promotes polymerization of monomers from initiator sites already attached to the catalytic surfaces [14]. This approach has been pronounced as a more promising and versatile method for preparing polymers.

Here we have developed a novel heterogeneous catalytic route through electron-rich carbon/silica composite for the synthesis of low molecular weight ketene polymer (LMKP) containing ester unit, which plays a major role in the nanoemulsion drug delivery system. Ketenes are derivatives of carboxylic acid, which contains the system of olefinic and carbonyl double bonds (C=C=O) [15]. The use of the ketene polymer would be advantageous because of its biocompatibility, biodegradability and the cost effectiveness, which is critical in the medical field. Controlled drug release from ketene based poly(ortho)esters was used for long term release studies [16].

Ketenes and dimethyl ketenes [17] are generally taken as the monomers for the synthesis of ketene polymers, but these substances are highly toxic in nature. But in this study we have used glycine, an aliphatic amino acid (a non-toxic source), as the starting material, and low molecular weight ketene polymers were successfully synthesized through the surface-initiated anionic polymerization.

2. Experimental

2.1. Materials

Glycine, polyvinyl alcohol, methanol, chloroform were purchased from E-Merck, Germany. CPH and GS were purchased from Sigma–Aldrich. The spin probe, 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (4-hydroxy TEMPO or TEMPOL), was obtained from Aldrich Chemical Company, Milwaukee, USA and used without further purification. The rice husk as the precursor material obtained from the agricultural industry was well washed with water several times for the removal of adhered starchy matter and used after drying in air oven at 110 °C for 6 h. The dried samples were then sieved to about 600-μm in size, and this fraction was used for the preparation of carbon/silica composite.

2.2. Preparation of electron rich activated carbon/silica composite

The carbon/silica composite (MAC_{800}) was prepared in two sequential steps: pre-carbonization and chemical activation. The washed rice husk was packed in an air-tight graphite crucible and heated at 400 °C for 4 h. The pre-carbonized material was activated, using ortho phosphoric acid (H_3PO_4) in the ratio of 1:2.3 (carbon:H_3PO_4) and sealed in an air-tight crucible. This was followed by heating to 800 °C at a heating rate of 5 °C/min using a temperature programmer and maintained at the final temperature for 1 h before cooling. Finally, the composite was washed with hot water to remove the excess phosphorus compounds until the wash water attained pH 7. The washed composite was dried at 110 °C to obtain the final product and it was labeled as MAC_{800}.

2.3. Characterization of the catalyst

The surface area and pore size distribution was determined from the N_2 adsorption–desorption isotherms. The N_2 adsorption–desorption isotherms of the composite was measured, using an automatic adsorption instrument (Quantachrome Corp. Nova-1000 gas sorption analyzer). The surface area of the activated carbon samples was calculated, using BET equation (m^2/g). The pore size distribution was determined, using BJH method. In addition, the *t*-plot method was applied to calculate the micropore volume and external surface area (mesoporous surface area).

Bruker X-band CW (EMX 102.7) spectrometer (Ettlinga, Germany) was used to obtain the ESR spectrum and spin density of MAC_{800}. Preliminary experiments were carried out with 4-hydroxy TEMPO (TEMPOL) as reference spin probe compound.

PerkinElmer model 1640 Infrared spectrometer (Massachusetts, USA) was used for the investigation of the surface functional groups. MAC_{800} was mixed with KBr of spectroscopic grade and made into pellets at a pressure of about 1 MPa. The sample was scanned in the spectral range of 4000–400 cm^{-1}.

2.4. Preparation of low molecular weight ketene polymer (LMKP)

About 100 mg of glycine (E-Merck, Germany) was dissolved in 2 ml of water and made up to 250 ml with methanol. The above mixture was fed into the spiral packed bed reactor at the flow rate of 1.0 ml/min. The reactor is comprised of 12 g MAC_{800}, which was maintained at the temperature of 10 °C (optimized). The solution collected at the outlet was subjected to conventional separation such as solar evaporation or vacuum distillation, whereby the alcohol escapes, leaving behind the LMKP. The LMKP in water was purified using extraction with chloroform.

2.5. Characterization of low molecular weight polymer

The ^1H, ^{13}C and DEPT nuclear magnetic resonance (NMR) spectra of the LMKP were recorded in $CDCl_3$ on Bruker AMX-400 MHz spectrometer (Germany). TMS was used as the internal standard.

The ATR-FTIR spectrum was recorded on Thermo Nicolat-320 spectrometer (AVATAR model), using the ATR attachment at room temperature. The sample was placed in a platinum liquid cell assembled in the ATR attachment. The spectrum was taken using EZ OMNIC 6.0 (Thermo Nicolat) software.

Number and weight-average molecular weights (M_n and M_w) and molecular weight distributions were determined by size exclusion chromatography (SEC), using a Waters 244 gel permeation chromatograph equipped with a refractive index detector. Polystyrene standards were used for calibration.

2.6. Preparation of nanoemulsion

The hydrophilic drugs CPH and GS, were formulated to nanoemulsion by the double emulsion (w/o/w) solvent evaporation method. About 100 mg of the LMKP was dispersed in 10 ml of water and homogenized for 2–3 min at 80 W in an ultrasonicator [Bandelin UW 2070]. About 10 mg of the drug was added slowly to the homogenized solution and again sonicated for 2–3 min to obtain a milky white suspension. The addition of chloroform to the above suspension forms a double layer of organic and aqueous phase. This was followed by continuous stirring with the addition of 20 ml polyvinyl alcohol (1%, w/v) and was homogenized for 2–3 min to result in the nanoemulsion.