Development of functionalized chitosan-coated carboxylated mesoporous silica: a dual drug carrier

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ABSTRACT
The principle aim of the present study is to synthesis a novel mesoporous silica-based dual drug delivery system (DDDS) with unique features for the delivery of amoxicillin (antibiotic) and thiamine hydrochloride (vitamin). The DDDS was characterized by Fourier transform infrared, X-ray diffraction, differential scanning calorimetry, scanning electron microscopy and atomic force micrographs analyses. The stimulus response behaviour of the drug carrier was studied by conducting swelling experiments as a function of pH and time. Drug release studies were carried out by mimicking gastric and intestinal conditions and found that the release of both drugs attained its maximum in the intestinal condition. The in vitro release data were analysed using Peppas's potential equation in order to predict the release mechanism. The release of antibiotic follows non-Fickian mechanism whereas that of vitamin was diffusion controlled. An in vitro cytotoxicity analysis was carried out on L929 cells and the results showed that the synthesized DDDS is safe. DPPH assay and antibacterial activity were also analysed. From the investigation, it is evident that the prepared DDDS has potential application as dual drug carrier.

1. Introduction
Drug delivery system (DDS) refers to different approaches, formulations, technology and systems for transporting a pharmaceutically active compound in the body as needed to achieve its desired therapeutic effect. They generally involve scientific site targeting within the body or facilitating systematic pharmacokinetics. It is a concept of heavily integrated dosage form and route of administration. DDS with single drug fails in the eradication of side effects of the drugs that has been introduced into the body, and there arises the need for dual drug delivery systems (DDDS). DDDS provides constant drug concentration within the therapeutic window and a method for the treatment of disease with minimum toxicity. Control release from DDDS has certain advantages like specific site delivery, low dosage frequency, zero premature release etc. But it remains as a great challenge for scientific community to develop a multi-drug loaded system capable of controlled release of each drug independently at its specific site with zero premature release. The combination of multiple drugs includes the most promising treatment strategies like reduction of side effects, suppressing drug resistance etc.

An efficient DDS should exhibit the qualities like biocompatibility, high loading of desired drug molecules, zero premature release, tissue specificity, ability of specific site direction and controlled release of drug with appropriate release rate. With these qualities in mind, many biodegradable materials, nanoparticles, dendrimers and liposomes have been synthesized and used as DDSs with controlled release of drugs followed by degradation of carrier at material certain chemical factors under physiological conditions. The major problem that came across was the difficulty to attain the zero premature release of drugs. Variety of structurally stable materials has been studied for drug delivery, among these silica materials with well-defined structures and surface properties are known for its biocompatible property with zero premature release of drugs.

Mesoporous silica nanoparticles (MSN) is widely used for controlled release of drugs now a days, because of its special properties like tuneable particle size, stable, rigid frame work, large surface area and the presence of dual functional surfaces (interior and exterior). The interconnectivity between the porous channels is absent in MSN due to its honey-comb like structure with cylindrical pores from one end of the sphere to the other. This property of unique porous structure of MSN helps in the zero premature release and degradation of drug to the
targeted site whether it is loaded with a single drug or dual drug. It is reported that mesoporous silica like MCM-41 and SBA-15 silicas are solids having hundreds of mesopores capable of encapsulating relatively large amount of biologically active molecules.[3] Studies were conducted and it is proved that silica is capable for storage and controlled release of therapeutically relevant drugs like antibiotics and so on.[13,14] Since MSN possess both internal and external surface, selective functionalization of both the surface with different moieties are possible.

MSN coated with biocompatible polymers helps to prevent the rapid degradation of the drug after its administration. The biocompatibility and biodegradable property of chitosan (CT) enhances its use in the field of drug delivery. [15–18] CT obtained from crustacean shells is a linear polysaccharide composed of randomly distributed β-(1–4) linkages between D-glucosamine units. It has been previously reported that CT is found to be responsive to the external pH due to the protonation of the amino groups in acidic pH. This property of CT assures that MSN coated with CT at low pH value remains stable even at physiological pH. Grafting of natural polymer will add new properties with minimum loss of its initial properties. Majority of the copolymers are synthesized by the graft polymerization of vinyl or acryl monomer on to the biopolymer.[19] Radical polymerization can be employed for the grafting of vinyl materials onto the polysaccharide. Modified polysaccharides with vinyl monomers could be applied for the design of stimuli responsive controlled system.[20]

Amoxicillin (AMO) is a widely used antibiotic that belongs to penicillin group of drugs. AMO is prescribed for the treatment of certain infections caused by bacteria.[21] AMO works by preventing the bacterial growth by killing them. Excessive intake of antibiotic causes severe stomach problems because of the imbalance of the harmless bacteria that synthesize vitamins in the intestinal lining. This leads to vitamin deficiency disorders. In order to overcome the side effects of antibiotics, vitamins are prescribed along with antibiotics.

Vitamins are micronutrients which are very much essential for growth and metabolism. Vitamins have traditionally been grouped based on their solubility in water or fat. Thiamine hydrochloride (THC) found in many vitamin B complex product as a combination with other B vitamins was first isolated from rice bran in 1926. THC is mainly used by type II diabetic patients to prevent kidney disease. The deficiency of THC causes beriberi characterized by pain, accumulation of body fluids and finally death.[22–24] Vitamins are not completely absorbed by the body, so a controlled release of vitamin is necessary in the intestinal region.

The present work involves the preparation of a highly stable, non-toxic and an effective solid support of MSN for drug storage and as a carrier for the controlled release of two drugs AMO and THC. Carboxyl functionalized MSN coated with CT is further modified with vinyl monomers to impart pH sensitivity. An investigation on compositional, structural and morphological analysis was carried out using techniques like Fourier transform infrared (FTIR), X ray diffraction (XRD), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and atomic force micrographs (AFM). Release profile of the two drugs was carried out at different pH (pH = 1.2 and pH = 7.4). The swelling studies were carried out in different pHs.

2. Materials and methods

2.1. Materials

(1-Hexadecyl)tri methyl ammonium bromide (CTAB), ethyleneglycoldimethacrylate (EGDMA) obtained from Alfa Aesar. CT, 3-cyanopropyl triethoxysilane, tetraethyl silicate (TEOS), AMO and cenic ammonium nitrate (CAN) were purchased from Sigma- Aldrich, USA. THC, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), methacrylic acid (MAA), sodium hydroxide, sulphuric acid and other solvents of analytical grade were obtained from E-Merck, Worli, India. L929 fibroblastic cell lines were purchased from NCCS, Pune. Trypsin in EDTA solution and Dulbecco’s Modified Eagle’s medium (DMEM) were obtained from HiMedia Laboratories, Mumbai, India. All the chemicals were used without further purification. Distilled water with specific conductivity less than 1 μΩ cm−1 was used throughout the study. L929 fibroblastic cell lines were purchased from NCCS, Pune. Trypsin in EDTA solution and DMEM were obtained from HiMedia Laboratories, Mumbai, India.

2.2. Synthesis of DDDS

2.2.1. Synthesis of carboxylated mesoporous silica (MSN-COOH)

First step involves the synthesis of cyano mesoporous silica (MSN-CN). About 1.00 g CTAB was dissolved in 250 mL water and adjusts the pH of the solution to alkaline by adding 0.1 N NaOH. Stir the solution for 30 min at 80 °C. TEOS (5 mL) was added drop wise with constant stirring (10 min). After 2 h, 0.4 mL 3-cyanopropyl triethoxysilane was added. The solution was kept under vigorous stirring for another 4 h at 60 °C. Obtained white precipitate was washed with distilled water and ethanol, and then dried. The MSN-CN thus obtained was then hydrolysed by treating with con. H2SO4 and refluxed for 24 h at 50 °C to obtain MSN-COOH. The product was extracted with acidic ethanol, centrifuged, washed with water and dried in vacuum.
2.2.2. Synthesis of poly(itaconic acid-copolymerized-methacrylic acid)-graft-chitosan-coated carboxylated mesoporous silica (P(IA/MAA)-g-CT MSNCOOH)

To about 0.4 g of MSN-COOH dispersed in 20 mL of distilled water, 0.15 g L⁻¹ of CT and 0.08 g of eDC were added and stirred for 3 h. To this solution, 2.193 g of CAN was added with constant stirring at 60 °C. After 30 min, the solution was cooled and 6.50 g IA, 4.3 mL MAA and 2 mL eGDMA were added. The reaction mixture was kept at 80 °C for 4 h. The precipitate was repeatedly washed with water to remove any homo polymers and dried.

2.3. Instruments for characterization

FTIR spectra of the samples were recorded with a Shimadzu FTIR spectrometer using KBr pellet technique. XRD patterns of the DDDS were recorded using X’Pert Pro X ray diffractometer using CuKα radiation. Thermal property of the samples was measured by DSC Mettler Toledo, DSC 822e. About 4–5 mg of the samples was heated at a rate 5 °C min⁻¹ in N₂ atmosphere. DSC expression curves were taken by heating the samples in the temperature range from −30 to 300 °C. SEM analysis was carried out using a JEOL JSM 6390 scanning electron microscope. This instrument uses cryo freezing method for taking photographs at 15 and 20 kV with a working distance of 6 mm and the frozen samples are coated with thin gold layer to make the surface conductive towards the electron beam. AFM of samples were taken with SCAN ASYST instrument by tapping mode. The concentration of THC and AMO in the supernatant solution was determined using JASCO-530 uV-visible spectrophotometer at λmax = 242 and 272 nm, respectively, using distilled water as reference. A μ processor Systronics pH metre (model 361) was used for all pH measurements. Drug release studies were conducted by using a temperature-controlled water bath shaker (Labline, India) with a temperature variation of ±1 °C.

2.4. Determination of carboxyl group by Boehm titration

Boehm titration is the most widely accepted and convenient method for the determination of the carboxyl group present in the system. About 0.15 g of MSN-COOH was shaken with 30 mL of NaHCO₃ at room temperature for 24 h. The contents were separated and the filtrate was collected. An aliquot amount of solution was mixed with excess of 0.05 N HCl and the CO₂ gas was boiled off and the solution was back titrated with 0.1 N NaOH using phenolphthalein as indicator.[25]

2.5. Swelling studies

Swelling studies of the drug carrier was performed in buffer medium. Swelling experiments were carried out by immersing 0.1 g of the dried P(IA/MAA)-g-CT MSNCOOH taken in a previously weighed dialysis bag in specific buffer volumes with pH = 1.2 and pH = 7.4. The weights of the swollen samples were collected at different intervals of time after wiping of the surface water with soft filter paper. The swelling (%) was calculated using the equation,

\[
\text{Swelling} (\%) = \frac{W_t - W_0}{W_0} \times 100
\]

where \( W_r \) and \( W_t \) are the weights of the swollen sample at a given time during swelling and the dry sample. Swelling (%) was also calculated as a function of pH by varying pH values from 1.0 to 10.0.

2.6. Loading of THC and AMO

About 0.1 g of MSN-COOH was added to 50 mL of THC solution having concentration 250 mg mL⁻¹ taken in stoppered bottles. The mixture was stirred at 1000 rpm for 12 h. The THC-MSNCOOH was collected by centrifugation at 10,000 rpm and the concentration of THC in supernatant was determined using UV-visible spectrophotometer at 242 nm. The drug loading efficiency (DLE) was calculated using the equation:

\[
\text{DLE} (\%) = \frac{\text{Amount of drug in supernatent}}{\text{Amount of drug used}} \times 100
\]

Similarly, the AMO was loaded by keeping 0.1 g of finely divided P(IA/MAA)-g-CT MSNCOOH onto drug solution (250 mg mL⁻¹). The concentration of AMO in supernatant was determined using UV-visible spectrophotometer at 272 nm and DLE was measured using above equation.

2.7. In vitro release of AMO and THC from P(IA/MAA)-g-CT MSNCOOH

Two different pH environments (pH = 1.2 and 7.4) were used to study the in vitro drug release of AMO and THC. The release experiments were conducted by preparing simulated gastric and intestinal fluid. About 0.5 g of the AMO and THC loaded P(IA/MAA)-g-CT MSNCOOH was added to 250 mL KCl-HCl solution (simulative gastric pH) and placed in a water bath shaker at 37 °C. At predetermined time intervals, 3 mL solution was taken out and the concentration of AMO and THC was determined using UV-visible spectrophotometer. In order to maintain a constant volume, equal volumes of fresh medium were replaced. The release studies were continued until no change in the concentration of the drugs was obtained in the solution. The same release experiment was carried out by using in 250 mL of Na₂HPO₄-KH₂PO₄ solution (simulative intestinal pH).
2.8. Cell viability of P(IA/MAA)-g-CT MSNCOOH on cultured L929 cell lines

Cytotoxic analysis of P(IA/MAA)-g-CT MSNCOOH was determined by MTT assay on L929 fibroblastic cell lines. Cells were maintained in DMEM medium supplemented with 10% FBS at 37 °C in humidified atmosphere with 5% CO₂ in an incubator. The cells were trypsinized for 2 min. Cells cultured were incubated with 6.25, 12.50, 25.00, 50.00 and 100.00 μg mL⁻¹ of P(IA/MAA)-g-CT MSNCOOH for 24 h. Cells were then washed with PBS and stained with 50 μL MTT solution. After 4 h, the medium was discarded and the formazan crystals were eluted with 200 μL isopropanol. The absorbance of the solution was measured at 570 nm using a microplate reader. Viability (%) was calculated using the formula

\[
\text{Viability} (\%) = \left( \frac{OD_{\text{test}}}{OD_{\text{control}}} \right) \times 100
\]

2.9. Determination of free radical scavenging activity

The free radical scavenging ability of P(IA/MAA)-g-CT MSNCOOH was evaluated by using DPPH assay method. Different concentrations of the P(IA/MAA)-g-CT MSNCOOH (1.25–20.0 μg mL⁻¹) were mixed with 0.1 mM DPPH solutions. The reaction mixture was incubated in dark at 37 °C for 30 min. The decrease in absorbance (A) of the DPPH solution after incubation was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as the standard control. The scavenging percentage was calculated using the formula:

\[
\text{Scavenging} (\%) = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100
\]

2.10. Antibacterial assay of P(IA/MAA)-g-CT MSNCOOH

The in vitro antimicrobial activities of AMO-P(IA/MAA)-g-CT THC MSNCOOH were tested against a Gram-positive bacteria (Staphylococcus aureus, S. aureus) and a Gram-negative bacteria (Escherichia coli, E. coli). Antibacterial activity was determined by using twofold serial dilution methods. A stock solution of DDS (10.0 mg mL⁻¹) was prepared and diluted to 3.15, 6.25, 12.5, 25 and 50 μg mL⁻¹. Test pathogens were spread on agar test plates and sterile wells. Each well was loaded with desired concentration of AMO-P(IA/MAA)-g-CT THC MSNCOOH and incubated overnight. The detection of clear inhibition zones (mm) around the well in the inoculated plates is an indication of antimicrobial activities of the carrier under study.

3. Results and discussion

3.1. Preparation of P(IA/MAA)-g-CT MSNCOOH

Synthesis of P(IA/MAA)-g-CT MSNCOOH, for the delivery of AMO and THC, involves multiple steps. The first step involves the synthesis of MSN-CN by using TeOS and 3-cyanopropyl triethoxysilane. Acid hydrolysis of MSN-CN using con. H₂SO₄ results in the formation of MSN-COOH (Scheme 1). The second step involves the synthesis of CT coated MSN-COOH (CT MSNCOOH) and free radical graft co-polymerization using vinyl monomers (Scheme 2). CT MSNCOOH was synthesized by simple EDC coupling reaction between carboxyl group of MSN-COOH and amino group of CT. In order to impart pH and thermo sensitive behaviour to CT MSNCOOH, it was further modified by free radical graft polymerization using two vinyl monomers IA and MAA using CAN as radical initiator and eGDMA as cross linker. On heating, Ce(IV) from CAN form complex with CT MSNCOOH then it reduces to Ce(III) with the release of a proton from primary hydroxyl group of CT to form CT MSNCOOH macroradical. These free radicals then react with the monomers to initiate the graft polymerization. The two monomers form radicals, the monomer with low molecular weight MAA gets attached first followed by IA. The presence of cross linking agent, eGDMA in the system will create three-dimensional interpenetrating polymer networks.

The amount of carboxyl functional groups in P(IA/MAA)-g-CT MSNCOOH was estimated by Boehm titration method and it is found to be 1.86 mequiv g⁻¹.

3.2. Characterization studies

The FTIR spectra of MSN-CN, MSN-COOH, THC-MSNCOOH, CT-THC MSNCOOH, P(IA/MAA)-g-CT MSNCOOH and AMO-P(IA/MAA)-g-CT THC MSNCOOH are given in Figure 1. In the case of MSN-CN, a peak at 2240 cm⁻¹ shows the stretching vibration frequency of –C≡N of cyano group and a peak at 1635 cm⁻¹ indicates the bending vibration of –OH group. Peaks at 1000, 1250, 3000 cm⁻¹ could be attributed to characteristic Si–O asymmetric stretching and Si–OH stretching vibrational frequency. The spectrum of MSN-COOH shows an additional absorption peak at 1725 cm⁻¹ which indicates the C–O stretching vibrational frequency of carboxyl group. A broad peak at 3369 cm⁻¹ shows the –OH stretching frequency due to the retention of water molecules on the siliceous material on the surface of MSN-COOH. The absence of peak at 2240 cm⁻¹ is due to the completion of hydrolysis of –CN groups to –COOH groups catalysed by H₂SO₄. A broader peak at 3362 cm⁻¹ in the spectrum of THC-MSNCOOH is the evidence for the successful encapsulation of THC in the interior pores. Peaks at 793, 1051 cm⁻¹ are due to Si–O–Si bending,
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X-ray diffraction (XRD) pattern verifies the porous nature of MSN-CN, as the silica particles have amorphous nature. The broad peak at 2θ ranging from 15° to 30° confirms the amorphous nature of MSN-CN. Both MSN-CN and MSN-COOH exhibit a distinct diffraction peak in the range 20°–30° indicating the characteristic diffraction pattern of the ordered hexagonal symmetry. This confirms the structural stability of the prepared mesoporous material. Hydrolysis of MSN-CN using H₂SO₄ only converts cyano group to carboxylate group without rupturing ordered structure. A single diffraction peak was observed at 22.7° for THC-MSNCOOH. The absence of characteristic crystalline peak for THC confirms the absence of vitamin on the surface of MSN-COOH. P(IA/MAA)-g-CT MSNCOOH give a XRD spectrum with new peaks at 2θ = 19.3°, 20.3°, 25.3°, 29.9° and 35.6°. The presence of these new peaks indicates the proper grafting of MAA and IA onto CT MSN-COOH.

Figure 3 shows the DSC thermogram of MSN-COOH, CT MSN-COOH and P(IA/MAA)-g-CT MSNCOOH. Pure MSN-COOH and CT MSNCOOH has sharp endothermic peak around 110–120 °C. This endothermic peak indicates the presence of water molecules entrapped on the porous structure. The glass transition temperature of pure CT is symmetric and asymmetric stretching vibrational frequency. In the spectrum of CT-THC MSNCOOH, peak at 1680 cm⁻¹ is due the presence of an amide group formed by the polymerization between MSN-COOH and the –NH₂ group of CT. A broad band at 3328 cm⁻¹ can be attributed to the presence of a primary –OH group. Besides, C–H stretching vibration is also present at 2980 and 2813 cm⁻¹. The absorption at 1494 cm⁻¹ is due to C–N stretching vibration. Peaks at 839 and 1228 cm⁻¹ show the presence of saccharide structure of CT. The graft polymerization of P(IA/MAA)-g-CT MSNCOOH could be attributed to the presence of peaks at 2929,1530 and 1360 cm⁻¹ which indicates the asymmetric stretching, asymmetric deformation and symmetric deformation of C–H and C–C group. A shift in the characteristic stretching frequency of –OH group confirms the existence of grafting in the sample. The spectrum of AMO-P(IA/MAA)-g-CT-THC MSNCOOH shows a peak at 3205 cm⁻¹ indicates the presence of intermolecular H-bonded –OH stretching. The presence of a sharp peak at 1700 cm⁻¹ indicates the presence of the carboxyl group in AMO.

Figure 2 shows the XRD patterns of MSN-CN, MSN-COOH, THC-MSNCOOH and P(IA/MAA)-g-CT MSNCOOH.

Scheme 1. Synthesis of MSN-COOH.
Scheme 2. Synthesis of P(IA/MAA)-g-CT-MSNCOOH.

Figure 1. FTIR spectra of (A) MSN-CN, (B) MSN-COOH, (C) THC-MSNCOOH, (D) CT-THC MSNCOOH, (E) P(IA/MAA)-g-CT MSNCOOH and (F) AMO-P(IA/MAA)-g-CT-THC MSNCOOH.

Figure 2. XRD patterns of (A) MSN-CN, (B) MSN-COOH, (C) THC-MSNCOOH and (D) P(IA/MAA)-g-CT MSNCOOH.
around 200 °C.[26] This peak was shifted to 194 °C in the CT MSN-COOH and confirms the proper modification of MSN-COOH with CT. From the DSC thermogram it is clear that some phase change occurred in the final polymer at 159 °C. The melting of final polymer at lower temperature compared to other two samples (MSN-COOH and CT MSN-COOH) is due to the presence of more hydrophilic IA and MAA, which absorb more water.[27] The endothermic peak at 176 °C in the final polymer indicates the decomposition/degradation of polymer. The DSC thermogram of P(IA/MAA)-g-CT MSN-COOH confirms the presence of some crystalline domains in amorphous material.

Figure 3. DSC thermogram of (A) MSN-COOH, (B) CT MSN-COOH and (C) P(IA/MAA)-g-CT MSN-COOH.
SEM was used to study the change in morphological features of MSN-CN, MSN-COOH, THC-MSNCOOH, CT-THC MSNCOOH, P(IA/MAA)-g-CT MSNCOOH and photographs are given in Figure 4. The surface morphology shows that each sample is entirely different from each other and thus confirms the proper modification on each step. The MSN-CN, MSN-COOH, THC-MSNCOOH has fluffy nature with decrease in size of particle after each modification. Loading with THC onto MSN-COOH completely removes the aggregation of particles. It indicates the successful encapsulation of THC in the MSN-COOH. The surface morphology of CT-THC MSNCOOH was distinct from that of MSNCOOH and THC-MSNCOOH. A layer of CT was present on the surface of THC-MSNCOOH. SEM image of P(IA/MAA)-g-CT MSNCOOH was found to be flaky in nature with larger surface area.

Figure 5 represents the AFM images of MSNCOOH, CT MSNCOOH, P(IA/MAA)-g-CT MSNCOOH. The application of AFM in drug developing is becoming more important with its qualities like minimal sample preparation and use in ambient conditions like the possibility to operate with high resolution in air and at the nanometre scale. AFM images were used to study the surface morphology of samples. AFM images of all samples appear to be distinct from each other with increased particle size confirms the structural modification in each stage. An AFM image of MSN COOH shows uniform distribution of particles with average particle size of 3.718 nm while CT coating MSN COOH decreases the peak height (5.14 nm). After graft copolymerization of IA and MAA onto CT MSNCOOH, the particle size increases to 24.16 nm.

Figure 4. SEM images of (A) MSN-CN, (B) MSN-COOH, (C) THC-MSNCOOH, (D) CT-THC MSNCOOH and (E) P(IA/MAA)-g-CT MSNCOOH.

Figure 5. AFM images of (A) MSNCOOH, (B) CT MSNCOOH and (C) P(IA/MAA)-g-CT MSNCOOH.
3.3. Swelling studies

3.3.1. Effect of pH

The pH responsive behaviour of P(IA/MAA)-g-CT MSNCOOH was carried out by placing 0.1 g of the drug carrier in buffer solution with varying pH values from 1.0 to 10.0 at a temperature of 37 °C (Figure 6). From the graph it was observed that with an increase in pH there was an increase in percentage swelling also. The pKa values of IA are 3.85 and 5.45 and that of MAA is 4.66. The H+ ion strength will be high when the pH is less than pKa and this will effectively suppress the ionization of –COOH groups of both IA and MAA. Therefore, at acidic pH, the flexibility of the polymeric chain is very low due to the protonation of the carboxylate anions. But it was observed that with an increase in pH, both IA and MAA ionize and carboxylate anions (COO−) are formed in the polymeric structure. This led to an increase in anionic repulsion and an extensive swelling of P(IA/MAA)-g-CT MSNCOOH. The graft polymerization of CT MSNCOOH using monomers like IA and MAA introduces certain hydrophilic moieties like –COOH. These hydrophilic moieties increase the hydrophilicity of P(IA/MAA)-g-CT MSNCOOH which in turn results in a dramatic increase in the swelling at pH 7.0.

3.3.2. Effect of time

Figure 7 represents the results of time-dependent swelling experiments carried out at two different pH, 1.2 and 7.4 at a temperature of 37 °C. From the figure it is clear that the swelling (%) increases with increase in time up to 4 h and then it tends to equilibrium. A greater swelling at pH 7.4 than at pH 1.2 was observed due to the increase in anionic repulsion between COO− ions. The existence of hydrophilic moieties like carboxylic groups tends to increase the hydrophilicity of a polymeric material and consequently increases its swelling values attained at equilibrium. At acidic pH (pH = 1.2), the acid groups exist in undissociated form and this led to a decrease in swelling behaviour.

3.4. Drug release profiles

Before the in vitro release analysis of dual drugs (THC and AMO) from synthesized DDSs, we measured the DLE (%) for THC onto MSN-COOH and AMO onto P(IA/MAA)-g-CT MSNCOOH. DLE (%) was found to be 80.59% for THC and 68.46% for AMO.

In order to mimic the gastrointestinal condition, we prepared two buffer solutions of different pHs (pH = 1.8 and pH = 7.4) for the release of both AMO and THC. The drug release (%) of both the drugs is shown in Figure 8 for AMO and THC. The release % of AMO was found to be 44.4 and 89.4% for the pH of 1.8 and 7.4, after 7 h. Similarly, the release % of THC at pH of 1.8 and 7.4 was found to be 46.1

![Figure 6. Swelling of P(IA/MAA)-g-CT MSNCOOH as a function of pH.](image)

![Figure 7. Swelling of P(IA/MAA)-g-CT MSNCOOH as a function of time.](image)

![Figure 8. Cumulative release of AMO and THC from P(IA/MAA)-g-CT MSNCOOH at pH 1.8 and 7.4.](image)
and 98.5%, respectively, after 24 h. From the release % values it was clear that the drug release from P(IA/MAA)-g-CT MSNCOOH is highly pH dependent. As the pH increases, there was a rise in the drug release which could be attributed to the increased swelling capacity of the polymer. This leads to the ionization of both the –COOH groups and effectively reduces the H-bonding interaction. From the figure it is clear that with increase in time there was an increase in the release % which attains maximum at 7 h for AMO and 24 h for THC.

The in vitro release data have been analysed using Peppas’s potential equation [28] to understand the release mechanism.

\[
M_t = M_\infty k t^n
\]

(5)

where \( M_t \) and \( M_\infty \) is the amount of drug released (%) at a time \( t \) and the amount of drug released completely, \( k \) is the apparent release rate and \( n \) is the diffusion exponent which explains the nature of the diffusion mechanism.

For identifying release mechanism, the release exponent \( (n) \) value can be used. When \( n = 0.43 \), Fickian diffusion dominates in which the diffusion of drug play an important role in the release process. The value of \( n = 0.85 \) indicates a swelling controlled release mechanism and the main reason for the drug release is the expansion of the polymer. The values of \( n \) between 0.43 and 0.85 indicate the non-Fickian drug diffusion mechanism, where the overall drug release rate is controlled by both diffusion and swelling.[29]

Plots of \( M_t/M_\infty \) versus time for both AMO and THC at pH 7.4 are shown in Figure 9. The plots are good fit with \( R^2 = 0.989 \) and 0.997 for both AMO and THC. The kinetic parameters \( k \) and \( n \) calculated from the graph and was found to be 0.703 and 0.013 for AMO and 0.437 and 0.002 for THC, respectively. From release kinetic model study, the \( n \) value (0.703) obtained for AMO at pH 7.4 clearly shows that the drug release from the drug carrier is a combined

**Figure 9.** Release kinetics of AMO and THC from P(IA/MAA)-g-CT MSNCOOH at pH 7.4.

**Figure 10.** In vitro cytotoxicity of P(IA/MAA)-g-CT MSNCOOH on L929 Cells after 24 h incubation.

**Figure 11.** DPPH scavenging activity of P(IA/MAA)-g-CT MSNCOOH.
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MSNCOOH was compared with ascorbic acid as standard. The inhibition (%) was found to be increased with increase in concentration of P(IA/MAA)-g-CT MSNCOOH (Figure 11). 20 μg mL−1 P(IA/MAA)-g-CT MSNCOOH show approximately 48% inhibition percentage. This lower IC50 confirms that the synthesized P(IA/MAA)-g-CT MSNCOOH have excellent free radical scavenger property.

3.7. Antibacterial activity of P(IA/MAA)-g-CT MSNCOOH

In vitro profiling of antibacterial activity of AMO loaded dual drug carrier was studied using five different concentrations ranging from 3.0 to 50.0 μg mL−1. Antibacterial properties were studied against E. coli (gram negative bacteria) and S. aureus (gram positive bacteria) by plate assay and formation of clear zone was measured. Figure 12 clearly indicate that AMO-P(IA/MAA)-g-CT-THC MSNCOOH has antibacterial activity against only E. coli and not towards Staphylococcus. The zone of inhibition was 1.1 mm for E. coli at 50 μg mL−1 concentration. The inhibition zone in the range of 1.0–2.0 mm obtained in the present study may be due to the low concentration of AMO-P(IA/MAA)-g-CT-THC MSNCOOH used for analysis. Thus, minimum inhibitory concentration of AMO-P(IA/MAA)-g-CT-THC MSNCOOH for E. coli was found to be 50 μg mL−1. The AMO and THC loaded dual drug carrier can be particularly active towards gram negative bacteria compared to gram positive bacteria. This result indicates that the prepared AMO-P(IA/MAA)-g-CT THC MSNCOOH is highly selective in nature.

4. Conclusions

In the present work, we synthesized a novel MSNs-based DDDS with unique features like increased stability at various physiological conditions and release rate at specific process of both diffusion and polymer matrix expansion. The n value (0.437) obtained for THC at the same pH shows that the drug release is mainly diffusion controlled. The diffusion controlled release includes the penetration of liquid into the silica matrix and dissolves the drug which is encapsulated in the interior pores. The dissolved drug then diffuses out after 24 h to the exterior liquid. The release constant k values for AMO and THC are 0.013 and 0.002, respectively, shows the mild interaction between the drug and the polymer matrix. Prolonged release of the drug from the drug carrier is indicated by smaller values of k. Thus, the k and n values suggest the release of AMO at 7 h and THC after 24 h from the polymer matrix is controllable.

3.5. Evaluation of cytotoxicity on L929 cells

The results of cytotoxicity analysis of synthesized P(IA/MAA)-g-CT MSNCOOH was evaluated by MTT assay using L929 cells lines and it is essential to ensure the biocompatibility. L929 fibroblast cell lines were widely used as an in vitro model for toxicological studies. The result was shown graphically in Figure 10. P(IA/MAA)-g-CT MSNCOOH shows dose dependent cytotoxicity after 24 h incubation. The viability (%) was found to decrease with increase in concentration. 92.51 and 60.70% viability was found for 6.25 and 100.0 μg mL−1 of P(IA/MAA)-g-CT MSNCOOH. The prepared dual drug carrier shows greater than 60.0% viable cells for all concentration tested. This indicates that P(IA/MAA)-g-CT MSNCOOH has no significant toxicity and assures to use as a safe dual drug carrier.[30]

3.6. DPPH assay

The DPPH method was used to determine the antioxidant activity of P(IA/MAA)-g-CT MSNCOOH. The amount of DPPH radical formed on reaction with P(IA/MAA)-g-CT MSNCOOH was compared with ascorbic acid as standard. The inhibition (%) was found to be increased with increase in concentration of P(IA/MAA)-g-CT MSNCOOH (Figure 11). 20 μg mL−1 P(IA/MAA)-g-CT MSNCOOH show approximately 48% inhibition percentage. This lower IC_{50} confirms that the synthesized P(IA/MAA)-g-CT MSNCOOH have excellent free radical scavenger property.
pH condition. The materials obtained in each stage during the synthesis of the drug carrier were characterized using techniques like FTIR, XRD, DSC, SEM and AFM which provide clear information about the functional groups, modifications, morphologically and topologically based surface changes. The swelling capacity of the drug carrier was found to be varying with change in pH and time. It was evident from the in vitro drug release studies that the drug release was easier in intestinal region. The drug release kinetics for AMO follows non-Fickian mechanism in which AMO release from the drug carrier was found to be a combined process of diffusion and polymeric matrix expansion while the release of THC was found to be diffusion controlled. In vitro cell viability analysis on L929 cell lines after 24 h of incubation confirms the biocompatibility of prepared dual drug carrier. Free radical scavenger property of P(IA/MAA)-g-CT MSNCOOH was confirmed by DPPH assay. Antibacterial analysis shows AMO-P(IA/MAA)-g-CT-THC MSNCOOH is highly active towards gram negative bacteria compared to gram positive. Thus, the study presents a dual drug carrier to overcome side effects caused by the excessive intake of AMO by the parallel supply of THC.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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