Optimization of Sustained Released based Drug Delivery system for Destruction of Cancer cells and causative agents for Typhoid and COVID -19 using nano carrier entrapped herbs

Synopsis Submitted by

Pritha Pal

DOCTOR OF PHILOSOPHY (SCIENCE)

School of Materials Science & Nano Technology
Faculty of Interdisciplinary Studies, Law & Management
Jadavpur University
Kolkata-700032
India

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Synopsis

This thesis is focused to find the efficacy of sustained released herbal drugs componentsto inhibit the growth of *S.typhi* bacteria, causative agent of typhoid, to fight against the cytokine storm caused by spike protein induced pathological changes of COVID-19,to retard the growth of HepG2 cell line of hepatocellular carcinoma disease. The active phytochemicals are extracted from the herbal drugs. These phytochemicals are the most stable active ingredients which are capable of inhibiting the growth of the above mentioned diseases. The herbal drugs chosen here are *Aloevera* and garlic (*Allium sativam*) which have many phytochemicals capable of curingthe above mentioned diseases. For any sustained released drug delivery, carrier or matrix is important for entrapment and release of herbal phytochemicals in different pH media.

Literature review is done to get detail about nano materials,herbal drugs and the importance of phytochemicals present in the drugs, drug release kinetics models and different substrate for encapsulation and release of drugs. Based on the literature review two herbal drugs are selected, *Aloe vera* and garlic and two microbial diseases are chosen – typhoid (bacterial), covid – 19 (viral) and neoplastic disease (liver cancer). Introduction and literature review in chapter 1 also helped to identify the active and stable phytochemicals of selected herbs and their correlation to retard the diseases. All data are collected from books, review articles and journals to review properly and seta goal for the objective of the thesis.

The goal of this thesis is to synthesize silica nanoparticles with different morphology (irregular and sphere shaped) and nano clay as porous substrate to encapsulate herbal extract and release them in SBF & SGF are described in chapter 2. For this herbs are extracted with different solvents and entrapped within all the nano carriers. Then release kinetic study of herbal extracts from silica nanocarriers and nanoclay in both SBF & SGF medium is done to optimize the process parameters. And finally, in the application part, the efficacy of sustained released herbal extracts of *Aloevera* and garlic (*Allium sativum*) are studied for the treatment of typhoid (bacterial disease), Covid-19 (viral disease) and liver cancer (neoplastic disease).

Chapter 3 describes the entire process to achieve the objectives of the thesis. Detail process for synthesis of nano carriers, drug encapsulation and release in different pH and kinetic study is

elaborated. Operations of the characterizing tools and their working parameters are also mentioned. In the application part of the released drug, the procedures for antibacterial, antiviral and anti-cancer study are described.

In chapter 4 two types of SiO_2nanocarrierwith different porosity are synthesized by the sol-gel method with acid catalyst (for irregular shaped silica) and base catalyst (for sphere shaped silica). Synthesized silica nanoparticles are characterized byx-ray diffraction (XRD) to examine the nature of synthesized carriers. Scanning electron microscopy (SEM) and Field-emission scanning electron microscopy (FESEM) is done to see the morphology of synthesized SiO_2. The active component of *Aloe vera* is Aloin, entrapped within all the SiO_2 nanocarriers and a detail kinetic study of the release of aloin in SBF & SGF is carried out. Fourier transform infrared spectra (FTIR) is done to confirm the entrapment of herbal components in the nanocarriers. The efficacy of released aloin as an antibacterial, antiviral, anticancer treatment is also evaluated.

Entrapment of the active component of garlic Diallyl disulphide (DADS)in all types of silica nanoparticles and the study of release kinetics of DADS in two different mediums (SBF & SGF) from the all the carriersare discussed in details in chapter 5. Antimicrobial, anticancer, and antiviral properties of released DADS are also evaluated in this chapter.

Modification of nano clay carrier is done with Na to exfoliate the clay for better entrapment of herbal drugs. XRDis done to examine the exfoliation of clay. FESEM and transmission electron microscopy (TEM) is done to see the structure and interlayer spacing of clay. Aloin and DADS are entrapped within modified nano clay and they are released in SBF and SGF. The release kinetics of the two herbal extractions in both media is studied and kinetic modeling is done. FTIR study confirms the entrapment of drugs in nano clay and UV-Vis spectrophotometer is used to study the kinetics. Finally the Highest release percentage of both drugs is considered for applicationin antimicrobial and anticancer agent as described in chapter 6.

Over all conclusion of the thesis work is drawn in Chapter 7. Comparisons of the two drugs in controlling the mentioned diseases are also discussed here.

Outcome of the work:

To achieve sustain release of drugs, both the drugs are extracted and entrapped with in bio compatible porous silica nano particles of different morphology and within nanoclay. Entrapment of the herbal extracts within nanoparticles is confirmed by FTIR plot. Presence of C=C bond and C=O bond of aloin which is the active component of *Aloe vera* and C-S and S-S bonds of DADS which is the active component of garlic in the FTIR plot of both drug entrapped silica nanoparticles and nano clay indicates the successful entrapment of the drugs in the nano carriers. Silica nano particles have two different types of morphology - irregular shape and spherical shape. In each type of nano silica nano particles, the molar ratio of the precursors i.e., TEOS, ethyl alcohol and water also control porosity of the silica nano particles. Spherical shaped silica nano particles are more porous as revealed from BET analysis with higher surface area. It is also inferred from BET study that with increase in ethyl alcohol percentage the gel porosity increases in case of acid catalyzed irregular shaped silica nano particle with maximum surface area is achieved at the molar ratio of TEOS: ethyl alcohol: water = 1: 6: 1. Whereas, in case of base catalyzed sphere shaped silica gel samples maximum surface is achieved at the molar ratio of 1: 2: 1 of TEOS: ethyl alcohol: water. It is observed drug loading efficiency of the nano carrier increases with increase in the surface area of the nano carriers. Thus, drug loading efficiency of spherical shaped nano particles (maximum 90.53% for aloin and 95.75% for DADS) is higher compared to irregular shaped nanoparticles (81.58% for aloin and 91.62% for DADS). Entrapped drug is released in two media- simulated body fluid (SBF, pH 7.4) and simulated gastric fluid (SGF, pH 1.2).Rate of drug release in the media depends upon factors like the nature of the drugs, pH of the medium and porosity of the nano carriers. Due to higher porosity, dissolution rate of both drugs are more from spherical shaped silica nano particles (81% for both aloin and DADS) compared to irregular shape silica nano particles (68% for aloin and 57% for DADS). Moreover, it is also observed that cumulative release rate of aloin is higher in SGF (68% from irregular shaped and 81% from spherical silica nano particles) as compared to SBF (52% for irregular shaped and 60% for spherical silica nano particles) due to acidic nature of SGF. So, the process parameters for the synthesis of silica nano carrier as a porous substrate for use in drug delivery is optimized at 1: 2: 1 of TEOS: ethyl alcohol: water with CTAB surfactant in basic medium.

Nano clay is also found to be a very good substrate for entrapment of both aloin and DADS with entrapment efficiency 87.17% for aloin and 78.82 % for DADS. Drug release from nano clay follows the same pattern as silica nano particles with higher release in SGF (79% aloin and 70% DADS) compared to release in SBF (69% aloin and 64% DADS).

Detail kinetic study helps to identify the kinetic models for release of aloin and DADS in SBF and GIF from all types of silica nano carriers and nano clay. Both burst release and sustained release of drugs from the nano carriers are considered for kinetics study.

Antibacterial study of released aloin and DADS against S. typhi proves the efficiency of the herbs as an antibacterial agent. MIC values of both pure aloin and DADS are evaluated as 2.5 mg/ml and 0.941 mg/ml respectively. CFU count of released aloin and DADS are found to be 56 (from aloin entrapped spherical silica, released in SGF) and 68 (from DADS entrapped spherical shaped silica, released in SGF) respectively. Released aloin is found to be more efficient in controlling *S. typhi* growth compared to released DADS and the best media for aloin release is found to be gastric fluid. CFU count for aloin and DADS released from clay are 57 and 62 respectively. Thus, clay can be considered as an efficient bio compatible substrate for drug delivery. Microscopic images reveal the deformation of the bacterial cells in presence of the released drugs. The cells are fused together in presence of both aloin and DADS.

In antiviral study, aloin in the preventive experimental sets in the chicken egg model can counteract and prevent damaging action of pro-inflammatory cytokines IL-6, IL-8 and IL-1β by increasing more gene expression of anti-inflammatory cytokine IL-10. It indicates that aloin could prevent the damage of different organs in cytokine storm, which is the main pathogenic factor in morbidity and mortality in SARS-CoV-2 infections, mediated mainly by the S-protein of the virus. IFNα and IFNβ are also mildly increased indicating a beneficial role in this condition.IL-1ß is increased and may be detrimental to healthy system but DADS Raw and DADS Release are capable of counteracting those detrimental changes by markedly increasing anti inflamatory cytokine gene IL-10 and thus it appears that both DADS Raw and DADS Release will give overall beneficial effect in SARS-CoV -2 pathological changes and appears to be an excellent agent in the management of corona virus infection.

In anticancer study, at first IC_{50} value of pure drugs is determined. For this both the drugs are diluted from 10 to 20 times from a stock concentration of 1mg/ml. From spectrophotometric analysis IC_{50} value is obtained as 12th dilution for aloin and 14th for DADS. This concentration is considered for methylene blue assay as it is regarded as the effective concentration against the hepatocellular carcinoma cell line. In case of aloin treatment this is prominent that 80-90% of cells are dead and more than 90% of cells are dead in DADS treatment as observed from methylene blue assay.

Future scope of work

- Study of pharmacokinetic behavior of pure drugs as well as drug entrapped nanocarriers of both silica and clay and compare their pharmacokinetic profile.
- Study of in vivo model of antimicrobial assay as well as anticancer assay with best results depending upon in vitro study.
- Study of histopathology after Covid-19 treatment with both drugs and observe the tissue damage after this viral infection.

Pritha Pal
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Mahua Ghosh Chaudhuri.
2/5/23

Dr. (Ms) Mahua Ghosh Chaudhuri
Associate Professor
School of Materials Science and Nano Technology
Jadavpur University
Kolkata-700 032, India

04/05/2023

Dr. Satadal Das
MBBS, MD, DCP, DBMS.