Development of Drug Delivery System Using Bio based Calcium carbonate Nanoparticles

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ABSTRACT

5-Fluorouracil (5-FU) is a well known anti-cancer drug which is commonly used for several cancer therapies. 5-FU is administered subcutaneously or intravenously to patients, and these results in low patient compliance. A better way to administer this 5-FU is in immediate need [1-2]. Calcium carbonate (CaCO3) nanoparticles are highly porous, biocompatible, biodegradable, and have pH-sensitive properties. These properties make CaCO3 nanoparticles one of the best candidates for a drug delivery system specially for 5-FU. In this research, the CaCO3 nanoparticles were derived from egg shells using sonochemical and mechanochemical methods. The glycerol and acetic acid were used as solvents in this process to avoid other toxic solvents. These nanoparticles were loaded with 5-FU drug by physical absorption. Oral administration of 5-FU can be beneficial to colon cancer patients as it could not only reduce the pain resulting in better compliance, but also mimic the physiological fate of 5-FU. A pill has been produced from 5FU-loaded CaCO3 nanoparticles. Ideally, these pills will be optimal for a successful delivery of 5-FU to the colon, after a coating with Eudragit S100 polymer. *In vitro* release was studied and analyzed. The release profiles show that both nanoparticles release large amounts of drug initially, and then slow to release at a steady rate for 3 hours. After 3 hours, the nanoparticles were tested for remaining drug and found that (EDTA solution (pH 8.0)) large amounts of the drug still found within the nanoparticles. We also compare the drug release profile of these two nanoparticles. With these initial results we air to produce a
pill with 5FU-loaded CaCO3 nanoparticles that will deliver to the colon with minimum cytotoxicity to normal cells and optimum cytotoxicity to the tumor cells.

Keywords: 5-FU, drug delivery system, calcium carbonate

1 INTRODUCTION

Modern day technologies such as nanotechnology shows applications of new materials for multiple functions with high efficiency. This is a field that has been widely researched, but still provides new discoveries. The term 'nanotechnology' refers to the creation of materials with at least one component on the nanometer scale [3]. Nanomaterials have a wide range of applications, including medical diagnostic and therapeutic interventions such as diagnostic imaging, photothermal therapy, nucleic acid delivery, implantable devices, and drug delivery. The main focus of this research is to create a drug delivery system (DDS) which is suitable to deliver the drug at desired location. There are several criteria that must be tested before DDS is designed such as the effectiveness and safety of materials for medical applications, the interaction between the material and a biological system etc. The biocompatibility of the material is one of the most desired requirement. It is important to ensure a safe drug release and minimize cytotoxicity to normal cells or healthy organs within the body. The biocompatibility and biodegradability of the DDS is also considered at most important factor in this development [4].

In this study, calcium carbonate ($CaCO_3$) nanoparticles are used as a potential DDS for 5-fluorouracil (5-FU). Here we report a preliminary results of *in vitro* release profile of 5-FU drug. The further studies are in progress to design a pill of 5-FU-loaded $CaCO_3$ nanoparticles for animal studies.

2 EXPERIMENTAL

2.1 Materials

The egg shells were obtained from American Dehydrated Foods Inc. (Social Circle, GA). Egg shells have valuable organic and inorganic components which can be used for different applications. The outside layer of the egg shells is mainly composed of $CaCO_3$. $CaCO_3$ is 95% of the shell and the remaining 5% includes calcium phosphate, magnesium carbonate, soluble and insoluble proteins. Egg shells were chosen for this research because they are highly porous, low cost, easily accessible, recyclable, and bio-compatible with the human body.

Polypropylene Glycol (PPG), Ethanol, and Glycerol were obtained from Sigma Aldrich (St. Louis, MO). PPG was used in the ball milling fluid for the $CaCO_3$ nanoparticles synthesis. PPG was chosen because it is highly viscous and aided in the ball milling synthesis of the nanoparticles. Glycerol is used as solvent in the sonochemical synthesis because of its biocompatibile nature.

2.2 Synthesis of $CaCO_3$ nanoparticles

The synthesis of $CaCO_3$ nanoparticles was carried out by initial cleaning, mechanochemical milling and

sonochemical irradiation. The egg shells were boiled for 24 hours. After the egg shells were cleaned, they were placed into a blender for initial grinding. The ground egg shells were dispensed in a water bath for 24 hours and finally then washed with fresh water and dried in oven. After the particles were dry, 1000 mg of the particles were placed in a ball mill canister with 5 ml of polypropylene glycol (PPGYL) and were ball milled for 10 hours. After the egg shells were ball-milled, they were washed three times with ethanol and dried for 24 hours in a vacuum oven. After the particles were dry, they were sifted through a 20µm sifter for uniform size particles. These particles were further irradiated with high intensity ultrasonic horn to reduce the particle sizes to nanoscale [5-6] for three hours in 50 ml of glycerol. The particles were then washed three more times and dried for 24 hours in a vacuum drier, and later dried again in a vacuum oven at 100°C for 24 hours.

2.3 Loading 5-FU into $CaCO_3$ nanoparticles

$CaCO_3$ nanoparticles are highly porous and have large surface areas. These unique qualities enable $CaCO_3$ nanoparticles to be loaded by physical absorption. Our targeted drug loaded concentration in this study is ~100 µM for each tablet. A stock solution of 5-FU with a concentration of 100 mM has been created with deionized water. 1000 µl of dH_2O was combined with 100 µl of 5-FU from the stock solution and then mixed for 24 hours with 50 mg of nanoparticles. After mixing was complete, the sample was frozen for 24 hours and then placed into a freeze dryer for 24 hours.

2.4 Characterization

2.4.1 X-ray Diffraction (XRD)

X-ray diffraction (XRD) was used to determine the structure and purity of the sample. XRD tests carried out using a Rigaku D/MAX 2200 X-ray diffractometer. XRD was studied for a sample of glycerol-sonicated $CaCO_3$ nanopartices and acetic acid-sonicated $CaCO_3$ nanoparticles.

2.5 Release Studies

For the release studies, phosphate buffer saline (PBS), with a pH of 7.4, was used as the media to represent the physiological pH of the biological fluids within the body. The 5FU-loaded $CaCO_3$ nanoparticles were placed into 1 mL of PBS. These samples were further placed into an incubator shaker. The supernatant were taken at 0, 1, 2, 3, and 4 hours and tested for their concentration using a UV absorbance. After the samples were taken from the supernatant, all supernantant was decanted and replaced with 1 ml fresh PBS. These samples were placed back into the incubator shaker. The results are displayed in two separate forms for better understanding.

3 RESULTS

3.1 XRD

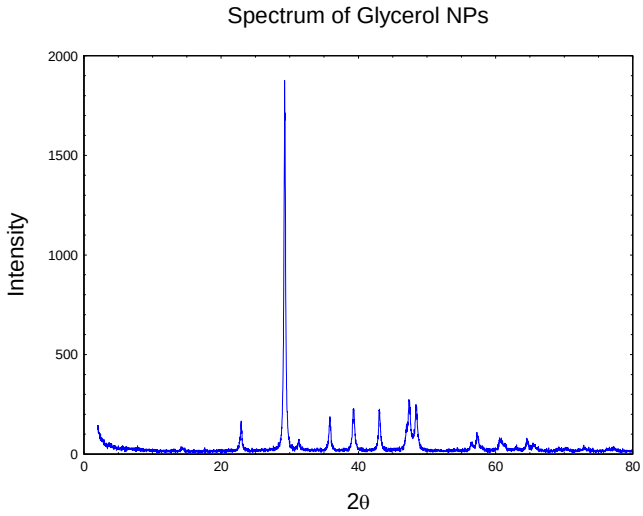

Figure 1: XRD spectrum of Glycerol-Sonicated Nanoparticles

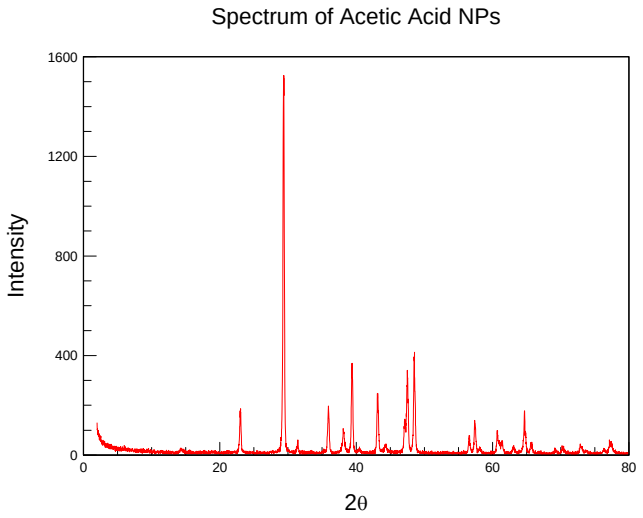

Figure 2: XRD spectrum of Acetic Acid-Sonicated Nanoparticles

The XRD patterns were studied for glycerol and acetic acid-sonicated nanoparticles. Figure 1 and 2 are the XRD spectra for both types of nanoparticles. These results show that both particles are highly crystalline and all the peaks match very well with the diffraction peaks of calcite $CaCO_3$ (JCPDS card No. 47-1743). No other impurities were observed.

3.2 Release Studies

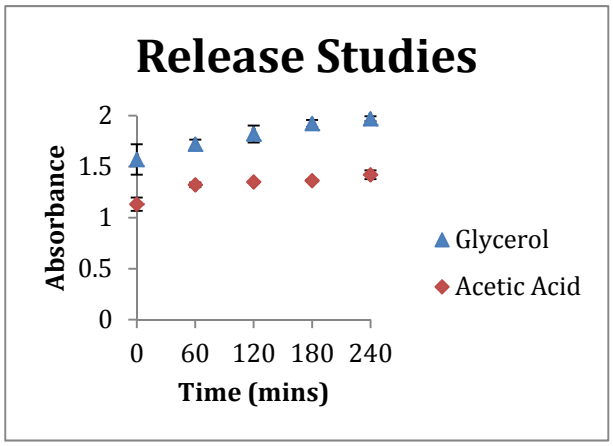

Figure 3: Release studies in absorbance units

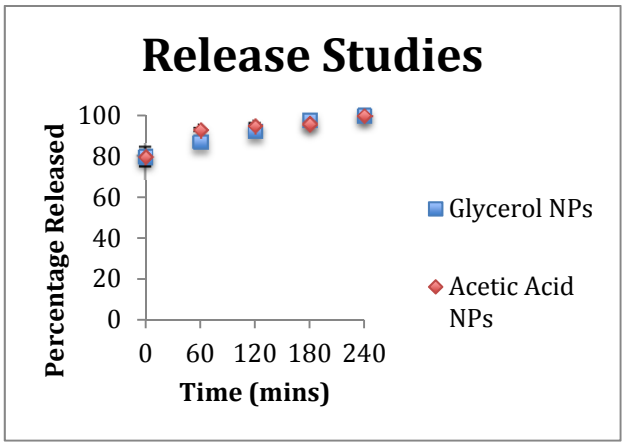

Figure 4: Release studies in percentage

Glycerol and acetic acid-sonicated nanoparticles were examined for release profiles. Figure 3 shows the release values in reference to their observance units. This graph shows that the Glycerol-sonicated nanoparticls show a more stable release than acetic acid-sonicated nanoparticles. Figure 4 shows the release studies in reference to the percentage of the total released overall. Both, glycerol and acetic acid-sonicated nanoparticles released 80% of their drug content in the first '0' minutes when they were placed in PBS. This shows that the nanoparticles are not completely safe from releasing the drug content, so a good coating system should be placed onto the nanoparticles in order to hold the drug content within. After each batch of nanoparticles reached 3 hours of their drug content, they were placed into ethylenediamine tetraacetic acid (EDTA) and then the absorbance reading was taken of the supernatant after all the particles were dissolved. Glycerol-sonicated nanoparticles had the most drug content remain. This further confirms that the glycerol-sonicated nanoparticles have a better controlled release rate than the acetic acid-sonicated nanoparticles.

4 CONCLUSIONS

The $CaCO_3$ nanoparticles were derived from egg shells using sonochemical and mechanochemical methods. The XRD results show that the as-prepared particles are highly crystalline and no impurities were observed. The glycerol and acetic acid were used as solvents in this process to avoid other toxic solvents. These nanoparticles were successfully loaded with 5-FU drug and release studies show that $CaCO_3$ nanoparticles are highly pH sensitive. The further studies are in progress to coat these drug loaded $CaCO_3$ nanoparticles with pH sensitive polymer.

REFERENCES

[1] Thomas, A., Kapanen, A., Hare, J., Ramsay, E., Edwards, K., Karlsson, G., & Bally, M. (2011). Development of a liposomal nanoparticle formulation of 5-fluorouracil for parenteral administration: Formulation design, pharmacokinetics and efficacy. *J. Control. Release.*, *150*, 212-219.

[2] Yang, Y., Lee, J., Kim, I., Jung, Y., & Kim, Y. (2007). Synthesis and properties of n-nicotinoyl-2-(5-fluorouracil-1-yl)-d,l-glycine ester as a prodrug of 5-fluorouracil for rectal administration. *Eur. J. Pharm. Biopharm.*, *66*, 260-267.

[3] Sahoo, S., Parveen, S., & Panda, J. (2007). The present and future of nanotechnology in human health care. *Nanomedicine: Nanotechnology, Biology and Medicine, 3*, 20-21.

[4] Naahidi, S., Jafari, M., Edalat, F., Raymond, K., Khademhosseini, A., & Chen, P. (2012). Biocompatibility of engineering nanoparticles for drug delivery. *Journal of Controlled Release, 166,* 182-194.

[5] Rangari, V. K., Hassan, T. A., Mayo, Q., Jeelani, S. (2009) 'Size reduction of WO3 nanoparticles by ultrasound irradiation and its applications in structural nanocomposites', *Composite Science and Technology,* Vol. 69, pp.2293-2300.

[6] Rangari, V.K., Mohammad, G.M., Jeelani, S., Hundley, A., Vig, K., Singh, S.R., Pillai, S. (2010) 'Synthesis of Ag/CNTs hybrid nanoparticles and fabrication of their Nylon-6 polymer nanocomposite fibers for antimicrobial applications', *Nanotechnology*, Vol. 21, pp.95-102.