Synthesis of Unstable Vaterite Polymorph of Hollow Calcium Carbonate Nanoparticles and Encapsulation of the Anticancer Drug Cisplatin

S. P. Dunuweera¹ and R. M. G. Rajapakse¹*

¹Department of Chemistry, University of Peradeniya, Peradeniya 20400, Sri Lanka.

Authors’ contributions

This work was carried out in collaboration among both authors. Both of them contributed equally towards assimilation, organization and writing up of the manuscript. Author SPD carried out all the experiments, planned them and analyzed results quite independently with minimal supervision from author RMGR. Both authors read and approved the final manuscript.

ABSTRACT

Cisplatin is the first generation platinum coordination complex-based anticancer drug developed to treat many different types of cancers. However, cisplatin is associated with numerous side effects. Most of these side effects could be minimized if cisplatin can be encapsulated in a suitable host material for its slow-release only at the cancer cells. In this research, we developed a convenient and simple wet chemical route to synthesize spherical nanoparticles of the vaterite polymorph of calcium carbonate (CCNP) with hollow and porous structures. This is achieved by the soft molecular template based synthesis of vaterite using the template obtained from hydrogen bonded ethylene glycol-water network structures. In these templates, vaterite is synthesized from calcium acetate and sodium hydrogen orthophosphate in the aqueous dispersion. Cisplatin is then encapsulated within these nanoparticles and the materials synthesized are well characterized by several independent analytical methods. The presence of cisplatin in its proper molecular form within the confined environment of vaterite is proven. Taking the advantage of the acidity of...
cancerous cells, these nanoparticles can be selectively and slowly dissolved only within cancerous cells thus releasing the drug only to the cancerous cells. This would minimize the adverse effects of cisplatin to healthy normal cells while increasing the bioavailability and efficacy of the drug and reducing its dosage.

GRAPHICAL ABSTRACT

Keywords: Vaterite; porous nanoparticles; cisplatin; encapsulation; targeted delivery; slow-release.

1. INTRODUCTION

Calcium carbonate particles have widespread technological applications in building, paper, pharmaceutical, textile, rubber, plastic, paint, cosmetic, toothpaste, glove, cosmetic, food and beverage, sealing wax, ink, plastic film manufacturing and many other industries. Such a widespread use of calcium carbonate in many industries is due to several reasons which help: increasing product volume, improving processing performance such as regulating viscosity, rheological properties and curing properties, decreasing production cost, improving
reinforcement properties and dimensional stability of products, improving appearance such as gloss finish, whiteness and enhancing abrasion resistance and flame-retardant properties of products. It is an essential ingredient in the construction industry. It is used in the extraction of iron in iron industry where calcium oxide produced from heated calcium carbonate in the blast furnace produces slag with impurities. Calcium carbonate is added to drilling fluids to improve formation-bridging, filter cake-sealing and density of the fluid, to swimming pools to adjust pH of water, and is also used in refining of raw sugar. Microporous ground calcium carbonate (GCC) particles are an essential ingredient in diapers and precipitated calcium carbonate (PCC) micro particles are used in latex and nitrile-butadiene-based glove manufacturing. In medicine and healthcare products, calcium carbonate is used as inexpensive dietary calcium supplement, phosphate binder for the treatment of hyperphosphatemia in patients with renal failure and also as an inert filler of tablets and pharmaceuticals [1,2]. It is quite astonishing to note that in some products such as plastic films, bottles etc. up to about 20% by mass is calcium carbonate and ceramic tile adhesives may contain up to 80% of calcium carbonate. In all these practical applications, if macro or micro particles that are currently used could be replaced by respective nanoparticles then the material requirement can be drastically reduced since nanoparticles have extremely large surface area to volume ratio. For instance 1 kg of 1 µm³ particles have the same surface area as 1 mg of 1 nm³ particles. Hence, in terms of surface area matching the material requirement can be reduced million-fold if 1 µm³ particles could be replaced by 1 nm³ particles, at these extreme ends.

Calcium carbonate minerals, in their pure form, such as calcite are readily available throughout the globe but calcium carbonate also exists in mixed mineral forms such as dolomite where it is approximately 1:1 CaCO₃ and MgCO₃ with SiO₂ impurities. We have designed and developed methods to separate calcium, magnesium and silica components using simple wet chemical procedures and we are currently engaged in scaling up of these processes. We have also developed procedures to prepare PCC nanoparticles and hydroxyapatite nanoparticles through bottom up approach of synthesizing them chemically from respective ions [3-12]. We then extended the procedures to develop various morphologies and phases of CaCO₃ nanoparticles, MgO nanoparticles and their nanocomposites with various polymers. However, almost all of the current synthetic methods developed to prepare calcium carbonate nanoparticles usually give either amorphous calcium carbonate or calcite since these two forms are the thermodynamically most stable forms of calcium carbonate. However, as far as some specific applications are concerned, calcite has some drawbacks; the major one being its rhombohedral shape. Vaterite is the least stable polymorph of calcium carbonate but it is spherical in shape and its synthesis requires special techniques such as use a very specific polymers like polyaniline [8] for stabilization. In this research, we used a water-ethylene glycol soft molecular template to prepare hollow nanoparticles of vaterite. The well characterized vaterite nanoparticles were used to encapsulate the anticancer drug cisplatin which is cis-diamminedichloroplatinum(II) with IUPAC name of (SP-4-2)-diamminedichloroplatinum(II). The chemical formula of cisplatin is given below.

![Chemical formula of cisplatin: (SP-4-2)-diamminedichloroplatinum(II).](Image)

Cisplatin also known as cisplatinum, platamin, neoplatin, with the chemical formula of cis-diamminedichloroplatinum(II) (CDDP) is a first generation of platinum coordination compound-based anticancer chemotherapeutic drugs that is used to treat small cell lung cancer, ovarian cancer, lymphomas, bladder cancer, cervical cancer and germ cell tumours [13,14]. It is found that cisplatin is particularly effective against testicular cancer with a cure rate of 10-85%. The cis configuration enables the binding of the coordination complex to two DNA strands and thereby crosslinking the DNA strands which triggers the cells to die in a programmed manner. However, cisplatin is associated with numerous side effects which include nephrotoxicity [15], neurotoxicity [16], nausea and vomiting [16], ototoxicity (hearing loss) [16], electrolyte disturbance and haemolytic anaemia [17]. In order to circumvent these toxic effects of cisplatin, we have developed a novel method in which cisplatin is encapsulated in porous CCNP and the main hypothesis of this work is taking the advantage of low pH conditions prevailing in cancerous cells. Hence, cisplatin will be released
slowly at the vicinity of the cancerous cells only. This way, only the minimum dosage required can be directed in the targeted manner to the cancer cells beside, avoiding its toxic effects to healthy normal cells and increasing the bioavailability and the efficacy of the drug.

2. MATERIALS AND METHODS

2.1 Materials

All the chemicals except cisplatin were purchased from Sigma Aldrich and they were of analytical grade and used without further purification. Cisplatin injection bottles containing 1% cisplatin in saline water was purchased from Sri Lanka Pharmacy, Kandy, Sri Lanka.

2.2 Methods

Hollow CaCO$_3$ nanoparticles were synthesized by the reaction between Ca(CH$_3$COO)$_2$ and NaHCO$_3$ in a solvent mixture of H$_2$O and ethylene glycol (EG). This method is a modification and improvement of a similar method developed by Zhao et al. [18]. Solution A is a mixture of Ca(CH$_3$COO)$_2$ (25.0 mL, 0.50 M), H$_2$O (10.0 mL) and EG (25.0 mL). Solution B is a mixture of NaHCO$_3$ (25.0 mL, 0.50 M), H$_2$O (10.0 mL) and EG (25.0 mL). Then, the solutions A and B were mixed slowly in dropwise manner under stirring. Stirring was continued for further 48 h and the resultant suspension was filtered. The solid mass obtained was washed first with ethanol and then subsequently with distilled water and dried at 100°C for 4 h in a vacuum oven. The CaCO$_3$ particles obtained were characterized by Laser Light Scattering based Particle Size Analysis (CILAS Particle Size Analyser NANO DS), XRD (Siemens D5000 X-ray powder diffractometer), XRF, FT-IR (Shimadzu IR-Prestige 21 Instrument with the KBr pellet method and SEM (Environmental SEM with EXAS Facilities) studies. The BET surface area was determined by the novel BET instrument constructed in our research group. This BET system is analogous to standard BET equipment but it contains advanced detection system based on electronic circuits and purpose-built software. A detailed description of this system will be published elsewhere. In the encapsulation of cisplatin drug into the hollow CaCO$_3$ nanoparticle, 0.50 g of CaCO$_3$ nanoparticles were dispersed in a 100 mL of cisplatin injection solution and kept stirring for 24 hours to facilitate cisplatin encapsulation. The product was subsequently separated by centrifuging followed by suction filtration and washed several times with distilled water. The encapsulation of cisplatin was confirmed by the FT-IR and XRF analyses of the products obtained.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, particle size analysis shows that the colloidal solution contains particles in the nano-range between 20 nm to 80 nm with average particle size of 36.9 nm in 90% confidence interval. It is interesting to note that the wet chemical synthesis gives such a narrow distribution of non-aggregated nanoparticles of calcium carbonate which is not usually straightforward. Therefore, the soft molecular template of hydrogen bonded network of more ethylene glycol molecules and fewer water molecules form cavities of this size [19].

Ethylene glycol-water mixture forms hydrogen-bonded cage-like structures in which relative proportions of EG and water molecules depend upon the molar ratio of EG:water used. An extensive review of molecular templates and their applications in synthesizing supra-molecular architectures can be found in an excellent review of Hubin et al. [19]. Chen et al. [20] have used near Infrared spectroscopy to determine molecular structures formed in pure ethylene glycol and ethylene glycol water mixtures. They found that in pure ethylene glycol only intermolecular hydrogen bonding exists and that there are no intra-molecular hydrogen bonding. As such, hydrogen bonded cage-like structures are present in pure ethylene glycol. In EG:water mixtures the relative ratio of EG:water in intermolecular hydrogen bonded structures depend on the EG:water molar ratio used. At high EG content more EG molecules than water molecules form cavities of this size [19].

The cavity size of these cages depends upon the number of molecules present in these cage-like structures. These cage-like structures present in EG:water mixtures can act as soft molecular templates for controlled growth of nanoparticles. Since, the cavities in these structures are mandatorily spherical in size, these soft templates can be used to synthesize spherical nanoparticles. This is the reason for the formation of spherical nanoparticles of calcium carbonate in its vaterite phase despite the fact that vaterite is not as stable as calcite in terms of
thermodynamic considerations. Ethylene glycol molecules also reside inside the nanoparticles formed. Upon removal of them cavities are formed within the nanoparticles.

The SEM images of such hollow nanoparticles of vaterite formed are shown in Fig. 2. The SEM images clearly show that the sample contains spherical particles of nano-range but clustered into aggregated particles. This clustering might have happened during deposition of particles onto glass surface for SEM analysis. As is evident from particle size determination of the suspension, discrete particles are present in the suspension. The morphology of the particles show a good porous nature. The BET surface area determination gives 1200 m$^2$/g indicating that the particles have a huge surface area possibly due to their porous nature.

As evident from the FT-IR spectrum, provided in Fig. 3, absorption bands at 877, 745.8 and 1084 cm$^{-1}$ confirm the presence of the vaterite polymorph of CaCO$_3$. No absorption bands at 854, 712, 700 cm$^{-1}$ and 848, 714 cm$^{-1}$ corresponding to aragonite and calcite, respectively, are present. Since vaterite is the most unstable form of CaCO$_3$, it is very interesting that our procedure produces and stabilizes this unstable vaterite form rather than forming stable calcite form. The use of molecular soft templates formed by hydrogen-bonded EG and water molecules direct the vaterite formation allowing to crystallize in spherical manner.
XRF of the CaCO$_3$ nanoparticles synthesized detects only Ca in our sample since our XRF machine is incapable of detecting elements with atomic number less than 13. Hence it shows that the product formed is free of impurities containing elements with atomic number higher than 13. We have further characterized the calcium carbonate nanoparticles prepared in this work using X-ray diffractometry and the X-Ray Diffractogramme obtained is given in Fig. 4. As can be seen from Fig. 4, the sample contains basically vaterite as the major phase. The XRD peaks of vaterite and calcite are indicated within the diffractogramme. As evident from the XRD, a very small fraction of calcite particles can also be observed though the percentage abundance is negligible compared to that of vaterite nanoparticles. Application of the Debye-Scherrer Equation to the major XRD peak gives the crystallite size to be 28.0 nm. This is very close to the average particle size obtained by the laser light scattering based particle size analysis. This may be the reason why both FT-IR and SEM techniques do not detect the calcite. The presence of a minute fraction of calcite is not surprising since it is the thermodynamically most stable phase of calcium carbonate and the presence of these nanoparticles have no adverse effect on the encapsulation of cisplatin.

The encapsulation of cisplatin within these hollow nanoparticles of vaterite was performed by stirring the nanoparticles in the cisplatin injection solution (cisplatin in saline solution) as explained in the Experimental Section. The presence of cisplatin in these nanoparticles was studied through FT-IR, X-Ray Fluorescence (XRF), XRD studies and Energy Dispersive X-Ray Spectroscopy (EDX) of SEM analysis.

The FT-IR spectrum of the encapsulated product obtained [Fig. 5] clearly show the presence of N-H antisymmetric and symmetric stretching vibration 3373 cm$^{-1}$ & 3597 cm$^{-1}$, N-H wagging together with CO$_3^{2-}$ vibrations, deformation vibrations (scissoring) 1650 cm$^{-1}$, 1590 cm$^{-1}$ and wagging oscillation broad transmittance band 800 cm$^{-1}$. It also contains all the bands that are reported for hollow nanoparticles of CaCO$_3$. In the FTIR spectrum, the absorption bands at 871 cm$^{-1}$ show the presence of vaterite polymorph. As such, the FT-IR data suggest the encapsulation of cisplatin in hollow CaCO$_3$.

The XRF spectrum of the cisplatin encapsulated CaCO$_3$ product shown in Fig. 6 clearly elicits the presence of Ca, Pt and Cl with the Pt:Cl atomic ratio of 1:2.5 showing that cisplatin maintains its identity in the confined environment also. Ideally, the ratio should be 1:2 but the presence of excess chloride in saline solution would encapsulate free chloride ions also thus increasing the chloride content. XRF data suggest the encapsulation of cisplatin in hollow CaCO$_3$.

The X-Ray Diffractogramme of the cisplatin encapsulated vaterite nanoparticles is shown in Fig. 7. It also contains vaterite as the major component with minute fractions of calcite and sodium chloride. The presence of cisplatin cannot be determined from the XRD since
cisplatin is present in molecular form within the confined environment of pores of vaterite nanoparticles in non-crystalline form. However, FT-IR and XRF studies complement this showing the presence of cisplatin in encapsulated nanoparticles. The encapsulation has not affected the crystallinity and crystallite size of vaterite nanoparticles. The crystallite size calculated using the major XRD peak is 29.2 nm which is same as that without cisplatin encapsulation. Hence cisplatin molecules occupy the free spaces of hollow nanoparticles of vaterite without changing the particle dimensions. The SEM images of the encapsulated product are shown in Fig. 8.

The SEM images are quite similar to those obtained for vaterite nanoparticles without cisplatin encapsulation. This shows that vaterite form of calcium carbonate is preserved even after the cisplatin encapsulation. It is interesting to note that the EDAX analysis does not show Pt, Cl or N in the cisplatin encapsulated vaterite nanoparticles though XRF clearly shows the presence of Pt and Cl and FT-IR gives evidence to the presence of N. Both XRF and FT-IR are bulk analytical techniques that measure the composition of the bulk sample. However, SEM analysis is a surface analytical technique detecting a few micrometer width from the surface and therefore EDAX detects the elements present close to the surface of the vaterite nanoparticles. Since the elements of cisplatin cannot be detected within this depth of the surface and bulk techniques detect them it can be concluded that cisplatin is present deep.
inside the particles at least deeper than 1 µm width from the surface. It is also intriguing that both the XRD and the light scattering based particle size analysis show 29 nm particle size the SEM show micrometer size particles. This is because in the absence of any protection these small nanoparticles attract to each other and form aggregates. When a large number of these nanoparticles assemble into aggregates cavities or hollow volumes are formed. These cavities act as hosts to reside cisplatin molecules. This is a clear evidence to prove that cisplatin is encapsulated in the centres of nanoparticles. As a rough estimate, we find that 85.3% of cisplatin is encapsulated from 50.0 mL of cisplatin injection solution within 0.5 g of vaterite nanoparticles. We are currently carrying out more detailed study on encapsulation efficiency and release kinetics in buffered solutions of different pH values.

Fig. 6. XRF spectra of cisplatin encapsulated CaCO$_3$ nanoparticles

Fig. 7. The XRD of cisplatin encapsulated vaterite nanoparticles
4. FUTURE WORK

Mechanistic study for administration of our nanoparticle formula in vitro and in vivo studies to confirm our hypothesis.

5. CONCLUSION

In this work, we developed a facile method for the synthesis of hollow spherical nanoparticles using precipitation reaction of NaHCO$_3$ with Ca(CH$_3$COO)$_2$ in H$_2$O/EG media. The colloidal solution prepared contain three sizes of particles in the nano-range with very narrow particle size distribution. These hollow nanoparticles were found to successfully encapsulate cisplatin molecules. Cisplatin retains its chemical identity within the confined environment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


