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Nano-antacids enhance pH neutralization beyond their bulk counterparts: synthesis and characterization[†]

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Abstract

Antacids are crucial in the treatment of gastro-esophageal reflux disease and peptic ulcers. Antacids based on the calcite phase of bulk calcium carbonate have been the standard for over fifty years. More recent research has shown that nanomaterial forms of such bulk materials often have improved properties. However, the metastable vaterite form of calcium carbonate is particularly difficult to synthesize as a nanomaterial, and thus has not been extensively studied. Here, we describe the synthesis of these particles and investigate them for antacid applications. Experimental and computational approaches show that nanoscale vaterite particles maintain neutral gastric pH values three times longer than commercial antacids.

1. Introduction

The vaterite phase of calcium carbonate exhibits high dissolution, dispersivity, and biocompatibility.^{1,2} As a widely used antacid, calcium carbonate has been studied extensively.^{3–6} It has three common polymorphs: calcite, vaterite, and aragonite. Calcite is the most stable, while vaterite is the least stable at room temperature and atmospheric pressure.⁷ Vaterite's thermodynamic instability converts it to calcite over time under normal conditions⁸ and also makes it difficult to study as an antacid. Much like other nanomaterials, CaCO₃ has different optical and mechanical properties than its bulk counterpart, as well as a higher surface area to volume size ratio, and different surface chemical properties. Several attempts have been made to synthesize the meta-stable vaterite form of calcium carbonate,

[†]Electronic supplementary information (ESI) available: Experimental plan; DLS size distribution; extended detail of material characterization tool (XRD); antacid profile compared with commercial counterpart; effect of stirring rate on antacid profile. See DOI: 10.1039/c6ra12856d

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Conflict of interest

All authors declare no competing financial interest.

however these particles are too big (>few micrometers),^{9–11} are not stable for an extended period of time,^{12,13} and have low phase purity.^{14,15}

This work develops a method for the synthesis of stable and monodisperse vaterite nanoparticles and investigates their antacid activity. Results are validated with both computational and experimental approaches.

2. Materials and methods

2.1. Synthesis and characterization of CaCO₃ nanoparticles

Stable vaterite nanoparticles were synthesized by reacting 0.1 M calcium chloride dihydrate (CaCl₂·2H₂O) and 0.1 M sodium bicarbonate (NaHCO₃). The rate of growth of the crystals was controlled by performing the reaction in water and ethylene glycol in a ratio of 1 : 5 (v/v). The solution was stirred at 700 rpm for 30 minutes at room temperature. The precipitate was then sequentially washed with ethanol, methanol and acetone to remove any water content. The final product was dried at 60 °C for an hour. The synthesized nanoparticles were characterized using Transmission Electron Microscope (TEM) for morphological studies, X-Ray Diffraction (XRD) for crystal phase study, X-Ray Photocorrelation Spectroscopy (XPS) for surface chemical composition and dynamic light scattering for stability studies.

2.2. Analysis of antacid profile

The antacid activity assay was done by converting both synthesized and purchased powdered antacids into liquids at different concentrations, 1 ml of which was added to 150 ml of deionized water at 37 °C. The round bottomed flask was first swirled thrice to evenly distribute the antacid and then stirred at 60 rpm to mimic the churning process in the stomach.¹⁶ The solution was titrated with hydrochloric acid having a pH of 1.2 (ref. 17) to an end point of pH 3, according to Fordtran's model.¹⁶ The pH was constantly measured using a pH meter, and values were recorded every minute. The antacid property of the vaterite nanoparticles was tested and compared with commercial chemicals having antacid properties, such as sodium bicarbonate, commercially available calcium carbonate, and TUMS® 500 regular strength chewing tablets. A comparative and extensive study on the dose response characteristics was done for the synthesized vaterite nanoparticles and TUMS chewing tablets.

3. Results and discussion

During multiple steps of the synthesis procedure, various amounts of ethylene glycol were added to the solution to increase viscosity and thereby reduce the molecular diffusion,² yielding particle sizes was about 100 ± 8.5 nm (Fig. 1). To achieve this particle size the optimal ratio of ethylene glycol to water was found to be 1 : 5. In addition to the role that viscosity plays in nanoparticle size control, solvent washing to remove water was found to be essential. Water encourages the particles to agglomerate as well as change the phase from vaterite to calcite.

The reaction proceeded as follows:

The nucleation and growth of the particles were studied by *ex situ* TEM (FEI Transmission Electron Microscope, 120 kV/LaB6 filament; 0.2 nm line resolution) to elucidate phase changes in the solution. The synthesis reaction mixture was sampled and transferred to TEM grids at different time points. Experiments show that vaterite formed through a multi-phase process, starting with the formation of amorphous calcium carbonate (Fig. 2A–F). Our findings of the nucleation and growth process are consistent with the multi-phase model reported earlier.^{9,18,19}

To confirm the crystal phase of the CaCO_3 nanoparticles, XRD patterns were obtained by using a Bruker D8 Advance X-ray Diffractometer (Bruker, USA) configured with a 1.5418 Å Cu X-ray tube for analysis of powder samples using a LYNXEYE_XE detector. The diffraction pattern in Fig. 3 shows a clear representation of the vaterite phase of CaCO_3 as shown in Fig. 3. Two small intensity peaks of aragonite were also observed, represented by the label (A) in the Fig. 3, although these peak intensities were non-significant.

Information about the molecular bonding and functionality of vaterite CaCO_3 were obtained with X-ray photoelectron spectroscopy (XPS, PHI 5000 VersaProbe II). XPS permitted directly probing the surface atomic compositions, showing chemical shifts of the C(1s), O(1s), and Ca(2p) peaks. Fig. 4 shows the XPS spectra of the Survey (S) scan, C1s, O1s, and Ca2p core levels of the CaCO_3 surface.

The binding energy of a standard C1s peak at 288.3 eV corresponded to CO_3 in the CaCO_3 surface, and an adventitious carbon peak occurred at 285.8 eV. The binding energy of 283.5 eV corresponded to a carbon centre originating from environmental carbon as a result of open environmental exposure. The high-energy resolution of the Ca(2p) spectra of the CaCO_3 samples indicated two binding energies, one for Ca2p_{3/2} (345.8 eV) and another for Ca2p_{1/2} (349.7 eV). The O1s core level for CO_3 of the CaCO_3 was observed at 530.2 eV.

The antacid activity time defines its effectiveness, and the greater the activity time, the greater the effectiveness. Fig. 5 shows that the antacid activity time for the vaterite particles is much longer than for other tested chemical substances. The enhanced antacid property of vaterite is attributed to its higher solubility than that of the calcite phase found in commercially available CaCO_3 and TUMS.²⁰ The higher solubility of vaterite results in a greater concentration of carbonate ions in the solution, which leads to a higher activity. Also, these small particles dissolve faster into the solution due to their high surface-to-volume ratio. The fast dissolution rate also increases the pH quickly and leads to faster relief when ingested. In Fig. 5 the activity times for TUMS are lower, or at best comparable to that of commercial CaCO_3 . The difference in activity time of TUMS compared to commercial CaCO_3 is likely a result of the TUMS tablets containing additional ingredients²¹ while the measured commercial CaCO_3 was 99% pure. Results indicated that as mass concentration increases (2 to 100 mg), antacid activity times was increased (2.8 minutes to ~25 minutes).

The experimental finding of antacid activity by vaterite nanoparticles was further validated by a computational simulation. The reactions that follow the addition of calcium carbonate to water are as follows

In simulation, a given solution containing a fixed amount of antacid is titrated using hydrochloric acid pH 1.2. The pH of such a solution would have the following electrical neutrality expression for all the ions present in the solution:²²

$$[\text{H}^+] + 2[\text{Ca}^{2+}] = 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{OH}^-] + [\text{Cl}^-] \quad (5)$$

Here, the carbonate ion concentration can be represented as

$$[\text{CO}_3^{2-}] = \frac{K_{a1} K_{a2} [\text{H}_2\text{CO}_3]}{[\text{H}^+]^2} \quad (6)$$

A similar equation can be derived for the bicarbonate ion concentration, and the hydroxyl ion concentration can be written as a function of the hydrogen ion concentration using K_w , the dissociation constant of water. In the simulation, as the hydrochloric acid was injected into the solution externally at a fixed flowrate (1 ml min^{-1}) and fixed concentration (0.063 M), the chloride ion concentration was a function of time as

$$[\text{Cl}^-] = \frac{0.063 \times \text{flowrate} \times \text{time}}{V_i + \text{flowrate} \times \text{time}} \quad (7)$$

where V_i is the initial volume of the antacid solution.

The calcium ion concentration was obtained using the solubility product equation of calcium carbonate; however, a restriction had to be set on the calcium ion concentration, which depends on the initial amount of antacid added to the solution. Thus, the electrical neutrality expression can be expressed solely in terms of a single variable, the hydrogen ion concentration. The equation is solved to determine the time required for the given solution to reach a pH end point of 3, and the results of the simulation, compared with experimental results, are as shown in Table 1.

The simulation is able to capture the neutralization times for small doses of the antacid, and some deviation from the experimental observation is seen for higher doses. However, the simulation gives a good approximation for the activity time of the antacid and can be used to predict the neutralization time for a given dose.

As we demonstrated previously in the use of nano- CaCO_3 for tumor therapy, a chemical compound used for biomedical application needs colloidal stability during storage, transport and delivery of the compound into a patient's body.²³ Colloidally stable particles may have further utility as pH neutralizers in other biological conditions.^{1,15,24} To determine vaterite's feasibility for pH neutralization *in vivo*, we studied the size and stability of these particles in different solvents, using time-resolved dynamic light scattering (TR-DLS) for 30 minutes. The duration was selected based estimated antacid activity.

The stability of vaterite nanoparticles in a variety of solvents, such as water, saline, bovine serum albumin, Dulbecco's modified Eagle's medium (DMEM), ethanol, methanol and phosphate buffer saline (PBS). Fig. 6 shows the results of stability tests that imply that albumin has a high affinity for calcium carbonate and hinders particle agglomeration, thus stabilizing the particles in solution. The PBS solution promotes precipitation of the particles by increasing their supersaturation,²⁵ as a result of added divalent ions (Mg^{2+} or Ca^{2+}). The particles are also quite stable in DMEM, facilitating their possible application in cell growth cultures requiring alkaline environments. Thus the synthesized particles would be useful for industrial applications in solvents such as ethanol, and would be good for biomedical antacid applications when dissolved in a solution of albumin.

4. Conclusions

In conclusion, this study describes a stable, rapid, and room temperature synthesis of vaterite phase nano- CaCO_3 . The diameter of the particles, determined by TEM, was about 100 ± 8.5 nm. *Ex situ* TEM-based nucleation and particle growth observations, along with XRD and XPS analysis, shows that vaterite phase CaCO_3 particles formed within 15 minutes of reaction, and remains stable up to 30 minutes of the synthesis reaction. The synthesized particles showed a considerably higher antacid profile than their bulk counterpart or commercial compounds used for gastric pH neutralization.

This work introduces nano-antacids with well controlled properties such as size, crystal phase and morphology. The gastric pH control of antacids are limited by its dissolution rate, dose concentration, and stability of neutralized pH. Currently, gastric-acidity patients use either high amount of sodium bicarbonate or calcium carbonate based compounds. Nano-scale engineering of calcium carbonate particles result in a high surface area to volume size ratio, and therefore expand the horizon of pH reutilization. Fundamental understanding of the synthesis mechanism allows us to make nanoparticles that dissolve at desired controlled rates. Dissolution rate of particles in acidic medium depends on the crystal phase, which is crucial for rapid pH neutralization and maintaining the buffering capacity for longer durations.

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References

1. Pellegrini P, Strambi A, Zipoli C, Hägg-Olofsson M, Buoncervello M, Linder S, De Milito A. Autophagy. 2014; 10:562–571. [PubMed: 24492472]
2. Parakhonskiy BV, Haase A, Antolini R. Angew Chem, Int Ed. 2012; 51:1195–1197.
3. Booth RE, Dale JK. J Am Pharm Assoc. 1955; 44:694–699.
4. Kapsner P, Langsdorf L, Marcus R, Kraemer FB, Hoffman AR. Arch Intern Med. 1986; 146:1965–1968. [PubMed: 3532984]
5. Smyth R, Herczeg T, Wheatley T, Hause W, Reavey-Cantwell N. J Pharm Sci. 1976; 65:1045–1047. [PubMed: 8626]
6. Washington N, Wilson C, Davis S. Int J Pharm. 1985; 27:279–286.
7. Turnbull AG. Geochim Cosmochim Acta. 1973; 37:1593–1601.
8. Rao MS. Bull Chem Soc Jpn. 1973; 46:1414–1417.
9. Coelfen, H., Antonietti, M. Mesocrystals and Nonclassical Crystallization. John Wiley & Sons; NY, USA: 2008.
10. Mihai M, Socoliuc V, Doroftei F, Ursu EL, Aflori M, Vekas L, Simionescu BC. Cryst Growth Des. 2013; 13:3535–3545.
11. Wang X, Kong R, Pan X, Xu H, Xia D, Shan H, Lu JR. J Phys Chem B. 2009; 113:8975–8982. [PubMed: 19496561]
12. Liu L, Zhang X, Liu X, Liu J, Lu G, Kaplan DL, Zhu H, Lu Q. ACS Appl Mater Interfaces. 2015; 7:1735–1745. [PubMed: 25578091]
13. Walsh D, Kim YY, Miyamoto A, Meldrum FC. Small. 2011; 7:2168–2172. [PubMed: 21626686]
14. Begum G, Rana RK. Chem Commun. 2012; 48:8216–8218.
15. Schüler T, Tremel W. Chem Commun. 2011; 47:5208–5210.
16. Fordtran JS, Morawski SG, Richardson CT. N Engl J Med. 1973; 288:923–928. [PubMed: 4693244]
17. Christensen CB, Soelberg J, Jäger AK. J Ethnopharmacol. 2015; 171:1–3. [PubMed: 26023029]
18. Nielsen MH, Aloni S, De Yoreo JJ. Science. 2014; 345:1158–1162. [PubMed: 25190792]
19. Van Driessche A, Benning L, Rodriguez-Blanco J, Ossorio M, Bots P, Gar ía-Ruiz J. Science. 2012; 336:69–72. [PubMed: 22491851]
20. Fawcett T, Faber J, Needham F, Kabekkodu S, Hubbard CR, Kaduk J. Powder Diffr. 2006; 21:105–110.
21. Upson, JG., Russell, CM. US5629013 A. 1997.
22. Sparks, DL. Env Soil Chem. Academic press; Cambridge, UK: 2003.
23. Som, A., Raliya, R., Tian, L., Akers, W., Ippolito, J., Singamaneni, S., Biswas, P., Achilefu, S. Nanoscale. 2016.
24. Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Donescu J, Sloane BF, Hashim AI, Morse DL, Raghunand N, Gatenby RA. Cancer Res. 2009; 69:2260–2268. [PubMed: 19276390]
25. Smeets PJ, Cho KR, Kempen RG, Sommerdijk NA, De Yoreo JJ. Nat Mater. 2015; 14:394–399. [PubMed: 25622001]

Fig. 1. TEM image of the synthesized particles when dispersed in methanol (agglomerates of the vaterite nanoparticle). Inset: scaled up image of a single vaterite particle (scale bar of 50 nm).

Fig. 2.

Ex situ TEM images showing size and growth of vaterite particles at different instants of time (A) between 0 and 1 min, (B) 1 min, (C) 5 min, (D) 10 min, (E) 15 min, (F) 30 min. The images show the rate at which the size of these particles increases with time during the synthesis process. The low rate of growth of these particles could be attributed to the fact that the rate of diffusion in a solution of ethylene glycol would be very less. Also, it is seen that the vaterite particle formation occurs within 15 minutes of the start of the synthesis.

Fig. 3.
XRD characterization of CaCO_3 nanoparticles.

Fig. 4. XPS characterization of vaterite phase CaCO_3 nanoparticles. Survey (S) spectrum along with high resolution C, O, and Ca spectra for the corresponding range of binding energy.

Fig. 5.

Antacid profile of vaterite nanoparticles and its conventional counterpart. Neutralization times for all the tested substrates at different concentrations of the liquid antacids (left).

Dose response profile of vaterite nanoparticles.

Fig. 6. Stability test of vaterite nanoparticles in various solvents measured by TR-DLS. Vaterite nanoparticles, the stability is in decreasing order as 2% albumin > ethanol > DMEM > methanol > PBS + MgCl₂ > water > saline.

Table 1

Antacid activity time obtained from simulations and experiments

Antacid amount (in mg)	Simulation time (in minutes)	Experimental time (in minutes)
2	3.08	2.82 ± 0.12
10	5.66	4.08 ± 0.21
20	8.88	6.83 ± 0.34
50	18.55	13.75 ± 0.56
100	34.66	24.5 ± 1.22