Antibacterial potential of biomaterial derived nanoparticles for drug delivery application

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Abstract
Hydroxyapatite (HA) nanoparticles possess a wide range of medical applications because of their biocompatibility and compositional similarity to bone calcium phosphate mineral. The aim of the present research was the synthesis of hydroxyapatite nanoparticles by using a cheap and natural biosource and to determine its role in drug delivery. The synthesized nanoparticles were loaded with vancomycin (drug) to improve the drug’s dissolution rate and its antibacterial activity. Hydroxyapatite nanoparticles were synthesized by microwave heating technique from eggshells. These particles were characterized by optical and microscopy techniques and were used to load vancomycin-HCl. The in vitro release profile of vancomycin loaded hydroxyapatite was compared with that of pure vancomycin-HCl. The antibacterial activity was explored against Escherichia coli and Staphylococcus aureus. The prepared nanoparticles exhibit hydroxyapatite phases with agglomerated rod-shaped morphology. Through FTIR, the characteristic peaks for HA were observed in treated eggshell powder showing the formation of hydroxyapatite. Loading content and encapsulation efficiency were 23.9% and 95.6% respectively. Moreover, the in vitro release rate of antibiotic loaded hydroxyapatite was increased as compared to antibiotic alone. The zone of inhibition of vancomycin loaded with HA for E. coli was 11.5 ± 0.5 mm and was 15 ± 0.4 mm for S. aureus. Against both bacterial strains, the antibacterial potential of synthesized hydroxyapatite nanoparticles was increased as compared to vancomycin. Based on these results, hydroxyapatite can be synthesized by using eggshells and can serve as a suitable drug delivery system to improve the drug properties.

1. Introduction
Infectious diseases cause by gram-positive and gram-negative bacteria possess serious mortality and morbidity threats to global health. A glycopeptide antibiotic vancomycin has been used to treat these bacterial infections by targeting cell wall synthesis in susceptible microorganism (McGuinness et al 2017). But in the systemic treatment, high concentration of antibiotic in the serum is needed for increased shelf life. Whereas, few side effects like antibiotic resistance, vomiting, scaling, nausea and reduction in micro-flora of gut have been reported (Uskoković and Desai 2014) which contribute to the failure of conventional antibiotic treatment. As there is deficiency in the development of new antibiotics, FDA has approved 16 antibiotics from 1983–1987 and only 6 new antibiotics have been approved between 2010–2016 showing the down fall in this field (Luepke et al 2017). So, it is necessary to improve the action of existing antibiotics for the enhanced accumulation, retention and penetration of antibiotic inside the bacterial cell.

The encapsulation of antibiotics in nanocarriers aids in the elimination of microorganism by releasing high antibiotic dose at target site before the development of resistance (Huh and Kwon 2011). In the recent years, the synthesis of nanostructured bioceramics is gaining importance to be used in medical application because of their small size, low density, permeability of surface, large pore volume and surface area and thermal and mechanical...
stability. Among these bioceramics, Hydroxyapatite (Ca\(_{10}\)(PO\(_4\))\(_6\)(OH)\(_2\)) is a calcium phosphate mineral (Hamidi et al 2017) and a major component of bone and teeth and exhibit noncarcinogenic, bioactive, biocompatible and osteoconductive properties and used in tooth and bone implantation, as scaffold and drug carrier to treat bone infections. HA is a compound having hexagonal structure with white solids (Azis et al 2018). HA nanoparticles less than 100 nm in size have potential to deliver the drug at difficult access sites by crossing the natural barrier and cell interfaces. For the treatment of osteomyelitis, antibiotics impregnations with porous HA have been used as implantable drug delivery system (Mohammad et al 2014). Hydroxyapatite is also extensively used as an adsorbent and catalyst due to their structure that is porous and also has heat resistance (Azis et al 2018).

HA can be synthesized synthetically from chemicals or from natural sources such as limestone, shellfish, cockle shell, bone, sea-shell and egg shells. As eggshells are considered as commonly available household waste for the synthesis of HA nanoparticles, the purpose of this study was to use chicken eggshells to produce nanoparticles. A glycopeptide antibiotic vancomycin was chosen for loading onto HA because of its broad-spectrum activity with the low degree of resistance till now and less toxicity for mammalian cells than other antibiotics (Duewelhenke et al 2007). S. aureus and E. coli were used as test micro-organism to compare the antibacterial activity of pure vancomycin and HA loaded vancomycin. The in vitro release kinetics of vancomycin loaded HA was compared with pure vancomycin to evaluate the sustained release.

2. Materials and methods

2.1. Sample collection
In this research, the chemicals which came into utilization were of high quality grade from the companies like Sigma-Aldrich, Fluka, BBI, Oxoid, Merck, Pharmacia and ICN. The renewable primary source of biomaterial-based nanoparticles was eggshells. The raw eggshells were collected and washed thoroughly first with tap-water and then with distilled water to remove dirt and organic matter. The adhesive membranes of eggshells were manually peeled off to avoid the disturbance during crushing. The cleaned eggshells were sun dried and grinded into powder by a domestic blender (Ibrahim et al 2015).

2.2. Synthesis of hydroxyapatite nanoparticles
For the synthesis of hydroxyapatite nanoparticles, a method given by Sajahan et al (2014) was used with modification (figure 1). A concentrated solution was made by dissolving the powder of chicken eggshells in 1:3 solution of hydrochloric acid and distilled water (25 g/100 ml). The prepared mixture was subjected to stirring for 24 hours using the magnetic stirrer. The extract was then filtered to remove eggshell residues. To the filtrate, 1850 ml of diammonium hydrogen phosphate (NH\(_4\))\(_2\)HPO\(_4\) solution was added and thoroughly stirred. The pH of the mixture was adjusted to 10–11 by using 35% liquor ammonia. The mixture was heated in an oven at 220 °C for 20 min and was centrifuged (3000 rpm) five times using distilled water to remove the ammonia remained in the mixture. The precipitates were collected and dried for two days at 37 °C and were ground into fine powder by mortar and pestle.
2.3. Characterization of hydroxyapatite nanoparticles

The characteristic analysis of sample powders was performed by spectroscopic and microscopic techniques.

2.3.1. Ultraviolet–visible absorption spectroscopy
UV–visible absorption spectrophotometer was performed for the absorption spectra of the powder samples. It is an absorption spectroscopy which makes the use of light that lies in ultra-violet region of 200–400 nm. Samples absorb the light at different wavelengths which are plotted to give spectra for sample identification. The powder was dissolved in ethanol and UV-visible absorbance spectra of the powder dispersions was recorded on Shimadzu UV-1800 spectrophotometer (Maleki et al 2017).

2.3.2. Fourier-transform infrared spectroscopy
FTIR spectra was recorded to obtain the bonding structures in the powder samples and for the evaluation of drug/carrier interaction in the drug loaded nanoparticles formulation. FTIR spectra of hydroxyapatite nanoparticles, eggshell powder, vancomycin and vancomycin loaded hydroxyapatite were recorded by placing a small amount of each powder sample on IR beam of FTIR spectrophotometer (Shimadzu 43000, Kyoto, Japan) in 400–4000 cm$^{-1}$ range at 4 cm$^{-1}$ spectral resolution (Maleki et al 2017).

2.3.3. Morphology and particle size
Scanning electron microscope was used to analyze the particle size and shape of hydroxyapatite nanoparticles. The dry sample powder was first sputter coated with gold and then bombarded with electron beam. The signal in the form of secondary electrons generated due to electron-sample interaction give information about morphology of sample (Swapp 2017).

2.4. Drug loading
The HA nanoparticles were used as delivery system for vancomycin-HCl. For drug loading, 5 mg of vancomycin-HCl was added to 20 ml PBS (0.1 M, pH:7) following with addition of 20 mg of HA powder. The prepared mixture was stirred for 24 h at room temperature by magnetic stirrer. After stirring, centrifugation of sample was done at the rotation speed of 3000 rpm for 10 min and the obtained pellet was dried at room temperature (Ye et al 2010).

2.4.1. Estimation of loaded drug
For the estimation of the drug loaded on HA nanoparticles a quantitative method of spectrophotometry was used. Vancomycin–loaded hydroxyapatite nanoparticles (0.02 g) were dissolved in PBS (0.1 M, pH:7) and the UV–visible spectrophotometer was used to measure the amount of vancomycin in the solution at 280 nm wavelength and quantification was done by comparing with standard curve obtained for vancomycin (Ye et al 2010).

2.4.2. Calibration curve of vancomycin
For the standard curve of vancomycin-HCl, the absorbance of known concentrations of vancomycin-HCl was determined. In 100 ml PBS (pH 7.0), 100 mg of vancomycin-HCl was dissolved to make a stock solution. From this stock, 2, 4, 6, 8, 10 ml were taken out in 100 ml volume flask and the remaining volume was adjusted by adding PBS to make the dilution of 20, 40, 60, 80 and 100 μg ml$^{-1}$. PBS (pH 7.0) was used as blank and absorbance reading of each sample at $\lambda_{max}$ of vancomycin-HCl (282 nm) was measured by UV-visible spectrophotometer. Standard curve was plotted against concentration and absorbance to quantify the drug amount (Tariq et al 2015).

2.4.3. Encapsulation efficiency (%)
Encapsulation efficiency is the drug percentage encapsulated successfully on nanoparticles calculated by following equation:

$$Encapsulation\ efficiency\ (%) = \frac{W_t - W_f}{W_t} \times 100$$ (1)

Where, $W_t$ is the the total drug weight and $W_f$ is the weight of free non-encapsulated drug (Isa et al 2016).

2.4.4. Loading capacity (%)
Loading capacity is the amount of loaded drug per unit nanoparticle weight, showing the weightage of nanoparticle mass due to drug encapsulation calculated by:
Drug loading(%) = \( \frac{W_t - W_f}{W_{np}} \times 100 \)  

Where, \( W_t \) is the total weight of drug added, \( W_f \) is the weight of non-encapsulated free drug, and \( W_{np} \) is the total weight of the nanoparticles (Isa et al 2016).

2.5. In vitro release

The release of vancomycin from hydroxyapatite nanoparticles was determined by using dialysis bag based on membrane separation technique to compare it with release rate of vancomycin. For this purpose, 0.5% solution of vancomycin and vancomycin loaded hydroxyapatite nanoparticles were separately placed in dialysis bags and immersed in PBS solution (7.4 pH) and allowed to stir at 150 rpm at 37 °C. At regular intervals, 3 ml was taken from test media and replaced with fresh PBS (7.4 pH). The release rate of drug from nanoparticles was determined by taking the absorbance values at variable time intervals by UV-visible spectrophotometer and compared with release rate of drug without nanoparticles (Simon et al 2013).

2.6. Antibacterial activity

The antibacterial activity of vancomycin loaded hydroxyapatite nanoparticles and vancomycin was tested against two bacterial strains, S. aureus and E. coli provided by department of zoology, Mirpur University of Science and Technology, Mirpur.

2.6.1. Agar disc-diffusion assay

Nutrient agar plates were inoculated uniformly by spreading suspension of bacteria with sterile cotton swab all over the plate in one direction. Sterile discs of filter paper 6 mm in diameter were impregnated with 20 µl of each solution and placed on agar plates. These petri plates were incubated overnight at 37 °C. The diffusion of antibacterial agent on agar takes place resulting in the inhibition of bacteria. The zone of inhibition of both the drug solutions was determined and compared by measuring the diameter in millimeters. The result was obtained as the mean of three replicate experiments (Roy et al 2010).

2.7. In vitro biocompatibility

2.7.1. Sample preparation

Different concentrations of HA were prepared by adding 0.25 mg, 0.5 mg, 1 mg, 2.0 mg and 4.0 mg of HA in 1 ml PBS separately.

2.7.2. Blood cell suspension preparation

Blood (5 ml) was taken in falcon tube from a healthy volunteer and centrifuged for about 5 min at 4000 rpm. The centrifugation separated the plasma from red blood cells. The supernatant was discarded and RBC in the pellet were diluted by adding 5 ml of fresh PBS (7.4 pH) and again centrifuged for 10 min at 3000 rpm. The obtained pellet was stored in refrigerator by dissolving in 15 ml PBS solution.

2.7.3. Hemolytic assay

Biocompatibility of HA was tested by hemolysis assay which determine the effect of HA nanoparticles on red blood cells. From each sample solution, 0.8 ml was taken and mixed with 0.2 ml of stored blood sample and incubated at 37 °C for 1 h. After the completion of incubation, all the sample tubes were centrifuged for 10 min at 3000 rpm to obtain the supernatant. The spectroscopic analysis of all the supernatant were done by placing in plate of 96 well and taking optical density at 570 nm. For the positive and negative control, triton X-100 and PBS was added in 0.2 ml prepared blood sample (Bandgar et al 2017). The extent of hemolysis of HA was calculated by:

\[
\text{Hemolysis (%) of RBCs} = (100 - \frac{\text{Absorbance of sample}}{\text{Absorbance of triton X - 100}} \times 100)
\]

3. Results and discussion

3.1. Nanoparticles preparation

HA powder was successfully synthesized from eggshells under the optimized conditions via microwave heating method and is shown in figure 2. Microwave synthesis is considered as convenient method resulting in high purity and yield. An important parameter in the completion of HA synthesis is alkaline pH. The mixture of eggshells solution and \((\text{NH}_4)_2\text{HPO}_4\) had the pH of 7–8 which was increased to 10–11 by liquor ammonia instead of ammonium hydroxide which prevent carbonate formation during synthesis process. Above the pH of 10,
crystalline HA have uniform morphology and narrow particle size whereas the pH less than 10 results in the formation of $\beta$-tricalcium phosphate and HA with irregular morphology. It was found by Palanivelu et al (2014) that at neutral pH of 7, synthesis of dicalcium phosphate and octa calcium phosphate (OCP) which are secondary phases of calcium deficient HA also occurred along with HA. This was due to the slow release of ions in the solution media between phosphate and calcium precursors and also caused the particles to be agglomerated (Palanivelu et al 2014).

3.2. Characterization of hydroxyapatite nanoparticles

The confirmation of HA nanoparticles preparation was performed by using UV-vis absorption spectroscopy, Fourier-transform infrared spectroscopy and scanning electron microscopic techniques.

3.2.1. Ultraviolet–visible absorption spectroscopy
HA powder showed the maximum absorption peak at 207 nm as shown in figure 3. The optical absorption of pure HA lies in the UV region of 200–340 nm showing a peak band below 247 nm. In a research conducted by Yadav et al (2017) HA were synthesized and they exhibited different peaks at 220 nm and 560 nm, same peaks were observed in figure 3. The obtained results are related to pure HA absorption spectra reported by De Araujo et al (2010). Due to the scattering of HA powder, an additional absorption was observed at ~274 nm. As a feature of colloidal particle size, the scattering pattern of light can vary (Yang et al 2016).
3.2.2. Fourier—transform infrared spectroscopy

The FTIR spectra of raw eggshells, HA nanoparticles are presented in figure 4. Many bands are present in raw eggshell powder from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\). In the spectra, carbonate has prominent peak of absorption at 1407 cm\(^{-1}\). The absorption peak for CaCO\(_3\) was observed at 873 cm\(^{-1}\). These results agree with eggshell powder characterization reported by Bashir \textit{et al} in which carbonate has peak at 876 cm\(^{-1}\) (Bashir and Manusamy 2016).

The characteristic peaks for HA were observed in treated eggshell powder showing the formation of hydroxyapatite. P–O bonds in phosphate group in HA exist in the form of four asymmetrical stretching vibration modes which are \(v_1\), \(v_2\), \(v_3\) and \(v_4\) (Nasiri-Tabrizi \textit{et al} 2013). Sample has the vibrating peaks of \(v_1\)–PO\(_4^{3-}\) vibrating peak at 964 cm\(^{-1}\) showing the weak vibration band. A significant \(v_1\) and \(v_4\) vibrating peaks are indicated at 1022 cm\(^{-1}\), 565 cm\(^{-1}\) and 602 cm\(^{-1}\) respectively. Vibration band \(v_2\) for PO\(_4^{3-}\) was not observed in the HA sample. These phosphate group FTIR characteristics confirm the HA phase. Two modes of structural OH exists in HA phase, liberation and stretching mode. A weak band representing the OH\(^-\) liberation mode can be observe at 630 cm\(^{-1}\) whereas the weak band at 3519 cm\(^{-1}\) correspond to stretching of structural OH\(^-\).

The eggshell HA sample showed the presence of carbonate group at 875 cm\(^{-1}\) and between 1410 cm\(^{-1}\) and 1450 cm\(^{-1}\) which is either because of eggshell calcium carbonate or because of CO\(_2\) as calcination product. Commonly biological apatite undergoes substitution of non-apatite ions like fluoride, carbonate and chloride which either replace OH or PO\(_4^{3-}\) groups depending on the type of apatite (type A and type B). The CO\(_3^{2-}\) group exhibited by the sample lies in the region of type B apatite. By increasing the heating time, substitution of other functional groups can be avoided (Sajahan \textit{et al} 2014). The important HA functional groups are summarized in table 1.

3.3. Morphology of nanoparticle

To study the morphology of HA, scanning electron microscope was used. Figure 5 represents the SEM images of prepared HA nanoparticles. The nanorods of HA were assembled hierarchically into mesoporous nanostructured microspheres. The HA nanorods have high degree of agglomeration. Azis \textit{et al} (2018) synthesize HA from eggshell by sol-gel method and reported the hydroxyapatite morphology in the form of agglomeration. The reason for agglomeration might be Ostwald ripening (Sagadevan and Dakshnamoorthy 2013).

![Figure 4. FTIR spectra of eggshell and hydroxyapatite.](image)

### Table 1. Functional Groups Assigned to HA Nanoparticles by FTIR Analysis.

| Functional groups | Wavenumber cm\(^{-1}\) |
|-------------------|------------------------|
| PO\(_4^{3-}\) \(v_1\) bending | 964 |
| PO\(_4^{3-}\) \(v_2\) bending | 1022 |
| PO\(_4^{3-}\) \(v_3\) | 565, 602 |
| OH\(^-\) | 630 |
| CO\(_3^{2-}\) | 3519, 1441 |

Figure 4. FTIR spectra of eggshell and hydroxyapatite.
surface is made up of atoms/molecules that are loosely bound to the particles other than ones in bulk. The smaller the particles, the larger is the weak bonding. As compared to larger particles, the smaller particles dissolve easily. With the time, the smaller particles/droplets dissolve and their molecules are diffused in bulk and deposit on the surface of larger particles. This process of disappearing the smaller particles and depositing on larger particles by dissolution is known as Ostwald ripening (Tadros 2013). The morphology of HA nanoparticles shown by SEM images are related with the reported results by Wei-Lin et al (Yu et al 2017).

3.4. Drug loading
During the process of drug loading, the molecules of vancomycin-HCl could be adsorbed on the porous HA surface by the reaction of −OH group present on HA surface and carboxyl group of vancomycin to form hydrogen bonding.

3.4.1. Confirmation of drug loading through FTIR
FTIR investigates the possible interaction between vancomycin-HCl and HA nanoparticles. Figure 6 shows the FTIR spectra of pure vancomycin-HCl, HA and drug loaded HA. The vancomycin-HCl showed the stretching peak at 1653 cm\(^{-1}\) of C=O, peak at 1490 cm\(^{-1}\) of C=C, 3279 cm\(^{-1}\) of OH group and 1230 cm\(^{-1}\) of phenolic hydroxyl group (Mohamed et al 2017). The characteristic bands of HA present in drug loaded sample are the indication of vancomycin attachment in HA nanoparticles. The bands in 1700–1200 cm\(^{-1}\) are because of vancomycin presence (Ye et al 2010). Based on this analysis, it was found that HA nanoparticles were loaded successfully with the vancomycin-HCl and the chemical structure of vancomycin was not affected during the loading in HA nanoparticles.

3.4.2. Calibration curve for vancomycin-HCl
The calibration curve for vancomycin HCl in PBS (pH 7.0) was obtained by plotting the value of concentration and absorbance. The calibration curve is shown in figure 7. The calculation of drug amount is facilitated by the straight-line equation from the absorbance value by regression analysis. A linear relationship existed between drug concentration and absorbance and the value of correlation coefficient (R\(^2\)) was found to be 0.9982 obtained from regression and linearity equation.

3.4.3. Loading content (LC%) and encapsulation efficiency (EE%)
The drug loading content (LC) and the encapsulation efficiency (EE) for HA usability as delivery system was evaluated by UV-vis results combined with equation given in above section. After the loading process, the concentration of drug in the supernatant estimated from calibration curve found to be 0.217 mg which represents the free non-encapsulated drug. Loading content (LC%) of vancomycin into HA particles found to be 23.9% and the encapsulation efficiency (EE%) was 95.6% which shows the minimum loss of vancomycin during loading process.
3.5. *In vitro* release study

The release of vancomycin from HA particles takes place by diffusion-controlled mechanism. The solvent from the surrounding media entered through the semi-permeable dialysis membrane to the inner of material allowing the drug to be slowly dissolved in release media (PBS). Under the controlled conditions, the drug release kinetics was determined by dissolution method for 90 min. Figure 8 shows the release pattern of pure vancomycin-HCl and vancomycin loaded hydroxyapatite nanoparticles in PBS solution having 7.4 pH. Cumulative drug release (%) refers to the drug amount in PBS at any time interval plus the drug amount discarded from sample by taking out the sample and replacing with the same amount withdrawn. The initial rate of drug release is low and with the time increased linearly. The amount of vancomycin-HCl release in 90 min is 57.02% and for pure vancomycin is 65.41%.

As compared to pure vancomycin, the drug release of vancomycin loaded HA is relatively slow. This was possibly due to association between HA and vancomycin HCl which resulted in slow drug release. These results suggest that loading the drug with HA carrier provide sustained drug release as compared to drug alone. Release time can be further prolonged by using the polymers such as chitosan, poly (ethylene glycol), collagen etc (Galindo *et al* 2019).
3.6. Antibacterial activity

Disc diffusion test is a common method for the determination of antibacterial activity. The antibacterial activity of vancomycin-HCl and vancomycin loaded HA nanoparticles was performed against gram negative bacteria *E. coli* and gram-positive *S. aureus*. Vancomycin is well known antibiotic having antimicrobial activity against several gram positive and negative bacteria. The antibacterial activity of both the samples for *E. coli* and *S. aureus* is shown in the figure 9.

The mean diameter of zone of inhibition of vancomycin for *S. aureus* was 13 ± 0.7 and for *E. coli* was 10 ± 0.5 mm and the zone of inhibition of vancomycin loaded HA for *E. coli* was 11.5 ± 0.5 mm and was 15 ± 0.4 mm for *S. aureus* as represented in table 2. This result suggests the improved antibacterial activity of vancomycin loaded HA as compared to antibiotic alone for both the bacteria. The enhanced antibacterial activity due to loading on HA may be due to the presence of ions in HA structure.

![Figure 8. Comparison of cumulative drug release (%) of vancomycin and vancomycin loaded HA.](image)

![Figure 9. Anti-bacterial activity of vancomycin loaded HA and vancomycin against (A) *S. aureus*, (B) *E. coli*.](image)

| Samples            | Zones of inhibition (mm) |
|--------------------|--------------------------|
|                    | *S. aureus* | *E. coli* |
| Vancomycin         | 13 ± 0.7     | 10 ± 0.5   |
| Vancomycin-HCl     | 15 ± 0.4     | 11.5 ± 0.5 |

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3.7. Hemolytic assay

The HA nanoparticles in different concentrations were prepared to screen their hemolytic effect on normal human RBCs. The material is said to be non-hemolytic if the percentage of hemolysis is less than 2%, if the percentage is above 5% it is said to be hemolytic and slightly hemolytic if hemolysis is between 2 and 5% (Bandgar et al 2017).

No hemolytic activity was exhibited by the samples up to 3 mg ml\(^{-1}\) concentration because they all show the hemolysis percentage below 2% as represented in figure 10. It was noticed that by increasing the dosage of hydroxyapatite hemolytic activity was also increased. Maximum hemolysis shown by HA was found to be 1.4%. The obtained results are in correspondence with the work conducted by Laranjeira et al (2016) in which pure hydroxyapatite was tested for hemolytic activity at different concentrations. No hemolytic activity was observed at higher concentration of 4 mg ml\(^{-1}\) but showed slight toxicity to erythrocytes when doped with iron (Laranjeira et al 2016). Hence these results suggest that exposure of erythrocytes to HA nanoparticles induce no toxicity.

4. Conclusions and recommendations

The eggshells can be utilized in a useful way to produce hydroxyapatite, calcium carbonate and \(\beta\)-tricalcium phosphate etc which have the wide range of pharmaceutical and medical applications. This work provides an interesting route for bioceramics production from a natural material i.e. chicken eggshell. Microwave irradiation method used in this research for the hydroxyapatite synthesis, proves to be an effective approach to obtain the HA particles with the different morphologies and characteristics which can be identified by various art techniques. This work demonstrated the sustained release and increased antibacterial activity of antibiotic after loading onto HA nanoparticles. Loading of vancomycin-HCl with the hydroxyapatite showed that due to the porous structure of hydroxyapatite it can act as a nanocarrier for the loading of number of drugs. The hydroxyapatite nanoparticles can be used to load other drugs as well and expected to enhance their efficiency against the different microbes.

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Conflict of interest

Authors declare that they do not have a conflict of interest in any capacity including competing or financial.
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