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ORIGINAL ARTICLE

Magnesium incorporated hydroxyapatite nanoparticles: Preparation, characterization, antibacterial and larvicidal activity

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Abstract Mosquito-borne diseases cause several deaths every year in tropical and subtropical climate countries. Control of vectors is an alarming problem in today's world due to the resistance matters. In this study magnesium incorporated hydroxyapatite nanoparticles have been synthesized by microwave irradiation method. Magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$) calcium nitrate tetra hydrate (Ca $(NO_3)_2{\cdot}6H_2O$) and disodium hydrogen phosphate (Na_2HPO_4) were used as magnesium, calcium and phosphorous sources to prepare hydroxyapatite nanoparticles. The FT-IR studies show the presence of hydroxyl and phosphate functional groups. The structural properties have been studied using X-ray diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM) and Transmission Electron Microscope (HRTEM). The energy dispersive X-ray analysis (EDAX) revealed the presence of Ca, Mg, P and O in the prepared samples. The antibacterial activity of the as-synthesized nanoparticles was evaluated against two prokaryotic strains, the gram negative bacteria *Escherichia coli* for three different concentrations of as-synthesized nanoparticles and they showed excellent antibacterial activity. The as-synthesized Mg-HAp nanoparticles were tested against fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* and the nanoparticles exhibited significant mortality against the selected mosquitoes. The observed results suggest that the magnesium incorporated hydroxyapatite nanoparticles have the potential to be used as an effective mosquito larvae control agent against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*. From the detailed literature review it has been observed that no work has been

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carried out so far on the larvicidal activity using hydroxyapatite (HAp) nanoparticles and magnesium substituted hydroxyapatite (Mg-HAp) nanoparticles.

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1. Introduction

Mosquitoes cause severe disease such as dengue, malaria, chikungunya, filariasis, Japanese encephalitis and leishmaniasis which are endemic in more than 100 countries. Due to mosquito borne disease more than two million people have died throughout the world and 2100 million people are at risk every year (WHO, 2009; Rajakumar and Abdul Rahuman, 2011). *Aedes aegypti* is recognized as a dengue vector and two-fifth of the world population is under risk of dengue (WHO, 2009, 2010). According to the WHO, 2012 survey report 50–100 million people are affected by dengue every year. *Anopheles stephensi* is solely responsible for transmission of malaria which causes morbidity and mortality with nearly three million cases every year (Sharma et al., 2009). Especially, peoples from Middle East and Indian subcontinent were affected by malarial infection severely. *Culex quinquefasciatus* is responsible for lymphatic filariasis commonly known as elephantiasis. In India, more than 553 million people are at risk of infection and are widely distributed in 17 states and 6 union territories (Das et al., 2000). Moreover, 120 million people were affected by lymphatic filariasis (WHO, 2013). Hence Mg-HAp nanoparticles which are less likely to cause ecological damage have been identified as a possible replacement of present insecticides such as permethrin, dieldrin and fenitrothion.

Disease causing microbes of gram negative and gram positive bacteria have become resistant to drug therapy and cause death more than 90 million people every year which is an alarming public health problem nowadays (Stevanovic et al., 2012). Hence there is an urgent need to develop novel bactericides. The antibacterial properties of HAp and Mg-HAp based compounds have been studied (Chung et al., 2006). Since the ion exchanging properties of HAp and Mg-HAp are known to be highly toxic to microbes and exhibit strong biocidal effects, it is currently used to control the bacterial growth and a number of other applications (Ragab et al., 2014). Many researchers have reported the antibacterial activity of HAp and Mg-HAp recently such as Zhao et al., 2016; Jiang et al., 2015; Liu et al., 2012.

Hydroxyapatite (HAp) [$Ca_{10}(PO_4)_6OH_2$] is a material of choice for various biomedical applications such as orthopedic, dentistry, antimicrobial and drug delivery, because of its similarity of composition to mineral phase of the bone, excellent biocompatibility, and ability to promote cellular functions and expression and osteoconductivity. HAp has the ability to integrate in bone structure and support bone in-growth, without breaking down or dissolving (Thamariselvi et al., 2006). Owing to its bioactive property, HAp is widely used in medicine and dentistry as a material for metallic implant coatings, or for bone cavity fillings (Currey, 2001; Thompson et al., 2001). In recent years the application of HAp has extended to various fields due to its excellent properties such as anti-microbial activities (Ragab et al., 2014). However hydroxyapatite does not have good mechanical strength (De With et al., 1981) and has low bioactive property due to its low resorbability (Maria and Daniel, 2005). The HAp lattice can easily accommodate a variety of substituents both cationic and anionic, inducing modifications in the crystallinity, morphology, lattice parameters and stability of the apatite structure. This feature can be used as a powerful tool to prepare materials with specific characteristics mimicking the biological apatites, which are non-stoichiometric and contain structural imperfections and defects as well as foreign ions (Rey, 1998; LeGeros, 1991). Therefore synthetic hydroxyapatite ceramics are doped with small amounts of additives (Mg^{2+}, Zn^{2+}, F^-, Mn^{2+} and CO_3^{2-} ions) in order to improve the mechanical and bioactive property of the implants. The incorporation of magnesium ions into the hydroxyapatite structure leads to significant improvement in the anti-microbial and larvicidal properties of HAp.

There are various methods available to produce nanosize HAp as reported in the literature including sol–gel, hydrothermal, precipitation, spray pyrolysis, solid state reaction method, etc. (Sun et al., 2006; Koumoulidis et al., 2003; Wang et al., 2006; Han et al., 2006; Tas, 2000; Kalita and verma, 2010). In the present study microwave irradiation method has been used to synthesize HAp due to its advantages such as fast reaction rate, simplicity, short reaction time, high reaction selectivity and energy saving. Microwave is an electromagnetic wave of high frequency which consists of alternate magnetic field and electric field. Microwave excitation heats the core and surface of the material homogeneously because microwave energy introduces thermal energy by means of collision between rotating molecules (Siddharthan et al., 2006). Microwave heating has thus been found to be a very convenient thermal source not only in the kitchen but also in a chemical laboratory. Chemists have explored the possibility of the application of a conventional microwave oven to carry out chemical reactions (Rashmi Sanghi, 2000). In the present work, for the first time we have made an attempt to investigate the anti-bacterial and larvicidal activity of microwave synthesized pure HAp and Mg-HAp.

2. Materials and methods

2.1. Chemicals

The chemicals used to synthesize HAp and Mg-HAp are calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}6H_2O$, 98%, Otto), magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$, 97%, Hi-Media), disodium hydrogen phosphate (Na_2HPO_4, 99%, Merck) and ammonium hydroxide (NH_4OH, 25%, Merck). All reagents used were of analytical reagent grade and were used without further purification and deionized water was employed as the solvent throughout the experiments.

2.2. Synthesis

Pure HAp and Mg-HAp were synthesized by microwave irradiation method. In a typical procedure for the synthesis of pure HAp, 10 mL of 0.15 M sodium dihydrogen phosphate was added to 10 mL of 0.25 M calcium nitrate tetrahydrate drop by drop to produce a colloidal solution while Ca/P ratio was maintained at 1.67. The molar ratios of the chemical elements of reactants used in the preparation of the powders are reported in Table 1.

Table 1 Molar ratios of the reactants used in the preparation.

Sample name	Ca	P	Mg/Ca	(Ca + Mg)/P
HAp	1.09	0.53	0	1.62
Mg-HAp	0.37	0.38	0.56	1.31

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