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ZEIN BASED MAGNESIUM OXIDE NANOWIRES: EFFECT OF ANIONIC CHARGE ON SIZE, RELEASE AND STABILITY

G. H. NAGUIB[a*], A. H. HASSAN[b], F. AL-HAZMI[c], M. KURAKULA[d], A. AL-DHARRAB[e], H. M. ALKHALIDI[f], A. M. AL-AHDAL[f], M. T. HAMED[g], D. H PASHLEY[h]

[a]*Department of Conservative Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia*
[b]*Department of Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia*
[c]*Department of Physics, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.*
[d]*Polymer Science Group, Department of "Giacomo Ciamcian" Chemistry, University of Bologna, Italy*
[e]*Department of Oral Rehabilitation, Faculty of Dentistry King Abdulaziz University, Jeddah, Saudi Arabia*
[f]*Department of Clinical Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*
[g]*Department of Oral Rehabilitation, Faculty of Dentistry King Abdulaziz University, Jeddah, Saudi Arabia*
[h]*Department of Oral Biology, The Dental College of Georgia, Augusta University, Georgia, USA.*

Magnesium oxide (MgO) is a vital mineral with competence in strong inhibition against resistant bacterial strains. Dosage form design for the MgO particles delivery is limited both due to the fact of poor solubility and instability by agglomeration on storage. Redesigning MgO nanostructured carrier coated by zein having anionic charge as stabilizer would create great prosthetic having antibacterial significance. We expect this innovative technique paves way not just to modify the release of MgO nanowires but can even overcome both the limitations. MgO nanowires were synthesized using microwave hydrothermal technique. Zein coating has exhibited significant effect over physicochemical properties of MgO. The pure MgO nanowires alone exhibited an average size of 150 nm but tend to aggregate increasing their size to 800 nm contrastingly to the zein coated MgO nanowires those average size was found 230 nm with no further size increase on exposure in aqueous dispersion over 1 h. This was supported with the scanning and transmission electron microscopy images that indicated grouped or aggregation of pure MgO nanowires while as highly sharp distinct MgO nanowires when coated with zein. Pure MgO and zein coated nanowires exhibited a surface charge of 11 and -22 mV at pH of 1.2 alternatively -15 and -18 mV at pH 6.8, respectively. X-ray crystallography of zein based MgO nanowires was highly pure and crystalline in nature. The FTIR spectra indicated distinct peaks with no possible interaction or formation of new product during synthesis or with zein. Interestingly, *in-vitro* dissolution was found prolonged about 20 % up to 12 h for the zein stabilized MgO nanowires at pH 1.2 with respect to pure MgO and zein coated MgO nanowires prepared at pH 6.8 indicating effective adsorption of zein. Degradation temperatures did not indicate any significant changes in physiochemical properties indicating the effect of zein over enhancement of nanowires stability. The use of zein has provided sufficient electrostatic and steric hindrance between nanowires preventing aggregation with altered MgO release. Fabricated zein-coated MgO nanowires are biocompatible and can be promising antibacterial agents in dental materials.

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[*]Corresponding author: beautsway@yahoo.com

1. Introduction

Bacterial contamination that cause inconvenience to public health is of great concern across the globe. The infections could be of many types and significant.[1] Dental hygiene care includes the use of several antibacterial agents in periodontal therapy. Antibiotic prophylaxis is not always an option for the patients those have a risk of hematogenous infections. Therefore, there is always an urge to develop a physical material as effective oral antibacterial agent. [2] Organic type of bactericidal agents such as weak acids or essential oils have exhibited greater limitation due to their low resistance to processing conditions. As an alternative, inorganic agents have been emerged due to their stability on harsh processing conditions and even for their approval as safe for human use. [3,4] Use of metal oxides as substitute of antibiotics can be of great value in safety and irrational use. [5] Metal oxides as inorganic agents have gained interest as antimicrobial agents because of their ability to bind and damage bacterial membranes inhibiting their growth.[6] In recent years, oxides of metals such as Zinc (ZnO), Titanium (TiO_2), Calcium (CaO), Magnesium (MgO) have been reported as effective bacteriostatic agents. However, their biocompatibility and studies for human use is still underway. [7-12]

Magnesium Oxide (MgO) is a vital mineral in bone regeneration [13] and that is recently found promising in tumor treatment [14] apart from its use as antacid [15] . MgO nanoparticles can be synthesized using different techniques but with limitations for use. One dimensional nanostructuring over conventional particle synthesis of metal oxide can be beneficial. [16-18] In our previous studies, nano-structured MgO such as nanowires had exhibited fair bactericidal activity, which was highly dependent on the particle size and concentration. [19-22] Unfortunately, MgO nanowires were found relatively insoluble and tend to agglomerate, deterioting the antibacterial properties of the nanowires.[23] This made us to consider a surfactant based stabilizer for MgO nanowires that not just prevents the agglomeration but even facilitates their dissolution to obtain optimum properties.

Zein is a natural alcohol-soluble protein present in the endosperm tissue of corn.[24] The unique properties such as biocompatible and biodegradability makes the polymer a promising candidate in formation of micro or nanospheres and even film coatings to modify the gene delivery.[25,26] Zein has wide pharmaceutical applications especially coating the tablet over sugar due to their high resistance to heat, humidity, abrasion and capacity to mask strong odor or unacceptable taste. Due to water insolubility and swelling properties zeins was even explored for its use in control or sustain the drug release from the dosage form. [27, 28] Zein is an amorphous polymer having glass transition temperature (Tg) at 165 °C, that exhibits plasticizing viscoelasticity. This inherent property helps zein to stabilize particles against aggregation by greatly reducing the hydrophobic attraction and increasing steric repulsion at molecular level that can be of great advantage. [29]

There are very few or no studies that have reported the use of zein as stabilizer in fabrication of one dimensional nanostructured metal oxide such as MgO. Therefore, in the present study we aim to prepare, characterize zein based MgO nanowires and examine the influence of anionic charge effect of zein over the formation, physiochemical, *in-vitro* dissolution and stability of MgO nanowires.

2. Material and methods

2.1. Facile synthesis of MgO nanowires:

All chemicals were Analytical grade and were obtained from Sigma-Aldrich, USA. MgO nanowires were synthesized by directly reacting magnesium acetate ($Mg(CH_3COO)_2$) and urea (CH_4N_2O) using the microwave hydrothermal technique [23]. A mixture of magnesium acetate and water (6.4 g / 75 mL) was stirred for 30 min at controlled temperature of 25 °C. Few drops of urea were added into water under continuous stirring conditions (1.2 g / 25 mL). Further, the solution was then transferred into a Teflon-lined autoclave (100 mL) that was sealed and maintained at 180 °C for 15 min. The autoclave was supported with a microwave furnace (1000 W). After completion of the reaction, the autoclave was left to cool. The products were collected and filtered using distilled water, rinsed with ethanol, and finally dried at 60 °C for 24 h. The obtained product was then calcinated at 600 °C for 1 h.[13]

2.2. Coating and stabilization of MgO nanowires with zein:

Zein approx. 0.02 g was weighed and dissolved in a mixture of ethanol (C_2H_5OH) and 0.1 sodium hydroxide (NaOH) solution (93.7 % (v/v)). Another aqueous solution was prepared containing approx. 0.02 g of MgO and polyvinyl alcohol (PVA) (0.9 % (w/v)). This solution was subjected to ultrasonic shear (750W and 20 kHz frequency) and the previous zein solution was carefully added drop wise. The temperature of the aqueous phase was controlled and maintained at 10 °C using an ice bath. Further, the zein based MgO suspension was kept under constant stirring at 500 rpm in order to evaporate the ethanol. After the complete evaporation of ethanol, the nanowires were washed with distilled water in order to remove the excess PVA. The aqueous suspension of zein nanowires were further purified by two cycles of differential centrifugation (3,000 rpm for 45 min). After centrifugation, the supernatant was discarded and the sediment was redispersed in 5 ml of buffer. Lastly, trehalose approx. 2 % (w/v) was added to the suspension and lyophilized. (Virtis ^{TM}, Bench Top model, USA). [30]

3. Characterization

3.1. Size and Zeta potential of nanowires

The particle size distribution of colloidal dispersions containing nanowires was measured by dynamic light scattering (Nano-ZS, Malvern Instruments, UK). The Z-average diameter was calculated from the measured distributions. The surface charge of the nanowires in the colloidal dispersions was determined using a micro-electrophoresis device (Nano-ZS, Malvern Instruments, UK). Samples were diluted with the same pH adjusted water or buffer solution prior to measurements to avoid multiple scattering effects.

3.2. Surface Morphology of the nanowires

Transmission electron microscopy (TEM) was performed using a JEOL 2011 electron microscope at an acceleration voltage of 100 kV. The MgO nanowires in ethanol were dropped onto carbon-coated copper grids and allowed to dry under ambient conditions. Scanning electron microscopy (SEM) was even performed using Carl Zeiss Meditec AG, Jena, Germany. The nanowires were mounted on to the metallic stub and sputter gold coated. The samples were examined at an acceleration voltage of 15 kV. The specimens were then examined directly without any further treatment.

3.3. X-ray diffraction (XRD) of the nanowires

XRD measurements of the MgO nanowires were performed using a Shimadzu XRD-6000 X-ray powder diffractometer using Cu Kα radiation (0.15406 nm at 15 kV and 30 mA for the X-ray tube). For every measurement, the phase type and content were analyzed at 5° min^{-1}. A complete 2θ scan was performed between 10° and 80°.

3.4. Differential scanning calorimetry (DSC) of the nanowires

Thermogravimetric analysis of the MgO nanowires was performed using a Shimadzu differential scanning calorimeter DSC-60 with airflow (flow rate of 50 mL min^{-1}). Dry MgO nanowires (1–2 mg) were sealed in aluminum pans. Empty sealed pans served as the controls. Specimens were scanned from 25 °C to 800 °C at a rate of 10 °C min^{-1}.

3.5. Fourier transform infrared spectroscopy (FTIR) of the nanowires

FTIR spectra of MgO nanowires, the zein polymer, and MgO-zein nanowires were obtained using the KBr pellet method using Nicolet spectrophotometer enabled with a DTGS TEC detector. The spectra were recorded from 4000 to 400 cm^{-1}.

3.6. In-vitro dissolution studies of the nanowires

In order to understand the role of zein over the modifying the solubility or release of MgO nanowires, dissolution studies were performed. Three different formulations were examined i.e. pure MgO, zein based MgO nanowires prepared at pH 1.2 and 6.8. The formulations were equivalently weight and taken in a glass vial (10ml). Phosphate buffer having pH 1.2 was used as the dissolution medium at 37^{o}C. The sampling times were 0.5,1,2,4,6,12 h respectively. Aliquots

were removed and immediately replaced with fresh buffer in order to maintain sink conditions. The samples were analysed using high performance liquid chromatography (HPLC).

3.7. Stability studies

The thermal decomposition at varied temperatures has an impact over the physiochemical as well biological response of the MgO nanowires. Therefore we conducted stability studies of the prepared nanowires at 25, 40 and 50^{o}C temperatures. The possible changes within the properties and in-vitro dissolution before and after the temperatures were recorded.

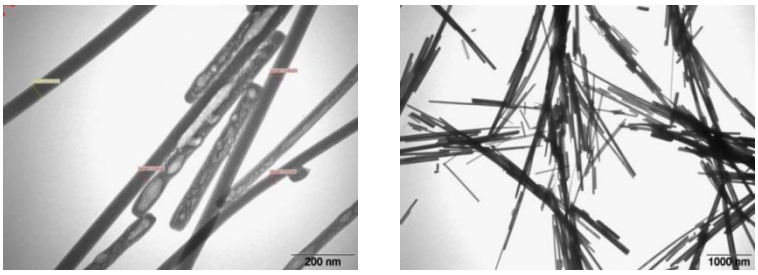
4. Results

4.1. Size and charge of nanowires

The pure MgO nanowires alone exhibited an average size of 150 nm but tend to aggregate increasing their size up to 800 nm on exposure in aqueous dispersion over 1 h. Contrastingly to the zein coated MgO nanowires those average size was found 230 nm with no further size increase even after exposure up to 12 h in water. This inhibition of further increase in size is due to the steric effect of zein, which has prevented the aggregation of nanowires.

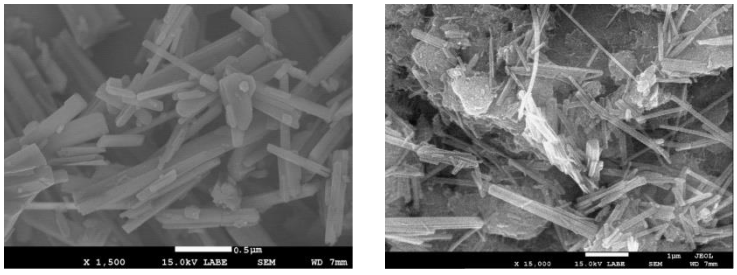
4.2. Surface morphology of nanowires

The TEM images indicated that nanowires were about 30-60 nm wide and 2-3 μm long. [Fig. 1] This result was in agreement with the XRD and SEM results (vide infra). The SEM images of pure MgO nanowires revealed size about 50–60 nm wide and 500–1000 nm long, appearance in the state of aggregation [Fig. 2 (a)]. When the MgO nanowires were coated with 1% zein polymer, nanowires appeared to be longer, individually distinct. It was even observed from the images that few nanowires even in aggregated state resulting formation of plates of 1-2 μm length and 0.1 μm in thickness. [Fig. 2 (b)]



(a) (b)

Fig. 1. Transmission Electron microscopy images of pure MgO nanowires



(a) (b)

Fig. 2. Scanning Electron microscopy images (a) pure MgO nanowires (b) zein coated MgO nanowires

4.3. X-ray diffraction of nanowires

The existence of strong and sharp diffraction peaks located at 2θ values of 36.2°, 45.4°, and 66.2° corresponding to the (111), (200), and (220) planes, respectively, indicating that the

powders are highly crystalline. In addition, the results even proved that the MgO nanowires were highly pure because there were no other diffraction peaks other than those for MgO. [Fig. 3]

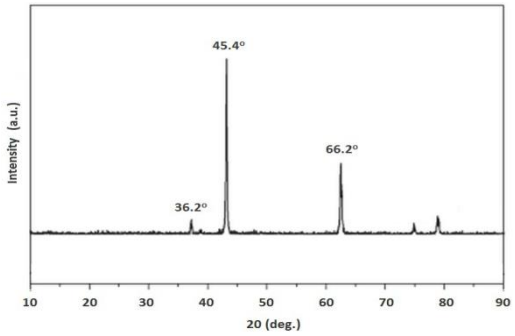
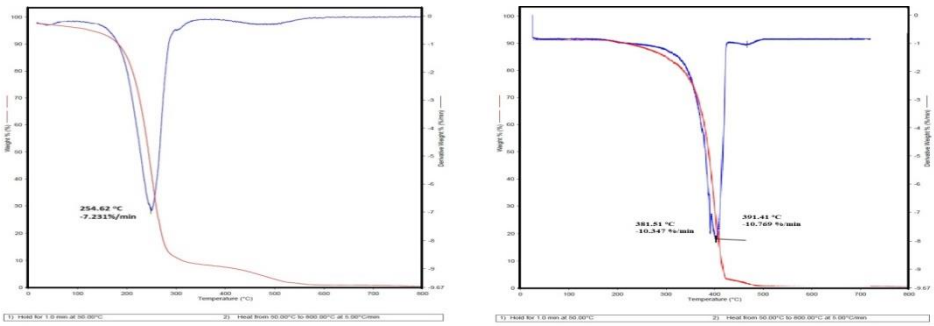


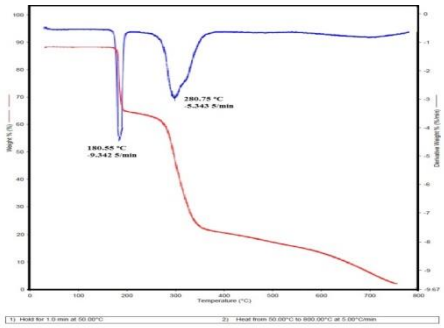
Fig. 3. X ray diffraction spectra of pure MgO nanowires

4.4. Differential scanning calorimetry of nanowires

The DSC spectra showed an endothermic single peak at 254 °C for the MgO nanowires [Fig. 4 (a)]. The peak was distinct indicating that the nanowires were pure. The scan of pure zein polymer resulted in single endothermic peak at 381.5 °C [Fig. 4 (a)]. Interestingly, zein stabilized MgO nanowires scan showed two distinct peaks at 180.6 °C and 280.8 °C respectively, indicating the shift of endothermic peaks towards the lower values. [Fig. 4 (a)] Even the melting point of MgO nanowires was lowered from 254 °C to 180.6 °C, while that for the zein polymer was decreased from 381 °C to 280.8 °C.



(a) (b)



(c)

Fig. 4. Differential scanning calorimetry spectra (a) pure MgO nanowires (b) pure Zein (c) Zein based MgO nanowires

4.5. Fourier transform infrared spectroscopy of nanowires

The FTIR spectra of the MgO nanowires showed IR absorption peaks at 3670 cm^{-1} and 3450 cm^{-1}. This might be corresponding to the O–H stretching mode of hydroxyl groups present on the surface of the nanowires (due to moisture). The peak at 1670 cm^{-1} was attributed to the bending vibration of water molecules. The major peak at 675 cm^{-1} confirmed the presence of Mg-O vibrations [Fig. 5(a)]. The spectra of zein based MgO nanowires indicated a band at 1660 cm^{-1} and 2760 cm^{-1} that corresponds to characteristic asymmetric stretching mode of C=C and CH_2 groups, respectively [Fig. 5(b)]. Similarly, band at 3180 cm^{-1} corresponds to the hydrogen bond of the hydroxyl groups of zein and MgO [Fig. 5(c)].

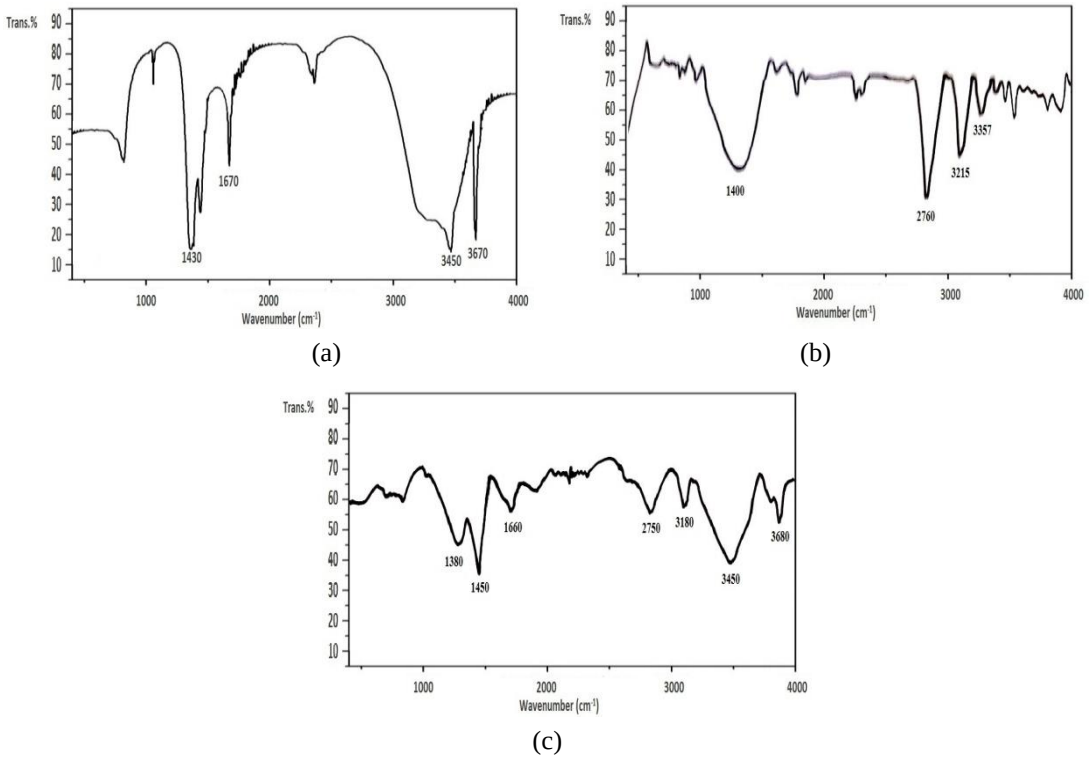


Fig. 5. Fourier transform infrared spectra (a) pure MgO nanowires (b) pure Zein (c) Zein based MgO nanowires

4.6 In-vitro release of nanowires

The nanowires of pure MgO indicated a sudden burst release of 90 % at 2 h indicating the high solubility of the nanowires in comparison to zein stabilized nanowires. The two different formulations prepared at different pH indicated contrasting release pattern. pH 6.8 stabilized MgO nanowires exhibited a prolonged release of about 60 % in comparison to 20 % release of MgO nanowires stabilized at pH 1.2.[Fig. 6] This characteristic can be attributed due to the fact of efficient coating of zein over MgO nanowires at pH 6.8 yielding a stable formula in comparison to the other two formulations. This feature can be highly beneficial in designing a prosthetic device of dental use.

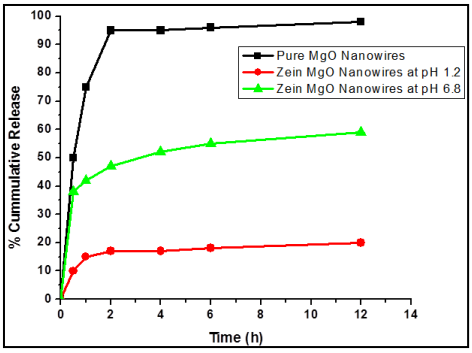


Fig. 6. Comparative In-vitro dissolution release of pure MgO nanowires and zein based MgO nanowires prepared at pH 1.2 and pH 6.8 respectively

4.7. Stability Studies of the nanowires

The stability studies conducted at three different temperatures did not indicate any significant changes in the physicochemical and release patterns. At temperatures of 50^{o}C, there was slight increase in the nanowire size surface charge and even in the release of the MgO nanowires prepared at pH 1.2 in comparison at 20 and 45^{o}C. This study revealed that MgO nanowires prepared at pH 6.8 are stable below 50^{o}C.

4. Discussion

MgO particles are reported for inhibitions against bacterial strains. [21, 22] However; the practical considerations indicate poor solubility and instability due to their tendency to agglomerate. One dimensional nanostructuring can greatly affect the wettability of MgO but still needs a suitable stabilizer that can sufficiently provide the steric or electrostatic repulsion among the particles aid in not just redispersibility but also altering its *in-vitro* behavior. [31,32] Natural polymers are proven ideal choice as stabilizer in the preparation of drug delivery systems or food products. [33] Zein (a corn polymer) is reported for desirable properties, such as a biocompatibility, low toxicity and high absorbability of the degraded end products *in-vivo*. [27] In our previous studies [21], we found that MgO particles in combination of zein portent a good antimicrobial activity that has actually encouraged us to evaluate further the anionic charge effect over the stability considerations. In the present study, the MgO size did not increase due to the complete adsorption of zein over the nanowires. This is supported due to the fact that the surface charge of the nanowires changed from 11 to -22 mV because MgO owes positive charge below its iso-electric point (pH 3), therefore, an electrostatic attraction occurred between positive and negative charges of MgO and zein, respectively, allowing efficient coating around the nanowires. [34] As per the XRD spectra, crystal phases and crystallinity of the synthesized MgO nanowires were crystalline and highly pure. This was similar to our previously synthesized pure MgO nanowires. [21] The DSC thermogram indicates shift of the endothermic peaks to lower temperatures possibly due to significant physical interaction but no new component formation similar to the studies that used zein in the synthesis of nanoparticles.[35] FTIR study provides valuable information about the way how oxygen is bound to the physical metal oxides and as well the phase compositions. The sharp peak at 3670 cm^{-1} confirms the formation of MgO nanowires.[36] FTIR spectra of the zein coated MgO nanowires showed well-defined bands that correspond to the bridging hydrogen-bonded hydroxyl group of zein and MgO. This hydrogen bond is essential for the stability of zein coatings around the metal oxide nanowires.[37,38] TEM images did not indicate planar or bending defects including twin crystals or stacking faults that can lead to instability of the nanowires as reported earlier . Even the SEM images indicate after zein-coating the MgO is present as homogenous nanowires in crystalline forms contrastingly to that of nanoflower morphology reported earlier. This type of distribution is a sign of stability for the metal oxide against any factor that may affect its state.[39] Zein being adsorbed on nanowires

aided increased wettability of the high surface area of MgO, further increasing the ability of MgO to be dispersed in solvents, which will lead to the formation of more homogenous nanowire. The release was also found prolonged about 12 h majorly due to the swelling mechanism of zein and can be advantageous in designing various dental formulations as reported.[40] The stability studies conducted over elevated temperatures did not indicate any marked changes in the crystalline state of MgO after coating with zein. This has indicated the efficiency of the coating procedure adopted in this study suitable for preserving the physicochemical properties. Unlike our final formulation, the other metal oxide nanowire in dry form allows them to remain stable during storage.

5. Conclusions

A novel generation of zein based metal oxide is reported in the present study. Coating of MgO using zein a corn extract, in the preparation of nanowires has improved the physiochemical properties in comparison to pure MgO nanowires. The MgO nanowires were highly crystalline with no possible interactions with zein. It was found that the extent of aggregation of nanowires was greatly minimized and even the dissolution was prolonged in case of MgO nanowires prepared at pH 1.2 in comparison to pH 6.8.

The stability was greatly enhanced at elevated temperatures indicating maximum efficiency of the coating procedure adopted. MgO nanowires coated with zein are biocompatible with competence in antibacterial effect, therefore can be safely used not just in dental materials but also in designing an effective drug delivery system. We anticipate to further use the zein as stabilizer for other physical metal oxide and explore their potential biological response.

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