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A Comparative Study of an In Vitro Release Patterns of Ceftaroline Fosamil from Chemically-Prepared Coated Hydroxyapatite Nanoparticles

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ABSTRACT
This research aimed to chemically synthesize four different sized hydroxyapatite (HA) compound nanoparticles, then coating them with trehalose sugar. After that, a drug release study in an *in vitro* model was achieved to evaluate the release of ceftaroline fosamil drug from HA different sized coated powders in a simulated body fluid.Calcium nitrate tetrahydrate and diammonium hydrogen phosphate were employed by sol-gel method and wet chemical method to produce the four different sized nanoparticles of hydroxyapatite compound. Identification of the produced HA nanoparticles was implemented using FTIR spectroscopy and X-ray diffraction. Additionally, scanning electron microscopy was utilized to check the morphology and particle size of the synthesized nanoparticles. These nanoparticles were coated by trehalose disaccharide. An *in vitro* release study was carried out to evaluate the release of ceftaroline fosamil, a low bacterial resistant 5th generation cephalosporin antibiotic, from the four HA coated nanoparticles, at a temperature of 37 ^{O}C for 4.5 hours with a time interval of 7.5 minutes, employing simulated body fluid as a releasing medium. The U.V. spectroscopy at λmax of 245.2 nm was used to check the loading amounts and to follow the release of ceftaroline from the synthesized coated HA nanoparicles. The loading percents of ceftaroline on the four identified HA nanoparticles were 59.6% w/w, 53.35% w/w, 38.21% w/w and 32.23% w/w. A percent of 75.84% w/w ceftaroline fosamil was released within 37.5 minutes from the coated hydroxyapatite nanoparticles that was formulated by sol-gel method with sintering for 12 hours. This release remained with a median of 74.95% w/w till the end of the 3rd hour, after that it started to decrease.The release of ceftaroline from HA nanoparticles that was formulated by sol-gel method with sintering for 12 hours, was the fastest to reach the steady state and the highest one during the study time than all other releases, so it is considered effective for futuristic therapeutic uses. The trehalose coating was expected to greatly diminish the hydroxyapatite-ceftaroline ionic interactions, resulting in increased drug release proficiency.

Keywords: hydroxyapatite, ceftaroline, drug delivery, nanoparticles.

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INTRODUCTION

There are a number of challenges that face the use of antibiotics for solid skeleton of mammalians. The limited penetration of antibiotics in these sites and the increased microbial resistance are of the important challenges that had been noticed in this area [1]. Day after day, the need becomes much greater for new items, techniques and approaches to overcome such obstacles and to open doors for new progress ways in this medical field.

Drug delivery, which usually concerns with formulations, technologies and systems for transporting pharmaceutical compounds, opened new amplitudes in developing orthopedic and dental medication [2]. In addition to the reduction of the side effects of some drugs and improving of pharmacokinetics for others, drug delivery contributed to the provision of high concentrations for many pharmaceutical agents in the desired site of action in bony compartments by targeting these agents locally to that site [3].

One of the important approaches that have been followed to confront microbial resistance in osseous tissue, as well as other tissues, is the use of the new antibiotics [4]. Much work had been exerted to develop new effective antibiotics in many categories, and the cephalosporins took much interest in these studies [5]. Ceftaroline fosamil is recently administered methicillin-resistant 5th generation cephalosporin antibiotic. It has good eradication ability against gram-positive bacteria. Also, studies have proven that this well-tolerated antibiotic possesses broad antibacterial spectrum for the gram-negative bacteria [6].

Hydroxyapatite is one of the calcium phosphate compounds, and it had been used for a variety of medical purposes. It's utilized in bone tissue engineering, as bone filler, as a coating biomaterial and in dental and orthopedic implant and as remineralizing agent in toothpaste [7, 8]. In addition to that, the hydroxyapatite is widely used in drug delivery [9]. It is considered one of the fascinating compounds that had used as a substrate to carry drugs into definite sites in body solid skeleton. It is characterized by good biocompatibility and osteoconductivity; also it has non-toxic and non-inflammatory characters [10]. Hydroxyapatite compound has fabulous bioactivity which means that it can make a direct bond with the osseous tissue [11]. In comparison with the other calcium phosphates, hydroxyapatite has the highest stability under physiological environment [10].

The scientific access to the stage of nanotechnology applications had led to a paradigm shift in many scientific disciplines including the medical field [12]. Drug delivery was one of the biggest areas that make full use of this nanotechnology, which has made great strides and still grows [13]. During this period of progress revolution, HA has witnessed a prominent place of interest by the researchers [14]. With the nano-hydroxyapatite (nano-HA), wide scopes had been paved for redesigning the previous medical applications of HA, primarily the drug delivery in areas of orthopedics and dentistry. New approaches, formulations and even sites of action for this compound were opened for the drug delivery by HA [15].

The present study deals with the chemical synthesis of four different sized HA compound nanoparticles and the subsequent identification of the HA compound. After sugar-coating of the nanoparticles, evaluation of ceftaroline fosamil release from HA different sized coated powders in a simulated body fluid was conducted in an *in vitro* model

EXPERIMENTAL WORK

Chemicals

The chemicals in this study were all of analytic grade. Ceftaroline fosamil was from AstraZenca pharmaceutical company. Calcium nitrate tetrahydrate, diammonium hydrogen phosphate, Sodium sulfate, tris-(hydroxymethyl) aminomethane and calcium chloride hydrate were provided by Fluka Co. Sodium chloride, sodium bicarbonate, Sodium hydroxide and potassium chloride were from Sigma Aldrich Co. Ltd. Ammonium hydroxide is supplied by B.D.H. Co. Sodium hydrogen phosphate dihydrate, and magnesium chloride hexahydrate were from Merck Co. Ethanol was provided by Scharlab S.L. The hydrochloric acid (37%) was supplied by Carlo-Erba, whereas distilled water was obtained from College of pharmacy / University of Basra and the deionized water was provided by College of Science / University of Basra.

Instruments

Different instruments were used in this study. Zeiss Supra 55VP, Germany, Field Emission Scanning Electron Microscope, Muffle Furnace, size 3, Gallenkamp Co., U.K., Hotplate, Heidolph Instruments Co., Germany, X-ray diffractometer, PANanalytical Co, EMCLAB GmbH Co., Germany, Netherlands, FTIR spectrophotometer, IR Affinity-1, SHIMADSU Co., Japan and UV-1100 Spectrophotometer were used in this research.

Preparation of calcium HA nanoparticles

In this study, hydroxyapatite nanoparticles with different sizes were prepared by two ways; sol-gel method and wet chemical method.

- by sol-gel method

Both of calcium nitrate tetrahydrate and diammonium hydrogen phosphate solutions with a concentration (0.5 molar) were prepared by dissolving each of the chemicals in 10 milliliters of distilled water. The pH of solutions was adjusted to a value of 11 by the addition of ammonium hydroxide. Then, these solutions were diluted by adding absolute ethanol in a quantity equal to the volume of these solutions. Diammonium hydrogen phosphate solution was added to the solution of calcium nitrate tetrahydrate upon continuous stirring. The reaction lasted for 2 hours at the temperature of 40 ^{O}C. Then, Filtration was done and the precipitate was washed 3 times with distilled water. The precipitate was equally divided into two parts and then kept in an oven at 40 ^{O}C separately for a period of 12 and 24 hours [16].

- by wet chemical method

Two hundred and fifty milliliters of (0.06 molar) calcium nitrate tetrahydrate solution with a pH of 7.4 were added drop by drop during 4 hours to a vigorously stirred 250 milliliters solution of diammonium hydrogen phosphate that already have a concentration of 0.04 molar and a pH equal to 4. The pH of the medium was maintained to be 10.8 throughout the reaction time by the addition of 0.1 molar sodium hydroxide solution. The stirring continued for 36 hours. Then, the same procedure was repeated, but for stirring time of 72 hours. The precipitates were dried, washed and sintered at 750 ^{O}C for 12 hours [17].

Fourier Transform Infrared analysis for HA powder

One milligram from each of the precipitates gained by sol-gel method and wet chemical method was carefully mixed with 100 milligrams of KBr and then compressed into a disk. The FTIR spectra were measured in the range of 400- 4000 cm^{-1}.

X-ray diffraction for HA powder

A quantity of two grams for each of the four resultant powders was subjected to X-ray diffractometry. The diffraction data were obtained in two theta range of 20^{0}-60^{o}C using diffractometer operating at a tension of 40kv and a current of 20Ma, producing Cu k (alpha) radiation with a wavelength of (1.54A^{0}) and a scan speed of 1/min [18].

Scanning electron microscopy for HA powder

Ten milligrams for each of the four HA powders were used as a sample to be examined by the scanning electron microscope (SEM).

Preparation of simulated body fluid (SBF)

Four liters of simulated body fluid were prepared. The weights and addition order are stated in table (1). The resultant concentrations, along with the plasma normal values are presented in table (2).

Table 1: The chemicals and their amounts constructing simulated body fluid [19].

Order	Reagent	Amount (Gram per 4L)
1	NaCl	26.188
2	$NaHCO_3$	9.072
3	KCl	1.492
4	$Na_2HPO_4.2H2O$	0.712
5	$MgCl_2$).6H2O	1.22
6	$CaCl_2.2H_2O$	1.472
7	Na_2SO_4	0.284
8	$(CH_2OH)_3CNH_2$	24.228

Deionized water was used for the preparation of simulated body fluid which has a concentration asymptotic to that of the human plasma. The additions of chemicals were done in such a sequence as in the table (1). Complete dissolving of

each chemical in 2800 milliliters deionized water is a compulsory issue for the second addition of a chemical. In order to avoid the occurrence of turbidity, 60 milliliters of 1molar HCl was added to the solution before the addition of calcium chloride dihydrate. After the completion of the final addition, i.e., *tris* (hydroxymethyl) aminomethane addition, the temperature of the resultant solution was set to be 37^{O}C. After that, 100 milliliters of 1 molar HCl was added to the solution to drive its pH to reach 7.4. The volume was completed to 4 liters by deionized water [20].

Table 2: Ion concentrations for both, prepared simulated body fluid and human plasma [21].

Sequence	Ion (mM)	Present work (mM)	Human plasma (mM)
1	Na^{+}	142.0	142.0
2	Cl^{-}	125.0	103.0
3	HCO^{-3}	27.0	27.0
4	K^{-}	5.0	5.0
5h	Mg^{+2}	1.5	1.5
6	Ca^{+2}	2.5	2.5
7	HPO_4^{+2}	1.0	1.0
8	SO_4^{+2}	0.5	0.5

Coating the HA with Trehalose Sugar

Trehalose solution has been prepared by dissolving 40 milligrams from trehalose disaccharide in 10 milliliters distilled water. To four volumetric flasks each of which contains 1000 milligrams of a definite form of the four hydroxyapatite powders, two milliliters of the trehalose solution have been added successively. After a tight closure of the volumetric flask, a vigorous shaking has been accomplished for 20 minutes. The contents of the flask have poured into a trough having water and the suspension was left for 60 minutes at a temperature of 25 ^{O}C with shaking from time to time. Then, each suspension has been centrifuged for 5 minutes at 2000 rpm. After centrifugation, the coated powders were collected and dried in the air [22].

Ceftaroline Drug loading on the coated HA nanoparticles

Four quantities of ceftaroline fosamil acetate, each weighs 600 milligrams were separately dissolved by 20 milliliters of the prepared simulated body fluid. Each of These solutions was added to a light-resistant container in which 1000 milligrams of each definite form of the trehalose-coated hydroxyapatite powders was found. The solutions in the containers were completed to 500 milliliters by adding sufficient volume of SBF. The containers were closed and left for 1 hour. Thereafter, the powders were filtered off and dried.

Ceftaroline release from the HA nanoparticles

Freshly prepared 500 milliliters of simulated body fluid were used to replace the loading media for each of the four loading containers. The release behavior of ceftaroline fosamil from the trehalose-coated HA powders was studied for 4.5 hours at 37^{o}C. The UV measurements were taken at 245.2 nm [23], which is the λmax of ceftaroline drug. These measurements were done at 7.5 minutes intervals for the entire time of the study.

RESULTS AND DISCUSSION

FTIR analysis of HA

Figures (1) and (2) represent the FTIR spectra of the hydroxyapatite prepared by sol-gel method with a sintering time of 12 hours (HAS12) and 24 hours (HAS24), while figures (3) and (4) refer to hydroxyapatite prepared by wet chemical precipitation method for 36 hours (HAW36) and 72 hours (HAW72).

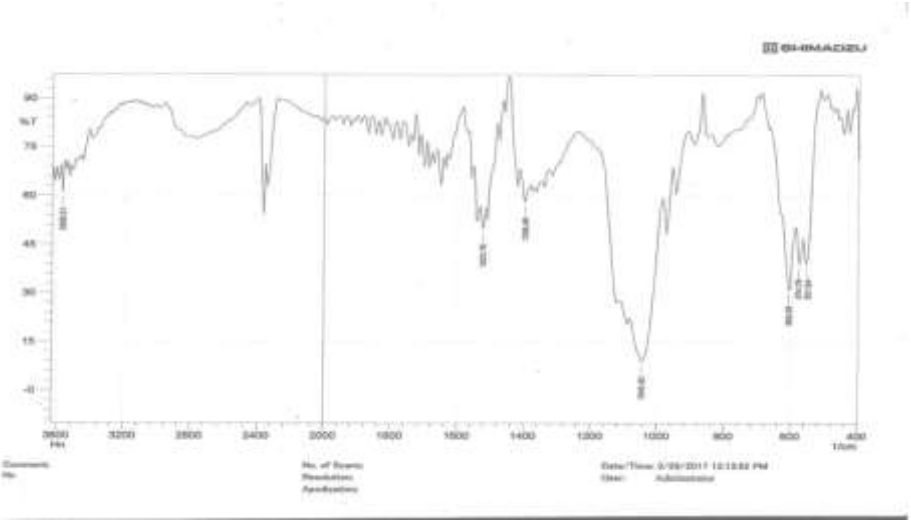

Figure 1: FTIR of HAS12.

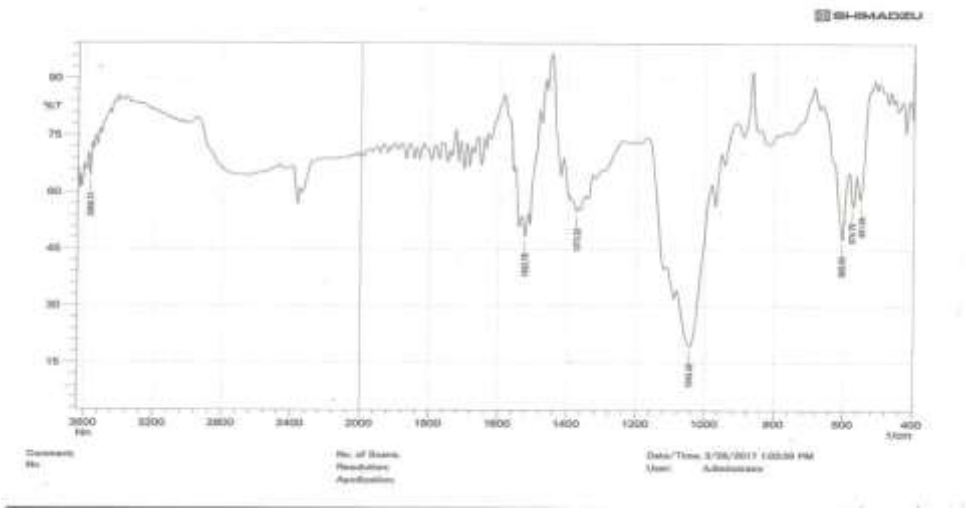

Figure 2: FTIR of HAS24.

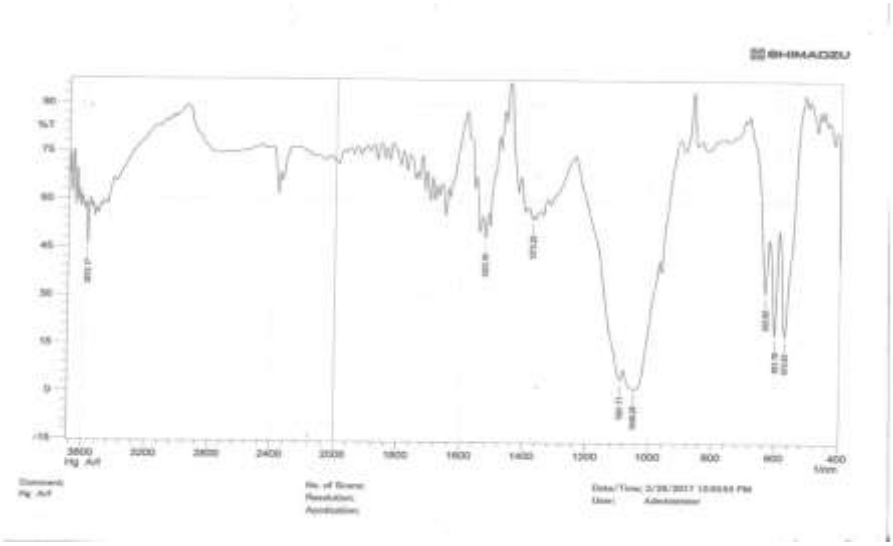

Figure 3: FTIR of HAW36.

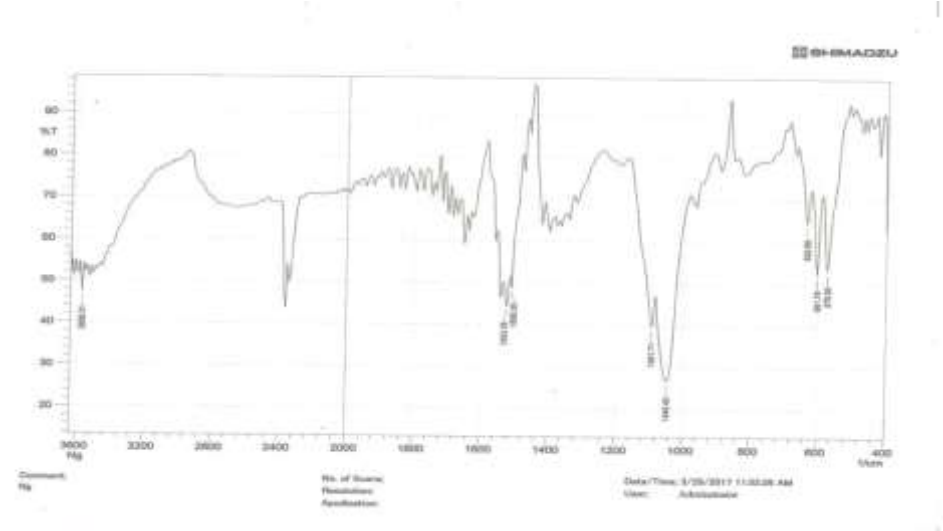

Figure 4: FTIR of HAW72.

The (O-H) stretching occurs at 3568.31- 3572.17 cm^{-1} for all FTIR spectra. Similarly, the (P=O) symmetrical stretching occurs at 1045.42-1049.28 cm^{-1}. The (P=O) deformation presents at 605.65 cm^{-1} in both figures (1) and (2), and at 632.65 cm^{-1} in figures (1) and (2). These data confirm the formation of hydroxyapatite [10].

Identification of HA by X-ray diffraction

The diffractograms of the four prepared nanoparticles are stated in figures (5, 6, 7, and 8). These diffractograms assured that the formation of the crystalline HA compound [18].

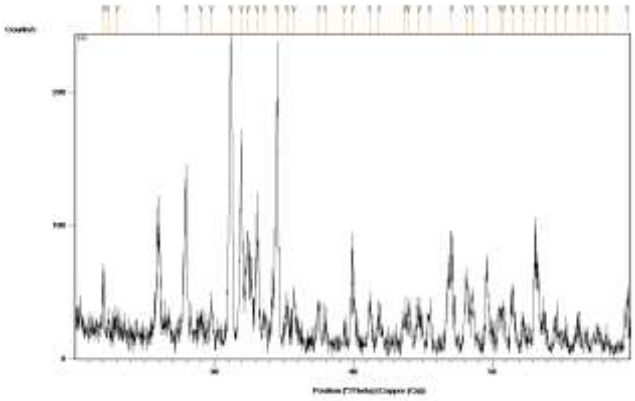

Figure 5: X-ray diffraction of HAS12.

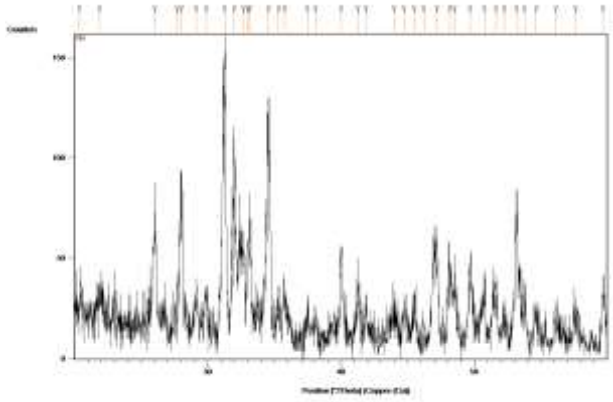

Figure 6: X-ray diffraction of HAS24.

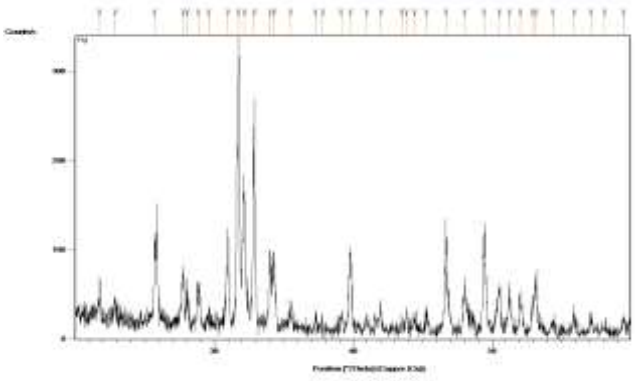

Figure 7: X-ray diffraction of HAW36.

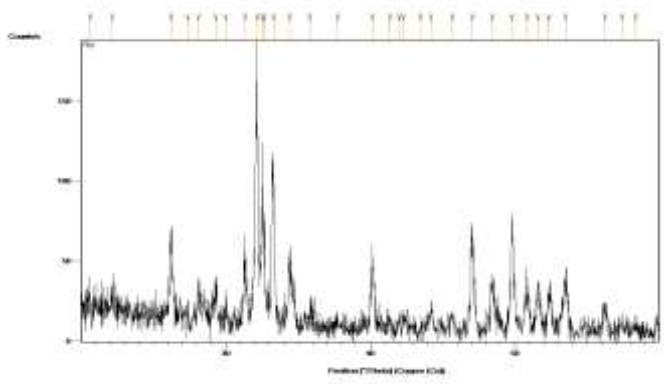

Figure 8: X-ray diffraction of HAW72.

Scanning electron microscopy for HA

Figures (9, 10, 11, and 12) represent images of the scanning electron microscopy that belongs to the four different texture HA nanoparticles (HAS12, HAS24, HAW36 and HAW72). These images revealed that all the four HA nano-powders resulted were in the scale of nano-size, but with a little difference in their particle sizes.

The sintering time usually has an effect in respect with particle size of the HA nanoparticles [24]. It is obvious that hydroxyapatite nanoparticles obtained by sol-gel method with sintering time of 12 hours (HAPS12) was really smaller than that heated for 24 hours (HAPS24), where the SEM stated that HAS12 had an average particle size equal to 172.8 nm, whereas the Average particle size for HAS24 was 262.73 nm.

The literature survey also correlates between stirring time and the nanoparticles size [24]. It was found that the size of particles produced by the wet chemical precipitation method with a stirring time lasted for 36 hours (HAW36) was less than that which took 72 hours (HAW72.). The Average particle size was 196.86 nm for HAW36, while it was 311.56 nm for HAW72. The morphology of the synthesized nanoparticles ranges from irregular to multi-faceted and slightly spherical shape.

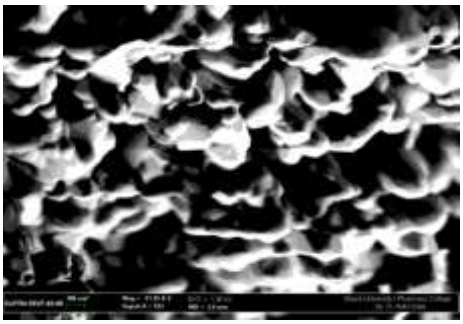

Figure 9: SEM of HAS12.

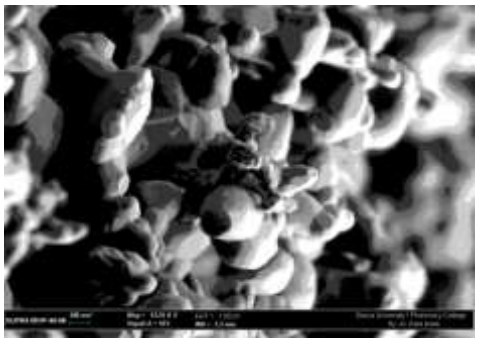

Figure 10: SEM of HAS24.

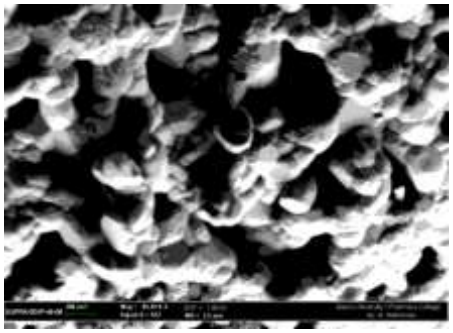

Figure 11: SEM of HAW36.

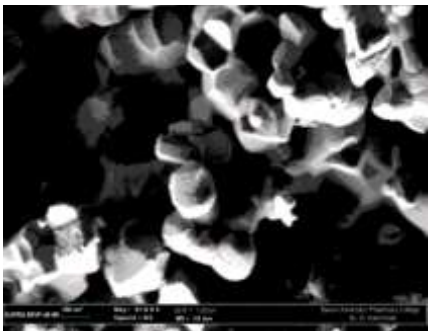

Figure 12: SEM of HAW72.

Quantities of ceftaroline fosamil loaded on the coated hydroxyapatite nanoparticles

UV spectroscopy has been used for estimating the loaded ceftaroline fosamil in the coated hydroxyapatite nanoparticles. The amounts of ceftaroline loaded in the HAS12, HAW36, HAPS24 and HAW72 were calculated to

be: 357.6 milligrams (59.6% w/w loading), 320.1 milligrams (53.35% w/w loading), 229.26 (38.21% w/w loading) and 193.44 milligrams (32.23% w/w loading) successively.

The release of ceftaroline fosamil from the HA coated

The release patterns of ceftaroline fosamil from the prepared coated hydroxyapatite nanoparticles formulas (HAS12, HAW36, HAPS24 and HAW72) were studied through UV spectroscopy. In this study, the release of ceftaroline was expressed in terms of the weight percent of the released drug to the weight of the drug that primarily loaded on the specific coated HA.

As it is shown in table (3), the release of ceftaroline fosamil from HAS12 attained (75.84% w/w) during the first 37.5 minutes of the releasing time, while the release from HAW36 reached (62.79% w/w) within 45 minutes. A time of 52.5 minutes was needed for the ceftaroline release from both HAS24 and HAW72 nanoparticles to get the steady state at (55.57% w/w) and (48.77% w/w) levels.

The median percent w/w releases from the four HAS12, HAW36, HAPS24 and HAW72 nanoparticles within the period of steady state condition were (74.95, 62.5, 55.6 and 47.5% w/w) respectively.

Moreover, the standard deviations for the release percent in the steady state were 0.603 for HAS12, 0.459 for HAW36, 0.641 for HAS24 and 0.535 for HAW72. It is obvious that in spite that the release approaches are somewhat convergent for the four HA formulations in respect with time needed to catch the steady state, but there's still a considerable difference regarding the extents of the release level within the steady state from the four HA nanoparticles in addition to the differences in median concentration for each release in the steady state.

The gained data state coherently that the particle size differences that accompanied the employment of two different methods for the production of HA nanoparticles, with a definite change in a particular production factor in each method, had been much reflected during the drug release pattern from the four different texture powders. Figure (13) illustrates that releases of ceftaroline antibiotic was greatly coincided with that phenomenon, where the general overview of the releases appears to link a lot to the differences of the powder surface areas, resulted from particle size difference.

After the steady state, the release from the four nanoparticles started to plummet. The release from HAS12 and HAW36 nanoparticles began to decrease after 180 minutes. On the other hand, 187.5 minutes was the slumping time for the ceftaroline drug release from HAS24 and HAW72 nanoparticles. This is may be related to ceftaroline stability behavior [25].

The decrease in the concentration of ceftaroline fosamil continued, and the drug concentrations from HAS12, HAW36, HAPS24 and HAW72 at the end of the study time were (71.95, 60.68, 51.33 and 45.54% w/w) respectively

Table 3: The release data of ceftaroline drug from the four different hydroxyapatite nanoparticles.

Time min	Time hr.	% w/w Release of drug from HAW72	% w/w Release of drug from HAS24	% w/w Release of drug from HAW36	% w/w Release of drug from HAS12
0	0	0	0	0	0
7.5	0.125	6.6	7.79	13.88	15.24
15	0.25	14.87	11.67	31.67	27.39
22.5	0.375	19.89	17.98	37.67	43.56
30	0.5	25.62	25.78	46.34	66.63
37.5	0.625	33.56	32.33	57.47	75.84
45	0.75	42.84	46.42	62.79	74.27
52.5	0.875	48.77	55.57	62.15	74.89
60	1	47.34	55.08	62.53	75.27
67.5	1.125	47.61	54.87	61.54	74.66
75	1.25	47.47	54.89	62.13	73.54
82.5	1.375	46.68	53.88	62.82	75.57
90	1.5	47.66	55.87	62.23	74.46
97.5	1.625	47.89	56.02	61.82	75.05
105	1.75	46.18	55.91	62.14	75.25
112.5	1.875	47.72	56	62.48	74.94
120	2	47.34	55.75	62.86	75.62
127.5	2.125	47.68	55.41	62.92	75.46
135	2.25	47.43	56.29	62.13	75.75
142.5	2.375	46.76	56.04	61.81	74.95
150	2.5	46.78	54.81	63.05	74.53
157.5	2.625	47.43	56.11	62.67	75.34
165	2.75	47.47	55.29	62.7	73.78
172.5	2.875	47.76	54.82	63.23	74.45

180	3	47.73	54.36	62.43	73.86
187.5	3.125	45.63	52.57	62.22	73.63
195	3.25	45.53	52.41	62.01	73.54
202.5	3.375	45.42	52.3	61.79	73.37
210	3.5	45.30	52.12	61.66	73.17
217.5	3.625	45.19	51.95	61.49	72.92
225	3.75	45.04	51.85	61.3	72.86
232.5	3.875	45.94	51.68	61.11	72.61
240	4	45.82	51.55	60.98	72.41
247.5	4.125	45.71	51.37	60.79	72.29
255	4.25	45.62	51.28	60.67	72.11
262.5	4.375	45.51	51.14	60.51	72.02
270	4.5	45.54	51.33	60.68	71.95

CONCLUSIONS

The release of ceftaroline drug from the hydroxyapatite nanoparticles formulated by sol-gel method with sintering for 12 hours (HAS12) was fabulously highest and fastest than all the other releases investigated in this study, whereas the release of the drug from hydroxyapatite nanoparticles produced by the wet chemical precipitation method whom the reaction continues for 36 hours (HAW36) was the most regular one during the steady state condition. Also, after 3 - 3.125 hours, all releases decreased due to drug stability problem. The overall release was largely expected to be enhanced, due to the trehalose coating which plays a role in preventing of any probable ionic interaction between the hydroxyapatite and the ceftaroline drug.

REFERENCES

1. Cornelia B. Landersdorfer, Jurgen B. Bulitta, Martina Kinzig, Ulrike Holzgrabe and Fritz Sorgel, Penetration of Antibacterials into Bone Pharmacokinetic, Pharmacodynamic and Bioanalytical Considerations, Clin Pharmacokinet, 48 (2), 89-124, (2009).
2. Benjamin D. Brooks, Sherry N. Davidoff, David W. Grainger, Amanda E. Brooks, Comparisons of release of several antibiotics from antimicrobial polymer-coated allograft bone void filler, International Journal of Biomedical Materials Research, 1(2), 21-25, (2013).
3. Lisa E. Cole , Tracy Vargo-Gogola , Ryan K. Roeder, Targeted delivery to bone and mineral deposits using bisphosphonate ligands, Advanced Drug Delivery Reviews, 99 12–27, (2016).
4. Julian Davies and Dorothy Davies, Origins and Evolution of Antibiotic Resistance, Microbiology and Molecular Biology Reviews, 74, 417–433, (2010).
5. Peter Davey, Jacqueline Sneddon and Dilip Nathwani, Overview of strategies for overcoming the challenge of antimicrobial resistance, Expert Rev. Clin. Pharmacol, 3(5), 667–686, (2010).
6. DeBellis and Tackett, Safety and Efficacy of Ceftaroline Fosamil in the Management of Community-Acquired Bacterial Pneumonia, Clinical Medicine Reviews in Therapeutics, 6, 1–6, (2014).
7. DA Wahl and JT Czernuszka, collagen- hydroxyapatite composites for hard tissue repair, European Cells and Materials, 11, 43–56, (2006).
8. Claudio Poggio, Maria Mirando, Davide Rattalino, Matteo Viola, Marco Colombo and Riccardo Beltrami, Protective effect of zinc-hydroxyapatite toothpastes on enamel erosion: An in vitro study, J Clin Exp Dent, 9 (1), 118-122, (2017).
9. Amit Kumar Nayak, Bibek Laha and Kalyan Kumar Sen, Development of hydroxyapatite-ciprofloxacin bone-implants using »Quality by design«, Acta Pharm. 61, 25–36, (2011).
10. Sudip Mondal' Biswanath Mondal, Apurba Dey, Sudit S. Mukhopadhyay, Studies on Processing and Characterization of Hydroxyapatite Biomaterials from Different Bio Wastes, Journal of Minerals & Materials Characterization & Engineering, 11, 55-67, (2012).
11. Gerrard Eddy Jai Poinern, Derek Fawcett, The Manufacture of a Novel 3D Hydroxyapatite Microstructure Derived from Cuttlefish Bones for Potential Tissue Engineering Applications, American Journal of Materials Science, 3(5), 130-135, (2013).
12. Mihail C. Roco, The long view of nanotechnology development:the National Nanotechnology Initiative at 10 years, J Nanopart Res 13, 427–445, (2011).
13. Nelson A. Ochekpe1, Patrick O. Olorunfemi and Ndidi C. Ngwuluka, Nanotechnology and Drug Delivery Part 2: Nanostructures for Drug Delivery, Tropical Journal of Pharmaceutical Research, 8 (3), 275-287, (2009).
14. N. Saglam, E. Emul, S. Saglam, E. Yalcin, M. Sam and F. Korkusuz, electrospun nanofibrous scaffolds in green nanotechnology and nanomedicine, International Journal on Technical and Physical Problems of Engineering, 7, 55-59, (2015).
15. Roza Haghgoo, Farid Abbasi and Mohammad Bagher Rezvani, Evaluation of the effect of nanohydroxyapatite on erosive lesions of the enamel of permanent teeth following exposure to soft beer in vitro, Scientific Research and Essays, 6(26), 5933-5936, (2011).
16. Hadeel Alobeedallah, Jeffrey L. Ellis, Ramin Rohanizadeh, Hans Coster and Fariba Dehghani, Preparation of Nanostructured Hydroxyapatite in Organic Solvents for Clinical Applications, Trends Biomater. Artif. Organs, 25(1), 12-19, (2011).
17. Arunseshan Chandrasekar, Suresh Sagadevan and Arivuoli Dakshnamoorthy, Synthesis and characterization of nano-hydroxyapatite (n-HAP)

using the wet chemical technique, International Journal of Physical Sciences, 8(32), 1639-1645, (2013).

18. Greta Gergely, Ferenc Weber, Istvan Lukacs, Attila L. Toth, Zsolt E. Horvath, Judit Mihaly and Csaba Balazsi, Preparation and characterization of hydroxyapatite from eggshell, Ceramics International, 36, 803–806, (2010).
19. Dhiraj Mehta, Suja George, Anjuvan Singh, Assessment of Different Synthesis Route of Hydroxyapatiteand Study of its Biocompatibility in Synthetic Body Fluids, International Journal of ChemTech Research, 9, 267-276, (2016).
20. CuK neyt Tas, Synthesis of biomimetic Ca-hydroxyapatite powders at 37 ^{O}C in synthetic body fluids, Biomaterials, 21, 1429-1438, (2000).
21. Crook A. Martin., Clinical Chemistry and Metabolic Medicine, Hodder Arnold LTD, 7th edition, (2006).
22. Pavani Vengala, Sana Aslam & C.V.S. Subrahmanyam, Development and In Vitro Evaluation of Ceramic Nanoparticles of Piroxicam, Lat. Am. J. Pharm., 32 (8), 1124-1130, (2013).
23. Suneetha*, K. China Venkanna, S. Kathirvel, Development and Validation of UV Spectrometric Method For Quantitative Estimation Of Ceftaroline Fosmile In Bulk And Injection Form., International Journal of Pharmaceutical Research & Analysis, 2 (2), 63-67, (2012).
24. Y.X. Pang, X. Bao, Influence of temperature, ripening time and calcination on the morphology and crystallinity of hydroxyapatite nanoparticles, Journal of the European Ceramic Society, 23, 1697–1704, (2003).
25. Aurelie Melard, Laetitia G. Garcia, Debaditya Das, Raoul Rozenberg, Paul M. Tulkens, Francoise Van Bambeke and Sandrine Lemaire, Activity of ceftaroline against extracellular (broth) andintracellular (THP-1 monocytes) forms of methicillin-resistant Staphylococcus aureus: comparison with vancomycin, linezolid and daptomycin, J Antimicrob Chemother, 68, 648–658, (2013).