3

The *In Vitro* Release Study Of Ceftazidime drug From Synthesized Strontium Flourapatite And Strontium Hydroxyapatite Coated Particles.

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ABSTRACT

Two compounds, strontium flourapatite (SrFA) and strontium hydroxyapatite (SrHA), were synthesized using wet chemical precipitation method. These compounds were characterized by Fourier transform infrared spectroscopy and X-ray diffraction techniques. The morphology and particle size for the resultant particles of the synthesized compounds were checked by scanning electron microscopy. The average particle size of strontium flourapatite was larger than that of strontium hydroxyapatite by 4.55 times. The particles of each compound were coated by trehalose sugar to decrease the possible ionic interactions with these synthesized compounds. An *in vitro* release study of ceftazidime antibiotic from the strontium flourapatite and strontium hydroxyapatite was performed in a simulated body fluid at a temperature of 37 ^{o}C. This release was studied by UV spectrophotometer at λ_{max} of 258 nm for 12 hours. The results of the release study showed that the release of ceftazidime was faster from strontium flourapatite, but it was higher from strontium hydroxyapatite. The disparity in release rapidity and level was related to the considerable difference in the average particle size for the formed particles of the two synthesized compounds.

الدراسة المختبرية لاطلاق عقار السفتازديم من جسيمات السترونتيوم فلوراباتيت والسترونتيوم هيدروكسيباتيت المصنعة و المغلفة

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الكلمات المفتاحية: السترونتيوم فلورباتيت، السترونتيوم هيدروكسيباتيت، سيفتازيديم، الاطلاق الدوائي.

الخلاصة

تم تصنيع مركبين، السترونتيوم فلورباتيت والسترونتيوم هيدروكسيباتيت، باستخدام طريقة الترسيب الكيميائي الرطب. اجري التشخيص لهذين المركبين بواسطة تحليل فورييه الطيفي بالأشعة تحت الحمراء وتقنيات حيود الأشعة السينية. لقد تم فحص كل من المظهر الخارجي والحجم للجسيمات الناتجة من المركبات المصنعة بواسطة المسح الضوئي للمجهر الإلكتروني وكان متوسط حجم الجسيمات من مركب السترونتيوم فلورباتيت أكبر منه من السترونتيوم هيدروكسيباتيت وبنسبة 4,55 مرة. بعد ذلك، تم تغليف جسيمات كل من المركبين بسكر تريهالوز وذلك لتقليل التفاعلات الأيونية المحتملة مع هذه المركبات المصنعة. أجريت دراسة مختبرية لاطلاق المضاد الحيوي "سيفتازيديم" من كل من السترونتيوم فلورباتيت والسترونتيوم هيدروكسيباتيت في السائل المشابه لسائل الجسم عند درجة حرارة 37 درجة مئوية. لقد تمت دراسة هذا الإطلاق بواسطة طيف الأشعة فوق البنفسجية في اعلى امتصاص "258 نانومتر" لمدة 12 ساعة. أظهرت نتائج دراسة الاطلاق الدوائي بأن اطلاق عقار السيفتازيديم من السترونتيوم فلورباتيت كان أسرع، في حين ان الاطلاق من السترونتيوم هيدروكسيباتيت كان أعلى. كان التفاوت في سرعة الإطلاق ومستواه مرتبطا بالفارق الكبير في متوسط الحجم لجسيمات المركبين.

INTRODUCTION

The substitution of chemical species or chemical elements to the structure of calcium hydroxyapatite, which is the most occurring apatite in nature, has attracted many researchers to enhance the characteristics of the calcium apatite and to explore the effect of these new formulated compounds [(1)].

The strontium substituted apatite is one of the large varieties of substituted apatites [(2)]. When using the strontium substituted apatite (that replaces partly the biological calcium apatite), the strontium may result in an increased size of bone lattice because the ionic radius of the strontium is relatively larger than that of the calcium [(3)]. In spite of that, strontium apatite still has much interest in medical and pharmaceutical research fields [(4)]. It was found that strontium have a stimulant effect for bone tissue proliferation and differentiation [(2)]. The strontium flourapatite compound was investigated in many experimental works as cement for dental applications [(5)], while the strontium hydroxyapatite have found a position in orthopedic researches; where it was experimented for bone grafting and as coating biomaterial [(6)].

Many studies have utilized the calcium hydroxyapatite as a carrier in drug delivery [(7)]. The similarity in chemical structure of both the strontium flourapatite and the strontium hydroxyapatite with the structure of calcium hydroxyapatite made these substituted apatites liable to have a place in drug delivery scope [(8)].

As one of the approaches for the drug delivery, targeting of antibiotics to many body compartments including bones has been explored [(9)].Ceftazidime is a third

generation cephalosporin antibiotic. This dianionic antibiotic [10] is active against gram positive and gram negative bacteria. Also it has an activity against anaerobes. It's indicated for a variety of infectious diseases, including joint and bone infections [11]. Although the ceftazidime has fewer side effects in comparison with many other antibiotics, but there are still harmful side effects if ceftazidime is taken systemically (Clostridium difficile diarrhea is an example) [12]. As with other pharmacological agents that were experimented in targeted drug delivery, the ceftazidime delivery for a specific site of action is expected to lessen its side effects [13].

The aim of the present research is to study and to compare the release of ceftazidime drug from the trahalose-coated particles of synthesized strontium flourapatite and strontium hydroxyapatite, utilizing the simulated body fluid as a releasing medium, in an *in vitro* model at physiological conditions.

MATERIALS AND EXPERIMENTAL WORK

All the chemicals used in this study were of analytical grade. The strontium chloride hexahydrate, ammonium dihydrogen phosphate, ammonium hydroxide and strontium nitrate were from B.D.H. Co. The sodium chloride, sodium bicarbonate, potassium chloride and hydrochloric acid were provided by Sigma Aldrich Co. Ltd. The sodium hydrogen phosphate dihydrate, Ammonium fluoride and magnesium chloride hexahydrate are supplied by Merck Co. The sodium sulfate, tris (hydroxymethyl) aminomethane and calcium chloride dihydrate were provided by Fluka Co. The ethanol was from Scharlab S.L., the distilled water from Basra College of pharmacy and the deionized water from the Basra College of Science. The ceftazidime powder (pentahydrate) was from Clearsynth labs ltd, India.

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

Preparation Of Strontium Flouropatite

A 100 milliliters solution of 43.57 g diammonium hydrogen phosphate (0.33 mole) and 5.92 g ammonium fluoride (0.158 mole) was prepared. This solution was heated till boiling, with adjusting the pH of the solution at 9 throughout the reaction time by addition of diluted ammonium hydroxide solution. Strontium nitrate solution of 0.392 M was added drop wise into the hot solution with continuous stirring for three hours. The precipitate formed in the reaction was filtered and washed several times with distilled water, and then it was kept in a desiccator at room temperature for 12 hours. After that, the precipitate was heated in a furnace at 150 ^{o}C for 2 hours and then it was calcined at 1000 ^{o}C for another 2 hours in the same furnace [14].

Preparation Of Strontium Hydroxyapatite

500 mL solution of 1 M strontium chloride hexahydrate was prepared by dissolving 133.31 g in the specified volume. Similarly; 0.6 M, 500 mL of diammonium hydrogen phosphate solution was prepared using 39.61 g of the diammonium hydrogen phosphate. The strontium chloride hydrate solution was put in a container and heated at 80 ^{o}C on hotplate, maintaining the pH of the solution at value 10 by addition of diluted ammonium hydroxide solution. The solution of diammonium hydrogen phosphate was added to the resultant solution and the reaction proceeds in a reflex condenser for 3 hours at 80 ^{o}C. Filtration of the solution was then achieved and the precipitate was washed with distilled water several times and then it was cleaned with ethanol. The precipitate was dried for one hour at 100 ^{o}C, and then it was calcined at 1000 ^{o}C for 2 hours in a muffle furnace [15].

Preparation Of Simulated Body Fluid

Simulated body fluid (SBF), with a volume of 3 liters was prepared. The chemicals, their amounts and their addition order are illustrated in table (1).

Table (1): The Chemicals of the SBF preparation and their amounts [16].

Order	Reagent	Amount (Gram per 2L)
1	NaCl	19.641
2	$NaHCO_3$	6.804
3	KCl	1.119
4	$Na_2HPO_4.2H2O$	0.534
5	$MgCl_2.6H2O$	0.915
6	$CaCl_2.2H_2O$	1.104
7	Na_2SO_4	0.213
8	$(CH_2OH)_3CNH_2$	18.171

2100 mL of deionized water was used to dissolve the chemicals needed for the SBF preparation. The chemicals addition was carried out in an order identical to that sequence in table (1). The addition of each compound was carried out after that the previously added one was completely dissolved. Prior to the addition of calcium chloride dihydrate, 45 mL of 1 M HCl was added to prevent occurrence of turbidity in the solution. After finishing the addition of the chemicals, the solution temperature was maintained at 37 ^{o}C. The pH of the solution was kept at 7.4 by adding 75 mL of 1 M HCl, and then the volume of the solution was completed to 3 liters [17].

Coating The SrFA And SrHA With Trehalose

A concentration of 4mg/mL trhalose sugar solution was prepared. One gram of each of the strontium flourapatite and the strontium hydroxyapatite was placed in a 25-mL volumetric flask, and to each of which, 2 mL of the trhalose sugar solution were added. The contents of each flask were shaken vigorously for 20 minutes, and then it was transferred separately to a wide container filled with water of a

temperature equal to 25 ^{o}C, where intermittent shaking was done for one hour. Each suspension was centrifuged for five minutes at 2000 rpm, and then it was filtered and left to dry [18].

Scanning Electron Microscopy

About five milligrams of each of the strontium flourapatite and strontium hydroxyapatite were used to accomplish the scanning electron microscopy test for these compounds. This was achieved in the central laboratory of the College of Pharmacy, Basra University.

The X-Ray diffraction

X-ray diffraction technique was carried out using one gram of each of the two synthesized chemical compounds. The data was recorded in the two theta range of 10^0_70^0. The x-ray diffractometer, with a goniometer radius equal to 240 mm, was working at a tension of 40 kv and a current of 20 mA, producing Cu radiations of K-Alpha1 [Å] = 1.54060, K-Alpha2 [Å] =1.54443 and K-Beta [Å] =1.39225.

Fourier Transform Infrared Spectroscopy

A weight of about five milligrams each of the strontium flourapatite and the strontium hydroxyapatite were mixed with about 100 mg of KBr powder and grounded together. Then, the mixture was pressed into disc and then FTIR spectra were recorded for each compound.

Determination Of Ceftazidime λ_{max} In simulated Body Fluid.

Ceftazidime solution of 10 μg/mL concentration was prepared, utilizing SBF as a solvent. The λ_{max} of the ceftazidime in SBF was checked using UV spectrophotometric scan.

Drug Loading

One gram of strontium flourapatite was added into 250 mL glass container. The same weight of strontium hydroxyapatite was added into another 250 mL glass container. To each container, 200 mL of freshly prepared 0.5% w/v ceftazidime solution (SBF was used as a solvent) was added, and then container closed and left for 3 hours [19, 20].

Drug Release

Each of the loading mediums was replaced by new 250 mL of simulated body fluid. The release of ceftazidime antibiotic was studied for 12 hours under physiological conditions.

Each of the drug loading and drug release experiments was repeated three times.

RESULTS AND DICUSSION

Simulated Body Fluid

Table (2) represents the resulted concentrations of the ions for the SBF and the normal concentrations of these ions in the plasma.

Table (2): The concentrations of ions in SBF and human plasma [21].

Seq.	Ion (mM)	Conce. in Human plasma (mM)	Conce. in the present work (mM)
1	Na^{+}	142.0	142.0
2	Cl^{-}	103.0	125.0
3	HCO_3^{-}	27.0	27.0
4	K^{-}	5.0	5.0
5	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

Scanning Electron Microscopy

The figures (1) and (2) illustrate the photos of the scanning electron microscopy for the strontium flourapatite and the strontium hydroxyapatite respectively. The particles of the strontium flourapatite appear as multi-faces and multi-rims guises with satin appearance. The average size of these strontium flourapatite particles was 898.06 nm. The particles of the strontium hydroxyapatite presented as granulated multi-faces nanoparticles and the average particle size was 196.96 nm.

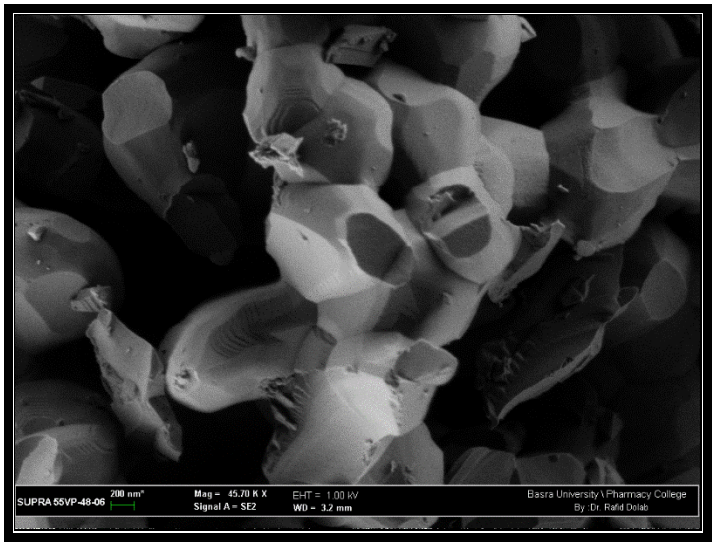

Figure (1): SEM of strontium flourapatite (SrFA)

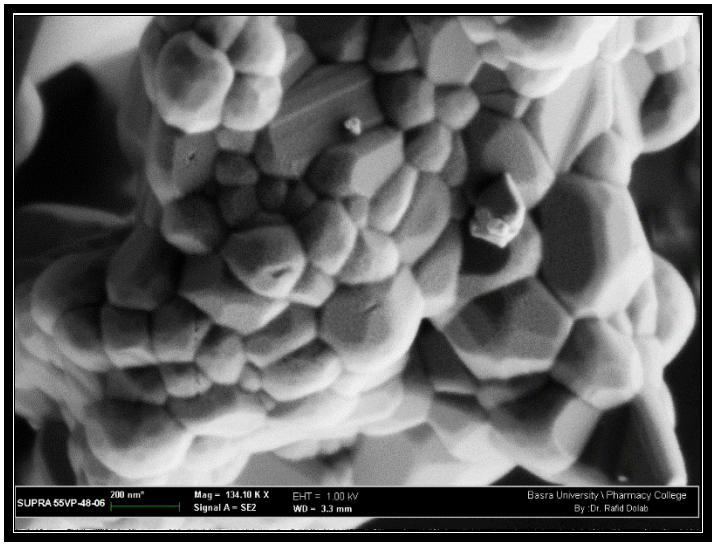

Figure (2): SEM of strontium hydroxyapatite (SrHA)

The X-Ray diffraction

The x-ray diffractiogram of the strontium flourapatite and the strontium hydroxyapatite are stated in figures (3) and (4). They stand in coincidence with that of the references obtained from the literature [22, 23].

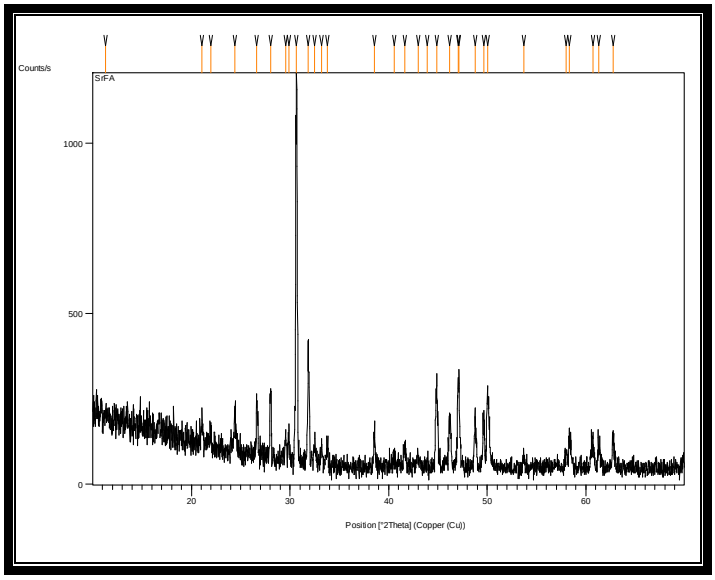

Figure (3): X-Ray diffraction of SrFA

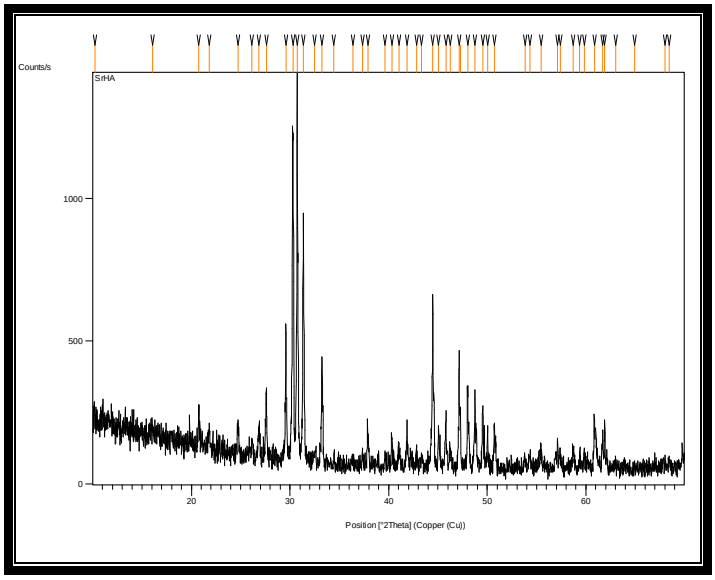

Figure (4): X-Ray diffraction of SrHA

Fourier Transform Infrared Spectroscopy

Figure (5) and figure (6) refers to the FTIR spectra of the strontium flourapatite and the strontium hydroxyapatite.

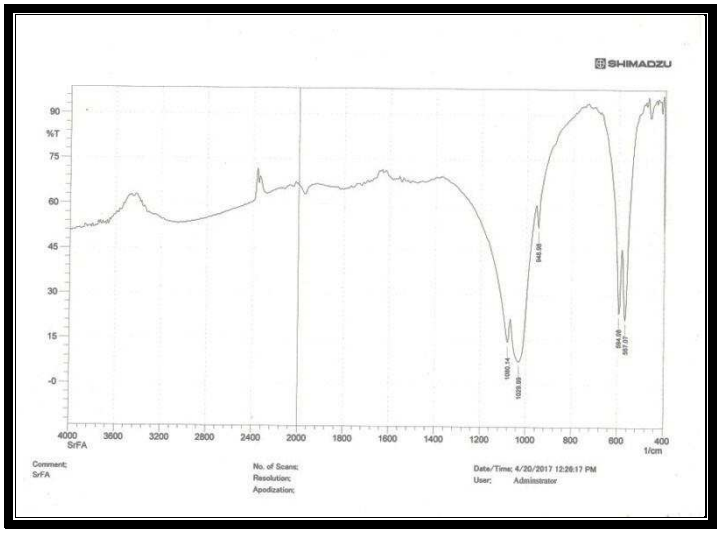

Figure (5): FTIR spectra of strontium flourapatite (SrFA)

The FTIR of strontium flouapatite (SrFA) shows (P=O) asymmetrical stretching at 1080.14^{cm-1} band and (P=O) symmetrical stretching at 1029.99^{cm-1} band. The P=O deformation appears at 567.07 $^{cm-1}$ band.

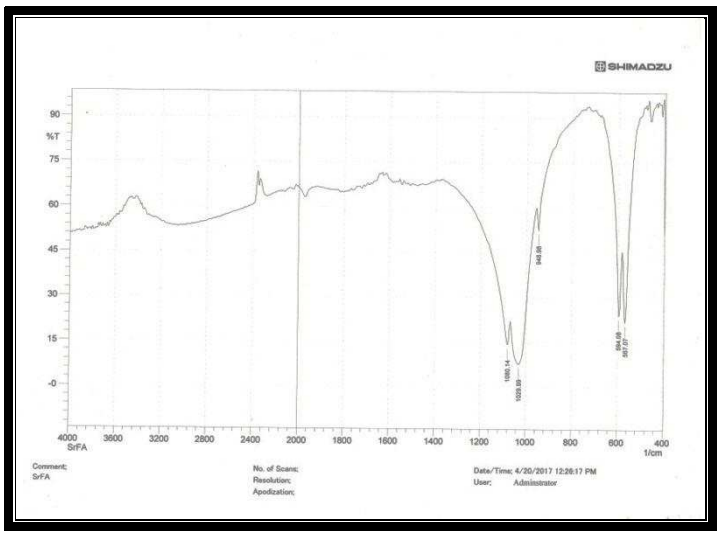

Figure (6): FTIR spectra of strontium hydroxyapatite (SrHA)

The FRIR spectra of the Strontium Hydroxyapatite (SrHA) states that the (P=O) asymmetrical stretching is present at 1072.42^{cm-1} band, while the band at 1029.99^{cm-1} refers to (P=O) symmetrical stretching. The appearance of a strong band at 567.07 $^{cm-1}$ is corresponded to (P=O) deformation. The (O-H) group is not very clear, but may occur at 3321.42 $^{cm-1}$ band.

The result of the FTIR corresponds to that of the references **(24, 25)**.

Amount Of Ceftazidime Loaded

The (λ_{max}) of the ceftazidime in SBF was checked and found to be 258 nm ([26]). The amount of ceftazidime antibiotic loaded on each compound was found by calculating the difference in concentration of ceftazidime in the SBF loading medium before and after loading using the UV spectroscopy. It was found that the mean amount of loaded ceftazidime on the SrFA for three experiments is 24.29 w/w% and that for SrHA is 84.57w/w%.

Ceftazidime Release

The figure (7) refers to the ceftazidime releasing fashion from strontium flourapatite and strontium hydroxyapatite compounds for first the 3 hours. Figure (8) presents the release for 12 hours.

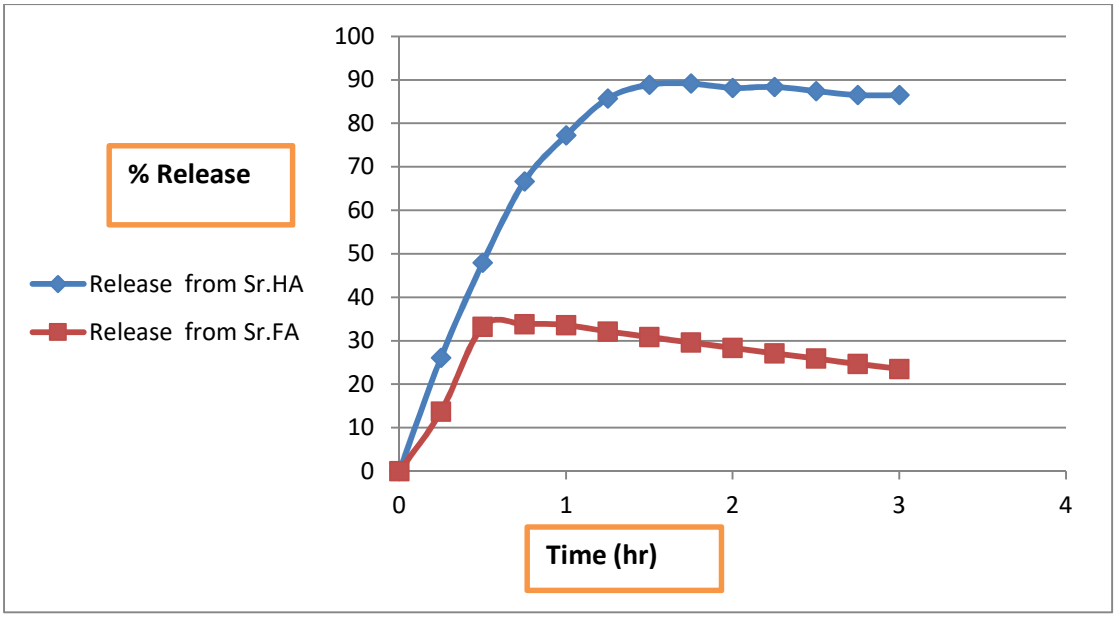

Figure (7): Ceftazidime release from strontium hydroxyapatite and strontium flourapatite for the first 3 hours

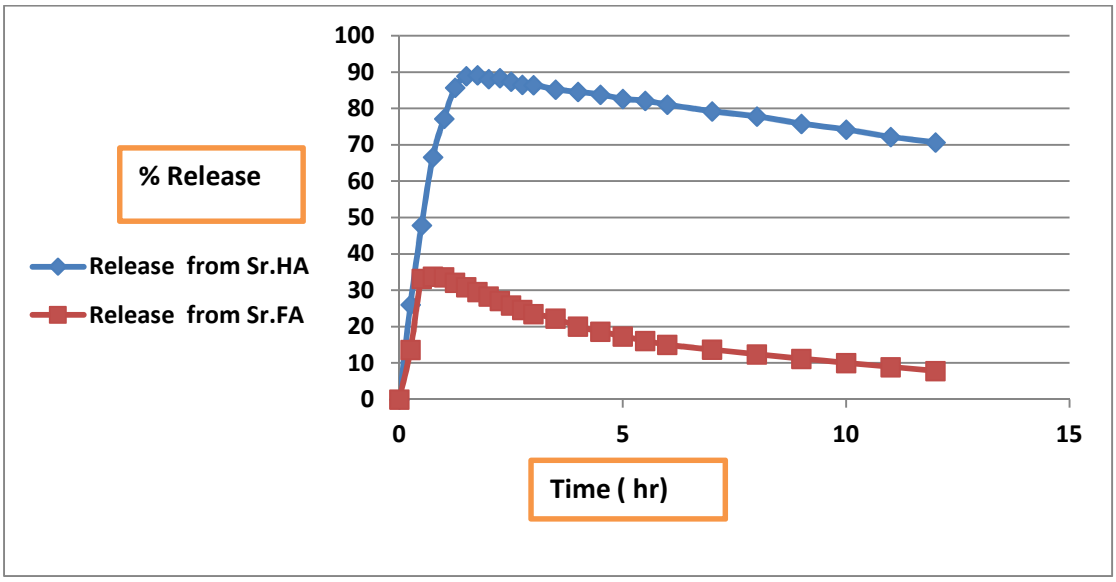

Figure (8): Ceftazidime release from strontium hydroxyapatite and strontium flourapatite for 12 hours

The release was experimented three times at 37 ^{o}C in a physiological medium, which is represented by the simulated body fluid. This simulated body fluid has provided a pH of 7.4 for the releasing medium, which refers to the physiological pH. The reported mean values, which were collected from three independent experiments, are presented in table (3).

Table (3): The release mean data from strontium hydroxyapatite and strontium flourapatite

Release from Sr.FA	Release from Sr.HA	time hours
0	0	0
13.66	26.09	0.25
33.18	47.89	0.5
33.82	66.61	0.75
33.59	77.20	1
32.12	85.70	1.25
30.86	88.88	1.5
29.57	89.14	1.75
28.31	88.15	2
27.07	88.38	2.25
25.88	87.41	2.5
24.67	86.48	2.75
23.52	86.48	3
22.22	85.23	3.5
20.02	84.55	4
18.63	83.81	4.5
17.34	82.67	5

16.12	82.13	5.5
15.02	81.07	6
13.68	79.23	7
12.39	77.81	8
11.19	75.82	9
10.05	74.21	10
8.92	72.19	11
7.84	70.69	12

The release from the strontium hydroxyapatite powder reached its highest level within 1.75 hours, when the concentration was 89.14% w/w. On the other side, the release from the strontium flourapatite arrived at its highest concentration, 33.82% w/w, within 0.75 hour. So, in gaining its highest level, the release from the strontium flourapatite was faster, but lower, than that from strontium hydroxyapatite.

The relative slowness of the release from the strontium hydroxyapatite is expected to be due to the higher entrapment of the ceftazidime molecules by the so-fine powder particles, as compared to that of the higher size particles of the strontium flourapatite.

On other side, figure (8) illustrates that the overall release from the strontium hydroxyapatite is much higher than that from strontium flourapatite. This is attributed to the larger surface area possessed by the strontium hydroxyapatite powder due to its smaller particle size, where the ceftazidime loading was higher, and consequently, the release was more.

Both of the releases undergone drops at early stages of the study. The release from the strontium hydroxyapatite started to fall clearly after 2.75 hours from the start of the study. The release from the strontium flourapatite decreased earlier, where it began to reduce after the first 1.5 hours of the study. This reduction in the release from the two powders, which continues till the end of the study, is expected to result from the stability characteristics of the ceftazidime antibiotic [(27)]. The general view states that the release drop from the strontium hydroxyapatite is straighter than that from the strontium flourapatite. The relative smaller size of the strontium hydroxyapatite particles might be a cause for this straight behavior.

Conclusion

Substituting the hydroxyl group by flour atom in the structure skeleton of the strontium apatite, have much affected the particle size of the resultant powders, and hence, the release modality of the ceftazidime drug. The ceftazidime release from the strontium hydroxyapatite can be considered as an efficient release if this release manner were the same *in vivo*. Long lasting and high concentration was obtained in this *in vitro* study.

Also, the trehalose coating for the particles of the synthesized strontium flourapatite and strontium hydroxyapatite has diminished the ionic interaction between the ions in those synthesized compounds and the ceftazidime dianionic antibiotic. This coating paves the way for the release of the antibiotic to follow a simple diffusion manner, which results in an orderly release style.

REFERENCES

[1] M Hidouri, K Bouzouita, F Kooli and I Khattech, (2003) Thermal behaviour of magnesium-containing fluorapatite, **Materials Chemistry and Physics**, 80 (2):496-505.

[2] A. R. Boyd, L. Rutledge, L. D. Randolph, B.J. Meenan, (2015) Strontium-substituted hydroxyapatite coatings deposited via a co-deposition sputter technique, **Materials Science and Engineering C.**, 46: 290-300.

[3] D. Bazin, A. Dessombz, C. Nguyen, H. K. Ea, F. Lioté, J. Rehr, C. Chappard, S. Rouzière, D. Thiaudière, S. Reguer and M. Daudon, (2014) The status of strontium in biological apatites:an XANES/EXAFS investigation, **J. Synchrotron Rad.**, 21:136-142.

[4] I. Pereiro, C. Rodríguez-Valencia, C. Serra, E. L. Solla, J. Serra and P. González (2012), Pulsed laser deposition of strontium-substituted hydroxyapatite coatings, **Applied Surface Science**, 258(23): 9192-9197.

[5] R.G. Hill, A. Stamboulis, R.V. Law, A. Clifford, M.R. Towler and C. Crowle, (2004) The influence of strontium substitution in fluorapatite glasses and glass-ceramics, **Journal of Non-Crystalline Solids**, 336: 223-229.

[6] G. X. Ni, K. Y. Chiu ,W. W. Lu ,Y. Wang, Y. G. Zhang, L. B. Hao, Z. Y. Li, W. M. Lam , S. B. Lu and K. D. K. Luk, (2006) Strontium-containing hydroxyapatite bioactive bone cement in revision hip arthroplasty, **Biomaterials**, 27 (24):4348-4355.

[7] Amit Kumar Nayak, Bibek Laha and Kalyan Kumar Sen, (2011) Development of hydroxyapatite-ciprofloxacin bone-implants using »Quality by design«. **Acta Pharm**, 61: 25-36.

[8] Z. Y. Li, W. M. Lama, C. Yang, B. Xu, G. X. Ni, S.A. Abbah, K. M. C. Cheung, K. D. K. Luk and W. W. Lu, (2007) Chemical composition, crystal size and lattice structural changes after incorporation of strontium into biomimetic apatite, **Biomaterials**, 28:1452-1460.

[9] Steven Gitelis and Gregory T. Brebach, (2002) The treatment of chronic osteomyelitis with a biodegradable antibiotic-impregnated implant, **Journal of Orthopaedic Surgery**, 10(1): 53-60.

[10] A. Bryskier, (2000) Perfecting the ring and extending the antibacterial spectrum, the multiple generations, **Cliln Microbiol lnfect**, 6 (3): 13-21.

[11] Sean C. Sweetman, (2009) **Martindale the Complete Drug Reference**, The Pharmaceutical Press, London, 36th edition: 235.

[12] Lawrence J. Eron, Robin I. Goldenberg, Choong H. Park and Donald M. Poretz, (1983) Ceftazidime Therapy of Serious Bacterial Infections, **Antimicrobial agents And Chemotherapy**, 23(2):236-241.

[13] Rainer H. Müller, Karsten Mäder and Sven Gohla, (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, **European Journal of Pharmaceutics and Biopharmaceutics**, 50(1):161-177.

[14] I. Nikcevic, V. Jokanovic, M. Mitric, Z. Nedic, D. Makovec and D. Uskokovic, (2004) Mechanochemical synthesis of nanostructured fluorapatite/fluorhydroxyapatite and carbonated fluorapatite/fluorhydroxyapatite, **Journal of Solid State Chemistry**, 177:2565-2574.

[15] Lin Yingguang, Yang Zhuoru, Cheng Jiang, Wang Lianshi, (2008) Synthesis, Characterization and Antibacterial Property of Strontium Half and Totally Substituted Hydroxyapatite Nanoparticles, **Journal of Wuhan University of Technology-Mater. Sci. Ed.**, 23:475-479.

[16] Dhiraj Mehta, Suja George, Anjuvan Singh, (2016) Assessment of Different Synthesis Route of Hydroxyapatite and Study of its Biocompatibility in Synthetic Body Fluids, **International Journal of ChemTech Research**, 9: 267-276.

[17] A. Cüneyt Tas, (2000) Synthesis of biomimetic Ca-hydroxyapatite powders at 37 ^{o}C in synthetic body fluids, **Biomaterials**, 21:1429-1438.

[18] Pavani Vengala, Sana Aslam & C.V.S. Subrahmanyam, (2013) Development and In Vitro Evaluation of Ceramic Nanoparticles of Piroxicam, **Lat. Am. J. Pharm**, 32 (8):1124-1130.

[19] Thirumala Govender, Snjezana Stolnik, Martin C. Garnett, Lisbeth Illum, Stanley S. Davis, (1999) PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug, **Journal of Controlled Release**, 57: 171-185.

[20] Anthony C Moffat, M David Osselton and Brian Widdop**,** (2011) **Clarke's Analysis of Drugs and Poisons**, Pharmaceutical Press, London, 4th edition: 1060.

[21] Crook A. Martin., (2006) **Clinical Chemistry and Metabolic Medicine**, Hodder Arnold LTD, London, 7th edition: 33.

[22] R. G. Hill, A. Stamboulis, R. V. Law, A. Clifford, M. R. Towler, C. Crowley, (2004) The influence of strontium substitution in fluorapatite glasses and glass-ceramics, **Journal of Non-Crystalline Solids**, 336:223-229.

[23] Yunfeng Li, Qing Li, Songsong Zhu, En Luo, Jihua Li, Ge Feng, and Jing Hu, (2010) The effect of strontium-substituted hydroxyapatite coating on implant fixation in ovariectomized rats, **Biomaterials**, 31(34):9006-9014.

[24] Chirantha Prageeth R., (2005) Synthesis and Characterization of Strontium Fluorapatite [**thesis**]. Las Vegas, NV: University of Nevada.

[25] Petre T. Frangopol, Aurora Mocanu, Valer Almasan, Corina Garbo, Reka Balint, George Boorodi, Ioan Bratu, Ossi Horovitz and Maria Tomoaia-

Costisel, (2016) Synthesis and structural characterization of strontium substituted hydroxyapatites. **Rev. Roum. Chim.**, 61(5): 337-34.

[26] Anthony C Moffat, M David Osselton, Brian Widdop and Jo Watts, (2009) **Clark's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem materials**. The Pharmaceutical Press, London, 14th edition: 1060.

[27] Carolina Alves dos Santos, Laura Oliveira-Nascimento, Marcos Camargo Knirsch, Marco Antônio Stephano, Adalberto Pessoa Júnior, Thereza Christina Vessoni Penna, (2012) Biological and physicochemical stability of ceftazidime and aminophylline on glucose parenteral solution, **Brazilian Journal of Pharmaceutical Sciences**, 48: 691-698.