Calcium and strontium hydroxyapatites: Microwave accelerated inorganic sol-gel synthesis, and potentiometric determining of the Point of Zero Charge (PZC) and isoelectric point (IEP)

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ABSTRACT

Calcium and strontium hydroxyapatites (HA) were synthesized via a microwave accelerated inorganic sol-gel method. The acid-base properties of synthetically prepared phosphates are investigated at ambient temperature, by potentiometric titrations. Potentiometric mass, time and surface charge titration techniques were used to achieve this purpose. Isoelectric point (IEP) is evaluated as the pH of the intersection point of the suspension or time titration curves. For both phosphates the point of zero charge (PZC) is equivalent to isoelectric point indicating no specific adsorption of ions other than the potential determining H^+/OH^- ions at the surface. PZC (IEP) values of 6.4 and 7.2 were found for Ca-HA and Sr-HA, respectively.

Keywords: Calcium, strontium, phosphate, Synthesis, Inorganic sol-gel processes, Microwave method, Potentiometric titration, PZC, IEP.

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INTRODUCTION

Calcium phosphate materials (CaP) such as hydroxyapatite (HA) are used extensively for biomedical and clinical applications. The successful uses of HA as biomaterials is due furthermore, to its biocompatibility, biodegradability, and nontoxicity. These compounds are used also as a carrier in drug delivery systems. Nevertheless, HA biomaterials have significant disadvantages such as brittleness, low strength, and dumping release of loaded drug at early stage of release process, and lack of the ability for stimulating osteogenetic process. Materials used as adsorbents should be characterized by small size as huge surface area produce high adsorption efficiency [1]. To improve hydroxyapatite biological performance and physicochemical properties, various metal elements are incorporated in hydroxyapatite [2, 3]. Among these elements, strontium is considered as beneficial for bone regeneration which stimulates formation, decreases bone resorption, and increases osteoblast activation [3-6]. Moreover, photo-induced luminescence is very powerful and useful in situ investigations that labeling using organic and inorganic fluorescent molecules has become a hot topic for biomedical applications [7]. Numerous apatite-type compounds are known to exhibit good luminescence properties [8] and are being widely investigated in both biological staining and diagnostics as luminescent-label material [1, 9]. Due to their luminescent properties and drug storage/release behavior, strontium –hydroxyapatite (Sr-HA) biomaterials are being suitable materials in this case [1]. In addition, Sr-HA has receive a lot of interest recently as optoelectronic compounds, nuclear waste immobilization matrix, catalyst, chemical sensor, ion exchanger, and fuel cell [10-12].

As discussed previously, several methods are reported for the synthesis of HA including furthermore, hydrothermal method, solid state route, precipitation and sol-gel techniques, but only few studies are dealing with the preparation of Sr-HA [13]. Recently, sol-gel route is widely used to synthesis HA biomaterials, and reported to be a convenient method for incorporation of organic and inorganic elements in bioactive phosphate materials [14-17]. This procedure provides a molecular-level mixing of the Ca and P precursors which results in specific homogeneous materials with preserving phase integrity of the gelled sample [18]. Nevertheless organic compounds are frequently

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used as calcium or phosphorus precursors which often, are diluted in organic solvents. So, calcinations of dried gel powder are then required to eliminate organic substances [15]. Further limitations are associated with these synthesis processes, due to slow reaction between Ca and P precursors in the sol phase that most of the sol gel methods require, a long time for hydrolysis reactions and strict pH control [18, 19].

Moreover, microwave synthesis is well established as a promising method for rapid heating, resulting in higher reaction rate, and dramatically reduced reaction time compared to conventional heating methods. Thus, this heating technology becomes a fast-growing area of research in synthetic chemistry involving the synthesis of CaP biomaterials [7, 20]. This process is found to be successful in a rapid synthesizing of pure and substituted calcium hydroxyapatite, via accelerated precipitation process [21, 22].

The phenomena occurring at the interface between various synthetic bioactive materials and the surrounding biological environments remain not totally controlled [23, 24], but it is assumed that the surface chemistry plays a crucial role in the interaction process involved in this case [21, 25, 26]. It is assumed that the surface of adsorbent materials is best described by the PZC and IEP electrochemical parameters [27]. For calcium phosphate CaP compounds, the surface charge is studied by potentiometric titration and measuring the zeta potential. A value of zero charge (iso-elctric) point of 5.6 to 8.7 is generally found for synthetic hydroxyaptite [28, 29]. While for strontium substituted phosphates, no PZC or IEP values are reported in the literature, but compared to Ca-hydroxapatite and according to suspension conditions, these materials exhibit higher negative or positive electrokinetic potentials [21, 30]. The higher zeta potential value shows the high associated surface charge of strontium substituted HA.

For these reasons, the present study aims to develop simple and rapid method for the preparation of strontium substituted hydroxyapatite. For these reasons, Sr-HA materials are synthesized via a microwave accelerated inorganic sol gel process, which requires no alkoxide precursors, or strict control of pH, Ca(II) and Sr(II) concentrations. The characterization of surface chemistry of obtained phosphates is carried out by electrochemical method. Thus, potentiometric mass titration, and potentiometric time titration are employed to evaluate the point of zero charge, and iso-electic point of various synthesized strontium phosphates.

MATERIALS AND METHODS

2.1. Solution preparation
All chemicals were analytical grade and used without further purification. Calcium phosphate and strontium-substituted hydroxyapatite (Sr-HAP) are synthesized by a microwave accelerated sol gel method, using phosphoric acid (0.3M), and 0.5M solutions of calcium hydroxide or strontium dichloride, as starting materials. Solutions with Ca/P and Sr/P ratio of 1.67are prepared by adding 0.3M H_3PO_4 to 0.5M solutions of $Ca(OH)_2$ or $SrCl_2$, respectively. The prepared Ca(II) and Sr(II) solutions are mixed at (Ca + Sr)/P molar ratio of 1.67 to obtain initial precursor solution with strontium percentages Sr/(Ca +Sr) varying between 0 and 100%. A starting hydroxyl ion concentration of the mixture is kept constant with adding NaOH. Obtained mixture is stirred at room temperature and the phosphate solution is added drop wise to it and then heated at 150°C for 1h. The obtained milky mixture is then transferred to a domestic type microwave oven (800w) and irradiated for 5 to 15 min. The gel formed is filtered off and dried overnight in air oven at 80°C.

The powder from dried xerogel is washed repeatedly to remove free ions. The X-Ray powder diffraction (XRD) analysis is performed on powder samples, in reflection mode with CuK☐ radiation. The IR spectra are performed using KBr pellets on IR spectrometer (Perkins model).

RESULTS AND DISCUSSIONS

3.1. Characterization of the strontium substituted calcium phosphates
The strontium substituted phosphates are characterized by Fourier Transform Infra-Red spectroscopy (FT-IR) and X-ray diffractometry (XRD).

Fig.1 shows the FT-IR spectra of various strontium substituted phosphates. From these results, all Sr-HA samples exhibit typical PO_4 bands of a hydroxyapatite phase. These bands shift to a lower wave number of FT-IR bands with increasing strontium amount, as found previously, [4, 31]. The bands of phosphate groups occur at 472, 563, 601, 987 cm^{-1}, and within a range 1000–1120 cm^{-1}. Weak bands associated with carbonate are observed at 873 cm^{-1} and within the range of 1412 to 1465 cm^{-1}. The peak at 873 cm^{-1} which may also be associated with HPO_4 groups, is absent in strontium phosphate [32]. Generally, the intensity of this band decrease as Ca/P ratio increases [33-35]. In

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addition, the bands at 3572 and 630 cm^{-1} assigned to the structural hydroxyl anions in calcium hydroxyapatite, are observed at 3590 cm^{-1}, and 537 cm^{-1} in strontium substituted hydroxyapatite.

Fig. 2 shows the XRD patterns of obtained phosphates with the different Sr/Ca molar ratios. All phosphates exhibit single HA phase with the mean at 2-theta (2θ) of 25.8, 31.8, 32.3 and 32.9º (JCPDS 860740). Similar results are obtained previously for strontium substituted phosphates [36]. One can note however that diffraction peaks corresponding to 40% substituted HA shift to higher 2 theta angles ,suggesting modifications of the lattice parameters of HA , as discussed before, [36, 37]. It is also shown that the crystallite size of apatite decreases with increasing Sr substitution from 0 to 50 mol%, and then increases as Sr continue to rise above 50 mol% , resulting then in more disordered apatite structure [37].

3.2. Potentiometric titration
The aim of the potentiometric titration is to determine the balance of adsorbed and desorbed protons on the surface of the phosphate materials as a function of pH, hydratation time and mass sorbent materials. The surface protonation/deprotonation reactions involved in the acid-base behavior of hydroxapatites are well described as reactions of an amphoteric acid-base surface groups according to:

$$\overline{> SOH} + H^+ \leftrightarrow \overline{> S(OH_2)^+} \qquad (1)$$

$$\overline{> SOH} \leftrightarrow \overline{> SO^-} + H^+ \qquad (2)$$

With $\overline{> SOH} \equiv \overline{> PO_4 H}$ and/or $\overline{> CaOH}$ $\overline{> SrOH}$ [39]
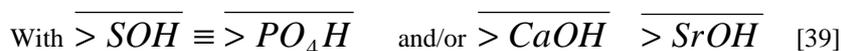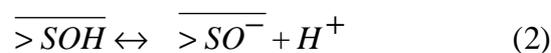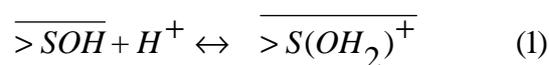
The on lined species belong to solid phase.
Potentiometric mass and time titrations designated respectively as PMT and PTT, developed previously for determining point of zero charge (PZC) and isoelectric point (IEP) of some sorbent materials [38, 39] are performed to evaluate PZC, and IEP for Ca-HA and Sr-HA materials. The PZC is identified as the common intersection point (CIP) of the potentiometric curve of the blank solution with the corresponding curves obtained for different t_h and suspensions, respectively. Whereas the IEP is identified as the CIP of suspension plots or hydratation time curves.
To achieve this purpose, PMT and PTT techniques are performed on KOH (0.05 M) suspensions obtained for Ca-HA or Sr-HA sorbent amounts of m= 0.5, 1.0 and 1.5 g/L, in the range : 12 H $\leq t_h \leq$ 72 H. Standard 0.1M HNO$_3$ solution is used as titrant.

3.3. Potentiometric time titration
Fig. 3 and 4 show titration results of blank and suspension of m= 1.0 g at t= 12, 24, 48, and 72 hours, obtained respectively for calcium and strontium hydroxyapatites. As found previously, equivalent volume (Ve) for acid-base titration of the suspensions is equilibrium time dependent, especially for Sr-HA sorbent [39]. This phenomenon is due to OH$^-$ consumption via deprotonation of predominant active surface sites as >CaOH$_2^+$, >SrOH$_2^+$ and >POH [39]. In this method the titration curves are dependent on hydration time of phosphate material at all pH values except that corresponding to the IEP. There is a common intersection point for the potentiometric titration curves obtained at different contact times and the CIP of titration curve of a blank solution and that recorded for a given duration. The pH of this common intersection point corresponding to both PZC and IEP value is ranging from 6.8 to 7.3, in all the cases.

3.4. Potentiometric mass titration
The PMT method is carried out at a constant contact time. The suspensions of m =0.5, 1.0, and 1.5g are equilibrated for 72 hours. As found above, the titration curves of a blank solution and suspension presented in Fig. 5 and 6 intersect at the PZC and EP value. The point of zero charge and isoelectric point are found to be about 6.4 and 6.8, for calcium and strontium phosphate respectively.

Titration curves, Q = f(pH)
To achieve precise determination of PZC and IEP, a complementary method is used. This is obtained from the variations of surface charge (Q) with pH, according to:

$$Q = \frac{FC \, \Delta V}{m} \qquad (Coulomb/g)$$

Where F is the faraday constant, C is the concentration of acid and base used in titration, ΔV is the difference in volume of acid used to reach the same pH in dispersion and blank solution, and m (g/L) is the suspension concentration of the solid particles.

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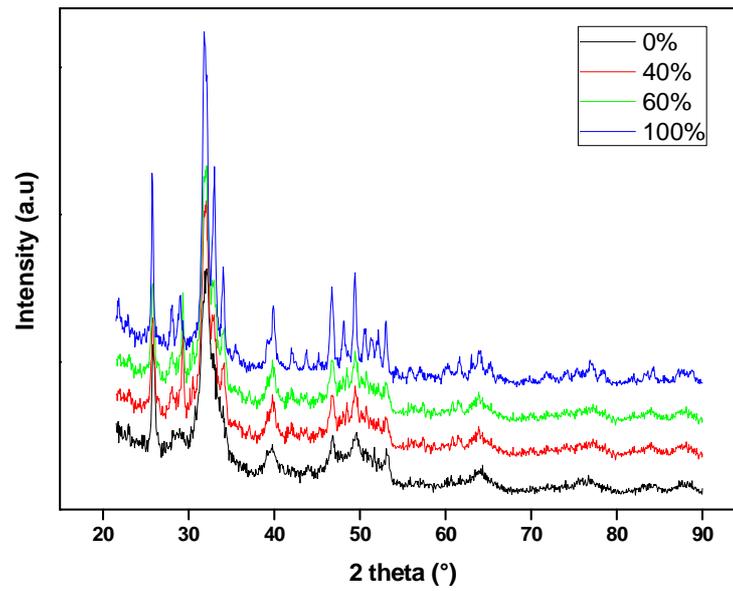
Figure 1: Typical XRD pattern of microwave synthesized for different percentage of Ca10-X SrX HA at 800 W microwave powers
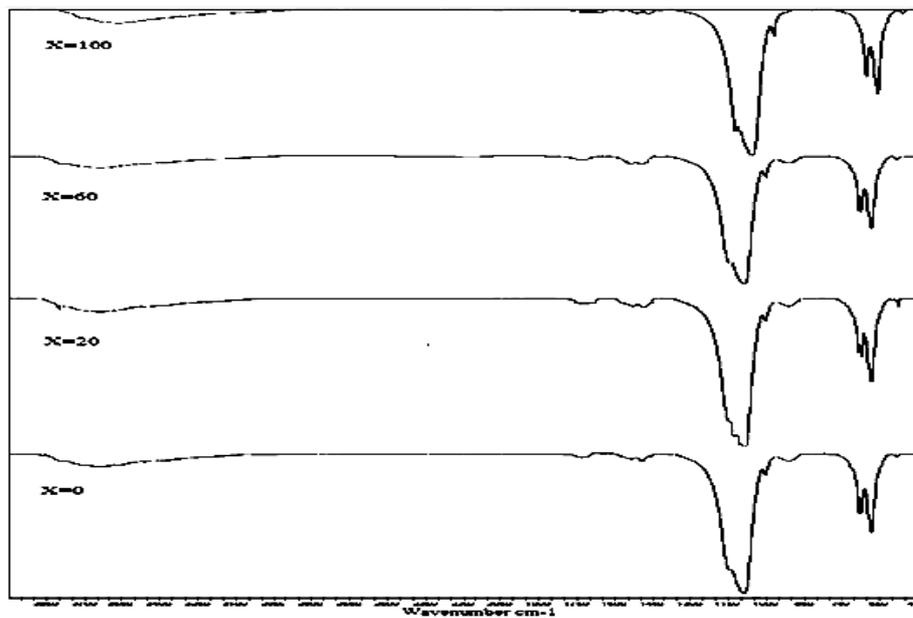
Figure 2: Typical FIR pattern of microwave synthesized for different percentage of Ca10-X SrX HA at 800 W microwave powers
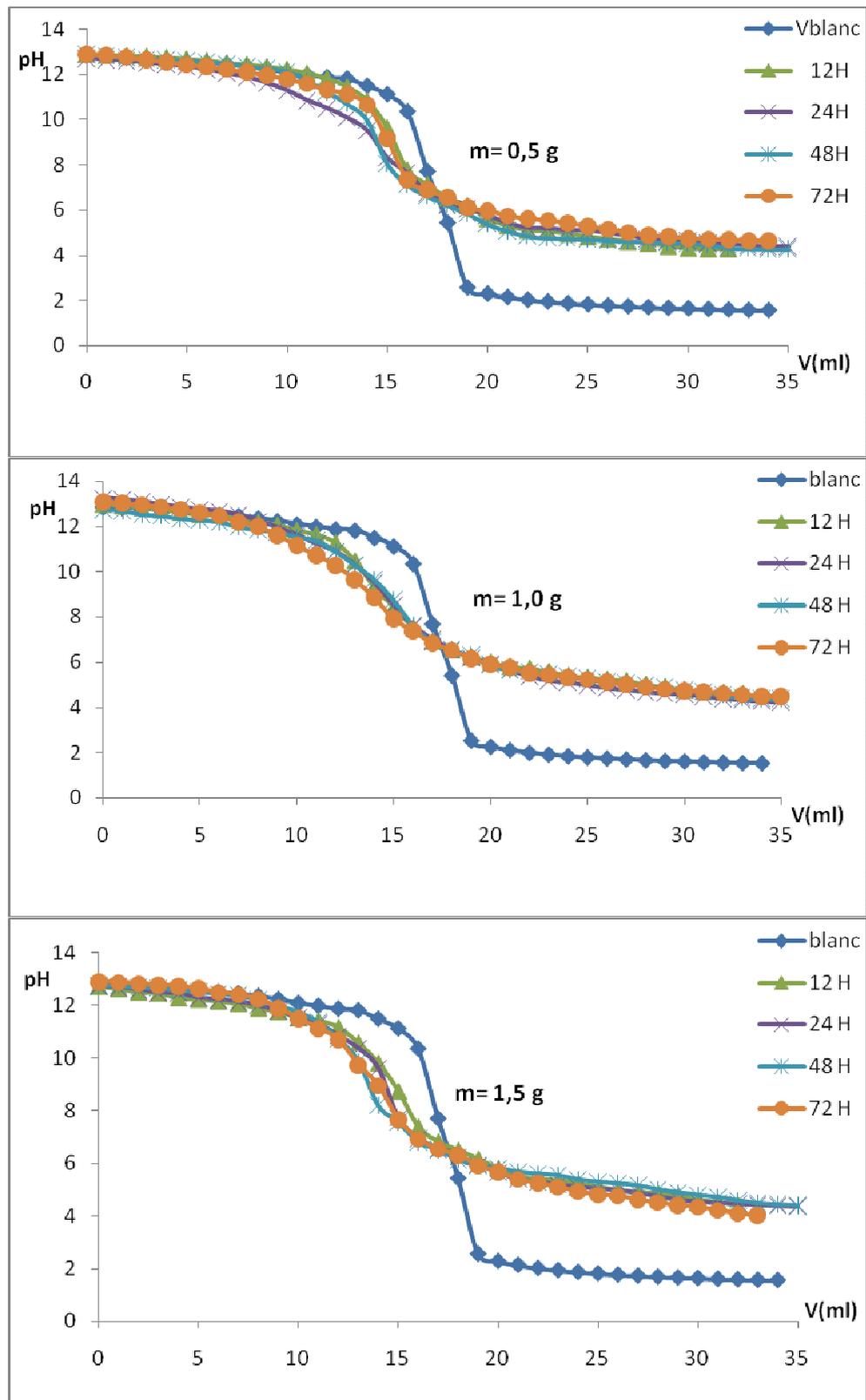
Figure 3: Titration of KOH (0.05 N) phosphate suspensions, obtained for various contact times, at different Ca-HA sorbent amounts of m= 0,5; 1,0 and 1,5 g/L, at t = 12, 24, 48, and 72 hours
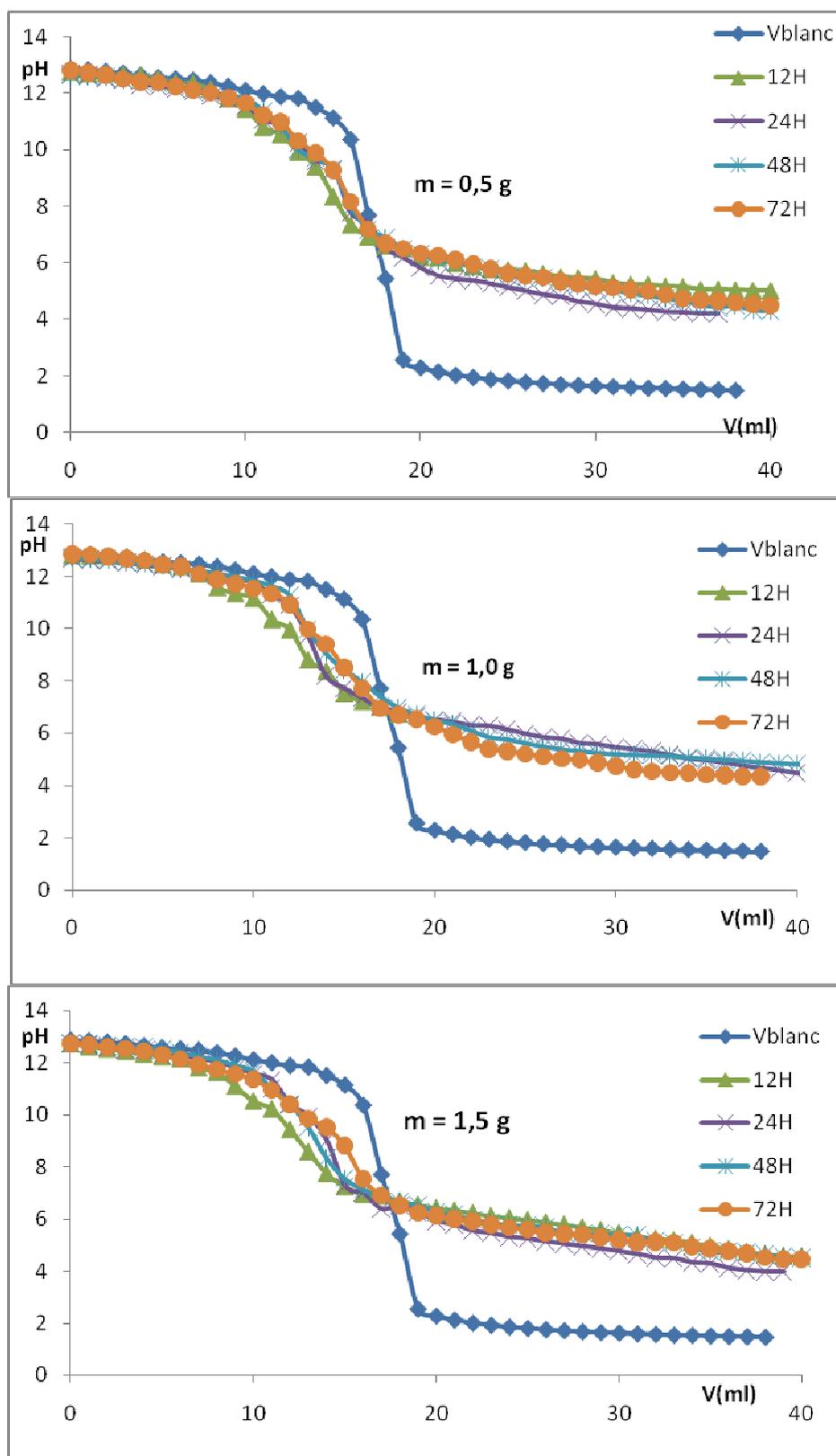
Figure 4: Titration of KOH (0.05 N) phosphate suspensions, obtained for various contact times, at different Sr-HA sorbent amounts of m= 0,5; 1,0 and 1,5 g/L, at t = 12, 24, 48, and 72 hours
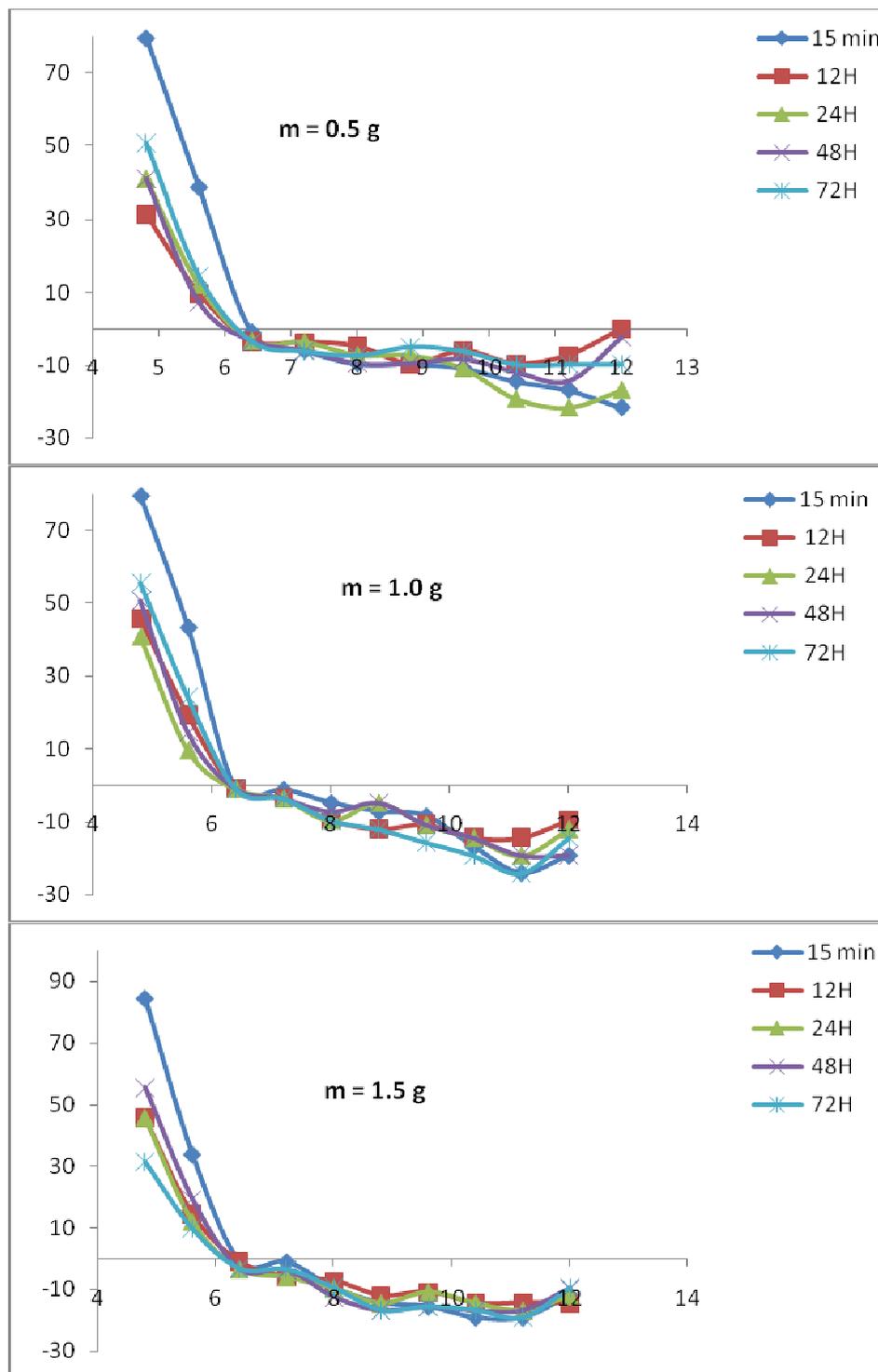
Figure 5:Variation of surface charge (Q) with pH obtained for various contact times, at different Ca-HA sorbent amounts of m= 0,5; 1,0 and 1,5 g/L

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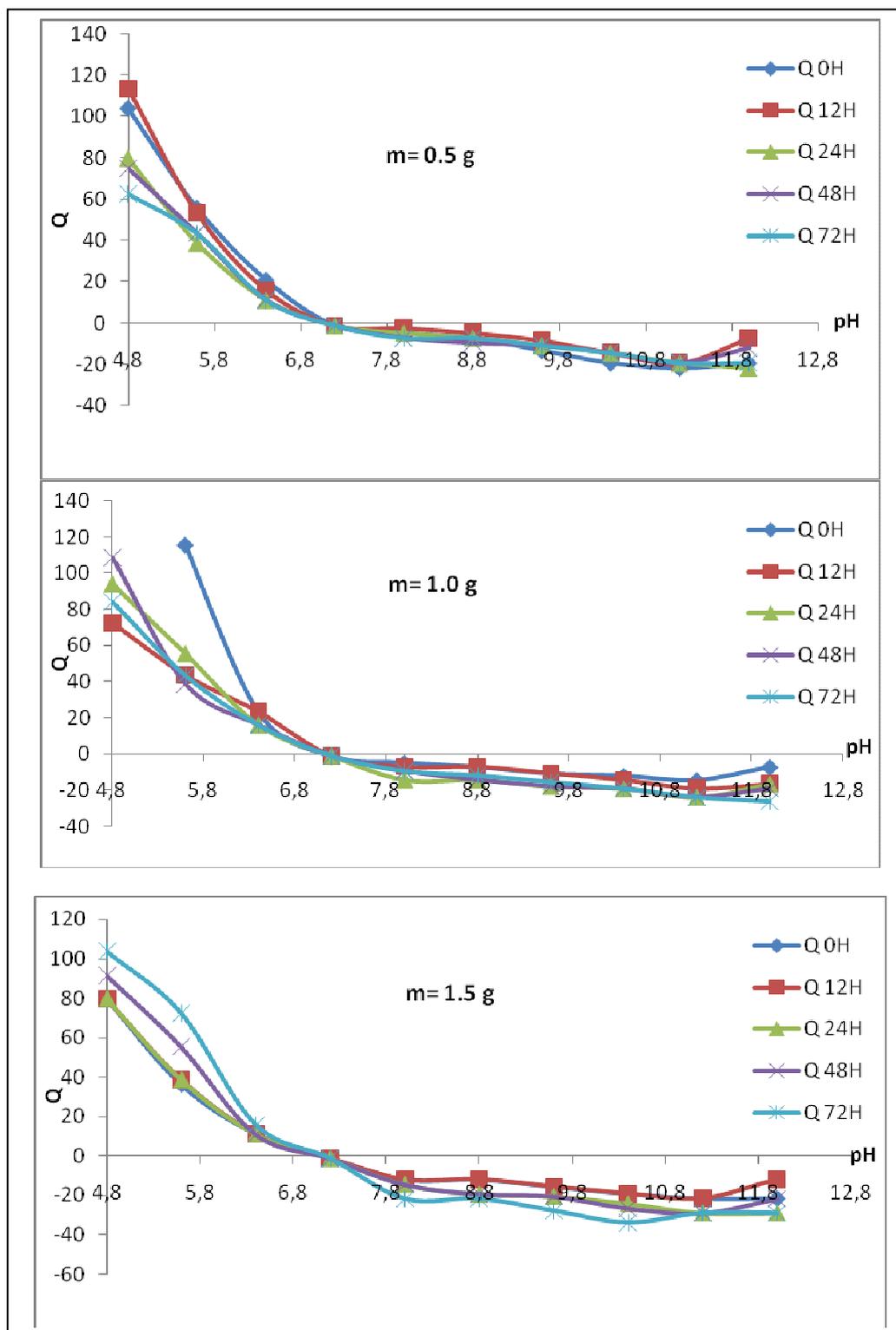
Figure 6: Variation of surface charge (Q) with pH obtained for various contact times, at different Sr-HA sorbent amounts of m= 0,5; 1,0 and 1,5 g/L

The titration curves Q = f(pH) obtained at m = 0.5, 1.0, and 1.5 g/L for different equilibrium times Fig. 5 and 6, intersect at Q = 0 (PZC) and IEP corresponding to pH 6.4, and 7.2 for Ca-HA and Sr-HA, respectively. Comparable PZC values of 6.35 and 6.5 are obtained previously for Ca-HA, while a value of 6.4 is obtained for octacalcium phosphate [39-41]. One can note a small variation at low negative surface charge values Fig.5, showing for Ca-HA Q oscillation around zero for pH ranging from 6.4 to 8. This may explain the consistency of PZC values of calcium hydroxyapatite observed in this pH range. However, PZC and   IEP values of Sr-HAP are not available for comparison with our results.

CONCLUSION

A low crystallized calcium and strontium hydroxyapatite powders are synthesized using microwave accelerated inorganic sol-gel method. The zero charge (PZC) and isoelectric (IEP) points of synthesized phosphates are determined by potentiometric titrations. PMT, PTT, and Q titration methods are performed in this case. It is found that Ca-HA and Sr-HA PZC is equivalent to IEP, in all the examined cases. Point of zero charge/isoelectric point values are of 6.4, and 7.2 for Ca-HA and Sr-HA materials, respectively.

REFERENCES

[1] A A El-Zahhara, N S Awwada, *J. Environ. Chem. Eng*., **2016**, 4, 633–638.
[2] M Kavitha, R Subramanian, K S Vinoth, R Narayanan, G Venkatesh ,N Esakkiraja, *Powder Techn*., **2015**, 271, 167–181.
[3] W Lou, Y Dong , H Zhang, Y Jin, X Hu, J Ma, J Liu, G Wu, *Int. J. Mol. Sci.,* **2015**, 16, 21070-21086.
[4] M D O'Donnell, Y Fredholm, A de Rouffignac, R G Hill, *Acta Biomaterialia*, **2008**, 4, 1455-1464.
[5] G Renaudin, P Laquerrière, Y Filinchuk, E Jallot, J M Nedelec, *J. of Mater. Chem.* , **2008**, 30, 3593-3600.
[6] J Abert, C Bergmann, H Fischer, *Ceramics Inter.,* **2014**, 40, 9195-9203.
[7] F Chen, P Huang, Y-J Zhu, J Wu, C-L Zhang, D-X Cui, *Biomaterials*, **2011**, 32, 9031-9039.
[8] B Ş Valise, R Truşcă, D Predoi , *U.P.B. Sci. Bull., Series B,* **2011**, 73 (4), 63-70.
[9] S L Iconaru, M Motelica-Heino, D Predoi. J. of Spectroscopy, Article ID 284285, **2013**, 10.
[10] J F Rakovan, J M Hughes, *The Canadian Mineralogist*, **2000**, 38, 839-845.
[11] A Balamurugan, G Balossier, P Torres, J Michel, J.M.F, *Mater. Sci. and Eng*. C, **2009**, 29, 1006-1009.
[12] M P Mahabole, M M Bahir, N V Kalyankar, R S Khairnar, *J. Biomed. Sci. and Eng*., **2012**, 5, 396-405.
[13] M Kavitha, R Subramanian, K S Vinoth, R Narayanan, G Venkatesh , N Esakkiraja, *Powder Techn*, **2015**, 271, 167-181.
[14] A Beganskienė , O Dudko, R Sirutkaitis , R Giraitis, *Mater. Sci*., **2003**, 9 (4), 383-386.
[15] M Bilton, S J Milne, A P Brown, Open J. of Inorg. *Non-metallic Mater*., **2012**, 2, 1-10.
[16] B A Allo, Engineering The University (Western Ontario, Canada, **2013**)**.**
[17] T Demirtaş, G Kaynak, M Gümüşderelioğlu, Mat*er. Sci. Eng. C Mater. Biol Appl*., **2015**, 49, (2015) 713-719.
[18] D-M Liua, T Troczynski , W J Tseng, *Biomaterials*, **2002**, 23, 1227-1236.
[19] M H Fathi , A Hanifi, *Mater. Lett.,* **2007**, 61, 3978-3983.
[20] Z-Y Cai , F Peng, Y-P Zi, F. Chen, Q-R Qian, *Nanomater*., **2015**, 5, 1284-1296.
[21] N D Ravi, R Balu, T S S Kumar, *J. Am. Ceram. Soc.,* **2012**, 95 (9), 2700-2708.
[22] T S Sampath Kumar, K Madhumathi, Y Rubaiya, M Doble, *Frontiers in Bioengineering and Biotechnology,* Article 59, **2015**, 3.
[23]T G Vladkova, *Inter. J. of Polymer Sci.,* Article ID 296094, **2010**, 22.
[24] A Barroug, H Noukrati, F Errassifi, S Sarda, S Cazalbou, C Combes, C Rey, M J Glimcher. J. Controlled adsorption and release onto calcium phosphates materials and drug delivery applications. MATEC Web of Conferences, **2013**, 5, 03002.
[25] K Rezwan, Swiss Federal Institute (Technology Zurich, Swiss, **2005**).
[26] E M Habraken, Radboud University (Nijmegen Medical Center, Nijmegen, Netherlands ,**2008**).
[27] K Suttiponparnit, J Jiang, M Sahu , S Suvachittanont , T Charinpanitkul, P Biswas, *Nanoscale Res. Lett*. doi:10.1007/s11671-010-9772-1.
[28] M Kosmulski, *J. Colloid and Interface Sci*., **2009**, 337, 439.
[29] WJanusz, E Skwarek, Annales Universitatis (Maria Curie-Sklodowska, Lublin, Polonia, Sectio AA, Vol. LXIV(2), **2009**, 11).
[30] A Anwar, University (College London, UK, **2014**).
[31] J Terra, E R Dourado, J G Eon , D E Ellis, G Gonzalez, A M Rossi, *Phys. Chem. Phys.,* **2009**, 11(3), 568-577.
[32] A R Boyd, L Rutledge, L D Randolph, BJ Meenan, *Mater. Sci. and Eng*. C, **2015**, 46, 290-300.
[33] Z Opre, Swiss Federal Institute (Technology (ETH) Zurich , Swiss, **2007**).
[34] S Belouafaa , H Chaaira, H Loukilib , K Diguaa , B Sallekc, *Mater. Res*., **2008**, 11 (1), 93-96.
[35] Z Gandou , A Nounah, B Belhorma, A Yahyaoui. *J. Mater. Environ. Sci*., **2015**, 6 (4), 983-988.
[36] D S Tavares, C X Resende , M P Quitan , *Mater. Res*., **2011**, 14 (4), 456-460.
[37] R Z Legeros, O R Trautz, J P Legeros, E Klein, W P Shirra, *Science* , **1967**, 155, 1409-1411.
[38] J LI, College of Engineering and Physical Sciences, University (Birmingham, UK, **2009**).
[39] S Koutsopoulos, *J. Biomedical Mater. Res.,* **2002**, 62, 600-612.
[40] K Ellouzi, A Elyahyaoui, S Bouhlassa, *Der Pharmacia Lettre*, **2015**, 7 (11), 152-159.
[41] K Skartsila, N Spanos, *J. Colloid Interface Sci*., **2007**, 308 (2), 405-412.