EFFECT OF SYNTHETIC ROUTE OF HYDROXYAPATITE-SODIUM ALGINATE COMPOSITE ON LOADING AND RELEASE PROFILES OF ANTICANCER AGENTS - DOXORUBICIN AND METHOTREXATE

ONOYIMA C. C[*1]; NWOYE E. E; MAHMOUD A. B[1]

[1]Department of Chemistry, Nigeria Police Academy, Wudil, Nigeria

Corresponding author email**: krissonoss@yahoo.co.uk**

08035982690

ABSTRACT

Processing parameters and mode of formation significantly influence the properties and interfacial characteristics of composites. Drug loading and release profiles of two hydroxyapatite-sodium alginates prepared through different routes were compared using two anticancer drugs – Doxorubicin (DOX) and Methotrexate (MTX). Drug loading was carried out at neutral pH, while *in vitro* drug release study was carried out in synthetic body fluid (SBF) at pH 7.4 and 37 ^{o}C. The major peaks from the FTIR spectra show that the two synthetic routes yielded composites with similar functional groups. The second synthetic route (2HASA) presented higher DOX loading efficiency than the first synthetic route (HASA), while for MTX 2HASA loaded higher only at lower sodium alginate contents (HASA-5%and HASA 20%). The release profiles for all the compared pairs have similarity factor (f_2) above 50, indicating that DOX release from composites prepared following the two synthetic methods have similar release profiles. The drug loading efficiency showed that the second synthetic route (2HASA) loaded higher than the first synthetic route (HASA), while the release profiles for the two synthetic routes are similar.

Keywords: alginate, composite, doxorubicin, Drug loading, methotrexate, release profiles.

INTRODUCTION

Chemotherapy is one of the most important cancer treatments currently available among the various approaches. The present status of chemotherapy is far from being satisfactory. Its efficacy is limited and patients suffer from serious side effects, some of which are life-threatening (Marques *et a*l., 2014). Conventional chemotherapy with anticancer drugs has no tumour selectivity and is randomly distributed in the body; resulting in severe side effects associated with anticancer drugs with low therapeutic index (Kakde *et al*., 2011).

The success of chemotherapy depends on the selection of an optimum carrier system. These carriers include nanoparticles, nanotubes, nanorods, dendrimers, liposomes, microspheres and so forth (Kakde *et al*., 2011). Polymers are used in drug delivery because they have the ability to successfully encapsulate drugs, avoid degradation of the drug, and enhance specificity to the diseased cell, leading also

to improved efficacy and reduced toxicity of the chemotherapeutic agent (Allermann *et al*., 1993).

Alginate has been widely exploited in many controlled drug delivery applications, protein delivery (Lee and Lee, 2009; Chan and Neufeld, 2010; Wells and Sheardown, 2009) wound dressing (Murakami *et al*., 2010; Balakrishnan *et al.*, 2006) cell culture (Bidarra *et al*., 2010; Wang *et al*., 2003) tissue engineering and cell delivery (Silva *et al*., 2008; Skaugrud *et al*., 1999; Thornton *et al*., 2004). Sodium alginate has the ability to delay the dissolution of drugs from tablets, capsules, and aqueous suspensions, and has been applied in the preparation of sustained release formulations (Priya *et al*., 2013).

It has been shown that the drug loading efficiency and controlled release behaviour can be enhanced because of the synergistic effect between biopolymer and inorganic materials (Devanand *et al*., 2011). HA/polymer nanocomposites have attracted much attention since such nanocomposite lead to improved properties (Khaled *et al*., 2014) as a result of improvement in the surface functionality of the apatite (Venkatesan *et al*., 2011). Such improvement has led to wide applications of HA polymer nanocomposite in many areas such as in drug delivery system (Raj *et al*., 2013).

Processing parameters and mode of formation significantly influence the properties and interfacial characteristics of composites. Therefore, suitable processing parameters must be carefully selected in order to yield the optimum composite products (Ku *et al*., 2011). The aim of this study is to evaluate the effect of changing the processing route of hydroxyapatite-sodium alginate composite on its loading and release profiles of two anticancer drugs – Doxorubicin and Methotrexate.

MATERIALS AND METHODS

Preparation of sodium alginate (SA) solutions

SA dissolves slowly in water. To prepare 1%wt of SA (based on the weight of hydroxyapatite used), 20 mg of SA was weighed in a 200 cm^3 beaker, and 100 cm^3 of distilled water was added onto it. The mixture was stirred vigorously using magnetic stirrer for about 30 minutes for complete dissolution of SA. Exactly 110 mg, 520 mg, 1.04 g, and 2.14 g of the alginate were subsequently measured in order to prepare approximately 5%wt, 20%wt, 33%wt and 50%wt of the alginate in the composite.

Preparation of hydroxyapatite-sodium alginate composite (HASA) (first synthetic route)

The *in-situ* preparation of HA in the presence of SA was done according to the method by (Rajkumar *et al*., 2010) with some modifications. The calcium solution (200 cm^3) was added in drops to a separately prepared 100 cm^3 of SA solution (1%wt) while stirring vigorously. This mixture was set on a magnetic stirrer, and 200 cm^3 of 0.06M diammonium hydrogen phosphate solution ($(NH_4)_2HPO_4$) was added into it drop by drop with continuous stirring. The stirring was continued for 24 h. The pH was maintained at approximately 10.5 throughout the experiment using 1 M sodium hydroxide. The suspension was

then stored for another 24h at room temperature for aging, after which the precipitate was separated by centrifugation, and subsequently washed with distilled water thrice. The resulting gel-like paste was dried at 60 ^{o}C for 24 h and then ground using agate mortar to obtain fine powder.

The same procedure was repeated using varying quantities of SA (5%wt, 20%wt, 33%wt, and 50%wt).

Preparation of hydroxyapatite-sodium alginate composite (2HASA) (second synthetic route)

To investigate the effect of order of polymer addition, the procedure in the preparation of HASA was repeated but with first adding SA to the phosphate solution before drop-wise addition to the calcium solution. The prepared phosphate solution (200 cm^3 of 0.06 M) was added in drop-wise manner to a 100 cm^3 separately prepared SA solution (1%wt) while stirring vigorously. The mixture was added drop by drop to 200 cm^3 aqueous solution of calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) (0.1 M) prepared earlier with continuous stirring for 24 h**.** The pH was maintained at approximately 10.5 throughout the experiment using 1M sodium hydroxide solution. The suspension was then stored for another 24h at room temperature for aging, after which the precipitate was separated by centrifugation, and subsequently washed with distilled water thrice. The resulting gel-like paste was dried at 60^{o}C for 24 h and then ground using agate mortar to obtain fine powders. The procedure was repeated using varying quantities of SA (5% wt, 20% wt, 33% wt, and 50% wt).

Drug loading and In-vitro drug release study

Drug loading was done according to the method by (Raj *et al.*, 2013). In-vitro drug release study was done in a freshly prepared synthetic body fluid (Kokubo *et al.*, 1990) at room temperature and pH 7. The procedure and calculations were as previously reported in our earlier work (Onoyima *et al.*, 2017).

RESULTS AND DISCUSSION

Comparison of Nanocomposites Prepared by the two synthetic routes

The two synthetic routes involved changing the order of addition of SA as described earlier. The first synthetic route was designated HASA, while the second synthetic route was designated 2HASA. Presented in Figure 1 are the FTIR spectra of composite prepared by the first synthetic route (HASA), and that of composite prepared by the second synthetic route (2HASA).

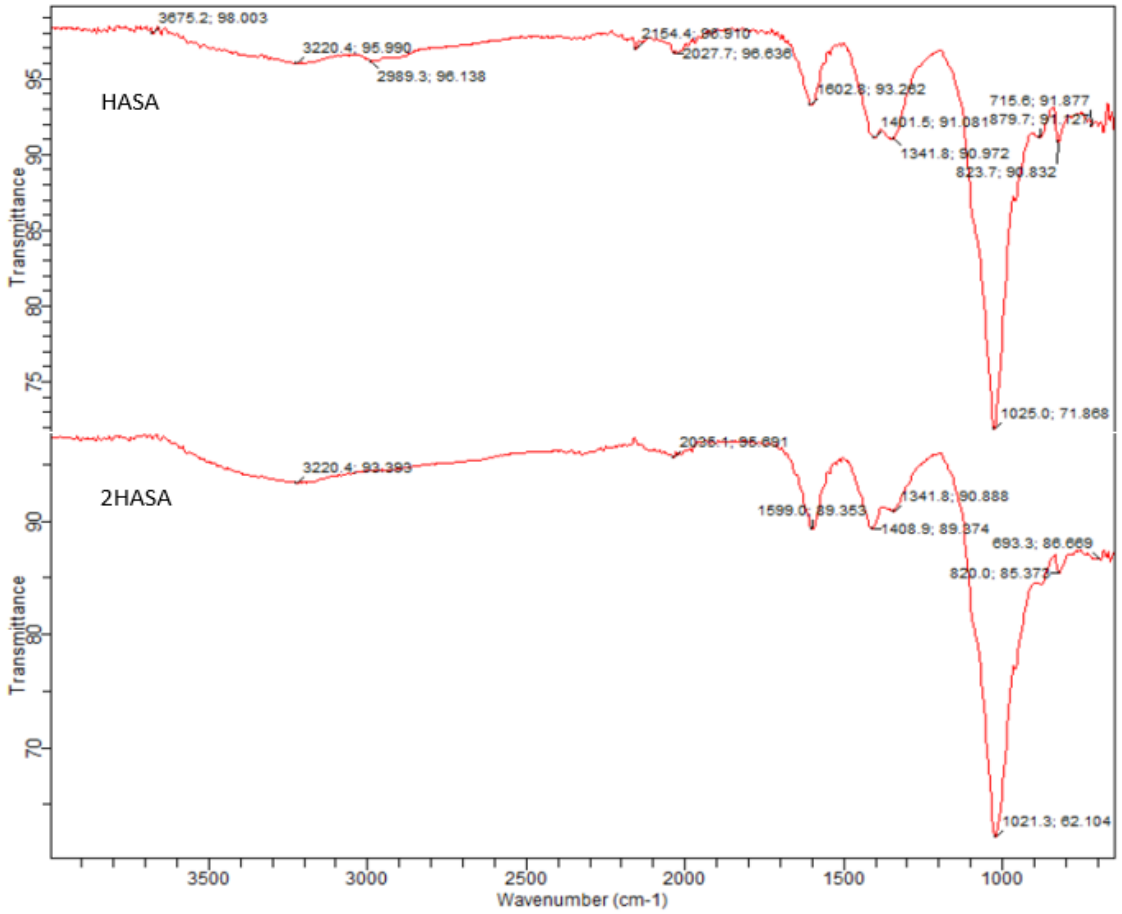

Figure 1: FTIR Spectra of HASA composite prepared by first synthetic route (HASA) and HASA composite prepared by the second synthetic route (2HASA)

From the FTIR spectra, it was observed that the major peaks, which includes the O-H stretching peak (3220.4 cm^{-3}), the C=O and C – O stretching peaks (1602.8 cm^{-1} and 1401.5 cm^{-1}), the O-Ca absorption peak (1341.8 cm^{-1}) (replacement of sodium ion in sodium alginate by a divalent metal (Azami *et al.*, 2010) and the peak due to PO_4^{3-} stretching (1025.0 cm^{-1}) were present in both formulations. This shows that the two synthetic routes yielded two nanocomposites with similar functional groups. In other words, the composites Synthesized from both routes can be said to be chemically the same.

The particle size and crystallite size of the composites prepared by the two routes are also shown in Figure 2. The crystallite size and particle size decreased with increase in the composition of SA for both synthetic routes. The addition of the polymer leads to inhibition on HA aggregation which leads to decrease in particles size. The decrease in the crystallite size of HA with addition of polymer has also been reported for HA/PVA (Rajkumar *et al.*, 2010).

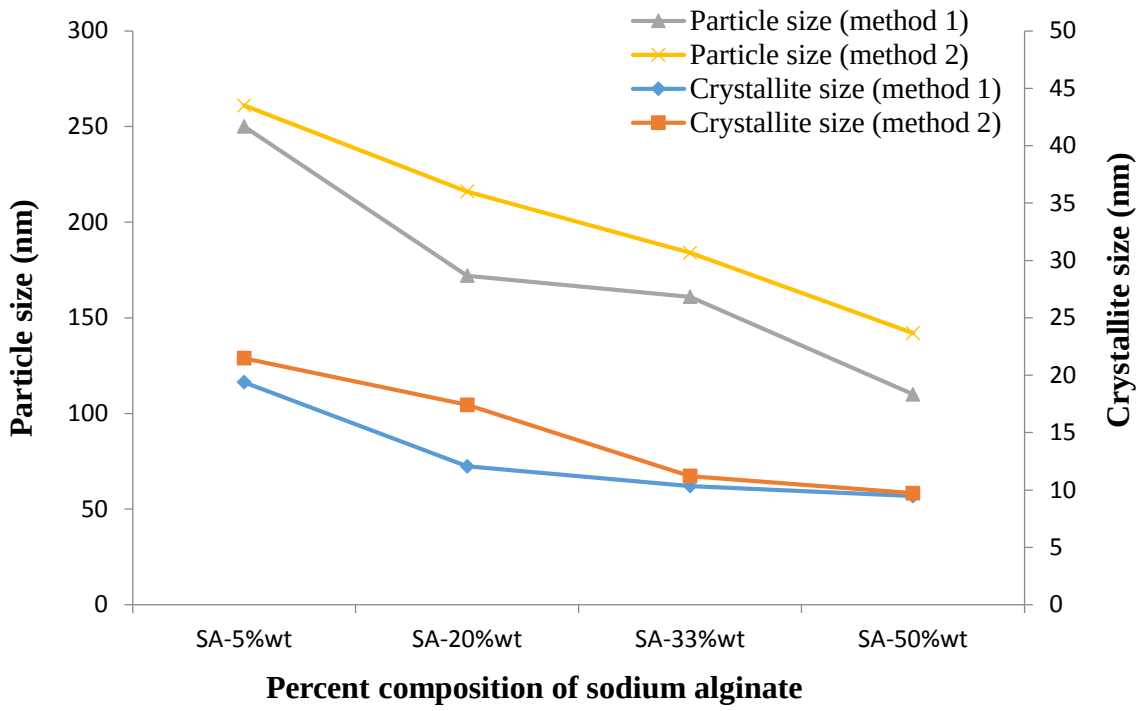

Figure 2: Comparison of the effect of synthetic route on particle size and crystallite size of Composites

The values ranged from 9.47 nm to 32.36 nm. There was also significant reduction in particle yield as particle size decreased**.** Materials with low crystallinity are preferred for biomaterial purposes due to their high in-vivo resorbability (Kucherov *et al.*, 2003)

Both crystallite size and particle size of 2HASA were consistently higher than those of HASA. However, the difference becomes less significant at higher sodium alginate (SA) composition (HASA-33%wt and HASA-50%wt). As was observed in HASA, morphological characterizations for all the formulations in 2HASA are similar. In addition, the variation in crystallinity and microstrain for both methods are similar. Microstrain was the dominant factor governing the solubility of carbonated apatite (Baig *et al*,. 1999). From these results, both methods yielded nanocomposites with similar chemical and physical characteristics except for slight variation in sizes.

The comparison of loading efficiencies for the two synthetic routes is shown in Figure 3 for DOX. For composites prepared with 5%wt of SA, the loading efficiency for the first synthetic route was 63.42%% while the second synthetic route was 69.39%; for composites prepared with 20%wt of SA, the loading efficiency for the first synthetic route was 74.92%, while the second synthetic route was 88.9%; with 33%wt of SA, the loading efficiency for the first synthetic route was 87.69%, while the second synthetic route was 92.63%, and with 50%wt of SA the first synthetic route was 92.03% while the second synthetic route was 94.74%.

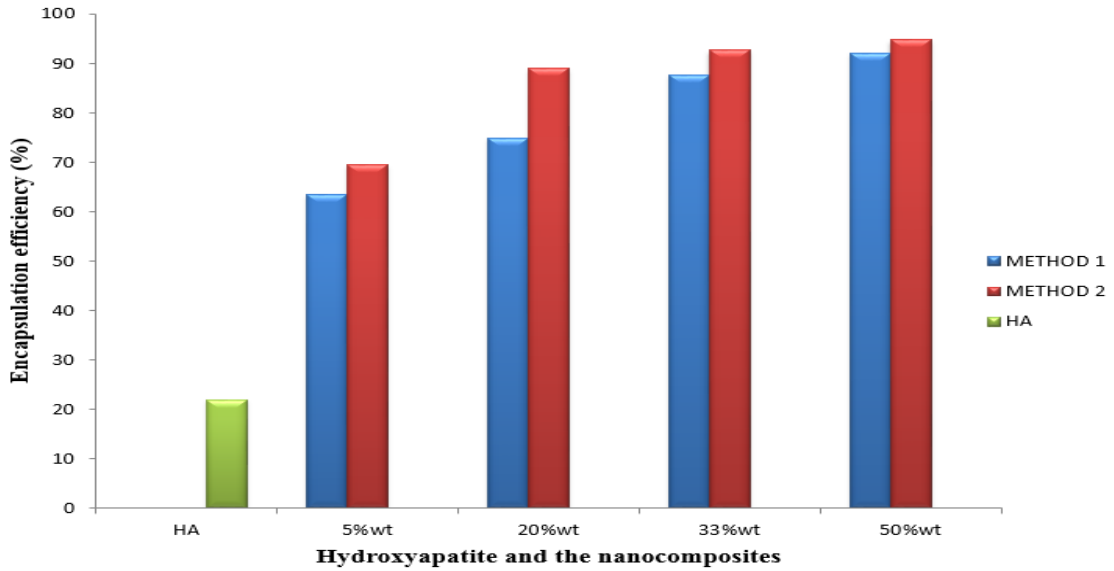

Figure 3: Comparison of loading efficiency of DOX in nanocomposites prepared by the two synthetic routes

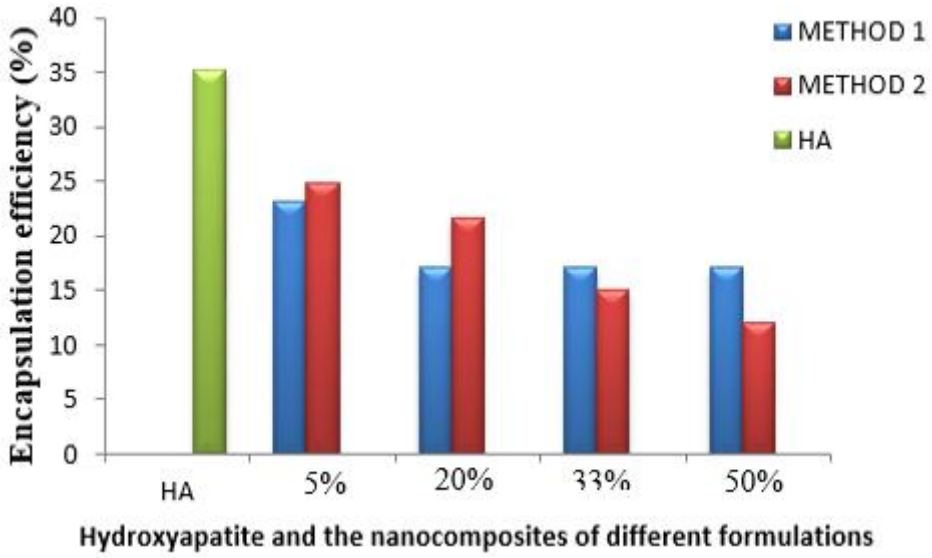

Figure 4: Comparison of loading efficiency of MTX in HA and the composites prepared by the two synthetic routes

For MTX (Figure 4), the loading efficiency for the first synthetic route using 5%wt SA was 23.15% while the second synthetic route was 24.89%; for composites prepared with 20%wt of SA, the loading efficiency for the first synthetic route was 17.09%, while the second synthetic route was 21.57%; with 33%wt of SA, the loading efficiency for the first synthetic route was 17.01%, while the second synthetic route was 15.02%, and with 50%wt of SA the

first synthetic route was 17.01% while the second synthetic route was 12.01%.

The second synthetic route (2HASA) presented higher DOX loading efficiency than the first synthetic route (HASA). However, for MTX (Figure 4) 2HASA recorded higher loading efficiencies at lower amount of SA (HASA-5%wt and HASA-20%wt), but as the quantity of SA was increased to HASA-33%wt and HASA-50%wt, HASA was found to load MTX more than 2HASA. DOX and MTX-loaded composites from both routes also displayed similar functional groups (Figure 5). This implies that both DOX and MTX interacted with the composites synthesized through both routes in similar manner

.

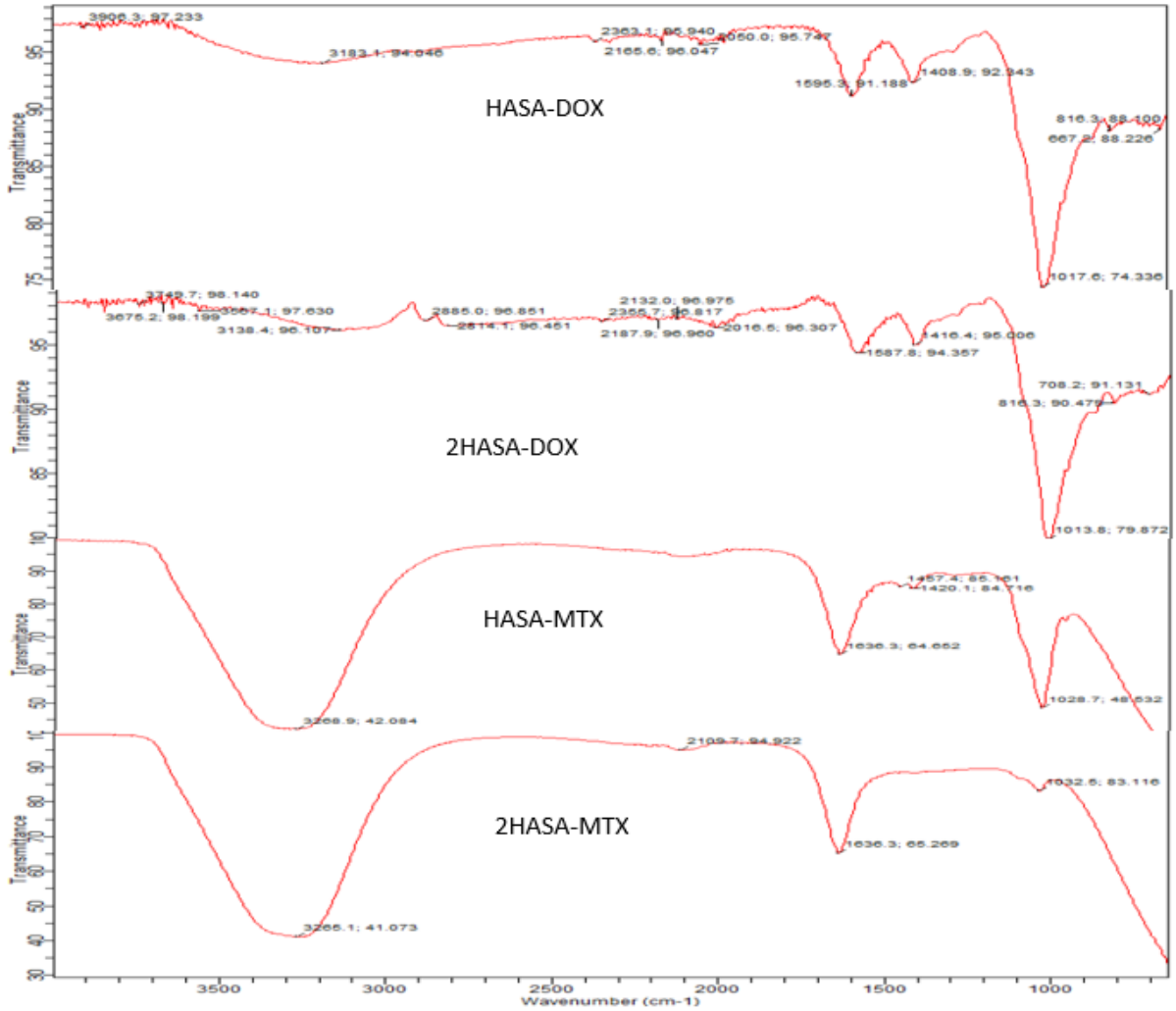

Figure 5: FTIR Spectra of DOX and MTX-loaded composites by the first synthetic route (HASA) and second synthetic routes (2HASA)

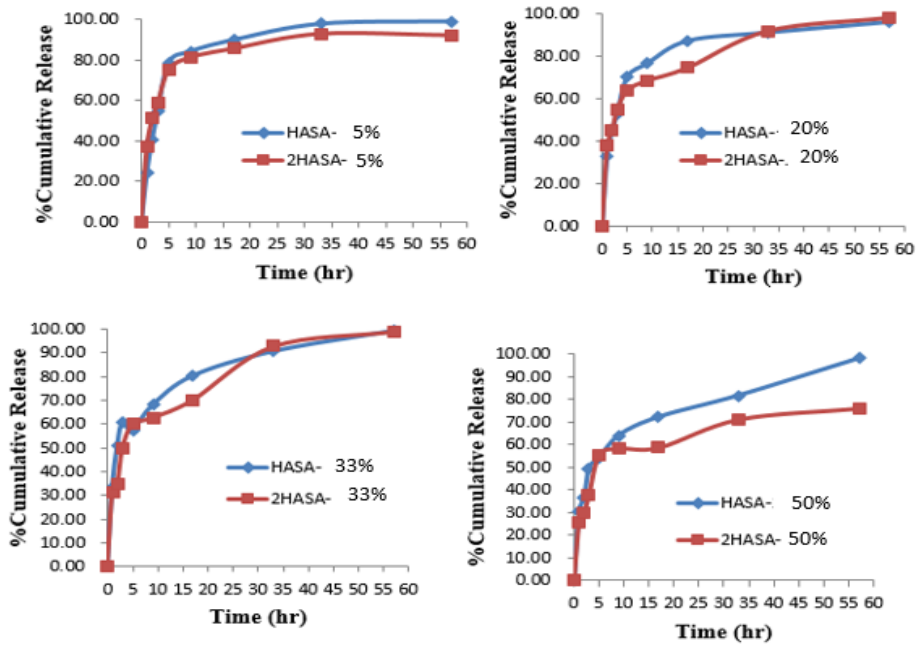

Figure 6: Comparison of the release profiles doxorubicin from hydroxyapatite-sodium alginate composites prepared from the two different methods.

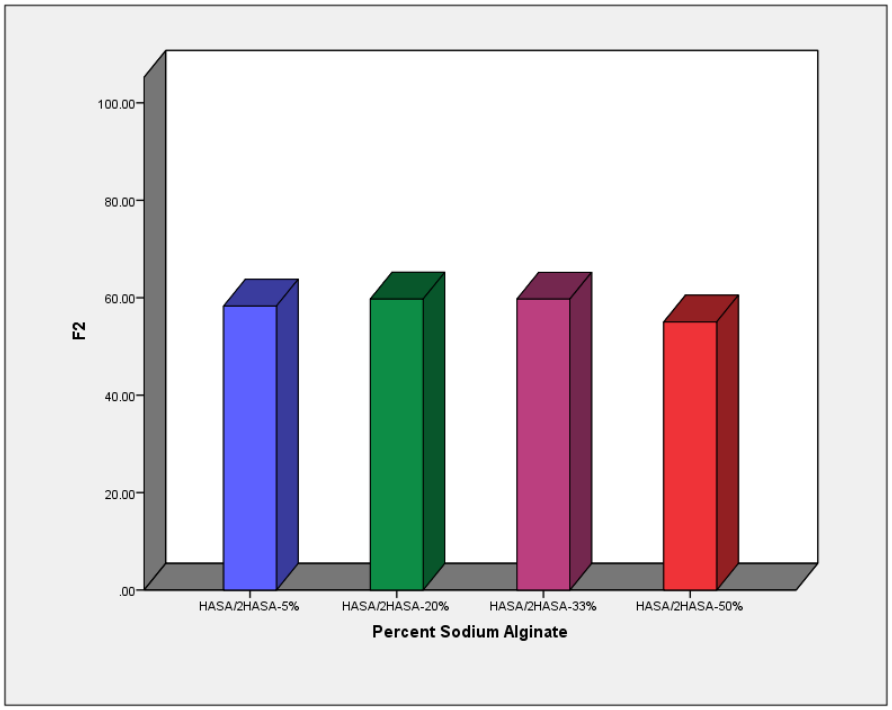

Figure 7: Comparison of DOX release profiles from composites prepared from first synthetic route (HASA) and second synthetic route (2HASA)

MTX has burst release from the composite, hence only DOX release profile was presented. DOX release profiles from composites synthesized from the first synthetic route (HASA) and second synthetic route (2HASA) are presented in Figure 6. The comparison was carried out for composites when the same quantities of precursors were used following the two different routes. Each pair of these profiles was subjected to similarity test (Zhang, *et al.*, 2010), in order to ascertain the statistical equivalence or otherwise of the profiles. The result presented in Figure 7 shows that the release profiles for all the compared pairs have similarity factor (f_2) above 50, indicating that DOX release from composites prepared following the two synthetic methods have similar release profiles. Two release profiles are similar if $50 \leq F_2 \leq 100$ (Zuo *et al.*, 2014). It has been reported that the similarity or otherwise of a HA-polymer composite depends of the percent weight of the polymer in the composite (Onoyima *et al.*, 2017).

CONCLUSION

Comparison of composites synthesized from both synthetic routes using FTIR shows that they are chemically the same, while method 1 had particles with larger sizes. The drug loading efficiency also showed that the second synthetic route (2HASA) loaded higher than the first synthetic route (HASA), while the release profiles for the two synthetic routes are similar.

REFERENCES

Allermann, E., Gury, R and Doelker, E. (1993). Drug-loaded nanoparticles-preparation methods and drugs targeting issues. *Europian Journal of Pharmacy and Biopharmacy*, 39, 173-191.

Azami, M., Samadikuchaksaraei, A and Poursamar, S.A. (2010). Synthesis and characterization of a laminated HA/gelatin nanocomposite scaffold with controlled pore structure for bone tissue engineering. *International Journal of Artificial Organ*, 33 (2): 86-95.

Baig, A.A., Fox, J.L., Young, R.A., Wang, Z., Hsu, J., Higuchi, W.I, Chnetty, A., Zhang, H and Otsuka, M. (1999). Relationship among carbonated apatite solubility, crystallite size, and microstain parameters. *Calif Tissue Internationals*, 64(5), 437 – 490.

Balakrishnan, B., Mohanty, M., Fernandez, A.C., Mohanan, P.V and Jayakrishnan, A. (2006). Evaluation of the effect of incorporation of dibutyryl cyclic adenosine monophosphate in an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials*, 27, 1355–1361.

Bidarra, S.J., Barrias, C.C., Barbosa, M.A., Soares, R and Granja, P.L. (2010). Immobilization of human mesenchymal stem cells within RGD-grafted alginate microspheres and assessment of their angiogenic potential. *Biomacromolecules*, 11, 1956–1964.

Chan, A.W and Neufeld, R.J. (2010). Tuneable semi-synthetic network alginate for absorptive encapsulation and controlled release of protein therapeutics. *Biomaterials*, 31, 9040–9047.

Devanand, V.G., Ramasamy, S., Ramakrishnan, V and Kumar, J. (2011). Hydroxyapatite – Alginate nanocomposite as drug delivery matrix for sustained release of ciprofloxacin. *Journal of Biomedical Nanotechnology*, 7, 1-9.

Kakde, D., Jain, D., Shrivastava, V., Kakde, R and Patil, A.T. (2011). Cancer Therapeutics- Opportunities, Challenges and Advances in Drug Delivery. *Journal of Applied Pharmaceutical Science,* 01(09), 01-10.

Khaled, R. M., Hanan, H. B and Zenab, M. E. (2014). In-vitro study of nano-hyroxyapatite/gelatine-chitosan nanocomposites for bio-application. *Journal of Advanced Research*, 5, 201 – 208.

Ku H., Wang, H., Pattarachaiyakoop, N., Trada, M. (2011). A review on the tensile properties of natural fiber reinforced polymer composites. Composites Part B: Engineering, 42 (4), pp 856 – 873.

Kucherov, A.V., Kramareva, N.V., Finashina, E.D., AKoklin, A.E and Kustov, L.M. (2003). Heterogenized redox catalysts on the basis of the chitosan matrix. Copper complexes *Journal of Molecular Catalalysis A*, 198, 377–89.

Lee, J and Lee K Y. (2009). Injectable microsphere/hydrogel combination systems for localized protein delivery. *Macromolecules and Bioscience*, 9, 671–676.

Marques, C., Ferreira, J. M., Andhenronescu, E., Ficai, D., Sonmez, M and Ficai, A. (2014). Multifunctional Material for bone cancer treatment. *International Journal of Nanomedicine*, 9, 2713 – 2725.

Murakami, K., Aoki, H., Nakamura, S., Nakamura, S., Takikawa, M., Hanzawa, M., Kishimoto, S, Hattori H, Tanaka Y, Kiyosawa T, Sato Yand Ishihara M. (2010). Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. *Biomaterials*, 31, 83–90.

Onoyima C. C, Okibe F. G, and Agbaji E. B. (2017). Preparation and in vitro evaluation of hydroxyapatite-sodium alginate nanocomposite for sustained delivery of doxorubicin. *African Journal of Pharmaceutical Research and Development*, 9(2), 154-16.

Priya, C., Rajkumari, and Ankita, K. (2013). Polymer: Boon to controlled drug delivery system. *International Research Journal of Pharmacy*, 4(4), 28 – 34.

Raj, M.S., Arkin, V.H., Adalarasu H and Jagannath, M. (2013). Nanocomposites Based on Polymer and HA for Drug Delivery Application. *Indian Journal of Science and Technology*, 6(5S), 4653 – 4658.

Rajkumar, M., Sundaram, N.M and Rajendran, V. (2010). In-situ preparation of HA nanorod embedded poly (vinyl alcohol) nanocomposite and its characterization. *International*

Journal of Engineering Science and Technology, 2(6), 2437 – 2444.

Silva, E.A., Kim, E.S., Kong, H.J and Mooney, D.J. (2008). Material-based deployment enhances efficacy of endothelial progenitor cells. Proc Natl Acad Sci USA, 105, 14347–14352.

Skaugrud, O., Hagen, A., Borgersen, B and Dornish, M. (1999). Biomedical and Pharmaceutical application of alginate and chitosan. *Biotechnology and Genetic Engineering Review*, 16, 23 – 40.

Thornton, A.J., Alsberg, E., Albertelli, M and Mooney, D.J. (2004). Shape-defining scaffolds for minimally invasive tissue engineering. *Transplantation*, 77, 1798–1803.

Venkatesan, P., Puvvada, N., Dash, R., Kumar, B.N., Devanand, S., Azab, B., Pathak, A., Kundu, S.C., Fisher, P.B and Mandal M. (2011). The potential of celecoxib-loaded HA-chitosan nanocomposite for the treatment of colon cancer. *Biomaterial*, 32, 3794 – 3806. doi:10.1016/j.biomaterials.2011.01.027

Wang, L., Shelton, R.M., Cooper, P.R., Lawson, M., Triffitt, J.T and Barralet, J.E. (2003). Evaluation of SA for bone marrow cell tissue engineering. *Biomaterials*, 24, 3475–3481

Wells, L.A and Sheardown, H. (2009). Extended release of high pI proteins from alginate microspheres via a novel encapsulation technique. *Europian Journal of Pharmacy and Biopharmacy*. 65, 329–335.

Zhang, Y., Huo, M., Zhou, J., Zou, Li.W. Yao, J and Xie, X. (2010). DDSolver: An-In Program for Modeling and Comparison of Drug Dissolution Profiles. *AmericanAssociation of Pharmaceutical Scientists Journal* **12**(3), 263 – 271. DOI: 10.1208/s12248-010-9185-1.

Zuo, J., GAO, Y., Bou-Chacra, N and Löbenberg, R. (2014). Evaluation of DDsolver Software application. *BioMed Research Internationl*, http://dx.doi.org/10.1155/2014/204925