



EFFECT OF SOLVENTS ON THE SYNTHESIS OF HYDROXYAPATITE SODIUM ALGINATE NANOCOMPOSITES FOR DOXORUBICIN DRUG LOADING AND DELIVERY

Onoyima C. C.,¹ Okibe F.G.^{2*} and Sholadoye, Q. O.¹

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

²Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

* Corresponding Author: fgokibe@gmail.com; fgokibe@abu.edu.ng +234-7039502969

ABSTRACT

In situ preparation of hydroxyapatite-sodium alginate nanocomposite (HASA) was carried out by the wet chemical precipitation method using water, ethanol, ethyl acetate and acetone as the dissolution medium. 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 °C. The FTIR results show similar functional groups for precursors and synthesized nanocomposites. The nanocomposite prepared in water medium has the highest drug encapsulation efficiency of 83.67 %. Doxorubicin release profile comparison using difference factor (f_1), showed that there is no significant difference between release profile of doxorubicin from nanocomposite synthesized in acetone and ethyl acetate ($f_1 = 3.92$), while other release profiles differed significantly. All the formulations follow first order release rate, while the n exponent in the Korsmeyer-Peppas equation indicates that the release mechanism is dominated by Fickian diffusion. The release profiles also indicate that nanocomposites synthesized from water medium is a better delivering agent for doxorubicin than the nanocomposites from the others solvents.

Keywords: nanocomposites, solvents, drug loading, hydroxyapatite, release profile

INTRODUCTION

Drug delivery system refers to the technology used to present the drug to the desired body site for release and absorption (Shargel *et al*, 2012). Because of their ability to overcome drawbacks of conventional dosage forms, these modified-release forms are increasingly replacing the conventional dosage forms (Dixit *et al*. 2013). Some categories of modified release delivery system are: sustained-release, delayed-release, and targeted-release drug delivery systems.

Targeted release drug delivery system is a system that selectively delivers drugs only to the required physiological sites, organs or cells, while reducing/avoiding delivery to the unwanted sites. Targeted delivery systems lead to improved therapeutic index and reduced side effects (Masayuk, 2005). Delayed release drug delivery system is a drug carrier that releases the drug at a time other than immediately after administration (Shargel *et al.*, 2012); while sustained release drug delivery system is a delivery system designed to achieve prolonged therapeutic effect by continuously releasing the therapeutic agent over an extended period of time after administration of single dose (Patnaik

et al, 2013). Sustained release drug delivery systems alter the pharmacodynamics and pharmacokinetics of the drug by modifying the rate at which the drug is being released into the system (Dixit *et al*, 2013). This leads to several advantages over conventional dosage forms, which include, improved patient compliance as a result of less frequent drug administration, reduction in fluctuation of plasma drug levels, maximal utilization of the drug, reduction in health care costs, and reduction in dose-dependent toxicity (Patnaik *et al*, 2013).

The aim of an ideal cancer chemotherapy is to deliver the correct amount of drug at controlled rate for sufficiently long time (sustained release) to the site of action (tumour cells) while minimizing contact with normal cells (targeted release). Conventional dosage forms result in fluctuation of drug concentration giving rise to peak and valley patterns. This leads to drug concentrations sharply rising above toxic level (maximum tolerated dose (MTD) or minimum toxic concentration (MTC) and within a short time falling below the minimum effective concentration (MEC). Figure 1 illustrates the comparison between the sustained drug release and conventional dosage forms.

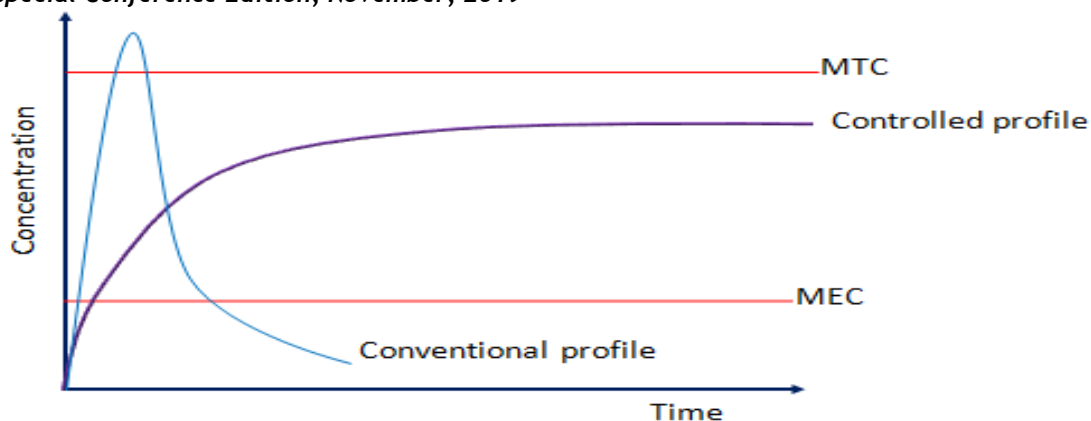


Figure 1: Schematic representation of conventional drug release profile and controlled release profile

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). Hydroxyapatite/polymer composites have attracted much attention since such composite 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 hydroxyapatite polymer composite in many areas such as in drug delivery system (Andronescu *et al.*, 2010; Venkatesan *et al.*, 2011; Sivakumar, and Raj *et al.*, 2013). Incorporation of hydroxyapatite into polymer structure has resulted in less burst release and more sustained release of the encapsulated drug (Devanand *et al.*, 2011; Krisanapiboon *et al.*, 2006; Cypes *et al.*, 2003; Yao *et al.*, 2010). Conditions used in the synthesis of drug carrier is an important factor in burst release.

Release of drug is governed by several factors such as nature and molecular weight of the drug, degree of cross linking, density, pore size of the matrix, solvent type etc. (Narayana *et al.*, 2011). Solvent plays an important role in a given chemical reaction. Solubility, equilibrium position and reaction rate are all affected by solvent. Solvent has direct effect on the particle size, surface morphology, drug encapsulation efficiency and release behaviour (Narayana *et al.*, 2011). Solvent system affected morphology and diameter of fibers, which directly affected ofloxacin loading and microbiological activity of the fiber (Karatas *et al.*, 2016). Morphology of core-shell nanoparticle depends on the nature of solvent chosen. (Joshi *et al.*, 2017). While solvents influenced morphological homogeneity and surface area of hollow nickel-silica composite (Umegaki *et al.*, 2014). Study by Mai *et al.* (2017), also showed that solvent has significant impact on the morphology of microcapsules.

Solvents affect morphology due to different ways solvents interact with the precursors (Khoza *et al.*, 2012). It is well known that the shape and size of the material strongly affect the properties and the applications of the material (Moloto *et al.*, 2009; Jun *et al.*, 2006; Tang *et al.*, 2000; Yang *et al.*, 2000). Klose *et al.* (2006) reported how porosity and size of the particles affect the drug release mechanisms from PLGA-based microparticles.

The presence of pores does not only increase the mobility of the drug molecules, but fundamentally alters the underlying drug release mechanisms. Generally, the release of encapsulated particles during the degradation process will be faster in the case of porous particles. Morphology influence the biodegradation kinetics of polymers which can dictate the release rate of the encapsulated drug (Badri *et al.*, 2014).

Solvent selection continues to be one of the most challenging issues for current synthetic chemistry since most of chemical or biochemical reactions on the earth occur in wet environment (Jiang *et al.*, 2014). The aim of this research is to evaluate the effect of using different solvents in the preparation of hydroxyapatite sodium alginate composite (HASA) on doxorubicin loading and release profiles. Based on toxicity profiles, water, ethanol, ethyl acetate and acetone were selected for this study.

MATERIALS AND METHODS

Preparation of HASA Nanocomposite in Different Solvents

Calcium nitrate tetrahydrate solutions (0.10M) (400 cm³) prepared in different solvents (water, ethanol, ethylacetate, and acetone) were separately added in drop-wise manner to different SA aqueous solutions (100 cm³) (50%wt) while stirring vigorously. The temperature of the one prepared in acetone solution was raised to above 37°C.

Special Conference Edition, November, 2019

This mixture was set on a magnetic stirrer, and 50 cm³ of 0.06 M diammonium hydrogen phosphate aqueous solution ((NH₄)₂HPO₄) was added to it dropwise 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 24 h at room temperature for aging, after which the precipitate was separated by centrifugation, and subsequently washed with distilled water three times. The resulting gel-like paste was dried at 60°C for 24 h and then ground using agate mortar to obtain fine powders. The nanocomposites were then used for drug loading and release study as previously reported (Onoyima *et al.* 2017).

Drug Release Kinetics and Mechanistic Study

In order to elucidate the release kinetics and the mechanism of drug release, the data was fitted into zero order model, first order model, Higuchi model, Korsmeyer-Peppas model, Hixon-Crowel model, and Hopfenberg model. This was done using a combination of DDSolver software and excel sheet.

Comparison of Drug Release Profiles

A release profile is a measurement of *in vitro* drug release from a preparation in receptacle media over a period of time. Similarities between the different release profiles were investigated using model-independent approach. Pairwise procedures were followed, while similarity factor (f_2) and the difference factor (f_1) were chosen for comparison (Zhang *et al.*, 2010)

$$f_1 = \left\{ \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} \right\} \times 100$$

$$f_2 = 50 \times \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

Where, n is the number of time points and R_t and T_t are the average percentage of drugs

released in reference and test products respectively at time t. The value of f_2 falls between 0 and 100, and two profiles are considered to be similar when f_2 ranges between 50 and 100 (Zuo *et al.*, 2014). f_1 is zero when the test and reference profiles are identical and increases proportionally with the dissimilarity between the two profiles. f_1 values above 15 are considered dissimilar (Moore and Flanner, 1996). In order to reduce calculation time and eliminate calculation errors, DDSolver program (excel plug-in program) was used for the calculations.

RESULTS AND DISCUSSION

Fourier-Transform Infrared Analyses of Precursors and the Synthesized Nanocomposites

Spectrum of SA (Figure 2) exhibited peaks which are in agreement with those reported in literature. The HA peak values (Figure 3) are similar to those reported for HA by Roul *et al.* (2013); Rajkumar *et al.* (2010); Nabipour *et al.* (2016). The formation of the nanocomposite was confirmed by the spectrum of HASA in Figure 4. It was observed that the peak at 3236.3 cm⁻¹ in alginate shifted to 3220.4 cm⁻¹, while the peaks at 1595.3 cm⁻¹ and 1405.2 cm⁻¹ shifted to 1599.0 cm⁻¹ and 1408.9 cm⁻¹ respectively. As divalent metal ions replace sodium ions in the SA, the charge density, the radius and atomic weight of the cation are different, creating a new environment around the carbonyl group, hence the observed peak shift (Azami *et al.*, 2010). This also leads to the formation of new peak at 1341.8 cm⁻¹. It has been documented that formation of chemical bond between carbonyl group and divalent metal leads to a new peak around 1340 cm⁻¹ (Kikuchi *et al.*, 2004; Itoh *et al.*, 2005; Azami *et al.*, 2010;).

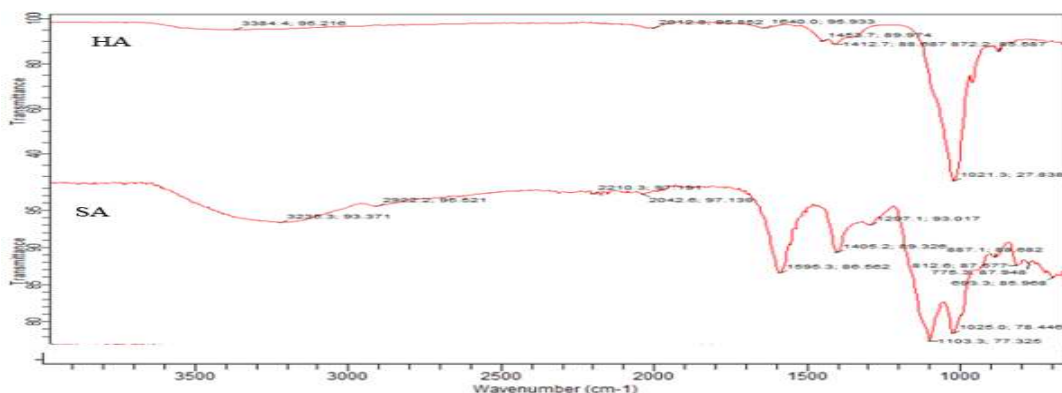


Figure 2: FTIR spectra of hydroxyapatite (HA) and sodium alginate (SA)

The FTIR study (Figure 3) indicated that replacement of water with organic solvent did not introduce additional functional group to the material as they had similar absorption peaks. That is to say that the differences in loading and release profiles was not due to presence/absence of chemical interaction but probably due to physical difference in microstructure of the nanocomposites.

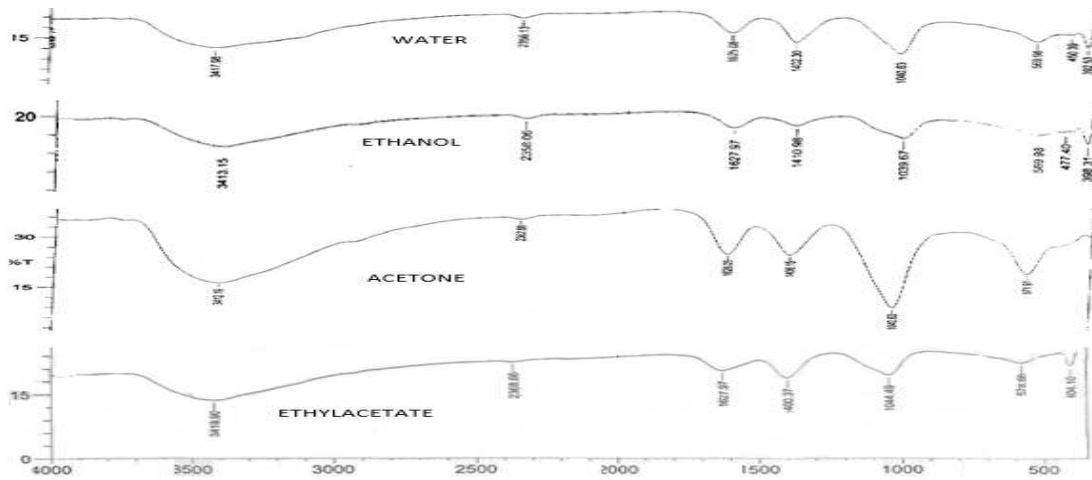


Figure 3: FTIR spectra of HASA synthesized under water, Ethanol, Acetone, and Ethyl acetate media

Effect of Medium of Synthesis on Drug Loading and Release

Nanocomposites prepared in different solvents were loaded with DOX to investigate the effect of these solvents on the loading and release of DOX. The result of the drug loading (Figure 4) showed that nanocomposites prepared using distilled water had highest DOX loading

efficiency of 83.67%. This was followed by nanocomposites prepared in ethanol medium with loading efficiency of 52.46%, while nanocomposites prepared in acetone medium and ethyl acetate medium had approximately equal loading efficiency of 47.35% and 46.50%, respectively.

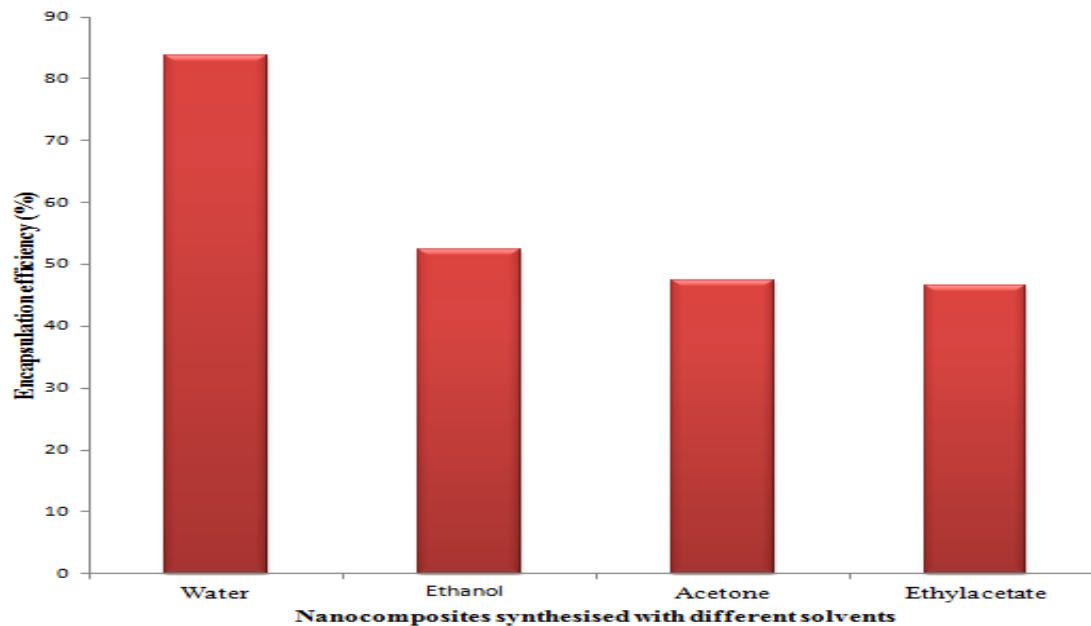


Figure 4: DOX encapsulation efficiency from nanocomposites prepared in different Solvents

From the drug release profile (Figure 5), it is interesting to note that nanocomposite prepared in aqueous medium which exhibited highest loading efficiency had slower release rate, hence more sustained release than other formulations. The release half time (t_{50}) (time required for releasing 50% of the loaded drug) for water formulation, ethanol formulation, acetone

formulation and ethyl acetate formulation were 16 hours, 8 hours, 4 hours and 4 hours respectively (Figure 4). Jalil, and Nixon (1990) has reported that increase in removal rate of organic solvents causes increase in porosity of the matrices, and consequently higher release rate.

Special Conference Edition, November, 2019

Hence, the higher release rate of the organic solvents formulations observed in this study may be attributed to higher porosity of the composites caused by higher removal rate of the organic solvents relative to water.

Profile comparison showed that water formulation and other organic solvent formulations are significantly different. The

difference factor (f_1) for water versus ethanol, water versus acetone, and water versus ethyl acetate are 23.96, 47.10, and 51.26 respectively. Among all the release profiles, only the profiles of acetone formulation and ethyl acetate formulation did not show significant difference ($f_1 = 3.92$) (Table 1)

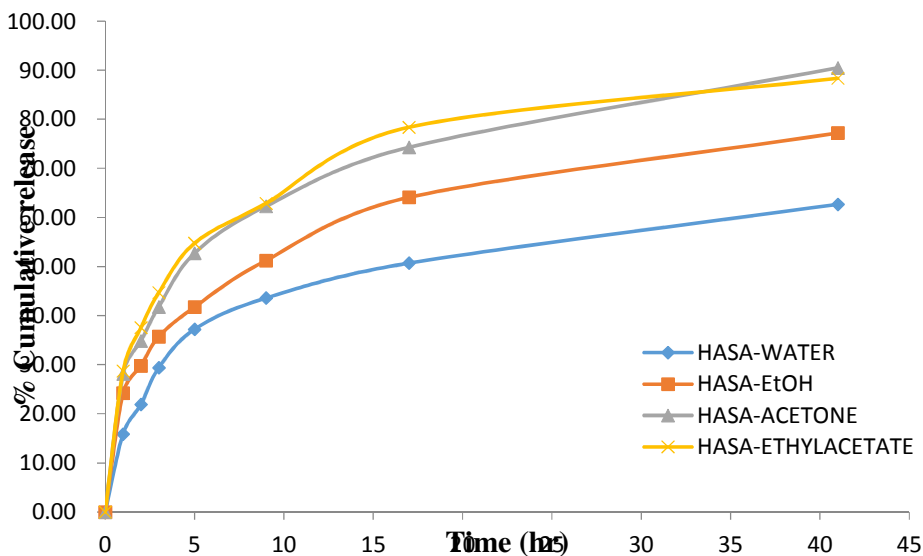


Figure 5: DOX release profiles from nanocomposites prepared in different media (solvents)

Table 1: Comparison of DOX release profiles from nanocomposites synthesised in different media (solvents) using difference factors (f_1)

	Water	Ethanol	Acetone	Ethylacetate
Water	0			
Ethanol	23.96	0		
Acetone	47.1	18.67	0	
Ethyl acetate	51.26	22.02	3.92	0

All the formulations followed first order release rate, while the n exponent in the Korsmeyer-Peppas equation indicates that the release mechanism was dominated by Fickian diffusion. In addition, the formulations with organic

solvents showed good fit with Higuchi model, Hixon-Crowel model, and Hopfenberg model, with Model Selective Criteria (MSC) above 2. (Table 2)

Table 2: Kinetic and mechanistic models of DOX release from HASA synthesized in different solvents

MODEL	PARAMETER	WATER	ACETONE	ETHANOL	ETHYLACETATE
Zero Order	R ²	0.7837	0.8244	0.8479	0.7729
	R ² -adj	0.7405	0.7892	0.8175	0.7275
	MSC	0.9597	1.168	1.312	0.911
	K ₀ (mol.L ⁻¹ s ⁻¹)	1.021	1.421	1.233	1.33
First Order	R ²	0.9297	0.9749	0.9501	0.9600
	R ² -adj	0.9157	0.9698	0.9401	0.9520
	MSC	2.083	3.112	2.426	2.646
	K ₁ (s ⁻¹)	0.224	0.0670	0.0370	0.0710
Korsmeyer-Peppas	R ²	0.9722	0.9916	0.9977	0.9864
	R ² -adj	0.9629	0.9888	0.9969	0.9818
	MSC	2.782	3.979	5.277	3.494
	N	0.4410	0.372	0.345	0.349
Higuchi	R ²	0.9222	0.9503	0.9638	0.9192
	R ² -adj	0.9066	0.9403	0.9566	0.9030
	MSC	1.982	2.428	2.748	1.944
	K _H	8.377	11.53	9.937	10.99
Hixon-Crowel	R ²	0.8314	0.9488	0.9196	0.9286
	R ² -adj	0.7977	0.9385	0.9036	0.9143
	MSC	1.208	2.399	1.949	2.067
	KHC	0.006	0.0160	0.0090	0.0180
Hopfenberg	R ²	0.8560	0.9747	0.9500	0.9599
	R ² -adj	0.7841	0.9621	0.9250	0.9398
	MSC	1.081	2.820	2.138	2.359
	N	648.9	399.4	928.2	1029

CONCLUSION

Based on the toxicity profiles of the solvents used and the loading and release study, it can be concluded that it is not necessary to use these organic solvents for the synthesis of the

nanocomposites. Nanocomposite prepared in aqueous medium had better loading and release profile, and water is non-toxic to the body system as compared to organic solvents.

REFERENCES

- Andronescu, E., Fikai, M., Voicu, G., Fikai, D., Maganu, M and Fikai, A. (2010). Synthesis and characterization of collagen/hydroxyapatite: magnetite composite material for bone cancer treatment. *Journal of Materials Science: Materials in Medicine*, 21(7), 2237-2242. doi:10.1007/s10856-010-4076-7
- 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.
- Badri, W., Eddabra, R., Fessi, H and Elaissari, A. (2014). Biodegradable Polymer Based Nanoparticles: Dermal and Transdermal Drug Delivery. *Journal of Colloid Science and Biotechnology*, 3, 141 – 149.
- Cypes, S.H., Saltzman, W.M and Giannelis, E.P. (2003). Organosilicate-polymer drug delivery systems: controlled release and enhanced mechanical properties. *Journal of Controlled Release*, 90, 163-169.
- 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.
- Dixit, N., Maaurya, S. D and Sagar, P.S. (2013). Sustained release drug delivery system. *Indian Journal of Research in Pharmacy and Biotechnology*, 1(3), 305 – 310.
- Huang, X and Brazel, C.S. (2001). On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *Journal of Controlled Release*, 73, 121–136.

Special Conference Edition, November, 2019

- Itoh, S., Kikuchi, M and Koyama, Y. (2005). Development of a novel biomaterial, HA/collagen (HAp/Col) nanocomposite for medical use. *Biomedical and Material Engineering*, 15, 29-41.
- Jalil, R and Nixon J.R. (1990). Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. *Journal of Microencapsulation*, 7, 297-325.
- Jiang, X., Wang, Y. and Li, M. (2014). Selecting water-alcohol mixed solvent for synthesis of polydopamine nano-spheres using solubility parameter. *Scientific Report*, 4, 6070; DOI:10.1038/srep06070
- Joshi D.P, Pant G, Arora N, and Nainwal S. (2017). Effect of solvents on morphology, magnetic and dielectric properties of (a-Fe₂O₃@SiO₂) coreshell nanoparticles. *Heliyon*, 3(2), 253.
- Jun, Y.W. Choi, J. S. and Cheon J. (2006). Shape control of semiconductor and metal oxide nanocrystals through nonhydrolytic colloidal routes. *Angewandte Chemie—International Edition*, 45(21), 3414-3439.
- Karatas A, Algan A.H, Pekel-Bayramgil N, Turhan F. and Altanlar N. (2016). Oflaxacin loaded electrospun fibers for ocular drug delivery: effect of formulation variables on fiber morphology and drug release. *Current Drug Delivery*, 13, 433 – 443.
- Khaled, R. M., Hanan, H. B and Zenab, M. E. (2014). In-vitro study of nano-hydroxyapatite/gelatin chitosan nanocomposites for bio-application. *Journal of Advanced Research*, 5, 201 – 208.
- Khoza P.B., Moloto, M.J and Sikhwivhilu L.M. (2012). The Effect of Solvents, Acetone, Water, and Ethanol, on the Morphological and Optical Properties of ZnO Nanoparticles Prepared by Microwave. *Journal of Nanotechnology*. doi:10.1155/2012/195106
- Kikuchi, M., Matsumoto, H.N., Yamada, T., Koyama, Y., Takakuda, K and Tanaka, J. (2004). Glutaraldehyde cross-linked HA/collagen self-organized nanocomposites. *Biomaterials*, 25, 63-9.
- Klose, D.; Siepmann, F.; Elkharraz, K.; Krenzlin, S.; Siepmann, J. (2006). How porosity and size affect the drug release mechanisms from PLGA-based microparticles. *International Journal of Pharmaceutics*, 314 (2), 198-206.
- Krisanapiboon, A., Buranapanitkit, B and Oungbho, K. (2006). Biocompatibility of HA nanocomposite as a local drug delivery system. *Journal of Orthopaedic Surgery*, 14(3), 315 – 318.
- Ma G. and Song C. (2007). PCL/Poloxamer 188 blend microsphere for paclitaxel delivery: influence of poloxamer 188 on morphology and drug release. *Journal of Applied Polymer science*, 104, 1895 – 1899.
- Mai Z. Chen, J., He T., Hu Y., Dong, X., Zhang, H., Huang, W., Ko F. and Zhou W. (2017). Electrospray biodegradable microcapsules loaded with curcumin for drug delivery systems with high bioactivity. *The Royal Society of Chemistry Advances*, 7, 1724-1734
- Masayuki Y. (2005). Drug targeting with nano-sized carrier systems. *Journal of Artificial Organs*, 8, 77-84.
- Moloto N, Moloto M.J, Coville N.J. and Sinha Ray S. (2009). Optical and structural characterization of nickel selenide nanoparticles synthesized by simple methods. *Journal of Crystal Growth*, 311(15), 3924-3932.
- Moore, J. W and Flanner, H. H. (1996). Mathematical comparison of dissolution profiles. *Pharmaceutical Technology*, 20(6), 64-74.
- Nabipour, Z., Nourbakhsh, M.S and Baniasadi, M. (2016). Synthesis, characterization and biocompatibility evaluation of HA-gelatin polyLactic acid ternary nanocomposite. *Nanomedicine Journal*, 3(2): 127-134. DOI:10.7508/nmj.2016.02.006
- Narayana K. L., Sonia A. and Dasan K. P. (2011). Morphology, in-vitro degradation and drug release profile of poly (ethylene-co-vinyl acetate)/microcrystalline cellulose acetate. *International Journal of Research in Ayurveda and Pharmacy*, 2(6), 1830 – 1833.
- 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.
- Patnaik, N.A., Nagarjuna, T and Thulasiramraju, T.V. (2013). Sustained release drug system: A modern formulation approach. *International Journal of Research in Pharmaceutical and Nano Sciences*, 2(5), 586 – 601.

Special Conference Edition, November, 2019

- 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.
- Roul, J., Mohaptra, R and Sahoo, S.K. (2013). Preparation, characterization and drug delivery behavior of novel biopolymer/HA nanocomposite beads. *Asian Journal of Biomedical and Pharmaceutical Science*, 3(24), 33 – 38.
- Shargel, L., Wu-Pong, S and Andrew, B. C. (2012). Applied Biopharmaceutics and Pharmacokinetics (Sixth Edition). Mc Graw Hill, USA.
- Sivakumar, M and Rao, K.P. (2002). Preparation, characterization and in vitro release of gentamicin from coralline HA–gelatin nanocomposite microspheres. *Biomaterials*, 23, 3175 – 3181.
- Tang, C. Fan S., De La Chapelle, M. L Dang, H. and Li P. (2000). Synthesis of gallium phosphide nanorods. *Advanced Materials*, 12(18), 1346–1348.
- Umegaki T., Watanuke Y., Xu Q, and Kajima Y. (2014). Effect of solvent on morphology of hollow nickel-silica composite sphere and their catalytic performance for hydrolytic dehydration of ammonia borane. *Journal of the Japan institute of Energy*, 93(8), 703 – 709.
- 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
- Yao B.L; Ni C.H; Xiong C, Zhu C.P and Hueng B. (2010). Hydrophobic modification of SA and its application in controlled drug release. *Bioprocess Biosystem Engineering*, 33, 457 – 463.
- Yang, J. Zeng, J. H., Yu, S. H. Yang, L., Zhou, G. E and Qian, Y. T. (2000). Formation process of CdS nanorods via solvothermal route. *Chemistry of Materials*, 12(11), 3259–3263
- Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, J and Xie, X. (2010). DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles. *American Association 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 International*, <http://dx.doi.org/10.1155/2014/204925>