# EFFECT OF LOADING METHODS ON RELEASE PROFILES OF METHOTREXATE AND DOXORUBICIN FROM ALGINATE-HYDROXYAPATITE COMPOSITES

Supported by tetfund TERTIARY EDUCATION TRUST FUND

**C. C. Onoyima[1], F. G. Okibe[2], I. B. Anweting[3] and J. N. Akoji[4]**
[1]Department of Chemistry, Nigeria Police Academy, Wudil, Nigeria
[2]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria
[3]Nigerian Institute of Leather and Science Technology, Zaria
[4]Department of Chemistry, Base University, Abuja
Corresponding author: krissonoss@yahoo.co.uk
**Received:**November 16, 2016 **Accepted:** March 02, 2017

**Abstract:** 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. Its success depends on the drug loading method employed. Four different methods (methods 1, 2, 3 and 4) of loading doxorubicin (DOX) and methotrexate (MTX) on hydroxyapatite-sodium alginate composite were investigated in this study. Doxorubicin was loaded well (above 80%) by all the four loading methods studied, while for methotrexate method 2 and 4 were better (39.98% and 37.10%, respectively) than method 1 and 3 (10.39% and 15.21%, respectively). Release study for doxorubicin, indicated that adsorption method (method 1) had faster release rate than other methods, and followed first order release rate with Fickian diffusion as the predominant release mechanism; while methods 2, 3 and 4 also followed first order release rate, but with a mixture of diffusion and degradation as the release mechanism. For methotrexate, only method 4 sustained the release of the drug for about 9 h, while other methods had high burst release effects, which provides insufficient release data for further kinetic study. These observations show that the success of a delivery system depends on the loading method.
**Keywords:** Drug loading, doxorubicin, methotrexate, composite, hydroxyapatite, sodium alginate

## Introduction

The ability of a drug delivery system to successfully load or encapsulate the chemotherapeutic agent indicates to a large extent its success as a delivery system. High drug loading capacity reduces the amount of matrix required for administration, and also reduces wastage of the chemotherapeutic agent (Bennet and Kim, 2014). Therefore, care must be taken to select the delivery system, loading method and conditions that ensure maximum loading capacity. There are basically two methods of drug loading – incorporation method and adsorption method.

In Incorporation method, drug is incorporated during the formation of nanoparticles/nanocomposites. Incorporation method has the advantage of higher loading capacity than the adsorption method (Ueda *et al*., 1998). The method also achieves better protection against environmental factors and more control over the encapsulation process (i.e. it is easier to reproduce) (Bennet and Kim, 2014). The major disadvantage, however, is that the preparation conditions for the nanocomposite can compromise the integrity of the drug, hence not suitable for labile drugs and proteins (Soppimatha *et al*., 2001).

Incorporation method is applied to both polymer-polymer composite and polymer-inorganic composite. In the case of polymer-inorganic composites, this method is mostly applied where the ex-situ preparation condition technique is used. This is probably due to incompatibility of the in situ preparation conditions with the drug. Raul *et al*., (2013) however incorporated ofloxacin during the in situ formation of hydroxyapatite in sodium alginate solution.

There are different ways of incorporating drugs into a nanocomposite. One of these is emulsion methods – double emulsion or single emulsion. Preparation of rimfapicin loaded chitosan-poly lactic was carried out by incorporation method following the water-in-oil-water (w/o/w) double emulsion technique (Rajan and Raj, 2013). w/o/w double emulsion was also used to synthesis 5-flurouracil-polylactic acid-montmorillonite nanocomposite (Seema and Datta, 2013). Double emulsion involves two steps; first formation of primary w/o emulsion and then the secondary w/o/w emulsion. The primary emulsion is stabilized by a hydrophobic surfactant while the secondary emulsion by a hydrophilic surfactant (Mora-Huertasa *et al*., 2010). Single emulsion has also been successfully used to encapsulate drugs into nanocomposites (Hua *et al*., 2010; Javid *et al*., 2014). In another approach known as solvent evaporation method, the drug solution is mixed with a solution containing the components and the solvent evaporated. This has been used to load/incorporate ofloxacin on gelatine/montmorillonite cloisite 30B nanocomposite (Sahoo *et al*., 2011); synthesize paclitaxel loaded gelatine/montmorillonite nanocomposite (Das *et al*., 2011); encapsulate curcumin onto chitosan-alginate/cloisite 30B nanocomposite (Malesu *et al*., 2011); load curcumin onto chitosan-poly vinyl alcohol/cloisite 30B nanocomposite (Parida *et al*., 2011); load curcumin onto starch-chitosan/montmorillonite nanocomposite (Mohanty *et al*., 2015).

Solvent evaporation method can leave free drug on the surface of the nanocomposite. In an approach to remove this shortcoming in a procedure for preparation of celecoxib-loaded hydroxyapatite-chitosan nanocomposite, celecoxib was first dissolved in ethanol and then added to the dispersion of hydroxyapatite in chitosan solution. The mixture was centrifuged and re-dispersed in ethanol to remove free celecoxib (Venkatesan *et al*., 2011).

A different approach was followed by Iliescu *et al*. (2011) in the preparation of carboplatin-mmt-alginate composite. The first step involved formation of carboplatin-mmt hybrid. This is followed by addition of carboplatin-mmt hybrid powder containing $CaCl_2$ to the alginate solution (ionotropic gelation) to form carboplatin-montmorillonite-alginate composite. Similar technique was applied in the preparation of irinotecan-montmorillonite-alginate composite (Iliescu *et al*., 2014).

Adsorption/absorption method involves adsorbing the drug on the nanocomposites/nanoparticles after the formation of the nanoparticles. The formed nanoparticles is stirred and incubated in a concentrated solution of the drug, after which the drug-bound nanoparticles is recovered and dried

(Soppimatha *et al*., 2001; Peer *et al.*, 2007; Sahoo and Nayak, 2013).

Various procedures have been reported in literature. In a study by Prasath *et al.* (2011), loading of amoxicillin into polycaprolactone/polyethylene-hydroxyapatite nanocomposite was carried out by incubating the nanocomposite in the antibiotic solution at 37$^{o}$C for 48 h, after which the drug-loaded nanocomposite was recovered and air-dried. Similar approach was followed by Zhao *et al*. (2007) to synthesize doxorubicin-chitosan-alginate multilayer microcapsules. The microcapsule-drug solution was incubated at 37$^{o}$C for 12 h, although the drying method was not reported. Adsorption of bovine serum albumin onto PHEMA-g-HAP was also carried out by shaking and incubating at 37$^{o}$C for 24 h (Bach *et al*., 2012).

Why some researchers omitted the incubation step (Liu *et al*., 2006; Chahatray *et al*., 2013; Sahoo and Nayak, 2013), others incubated the nanocomposite-drug solution at room temperature for varying periods of time (Sivakumar and Rao, 2002; Raj *et al*., 2013; Alimardan *et al*., 2014). Unlike other reported works, Sivakumar and Rao (2002) carried out adsorption of gentamicin onto coralline hydroxyapatite/gelatine nanocomposite in a phosphate buffer saline at pH 7.4. Other drying methods reported in literature include freeze-drying (Venkatasubbu *et al*., 2011) and vacuum oven drying (Alimardan *et al*., 2014).

As mentioned earlier, adsorption method of drug loading has lower drug loading capacity compared with incorporation method. Surface adsorption has been given as one of the major causes of high burst release in drug delivery systems (Huang and Brazel, 2001). However, the method also has several advantages. It is simple to use and does not affect the stability of the drug. Again, vital information can be obtained from the adsorption isotherm of the nanocomposite-drug delivery system which can be used to get the best possible formulation, the drug binding capacity onto the surface of nanocomposite, and the amount of drug adsorbed (Soppimatha *et al*., 2001).

Globally, cancer remains the second most common cause of death despite the advances in prevention, early detection and protocols of treatment (Marques *et al*., 2014). Chemotherapy is one of the most important treatments currently available among the various approaches. The aim of this research is to evaluate the effect of adsorption method and incorporation methods of loading methotrexate and doxorubicin on hydroxyapatite-sodium alginate composite on the drug encapsulation efficiency and release profiles.

## Materials and Methods

### *Materials*

Distilled water was used for the preparation of all the solutions used in this work. Sodium alginate (SA) was obtained from Fisher Scientific Company, USA, while the drugs DOX HCl and MTX were from Zuvius Life Science Ltd and Pharma Aid, respectively. Synthetic body fluidwas prepared following a method by Kokubo *et al.* (1990). All other reagents were of analytical grade and were used without further purification.

### *Preparation of drug solutions*

DOX: Lyophilized DOX HCl powder was used to prepare the solution. In order to prepare 2 mg/mL of the drug, 100 mg of the powder was completely dissolved in 50 mL beaker and quantitatively transferred to 50 mL volumetric flask and then made up to mark with distilled water. The solution was used immediately after preparation.

MTX: Each vial of MTX injection BP contains 50 mg/2 mL of MTX. To prepare 2 mg/mL, two vials containing a total of 100 mg of MTX were quantitatively transferred into a 50 mL volumetric flask, and made up to mark. The solution was used immediately after preparation.

### *Drug loading*

Method 1 is the adsorption method which involved adsorbing the drug in already prepared nanocomposites (Chandrasekar *et al*., 2013), while methods 2, 3, and 4 are different modifications of incorporation methods. In method 2 the dried hydroxyapatite was cross-linked with sodium alginate in the drug solution using calcium chloride solution; in method 3 the hydroxyapatite was first incubated in the drug solution before the cross-linking stage; while in method 4 the freshly prepared hydroxyapatite was cross-linked with sodium alginate in the drug solution.

The amount of drug loaded was determined by finding the difference in the concentrations in the aqueous solution before and after loading. The drug encapsulation efficiency (EE) was evaluated by measuring the absorbance of the supernatant using UV spectrophotometer. The wavelength of maximum absorbance ($\lambda_{max}$) for DOX (290 nm) and MTX (419 nm) were obtained by scanning a solution of the drugs with an Agilent 600 UV spectrophotometer within the wavelength range of 200 nm to 600 nm. A series of standards were prepared which were used to construct a calibration curve.

The EE of the nanocomposites were calculated according to the equations (Papadimitriou, *et al*., 2008):

$$E.E = \frac{W_t - W_f}{W_t}$$

**Where:** $W_t$ represents the total amount (mg) of the drug; $W_f$ is the amount (mg) of free drug in the supernatant. All measurements were performed in triplicate and the mean value reported.

### *Method 1*

This method is the traditional adsorption method. Drug loaded hydroxyapatite-sodium alginate nanocomposite was prepared by agitating ex-situ prepared hydroxyapatite-sodium alginate nanocomposite (100 mg) in the drug solution for 1 h. The nanocomposite/drug solution was then incubated for 12 h after which the mixture was centrifuged and the drug-loaded nanocomposite recovered for drug release study.

### *Method 2*

Hydroxyapatite (90 mg) was weighed into a 5 mL drug solution (2 mg/mL). Sodium alginate solution (3 mL) having concentration of 10 mg/ml was then added into the mixture. Solution (2 mL) of $CaCl_2$ (5.513 mg/mL) was then added in drops into the mixture with vigorous shaking. The shaking was continued for 1 h. The drug-nanocomposite solution was then incubated for about 12 h at room temperature, after which the drug loaded nanocomposite was recovered by centrifugation.

### *Method 3*

Hydroxyapatite (90 mg) was shaken in a 5 mL drug solution (2 mg/mL) for 1 hour and then incubated for another 6 hours at room temperature. 3 mL of sodium alginate (10 mg/mL) was then added with shaking followed by drop wise addition of 2 mL $CaCl_2.2H_2O$ (5.513 mg/mL). The shaking was continued for another 1 h. The mixture was then incubated at room temperature for 6 h and the drug-nanocomposite recovered by centrifugation.

### *Method 4*

Hydroxyapatite was prepared following the method by Chandrasekar *et al*. (2013). The freshly prepared HA was re-dispersed in a 5 mL drug solution. 3 mL of sodium alginate solution (10 mg/mL) was then added while stirring, followed by addition of 2 mL of $CaCl_2.2H_2O$ (5.513 mg/mL in drops. The mixture was shaken for 1 h and allowed to stand for 12 h. The drug-loaded nanocomposite was recovered by centrifugation.

***In-vitro drug release study***

The *in vitro* drug release study was carried out following a method reported by Sivakumar and Rao, (2002). In order to determine the drug release profile, 100 mg each of the drug loaded nanocomposite and drug loaded hydroxyapatite were introduced into a screw capped glass bottle containing 50 mL of synthetic body fluid (SBF) medium at 37°C and pH 7.4 under sterile conditions. Aliquots of 5 mL samples were withdrawn by a pipette at regular intervals and replaced immediately with 5 ml of fresh SBF medium (this was accounted for when calculating the amount released). Drug concentrations in the collected samples were measured using UV-VIS Spectrophotometer based on pre-designed standard curve.

***Drug release kinetics and mechanistic study***

In order to elucidate the release kinetics and the mechanism of drug release, the release experimental data was fitted into the following:

- Zero order model (Dash *et al*., 2010)

  $Q_t = Q_0 + K_0t$

  **Where:** $Q_t$ is the quantity released at time t, $Q_0$ is the initial quantity of drug and $K_0$ is the zero order release constant.
- First order model(Gibaldi and Feldman (1967) as reported by Chime *et al*. (2013)

  $\text{Log } C = \text{Log}C_0 - \frac{Kt}{2.303}$

  **Where:** C is the concentration at time t, and $C_0$ is the initial concentration, and K is the first order constant.
- Higuchi model (Higuchi, 1961)

  $Q = A\sqrt{D(2C - C_s)C_s}t$

  **Where:** Q is the amount of drug released in time t, A is the area of the matrix, D is the diffusivity of the drug (diffusion coefficient), $C_s$ is the drug solubility in the matrix media.
- Korsmeyer-Peppas model (Peppas and Korsmeyer, 1986)

  $\frac{M_t}{M_\infty} = Kt^n$

  **Where:** $M_t$ is the amount of drug released at time t, and $M_\infty$ is the amount of drug loaded. The value of the exponent n is used to indicate the type of release mechanism, where K is a constant which depends on diffusion coefficient and thickness of the film.
- Hixson-Crowell model (Dash *et al*., 2010)

  $W_t^{\frac{1}{3}} = W_0^{\frac{1}{3}} - Kt$

**Where:** $W_t$ is the weight (mg) of the drug released at time t, $W_0$ is the initial amount (mg) in the release material, and K is a constant.

- Hopfenberg model (Shaikh *et al*., 2015)

  $\frac{M_t}{M_\infty} = 1 - [1 - \frac{K_0 t}{C_0 a_0}]^n$

  **Where:** $M_t$ is the amount of drug released at time t, $M_\infty$ is the amount of drug loaded, $K_0$ is the erosion rate constant, $C_0$ is the initial concentration of the drug in the matrix, $a_0$ is the initial radius of the particle and n denotes the geometry

These were done using a combination of DDSolver software and excel sheet.

**Results and Discussion**

***Doxorubicin loading and release profiles***

The result of doxorubicin loading by the four different loading methods (Fig. 1) showed that the loading efficiencies for method 1, method 2, method 3 and method 4 were 88.11%, 87.47%, 89.64%, and 88.64%, respectively. This result implied that there was high doxorubicin loading efficiency for all the four different methods, and there was no significant difference in loading efficiency of doxorubicin by the four methods compared.

The release profiles for the four different loading methods (Fig. 2) show that after fifty seven h (57 h), the percent cumulative releases for different methods were as follows: Method 1 - 94.72%, Method 2 - 91.54%, Method 3 - 85.87% and Method 4 - 80.84%. That is to say that all the four different loading methods prolonged the release of doxorubicin for fifty seven h. To compare the release rate, the percent release half time ($t_{50}$) was used. It was observed that the $t_{50}$ values for method 1, method 2, method 3 and method 4 are 4, 18, 10 and 20 h, respectively (Fig. 2). This result indicated that method 1 released doxorubicin at a faster rate than other methods. The higher release rate observed in adsorption is because in adsorption, some drug molecules get trapped on the surface of the delivery system and are released immediately upon contact with the release medium (Huang and Brazel, 2001). Method 1 also followed first order release rate as indicated in Table 1, while the release mechanism from the Korsmeyer-Peppas exponent n is by Fickian diffusion. Drug release from systems with n < 0.45 is due to diffusion through matrix and water filled pores (Shende and Marathe, 2015).

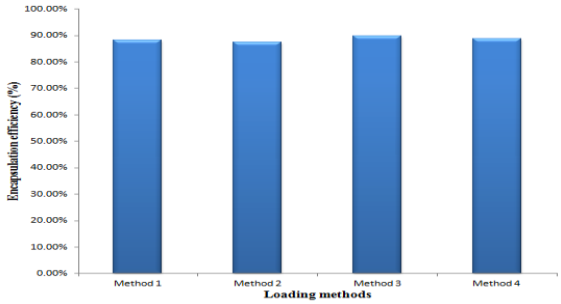


**Fig. 1:** Doxorubicin encapsulation efficiency from different loading methods

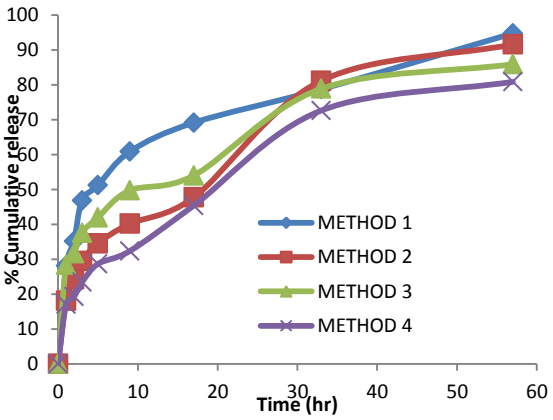


**Fig 2**: Release profiles of doxorubicin loaded into hydroxyapatite-sodium alginate nanocomposites by different loading methods

The release profiles for the incorporation methods (method 2, method 3, and method 4) display sigmoid biphasic phases. The first phase of these profiles indicates drug release due to diffusion. However, after about seventeen hours, there was rapid increase in drug release. This point indicates degradation of sodium alginate matrix leading to rapid release of the entrapped drug. This observation is corroborated by the high fit of these methods with Hopfenberg model (Table 1), which further shows that drug release was due to polymer degradation (Costaand Sousa-Lobo, 2001).

**Table 1: Kinetic and mechanistic models of doxorubicin release from Doxorubicin-loaded hydroxyapatite-sodium alginate nanocomposites prepared by different loading methods**

| Model | Parameter | Method 1 | Method 2 | Method 3 | Method 4 |
|---|---|---|---|---|---|
| Zero Order | $R^2$ | 0.818 | 0.934 | 0.903 | 0.933 |
| | $R^2$-adj. | 0.788 | 0.923 | 0.887 | 0.922 |
| | MSC | 1.203 | 2.211 | 1.831 | 2.199 |
| | $K_0(mol.L^{-1}s^{-1})$ | 1.124 | 1.308 | 1.027 | 1.195 |
| First Order | $R^2$ | 0.948 | 0.974 | 0.985 | 0.987 |
| | $R^2$-adj. | 0.939 | 0.970 | 0.983 | 0.985 |
| | MSC | 2.447 | 3.158 | 3.717 | 3.830 |
| | $K_1(s^{-1})$ | 0.065 | 0.037 | 0.034 | 0.029 |
| Korsmeyer-Peppas | $R^2$ | 0.895 | 0.983 | 0.988 | 0.976 |
| | $R^2$-adj. | 0.861 | 0.977 | 0.983 | 0.968 |
| | MSC | 1.458 | 3.277 | 3.583 | 2.933 |
| | N | 0.367 | 0.345 | 0.267 | 0.312 |
| Higuchi | $R^2$ | 0.931 | 0.975 | 0.982 | 0.978 |
| | $R^2$-adj. | 0.919 | 0.970 | 0.979 | 0.974 |
| | MSC | 2.169 | 3.170 | 3.522 | 3.300 |
| | $K_H$ | 10.266 | 11.444 | 9.172 | 10.477 |
| Hixon-Crowel | $R^2$ | 0.938 | 0.978 | 0.971 | 0.981 |
| | $R^2$-adj. | 0.928 | 0.974 | 0.966 | 0.978 |
| | MSC | 2.286 | 3.331 | 3.057 | 3.460 |
| | $K_{HC}$ | 0.014 | 0.009 | 0.008 | 0.008 |
| Hopfenberg | $R^2$ | 0.947 | 0.978 | 0.985 | 0.986 |
| | $R^2$-adj. | 0.926 | 0.969 | 0.979 | 0.981 |
| | MSC | 2.196 | 3.082 | 3.463 | 3.578 |
| | N | 568.170 | 3.195 | 524.384 | 226.062 |

**Note:** Kinetic study was not possible for methotrexate because of insufficient release data

***Methotrexate loading and release profiles***

The methotrexate loading efficiency by the four different loading methods (Fig. 3) shows that method 2 and method 4 recorded higher loading efficiency of 39.98% and 37.10%, respectively, than method 1 (10.39%) and method 3 (15.21%). The two methods that loaded higher amount of methotrexate are incorporation methods. Adsorption method did not load appreciable amount of methotrexate in the nanocomposite.

The release profiles of methotrexate-loaded nanocomposites prepared by the four different methods (Fig. 4) showed that, adsorption method (method 1) did not sustain the release of methotrexate. This was similar to observation by Huang and Brazel, (2001) for adsorption method, where surface adsorption was given as the reason for the high burst release. The release profile of method 3 is similar to method 1 as the two did not sustain the release of methotrexate even for 1 h. However, with method 2 (incorporation method), methotrexate release was sustained for three hours, while method 4 which has the best release profile, sustained the release of methotrexate for nine hours. As was observed by Ueda *et al*. (1998) drug particles are entrapped into the inner part of the carrier which leads to longer release time.

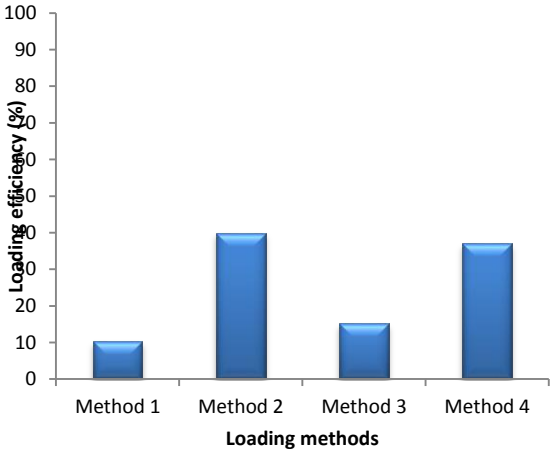


**Fig. 3**: Loading efficiency of methotrexate encapsulated using different methods

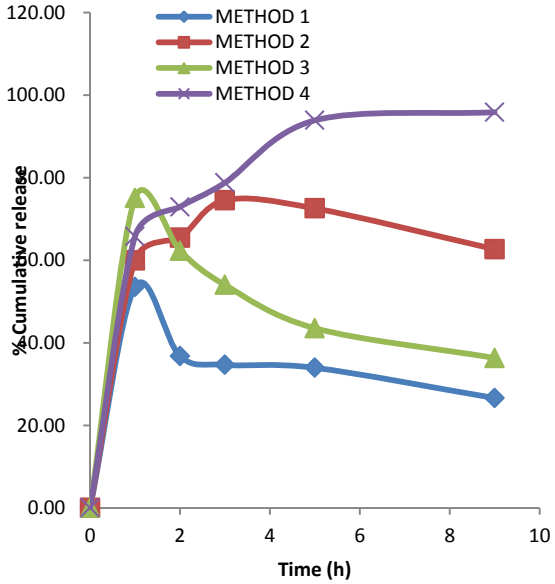


**Fig. 4:** Release profiles of methotrexate loaded into hydroxyapatite-sodium alginate nanocomposites by different loading methods

**Conclusion**

Both adsorption method and the incorporation methods achieved high loading efficiency for doxorubicin, with adsorption method showing higher release rate than other methods. Doxorubicin loaded by the adsorption method was released by diffusion mechanism following first order release rate, while other methods followed mixed mechanism of diffusion/degradation. For methotrexate, only method 4 was able to sustain the release of the drug for about 9 hours, while other methods showed high burst release. This study has shown that the efficiency of a material to load and release a drug depends, to a very large extent, on the loading method applied.

**References**


Alimardan Z, Darabi M &Natori R 2014. Drug delivery of indomethacin in use nature starch based nanocomposite hydrogel in vitro. *Int. J. Biosci.*, 5(11): 51-56. http://dx.doi.org/10.12692/ijb/5.11.51-56.

Bach LG,Islam MR,Mai TB, Nga TT,Niranjanmurthi L &Lim KT2012. *Synthesis, characterization, and protein immobilization study of phema grafted hydroxyapatite nanocrystals via surface initiated radical polymerization*. Paper Presented at the 15th European Conference on Composite Materials, Venice, Italy, 24-28 June, 2012.

Bennet D &Kim S 2014. Polymer nanoparticles for smart drug delivery. InTech, 257 - 310 http://dx.doi.org/10.5772/58422

Chahatray R, Sahoo D, Mohanty DP &Nayak PL 2013. Guargum-Sodium Alginate Blended with Cloisite 30B for Controlled Release of Anticancer Drug Curcumin. *World J. Nano Sci.& Techn.*, 2(1): 26-32.

Chandrasekar A, Sagadevan S &Dakshnamoorthy A 2013. Synthesis and characterization of nano-hydroxyapatite (n-HAP) using the wet chemical technique. *J. Physical Sci.*, 8(32): 1639 – 1645.

Chime SA, Onunkwo GC &Onyishi I 2013. Kinetics and mechanisms of drug release from swellable and non swellable matrices: A review. *Res. J. Pharmac., Bio.&Chem. Sci.*, 4(2): 97 – 103.

Costa P &Sousa-Lobo JM 2001. Modeling and comparison of dissolution profiles. *Eur. J. Pharmac. Sci.*, 13: 123–133.

Das PR, Nanda RM, Behara A &Nayak PL 2011. Gelatin blended with nanoparticles cloisite30B (MMT) for control drug delivery of anticancer drug paclitaxel. *Int. Res. J. Biochem.&Bioinformatics*, 1(2): 035-042.

Dash S, Murthy PN, Nath L &Chowdhury P 2010. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmac. – Drug Res.*, 67(3): 217 – 223.

Higuchi T1961. Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharmac. Sci.*, 50: 847-875.

Hua S, Yang H &Wang A 2010. A pH-sensitive nanocomposite microsphere based on chitosan and montmorillonite with in vitro reduction of the burst release effect. *Drug Devt.&Indu. Pharmacy*, 36(9): 1106–1114.

Huang X&Brazel CS 2001. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *J. Controlled Release*, 73: 121–136.

Iliescu RI, Andronescu E, Ghiţulică DC, Berger D &Ficai A 2011. Montmorillonite-alginate nanocomposite beads as drug carrier for oral administration of carboplatin – preparation and characterization. *U.P.B Sci. Bull. Series B*, 73(3): 3 – 16.

Iliescu RI, Andronescua E, Ghitulicaa CD, Voicua G, Ficai A &Hoteteu M 2014. Montmorillonite–alginate nanocomposite as a drug delivery system – incorporation and in vitro release of irinotecan. *Int. J. Pharmac.*, 463: 184– 192.

Javid A, Ahmadian S, Saboury AA, Kalantarc SM &Rezaei-Zarchid S 2014. Novel biodegradable heparin-coated nanocomposite system for targeted drug delivery. *Royal Soc. Chem. Advan.*, 4: 13719 – 13728.

Kokubo T, Kushitani H, Sakka S, Kitsugi T &Yamamuro T1990. Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W. *J. Biomed. Materials Res.*, 24:721–734.

Liu T, Chen S, Li J &Liu D 2006. Study on drug release behaviour of CDHA/chitosan nanocomposites: Effect of CDHA nanoparticles. *J. Controlled Release*, 112: 88–95.

Malesu VK, Sahoo D &Nayak PL 2011. chitosan–sodium alginate nanocomposites blended with cloisite 30b as a novel drug delivery system for anticancer drug curcumin.*Int. J. Appl. Bio.& Pharmac. Techn.*, 2(3): 402 – 411.

Marques C, Ferreira JM, Andhenronescu E, Ficai D, Sonmez M &Ficai A2014. Multifunctional Material for bone cancer treatment.*International Journal of Nanomedicine*, 9, 2713 – 2725.

Mohanty DP, Biswal SK &Nayak L2015. Preparation of starch-chitosan nanocomposites for control drug release of curcumin. *Int. J. Current Engr.&Techn.*, 5(1): 336 – 343.

Mora-Huertasa CE, Fessi H &Elaissari A 2010. Polymer-based nanocapsules for drug delivery. *Int. J. Pharmac.*, 385: 113 – 142.

Papadimitriou S, Bikiaris D, Avgoustakis K, Karavas E &Georgarakis M2008. Chitosan nanoparticles loaded with dorzolamide and pramipexole. *Carbohydrate Polymers*, 73: 44–54.

Parida UK, Nayak AK, Binhani BK &Nayak PL 2011. Synthesis and characterization of chitosan-polyvinyl alcohol blended with cloisite 30B for controlled release of the anticancer drug curcumin. *J. Biomate &Nanobiotech.*, 2: 414-425.

Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R &Langer R 2007. Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotech.*, 2: 751 – 760.

Peppas NA &Korsmeyer RW 1986. Dynamically swelling hydro gels in controlled release applications. In: N.A. Peppas (Ed.), Hydrogels in Medicine and Pharmacy, Vol. 3, CRC Press, Boca Raton, pp. 109–136.

PrasathPV, Senthil K &Ravichandran K 2011. Preparation and cytotoxicity study of polycaprolactone/polyethyleneglycol-hydroxyapatite nanocomposite for biomedical application.*Int. J. Innov. Res. Sci. & Engr.*

Raj MS, Arkin VH, Adalarasu &Jagannath M 2013. Nanocomposites based on polymer and hydroxyapatite for drug delivery application. *Indian J. Sci.&Techn.*, 6(5S): 4653 – 4658.

Rajan M &Raj V 2013. Formation and characterization of chitosan-polylacticacid-polyethylene glycol-gelatin nanoparticles:A novel biosystem for controlled drug delivery. *Carbohydrate Polymers*, 98: 951– 958.

Raul J, Mohaptra R &Sahoo SK 2013.Preparation, characterization and drug delivery behavior of novel biopolymer/hydroxyapatite nanocomposite beads. *Asian J. Biomed. Pharmac. Sci.*, 3(24): 33 – 38.

Sahoo S, Behera A, Nanda RM, Sahoo R &Nayak PL 2011. Gelatin blended with Cloisite 30B (MMT) for control release of Ofloxacin.*Amer. J. Scientific & Indu. Res.*, 2(3): 363-368.

Sahoo S &Nayak PL 2013. Synthesis and characterization of gelatin-chitosan blended with cloisite 30B nanocomposite for controlled release of the ophthalmic drug ketorolac. *J. Pharmac.& Biomed. Sci*., 30(30): 892-901.

Seema R &Datta M 2013. MMT-PLGA Nanocomposites as an oral and controlled release carrier for 5-Fluorouracil: A novel approach. *Int. J. Pharmacy & Pharmac. Sci.*, 5(2): 332 – 341.

Shaikh KH, Kshirsagar RV &Patil SG 2015. Mathematical models for drug release characterization: A review. *World J. Pharmacy &Pharmac. Sci.*, 4(4): 324 – 338.

Shende MA &Marathe RP2015.Formulation and development of baclofen gastro retentive mucoadhesive tablets using hibiscus esculentus and xanthan gum.*Indo Amer. J. Pharmac. Res.*, 5(9): 2925 – 2938.

Soppimatha KS, Aminabhavia TM, Kulkarnia AR &Rudzinski WE2001. Biodegradable polymeric nanoparticles as drug delivery devices. *J. Controlled Release*, 70: 1–20.

Sivakumar M &Rao KP 2002. Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite–gelatin composite microspheres. *Biomaterials*, 23: 3175 – 3181.

Ueda M, Iwara A &Kreuter J 1998. Influence of the preparation methods on the drying release behavior of loperamide-loaded nanoparticles. *Journal of Microencapsulation*, 15: 361–372.

Venkatasubbu GD, Ramasamy S, Ramakrishnan V &Kumar J 2011. Hydroxyapatite-Alginate nanocomposite as drug delivery matrix for sustained release of ciprofloxacin. *J. Biomed. Nanotechn.*, 7: 1 – 9.

Venkatesan P, Puvvada N, Dash R, Kumar BN, Devanand S, Azab B, Pathak A, Kundu SC, Fisher PB& Mandal M 2011. The potential of celecoxib-loaded hydroxyapatite-chitosan nanocomposite for the treatment of colon cancer. *Biomaterial*, 32: 3794 – 3806.

Zhao Q, Han B, Wang Z, Gao C, Peng C & Shen J 2007. Hollow chitosan-alginate multilayer microcapsules as drug delivery vehicle: doxorubicin loading and in vitro and in vivo studies. *Nanomedicine: Nanotechn. Bio. & Med.*, 3: 63– 74.