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PREPARATION AND IN VITRO EVALUATION OF HYDROXYAPATITE-SODIUM ALGINATE NANOCOMPOSITE FOR SUSTAINED DELIVERY OF DOXORUBICIN

ONOYIMA CC[1*], OKIBE FG[2], AGBAJI EB[2]

[1]Department of Chemistry, Nigeria Police Academy, Wudil, Nigeria
[2]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

This study was aimed at preparation of hydroxyapatite-sodium alginate nanocomposite and evaluation of its ability to sustain the release of doxorubicin and eliminate its dose-limiting toxicities. *In situ* preparation of hydroxyapatite-sodium alginate nanocomposite (HASA) was carried out by the wet chemical precipitation method. 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 ^{0}C. The result of drug loading study showed that the nanocomposites have high loading efficiency for doxorubicin, which increased with increase in sodium alginate composition reaching a maximum value of 92.03% for HASA-50%wt. There was sustained release of doxorubicin by the nanocomposites with sodium alginate composition of 5%wt and above for about 57 hours. The release rate decreased with increase in sodium alginate composition. The result of profile comparison indicates that the following release profiles are similar: HASA-5%wt and HASA-20%wt, HASA-20%wt and HASA-33%wt, HASA-33%wt and HASA-50%wt. The incorporation of sodium alginate to the nano-hydroxyapatite improved its ability to load and release doxorubicin.

KEYWORDS: Doxorubicin, Drug delivery, Hydroxyapatite, Nanocomposite, Sodium alginate,

INTRODUCTION

Burst release is a major problem in conventional drug delivery system. According to Martinho *et al*., burst releases lead to high toxicity for potent drugs and treatment of chronic diseases [1]. One of such diseases is cancer. Cancer is mainly treated by chemotherapy. The success of chemotherapy depends on the selection of optimum carrier system.

Polymers have been used extensively in drug delivery as a result of its exceptional properties which so far have not been attained by any other materials. Alginates can control the release rate of low molecular weight drugs [2], and because of their hydrophilicity, can contribute to longer in vivo circulation times and higher encapsulation of water soluble biomolecules [3, 4]. However, Marques *et al*., has cited that polymers/ceramics composites possess excellent, synergistic characteristics for drug delivery applications and have been popularly used for this purpose [5].

However, ionically cross linked alginate hydrogel has limited drug loading efficiency which limits its applications [6, 7]**.** Other major disadvantages of alginate beads are their fast disintegration, and their high porosity, which result in burst release. It has been shown that most of these defects can be reduced by the use of nanocomposites.

IUPAC Gold Book defined nanocomposite as 'a composition in which at least one of the phase domains has at least one dimension of the order of nanometre'. It has been reported by Devanand *et al*., that the drug loading efficiency and controlled release behaviour can be enhanced because of the synergistic effect between biopolymer and inorganic

***Corresponding author:** krissonoss@yahoo.co.uk; +2348035982690

materials [8]. According to Pongjanyakul, and Rongthong, water insoluble materials can be incorporated to alginate matrix in order to improve drug encapsulation efficiency and control drug release [6]. Hydroxyapatite/polymer composites have attracted much attention since such composite lead to improved properties [9] as a result of improvement in the surface functionality of the apatite [10]. Such improvement has led to wide applications of hydroxyapatite polymer composite in many areas such as in drug delivery system [11].

Venkatesan *et al*., carried out study on chitosan modified hydroxyapatite nanocomposite loaded with celecoxib. The anticancer nanocomposite showed high entrapment efficiency and sustained release profile [10]. Hydroxyapatite is a good biocompatible material, but when used as a carrier in pure form, impregnated drug can elute only for a short period [12]. In vitro drug release study by Raj *et al*., showed that normal hydroxyapatite showed a burst release in the initial stage. However, coating of hydroxyapatite by poly vinyl alcohol (PVA) showed sustained release of about 70% of the drug in 7 days 12 hours [11]. Addition of cloisite nanoparticles to poly (ethylene-co-vinyl acetate) resulted in slower release of dexamethasone [13], while modification of sodium alginate with hydrophobic poly (butyl methacrylate) led to the prolonged release of the model drug as compared with unmodified alginate gels [14]. By controlling the release kinetics of drugs, one can not only optimize the therapeutic effects of the drugs, but also influence their biological activity.

Doxorubicin are commonly used in the treatment of a number of diverse malignant tumours like acute leukemia, non-Hodgkin's and Hodgkin's lymphoma and several solid tumours. However the side effect of dose – dependent cardiotoxicity, myelosuppression as well as large distribution volume and low life time represent the limitations of its clinical use [15]. The aim of this study is to prepare hydroxyapatite-sodium alginate nanocomposite and to evaluate its ability to sustain the release of doxorubicin so as to eliminate its dose-limiting toxicities.

MATERIALS AND METHODS

Preparation of Hydroxyapatite/Sodium Alginate Nanocomposites

In-situ preparation of hydroxyapatite-sodium alginate nanocomposite was done according to the method by Rajkumar *et al.,* with some modifications [16]. The moles of the calcium and phosphate precursors were chosen so as to maintain a Ca/P mole ratio of 1.67, the stoichiometric amount of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. Aqueous solution of calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) (0.1M) was prepared using distilled water. The calcium solution was added in drop-wise manner to a separately prepared sodium alginate solution (1%wt) while stirring vigorously. Disodium hydrogen phosphate solution ($(NH_4)_2HPO_4$) (0.06M) was also prepared in distilled water. The phosphate solution was added in drops to the mixture prepared earlier with continuous stirring for 24h**.** The pH was maintained at close to 10.5 throughout the experiment using sodium hydroxide. The suspension was then stored for 24h at room temperature for aging, after which the precipitate was separated using centrifugation, and subsequently washed with distilled water three times. The resulting gel-like paste was dried at 60^0C for 24h and then ground using agate mortar to obtain fine powders.

The same procedure was repeated using 5%wt, 20%wt, 33%wt, and 50%wt of sodium alginate, designated as HASA-5%wt, HASA-20%wt, HASA-33%wt, and HASA-50%wt respectively. Similarly, the pure nano-hydroxyapatite was prepared using the above method without sodium alginate.

Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR analysis was conducted to identify the functional groups of the samples. Infrared spectra in the wavenumber range of 650-4000 cm^{-1} were recorded with Cary 630 Agilent Fourier Transform infrared spectrometer. The analyses were carried out with 8 scans at 16 cm^{-1} resolution, applying transmittance method.

Preparation of Drug-Loaded Hydroxyapatite and Drug-Loaded Hydroxyapatite/Sodium Alginate Nanocomposites

Drug loading was done according to the method by Raj *et al.* [11]. In order to load doxorubicin onto hydroxyapatite nanoparticles and the nanocomposites, the drug was dissolved in distilled water. A constant drug concentration of 2 mg/ml was used for all the formulations. Hydroxyapatite and hydroxyapatite/sodium alginate nanocomposites were added to the drug solution and stirred using magnetic stirrer for 40 min. Then the solution was left undisturbed overnight. The suspension was then centrifuged (2000 rpm, 5 min) and the supernatant and precipitate separated by decantation. The amount of the doxorubicin loaded

was determined by finding the difference in the concentrations in the aqueous solution before and after loading. Drug encapsulation efficiency (EE) and loading capacity (LC) were evaluated by measuring the absorbance of the supernatant at 485 nm using UV spectrophotometer.

The EE and LC of the nanoparticles were calculated according to the following equations [17]:

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

$$L.C = \frac{W_t - W_f}{W_n}$$

W_t represents the total amount drug; W_f is the amount of free drug in the supernatant; and W_n is the weight of nanoparticles. All measurements was performed in triplicate and the mean value reported

In-Vitro Drug Release Study

In-vitro drug release was used to study the doxorubicin release profiles from hydroxyapatite nanoparticles and the nanocomposites. 100 mg of the drug loaded-nanocomposite and drug loaded-hydroxyapatite was introduced into a glass bottle (screw capped) containing 50 ml of synthetic body fluid (SBF) medium at 37°C and pH 7.4 under sterile conditions. 5ml samples was withdrawn by a pipette at regular intervals and replaced immediately with 5 ml of fresh SBF medium, which was accounted for when calculating the amount released. The concentrations of the drug in the collected samples were evaluated by measuring the absorbance at 485 nm using UV-VIS Spectrophotometer.

The quantity of the drug released at any time point t was calculated using the equation below:

$$Q_t = C_t V_T + v\sum_{i=1}^{n-1} C_{ti}$$

while the percent cumulative release ($\%CR_t$) at any time point t is:

$$\%CR_t = \frac{Q_t}{Q_T} \times 100$$

where Q_t is the quantity of drug released at the time point t, Q_T is the quantity of the drug encapsulated into the material, C_t is the concentration at time t, V_T is the total volume of the release medium, v is the volume of the sample, $\sum_{i=1}^{n-1} C_{ti}$ is the summation of C from i = 1 to n-1. The second part of the right hand side of the equation, $v\sum_{i=1}^{n-1} C_{ti}$ is the volume correction, used to account for the amount of the drug discarded at every sampling point.

Comparison of Drug Release Profiles

A release profile is a measurement of in vitro drug release from a preparation in a receptacle media over a period of time [18]. Similarities between the different release profiles were investigated using model-independent approach. Pairwise procedures were followed, while similarity factor (f_2) was chosen for the comparison [19].

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

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 [20, 18].

In order to reduce calculation time and eliminate calculation errors, DDSolver program (excel plug-in program) was used for the calculations.

RESULTS AND DISCUSSION

Results of FTIR Study

The Hydroxyapatite-Doxorubicin (HA-DOX) spectra shown in Figure 1, is dominated by peaks from Hydroxyapatite, except the weak peaks at 1636 cm^{-1} and 704 cm^{-1} which are due to NH_2 bending and out-of-plane wagging respectively. The predomination of hydroxyapatite peaks may be due to low encapsulation efficiency of hydroxyapatite and the presence of very strong phosphate peak in hydroxyapatite which dominate the few doxorubicin vibration peaks.

Doxorubicin-loaded hydroxyapatite-sodium alginate nanocomposite (HASA-DOX) spectrum is presented in Figure 2. Prominent peaks in this spectrum include the peaks at 3183.1 cm^{-1} and 1408.9 cm^{-1} due to O-H stretching vibration and in-plane bending respectively, 1595.3 cm^{-1} due to COO^- asymmetric stretching, and 1017.6 cm^{-1} due to P-O asymmetric stretching. It is important to note from the spectrum that the O-H stretching peak shifted down to 3183.1 cm^{-1}, while the C=O peak (1718.3 cm^{-1}) and COO-Ca peak (1341.8 cm^{-1}) were not observed. These observations point to the possibility of chemical interaction between doxorubicin and the nanocomposite.

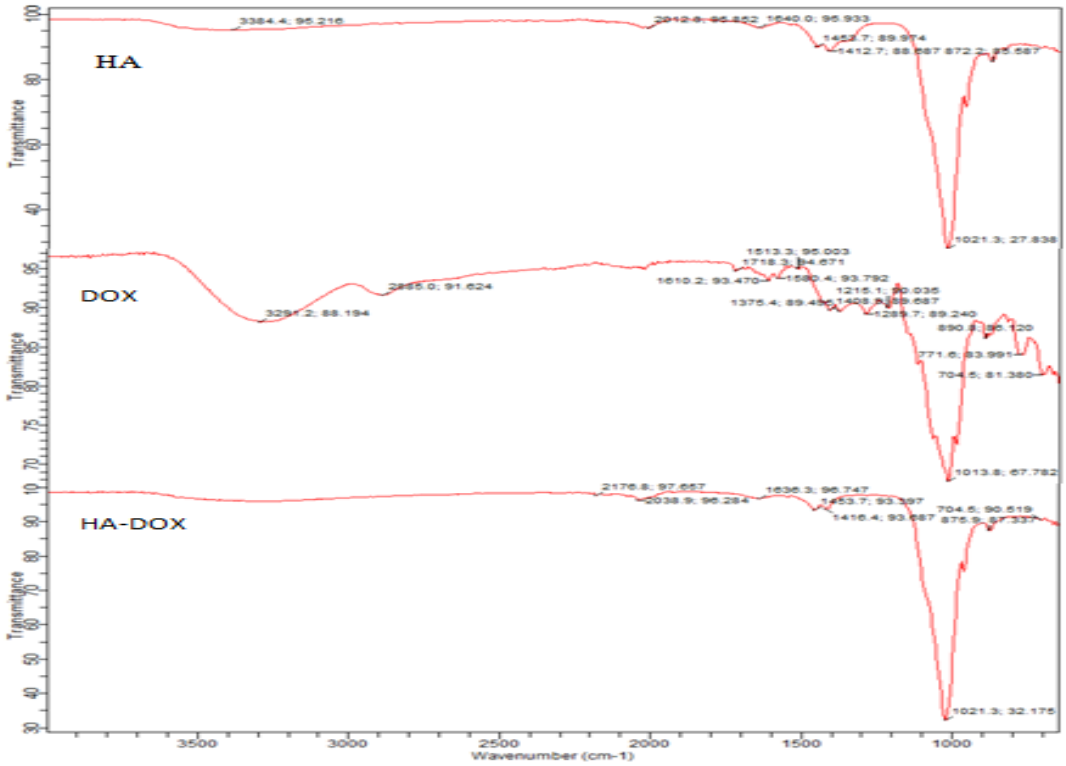

Figure 1: FTIR spectra of Hydroxyapatite (HA), Doxorubicin (DOX), and Doxorubicin-loaded Hydroxyapatite

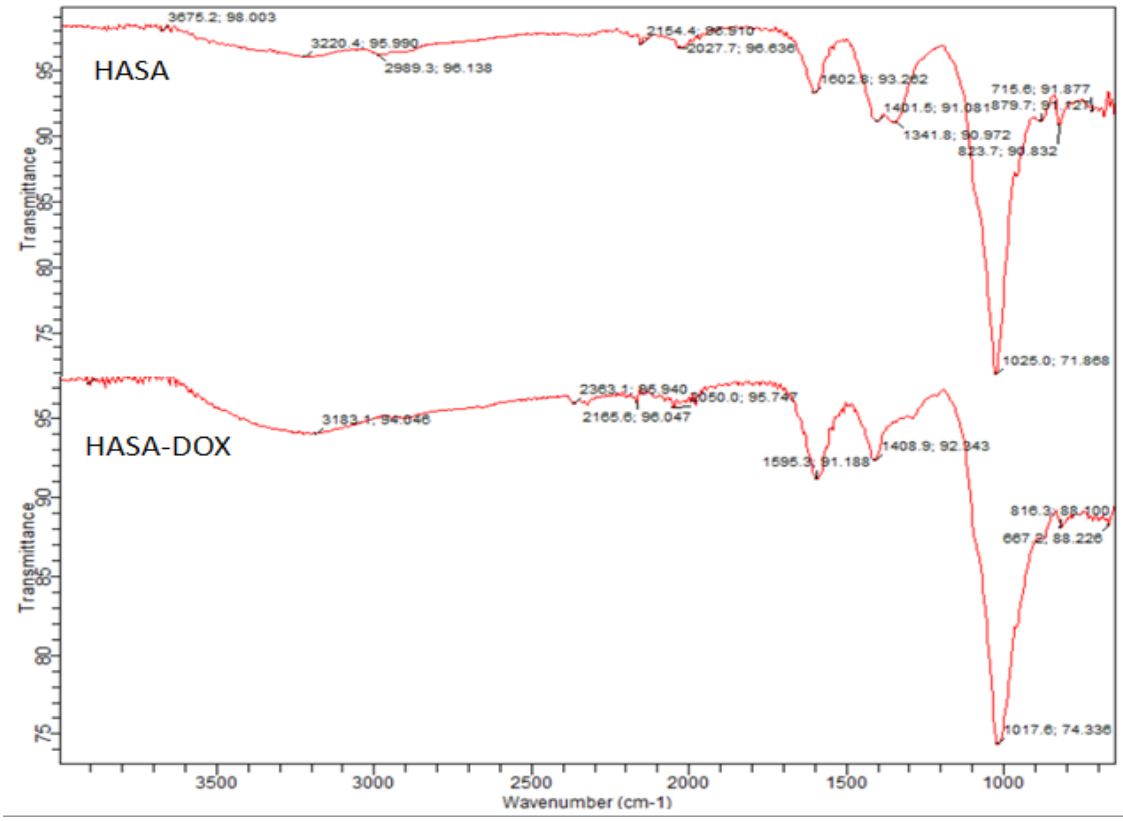

Figure 2: **FTIR spectra of Hydroxyapatite-sodium alginate (HASA), and Doxorubicin-loaded Hydroxyapatite-sodium alginate (HASA-DOX)**

Doxorubicin Loading

Figure 3 presents the results of Doxorubicin encapsulation into Hydroxyapatite and Hydroxyapatite- sodium alginate nanocomposite as a function of the amount of sodium alginate. It was observed that doxorubicin loading efficiency increased with increasing amount of sodium alginate. Pure hydroxyapatite recorded the lowest encapsulation efficiency of 21.93%, which increased as follows HASA-1%wt (26.29%), HASA-5%wt (63.42%), HASA-20%wt (74.92%), HASA-33%wt (87.69%), and HASA-50%wt (92.03%). This trend can be attributed to the observed chemical interaction between doxorubicin and hydroxyapatite-sodium alginate nanocomposite. The interaction was probably from the sodium alginate part of the nanocomposite as this interaction was not observed for pure hydroxyapatite, hence the higher the amount of sodium alginate, the higher the interaction and consequently the higher doxorubicin encapsulation efficiency.

The increase in doxorubicin loading with increase in sodium alginate quantity was not linear. From HASA-33%wt to HASA-50%wt, there was 100% increase in sodium alginate with only 4.34% increase in drug encapsulation. Hence, the optimal quantity of sodium alginate for doxorubicin loading is 33%wt. The loading capacity of this formulation is 9.42%. This means that 0.0942 mg of doxorubicin was loaded per milligram of Hydroxyapatite-sodium alginate nanocomposite. This relationship can be used to predict the amount of doxorubicin present in every known quantity of the nanocomposite prepared using 33%wt of sodium alginate.

Doxorubicin Release Study

The summary of Doxorubicin release profiles from the Hydroxyapatite and the nanocomposites are presented in Figure 4**.** The in-vitro release study was conducted for 57 hours. However, hydroxyapatite (HA), and HASA-1%wt released doxorubicin for 5 hours and 9 hours respectively, while for other formulations (HASA-5%wt, HASA-20%wt, HASA-33%wt, and HASA-50%wt), doxorubicin release was observed throughout the study period. The results indicate that after 33 hours, the percent cumulative release for these formulations – HASA-5%wt, HASA-20%wt, HASA-33%wt and HASA-50%wt are 95.15%, 88.70%, 85.82%, and 78.72% respectively.

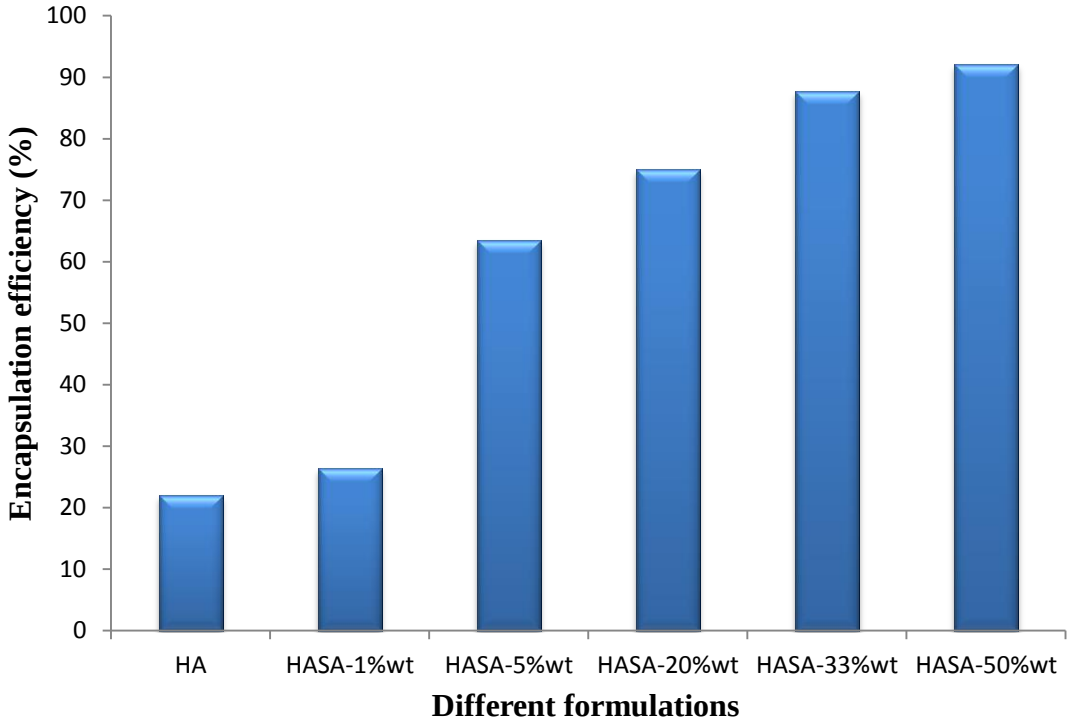

Figure 3: **Doxorubicin encapsulation efficiency by Hydroxyapatite-sodium alginate of different compositions**

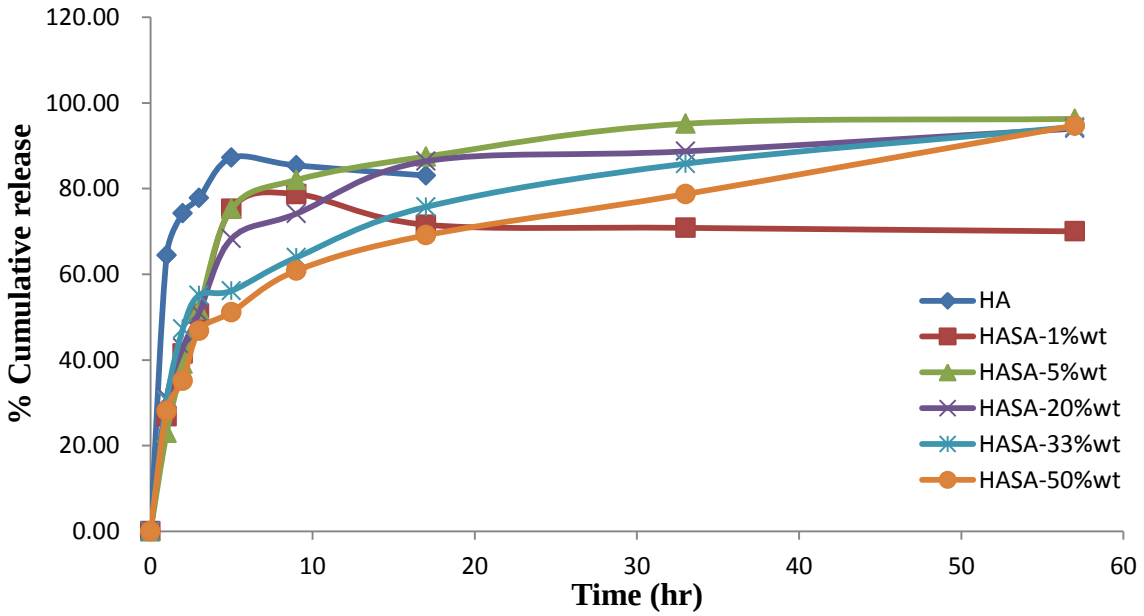

Figure 4: Doxorubicin encapsulation efficiency by Hydroxyapatite-sodium alginate (HASA) of different sodium alginate compositions

Table 1: Comparison of Doxorubicin release profiles from hydroxyapatite-sodium alginate (HASA) of different formulation using similarity factor (f_2)

	HA	HASA -1%wt	HASA-5%wt	HASA-20%wt	HASA-33%wt	HASA-50%wt
HA	100					
HASA-1%wt	32.39	100				
HASA-5%wt	31.59	43.92	100			
HASA-20%wt	33.13	46.75	**63.89**	100		
HASA-33%wt	31.42	44.46	48.15	**58.26**	100	
HASA-50%wt	26.84	43.45	43.01	49.26	**59.96**	100

The decreasing percent cumulative release is an indication of increase in more sustained release and decreasing burst release effect. That is to say that increase in the relative amount of sodium alginate increased the sustained release of doxorubicin. Higher polymer concentration in a composite material has been reported to give rise to more effective diffusion barrier leading to decrease in release rate **[4]**

The results of profile comparison (Table 1) indicate that the following release profiles are similar: HASA-5%wt and HASA-20%wt, HASA-20%wt and HASA-33%wt, HASA-33%wt and HASA-50%wt. The similarity factors (f2) for the similar profiles (indicated in bold in Table 1) are above 50. This means that the similarity of different profiles depends on the closeness of their sodium alginate

compositions. HA and HASA-1%wt did not show similar release profile with any other formulation.

CONCLUSION

Hydroxyapatite-sodium alginate nanocomposite recorded high encapsulation efficiency for the anticancer drug doxorubicin. The encapsulation efficiency increased with increase in sodium alginate composition which confirmed the drug-polymer interaction observed with the FTIR study. Hydroxyapatite alone showed burst release of the drug which reduced with increasing incorporation of sodium alginate. Doxorubicin encapsulation efficiency and release profile depended to a large extent on the quantity of sodium alginate, which shows that effective delivery of doxorubicin can be achieved by incorporating optimal quantity of sodium alginate into the hydroxyapatite.

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