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

C. C. Onyima¹, F. G. Okibe², I. B. Anweting³ and J. N. Akoji⁴
¹Department of Chemistry, Nigeria Police Academy, Wudil, Nigeria
²Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria
³Nigerian Institute of Leather and Science Technology, Zaria
⁴Department of Chemistry, Base University, Abuja

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 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 (Soppimath 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. Raull 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 acid 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-flourouracil-polyactic 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-Huertas 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 (Males 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 CaCl2 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°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°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°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 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 treatment 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 fluid was 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 (λmax) 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):

\[
E.E = \frac{W_t - W_s}{W_t}
\]

Where: \(W_t\) represents the total amount (mg) of the drug; \(W_s\) 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 CaCl₂ (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 CaCl₂·2H₂O (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 CaCl₂·2H₂O (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.
Effect of Loading Methods on Release Profiles of Methotrexate & doxorubicin

Doxorubicin loading and release profiles
The result of doxorubicin loading by the four different loading methods (Fig. 1) showed 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).

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](image1)

![Fig. 2: Release profiles of doxorubicin loaded into hydroxyapatite-sodium alginate nanocomposites by different loading methods](image2)
Effect of Loading Methods on Release Profiles of Methotrexate & doxorubicin

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

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
<th>Method 4</th>
</tr>
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<tr>
<td>Zero Order</td>
<td>$R^2$</td>
<td>0.818</td>
<td>0.934</td>
<td>0.903</td>
<td>0.933</td>
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<td></td>
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<td></td>
<td>$K_0$(mol.L$^{-1}$s$^{-1}$)</td>
<td>1.124</td>
<td>1.308</td>
<td>1.027</td>
<td>1.195</td>
</tr>
<tr>
<td>First Order</td>
<td>$R^2$</td>
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<td>0.974</td>
<td>0.985</td>
<td>0.987</td>
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<tr>
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<td>$K_1$(s$^{-1}$)</td>
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<td>0.034</td>
<td>0.029</td>
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<tr>
<td>Korsmeyer-Peppas</td>
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<tr>
<td></td>
<td>$N$</td>
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<td>0.345</td>
<td>0.267</td>
<td>0.312</td>
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<tr>
<td>Higuchi</td>
<td>$R^2$</td>
<td>0.931</td>
<td>0.975</td>
<td>0.982</td>
<td>0.978</td>
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<td></td>
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<td>11.444</td>
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<tr>
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<td>3.195</td>
<td>524.384</td>
<td>226.062</td>
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</table>

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
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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


Effect of Loading Methods on Release Profiles of Methotrexate & doxorubicin


