Evaluation of pH - Dependent Release of Doxorubicin from Hydroxyapatite-Sodium alginate Composites

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ABSTRACT

Cancer is one of the diseases where pH-sensitive delivery system is highly desirable because of the more acidic environment of tumour cells compared to normal cells. The effect of the pH of the release medium (pH 3, pH 5 and pH 7.4) on the release profile of hydroxyapatite-sodium alginate (HASA)-loaded doxorubicin, an anticancer drug, was studied. *In situ* preparation of hydroxyapatite-sodium alginate nanocomposite (HASA) was carried out by the wet chemical precipitation method. Drug loading and in-vitro drug release study was carried out using synthetic body fluid as the release medium. Doxorubicin (DOX) release rate was initially faster at acidic conditions than at physiologic condition. The cumulative percent release of DOX after five hours was 30.53% in pH 3.0, 33.64% in pH 5.0 and 18.21% in pH 7.4. After 33 hours, 38.56% of DOX has been released in pH 3.0, 36.42% in pH 5.0, and 55.43% in pH 7.4 There was preferential release of doxorubicin from the composite at acidic condition which indicates that the composite is a potential carrier for targeted release of DOX to cancer cells.

Key words: Doxorubicin, drug-loading, release profile, cancer cells

INTRODUCTION

In the words of Maeda *et al*. (2009), "traditional low-molecular weight anticancer drugs have very little tumour selectivity, and most of such drugs are widely distributed to normal organs and tissues as well as tumours. Consequently, these anticancer drugs provide insufficient therapeutic benefits and cause severe systemic toxicity, a so-called dose-limited toxicity." This problem can be avoided by development of tumour-selective anti-cancer agents (Maeda *et al*., 2012). 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). The mode of degradation of polymers can dictate the release rate of the encapsulated drug; for example, polyanhydrides and polyorthoesters degrade at the surface, resulting in a release rate that is proportional to the surface area of the drug delivery system (Joshi and Patel, 2012). Factors that influence the biodegradation kinetics of the selected polymer are: the chemical structure, size, shape, chain defects, ion exchange, ionic strength, pH, morphology (amorphous, semi crystalline, crystalline, micro-structure, residual stress), mechanisms of degradation (enzymatic, hydrolysis, microbial), molecular weight distribution, processing condition and sterilization process, annealing and storage history, route of administration, and site of action (Badri *et al*., 2014). However Priya *et al*. (2013) stated that majority of polymers used in controlled drug delivery undergo bulk erosion. One of the biggest challenges in cancer treatment with chemotherapy is the acquisition of drug resistance by the cancer cells. Cancer cells often develop multiple resistances to different functionally unrelated drugs. This is known as multi-drug resistance (Gottesman, 2002). Cancer multi-drug resistance is defined as the cross resistance or insensitivity of cancer cells to the cytostatic or cytotoxic actions of various anticancer drugs, which are structurally or functionally unrelated and have different molecular targets (Gottesman, 1993). The two mechanisms involved in cancer drug resistance are classified as cellular and non-cellular mechanisms. The non-cellular mechanism can occur in one or more of the following ways: reduced access of the drug to tumour cells due to poorly vascularised nature of tumour regions; ionization of basic drugs in acidic environment of the tumour which prevent their diffusion across cellular membrane (the pH around tumour tissues in the body is more acidic (~5.5 – 6.5), relative to physiological pH which is 7.4 (Magadala *et al*., 2008); reduced extravasations of drug molecules due to high vascular pressure and low microvascular pressure of the tumour site (Kakde *et al*., 2011). Hydroxyapatite-polymer composites have attracted much attention since such composite lead to improved properties (Khaled, *et al*., 2014) such as sustained drug delivery (Raj *et al.*, 2013), and pH-sensitive release offered by the polymeric component containing weakly acidic or basic group (Balamuralidhara *et al*. 2011).The objective of this study is to evaluate the release profiles of doxorubicin, an anticancer drug, at different pH, from hydroxyapatite-sodium alginate composite.

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MATERIALS AND METHODS

Preparation of hydroxyapatite sodium alginate composite

Exactly 2.14 g of sodium alginate was weighed in a 200 cm^3 beaker, and 100 cm^3 of distilled water was added onto it. The mixture was stirred vigorously using magnetic stirrer for about 30 minutes for complete dissolution of the sodium alginate.

Phosphate solution (200 cm^3 of 0.06 M) was added in drop-wise manner to a 100 cm^3 of the prepared sodium alginate solution while stirring vigorously. The mixture was added drop by drop to 200 cm^3 aqueous solution of calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) (0.1 M) 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 solution. 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 thrice. The resulting gel-like paste was dried at 60^oC for 24 h and then ground using agate mortar to obtain fine powders.

Drug Loading Study

Preparation of drug solutions

Lyophilized DOX HCl powder was used to prepare the drug solution. A 100 mg sample of the powder was completely dissolved in about 20 cm^3 distilled water inside a 50 cm^3 capacity beaker and quantitatively transferred to 50 cm^3 volumetric flask and then made up to mark with distilled water. The solution was used immediately after preparation.

Preparation of drug-loaded composite

Drug loading was done according to the method by Raj *et al.* (2013). In order to load the drugs on hydroxyapatite-sodium alginate (HASA) particles, 100 mg of the HASA was added to 10 cm^3 of the drug solution (2 mg/ml) 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 were separated.

Preparation of synthetic body fluid

For preparing 1 litre of Synthetic Body Fluid (SBF), the first five reagents were added according to the order given on Table 3.1 to 700 cm^3 deionized water in a 2 litre beaker with continuous stirring using magnetic stirrer. Preparation of 1 litre SBF required 40 cm^3 of 1 M HCl. Before the addition of the sixth reagent, 15 cm^3 of 1 M HCl was added to the solution. Then sixth, seventh and eight reagents were added subsequently and the temperature was raised to 37^oC. The remaining 25 cm^3 1 M HCl solution was added in drops until the pH was adjusted to 7.4 at 37^oC. Deionized water was added to make it up to 1000 cm^3. The prepared SBF was stored in a refrigerator to avoid degradation before us

Table 1: The composition of the reagents required to prepare 1 Litre of SBF (Kokubo *et al.* 1990)

Order	Reagent	Amount (g)
1	Sodium chloride (NaCl)	6.546 g
2	Sodium bicarbonate ($NaHCO_3$)	2.268 g
3	Potassium chloride (KCl)	0.373 g
4	Disodium hydrogen phosphate dehydrate (Na_2HPO_4).$2H_2O$	0.178 g
5	Magnesium chloride ($MgCl_2$).$6H_2O$	0.305 g
6	Calcium chloride dehydrate ($CaCl_2$).$2H_2O$	0.368 g
7	Sodium sulphate (Na_2SO_4)	0.071 g
8	Tris(hydroxymethyl)aminomethane $(CH_2OH)_3CNH_2$	6.057 g

Investigation of the Effect of Release Medium pH on Drug Release Profiles

Before the release study was carried out, freshly synthesized synthetic body fluid media were divided into three parts and the pH adjusted to 7.4, 5.0 and 3.0 respectively using 1 M HCl. Drug release studies were then carried out in each of them.

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 composite were introduced into a screw capped glass bottles containing 50 cm^3 of synthetic body fluid (SBF) medium at 37^oC and pH 7.4, 5.0, and 3.0 respectively under sterile conditions. Aliquots of 5 cm^3 samples were withdrawn using a pipette at regular intervals and replaced immediately with 5 cm^3 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.
The quantity of the drug released at any time Q_t was calculated using the equation 1: where Q_t is the quantity of drug (mg) released at the time point t;

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

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

$$\%Cr_t = \frac{Q_t}{Q_T} \times 100 \quad (2)$$

Where $\%Cr_t$ is the cumulative percent release at time t (h), and Q_T is the quantity of the drug (mg) encapsulated into the material.

C_t is the concentration (mg/cm^3) at time t, V_T is the total volume (cm^3) of the release

RESULTS AND DISCUSSION

Effect of the pH of the Release Medium on DOX Release Profile

The effect of the pH of the release medium on the release profile of DOX from HASA is shown in Figure 1

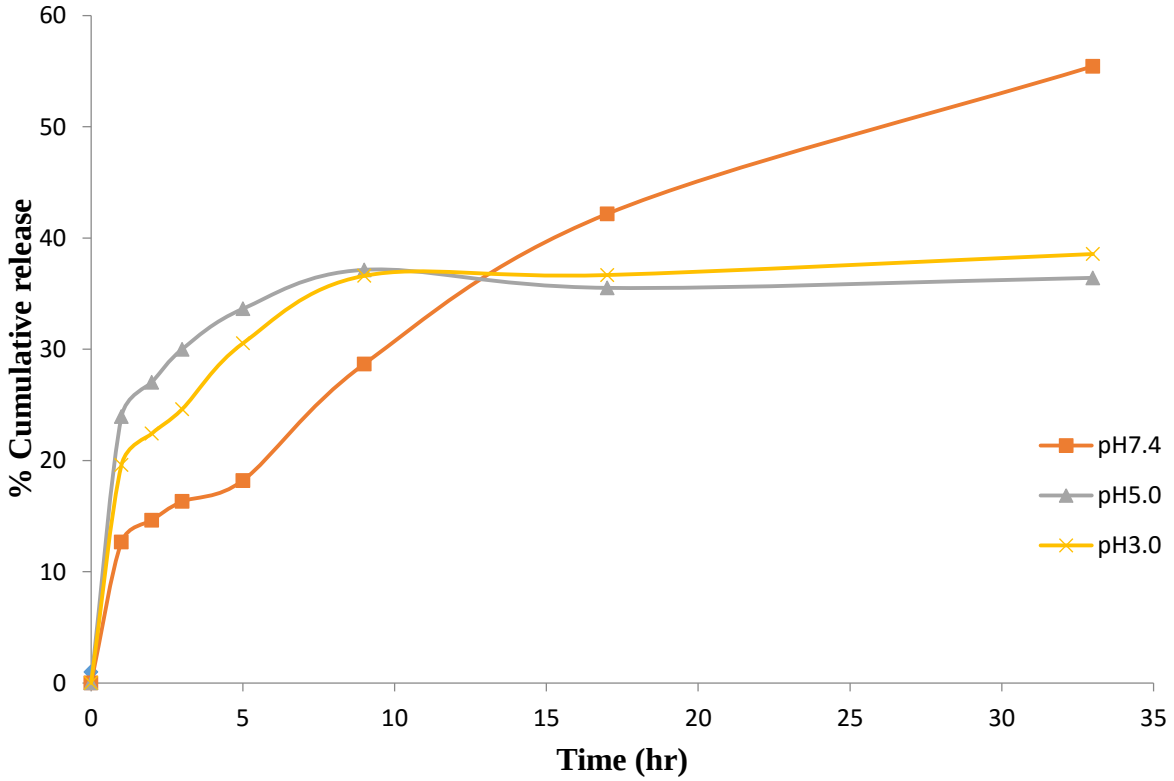

Figure 1: Release profiles of DOX-loaded HASA composites at different pH medium

The figure shows the release profile at pH 7.4 (the physiological pH), pH 5.0 (the extracellular environment of solid tumour, and pH 3.0 (the lysosomal pH). There was higher initial release for acidic pH (pH 3 and pH 5) which slowed down at the latter part of the release study. It was observed that at the initial time period (up to nine hours), DOX release rate was faster at acidic conditions than at physiologic conditions. Higher release of DOX at lower pH has been previously observed for other materials (Manocha and Margaritis, 2010; Kamba *et al*., 2013; Madhusudhan *et al*., 2014). Generally, pH dependent release from polymer or polymer inorganic composite has been attributed to presence of ionisable group. While the release of lidocaine-HCl and sodium salicyclate from sodium alginate was retarded in acidic medium, Guaifenesin, a neutral molecule, with absence of of any ionisable groups in the molecule showed pH independent drug release (Pork *et al.* 1998). DOX is a weak base with a pKa of 8.30 and can adsorb by electrostatic interaction (Seib *et al*., 2013).
In DOX-loaded HASA studied in this research work, the carboxylic group in sodium alginate might have interacted with the amino group of DOX. However, at acidic pH the carboxylic group ionizes thereby releasing the drug, hence the observed higher initial release at acidic pH.

Another possible reason might be due to increased solubility DOX at acidic pH. According to Wang *et al*., (2010), the increase in solubility of DOX at mildly acidic pH, could lead to higher release rate. It has also been previously observed that Solution pH and ionic strength have great influence on the stability of drug/polymer ionic complex, and consequently the drug release (Manocha, and Margaritis, 2010).

It was also observed in this study that after nine hours, the release rate of DOX at acidic medium decreased sharply while the drug continued to be released at physiologic medium. From the research by Davidovich-Pinhas and Bianco-Peled (2010), alginate shrinks at low pH, which makes the encapsulated drugs more difficult to be released under acidic conditions. Assuming that the observed decrease in release rate in this study was due to the shrinkage, we can infer that the shrinkage did not occur until after about nine hours, hence the observed initial fast release.

Cancer is one of the diseases where pH-sensitive delivery system is highly desirable because of the more acidic environment of tumour cells compared to normal cells. Therefore, preferential release at acidic pH will provide targeted release to cancer cells, which in turn reduces toxicity to normal cells.

CONCLUSION

The release profiles of doxorubicin-loaded hydroxyapatite-sodium alginate composite shows that it depends on pH of the release medium. The study shows that doxorubicin can be released from the composite preferentially at acidic pH which is similar to pH of the tumour cells. This preferential release can provide targeted release of the drug to tumour cells which can reduce toxicity of the drug to normal cells.

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