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

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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 (f1), showed that there is no significant difference between release profile of doxorubicin from nanocomposite synthesized in acetone and ethyl acetate (f1 = 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.
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 oflaxacin loading and microbiological activity of the fiber (Karatas et al. 2016). Morphology of coreshell 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 cm$^3$) prepared in different solvents (water, ethanol, ethylacetate, and acetone) were separately added in drop-wise manner to different SA aqueous solutions (100 cm$^3$) (50%wt) while stirring vigorously. The temperature of the one prepared in acetone solution was raised to above 37°C.
This mixture was set on a magnetic stirrer, and 50 cm$^3$ of 0.06 M diammonium hydrogen phosphate aqueous solution ((NH$_4$)$_2$HPO$_4$) 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)

$$f_1 = \left(\frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2\right)^{0.5} \times 100$$

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

Where, $n$ is the number of time points and $R_i$ and $T_i$ 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.
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.

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.
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)](image)

**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        |
|                    | $K_{HC}$  | 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.

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