

# Effect of pH Value and Doxorubicin/Hydroxyapatite Ratio on the Release of Doxorubicin from Hydroxyapatite Surface in Phosphate Buffered Saline (PBS)

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**Abstract**— Hydroxyapatite (HA) can be used as drug delivery material in cancer treatment by adsorption of cancer drugs such as doxorubicin (DOX) to hydroxyapatite surface. This experiment studied the effect of pH value and DOX/HA ratio on the release rate of doxorubicin from hydroxyapatite in phosphate buffered saline (PBS). The release of doxorubicin from hydroxyapatite surface was done by soaking DOX-HA powder into 350 mL of PBS solution at pH 5.6 and 7.4 in a beaker glass. The sample was taken from the suspension every two hours for 120 hours. The samples were analyzed using UV-Vis spectrophotometer to determine the DOX concentration released. The DOX-HA powder was characterized using SEM-EDX. At lower the pH value the higher DOX concentration released. DOX concentration released in the first 40 hours were maintained at 1.25 and 6.92 ppm for pH value 7.4 and 5.6 respectively. Beside that, the more hydroxyapatite used the higher DOX concentration released. The DOX concentration released in the first 40 hours at pH value 7.4 was maintained at 0.625, 3.541, 4.508 and 4.958 ppm when using 0.2, 0.3, 0.4 and 0.5 gram of hydroxyapatite respectively.

**Keywords**—hydroxyapatite, mass transport, doxorubicin, release, drug delivery

## I. INTRODUCTION

Cancer is one of the main issues causing death all over the world. In 2015, there were 8.7 million deaths caused by cancer where tracheal, bronchus, lung, and breast cancer was the leading cause of cancer deaths [1]. There have been many therapies developed for cancer treatment, one of them is using drugs like cyclophosphamide, methotrexate, doxorubicin, and fluorouracil [2]. Chemotherapy also damages healthy cells while damaging cancer cells which leads to side effects, such as decreased production of blood cells and hair loss [3, 4]. One of the methods developed to overcome these problems is the nanomedicine approach in the form of drug delivery systems (nano drug delivery). Nano drug delivery is a method of delivering drug

compounds directly to the targeted therapy [5]. The principle of this method is to conjugate the drug to nanoparticles as a drug carrier which is then inserted into the body. The advantages of nano drug delivery compared to conventional methods are its ability to treat specific targets in the body, reduce drug doses compared to oral use, reduce drug concentrations on non-target sites and reduce side effects caused by drug toxicity in non-target cells/tissues [6]. The purpose of a controlled drug delivery system is to achieve a constant, controlled and long-term release rate. This is the basic choice of treatment. Nano drug delivery is a very promising method to be a solution to various side effects caused by chemotherapy [7].

One of the suitable materials to use as drug delivery is hydroxyapatite [8]. Hydroxyapatite is the main component of bone as it contains 65-70% hydroxyapatite, 20-25% organic materials, and 5-8% water [9]. Calcium and phosphate are important inorganic components composing hydroxyapatite. The thermodynamic stability of hydroxyapatite in physiological conditions is the best among the calcium phosphate family [9]. Hydroxyapatite shows excellent bioactivity, biocompatibility, and high compressive strength making it suitable for use in cancer therapy [10]. Nanoparticles that can be used as drug delivery must have specific characteristics to achieve the desired target, have suitable combinations in terms of size, surface properties, method of drug adsorption or drug loading, drug release, hydrophobicity/hydrophilicity and long-term circulation in the blood [11].

## II. MATERIALS AND METHODS

### A. Materials

The materials used in this study were hydroxyapatite powder with 84% purity (Lianyungang Kede Chemical Industry Co., LTD.), distilled water, doxorubicin (DOX) HCl

2 mg/mL (Kalbe Company), and phosphate buffered saline solution (Biogear).

### B. Methodology

The procedures in this research divided into three parts: DOX adsorption on hydroxyapatite, DOX release from hydroxyapatite, and SEM-EDX analysis.

1) *DOX adsorption on hydroxyapatite:* DOX/HA ratio in this study was 1:200, 1:300, 1:400, and 1:500. Hydroxyapatite (0.2, 0.3, 0.4 and 0.5 gram) was dissolved in 10 mL DOX (100 ppm) then stirred using a hot plate and magnetic stirrer at a stirring speed of 250 rpm and a temperature of 37 °C for 24 hours. After that the suspension filtered using a vacuum dryer and left for 24 hours.

2) *DOX release from hydroxyapatite:* As much as 270 mg DOX-HA powder was soaked in 350 mL PBS solution (pH 5.6 and 7.4). The sample was taken as much as 10 mL every two hours for 120 hours then analyzed using a UV-Vis spectrophotometer.

3) *SEM-EDX Analysis:* SEM-EDX analysis was carried out to find out the surface morphology and elemental composition of hydroxyapatite, hydroxyapatite after DOX adsorption, and hydroxyapatite after DOX release.

## III. RESULTS AND DISCUSSIONS

### A. DOX adsorption and release

Hydroxyapatite which has adsorbed DOX changes color from white to pink. Then DOX was released again in a buffer solution release media, namely, phosphate buffered saline (PBS) solution to determine its ability as drug delivery. Fig. 1 shows the difference in the color of the initial hydroxyapatite, after DOX adsorption, and after DOX release on PBS. This color difference indicates that DOX has been successfully adsorbed and released back from hydroxyapatite.

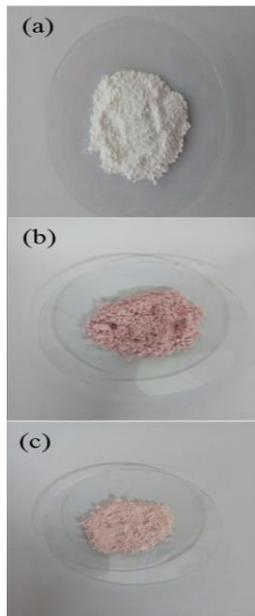


Fig. 1. Color differences between (a) initial hydroxyapatite (b) hydroxyapatite after DOX adsorption (c) hydroxyapatite after DOX release

### B. Effect of pH values on DOX concentrations released

The physiological pH in the bloodstream is 7.4 and the pH in the endosome is in the range of 5-6 [12, 13]. Based on these values, the DOX release study was carried out in PBS with pH 7.4 and 5.6. As shown in Fig. 2, rapid release occurs in the first 40 hours followed by slow release up to 120 hours. After initial rapid release, the DOX concentration released in PBS was maintained at around 1.25 and 6.92 ppm at each pH 7.4 and 5.6. The concentration released by DOX from HA at pH 5.6 is greater than pH 7.4. This happens because DOX on the HA surface can get protons and exchange amino groups (-NH<sub>2</sub>) into tertiary amines ((-NH<sub>3</sub><sup>+</sup>) in acidic medium that makes the hydrogen bond between DOX and HA cannot be formed [14]. Besides that the DOX protonation has a high solubility [15], consequently the DOX release rate increases with a decrease in the pH value of the buffer solution. The purpose of a controlled drug delivery system is to achieve a constant and controlled release of drug at a targeted zone within a certain period of time [16]. Therefore, in this study, the pH that were suitable for the purpose of the controlled drug delivery system was chosen which is pH 7.4.

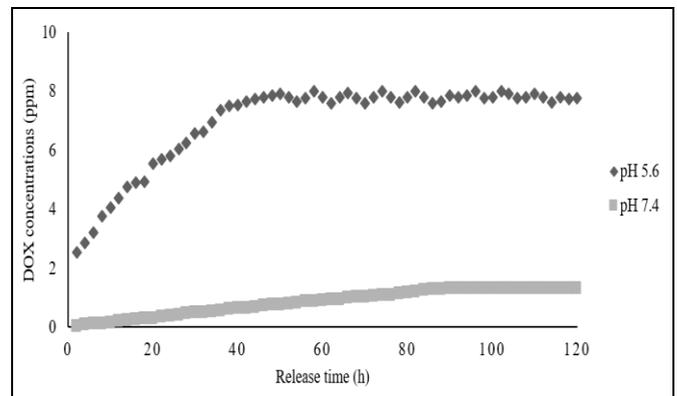


Fig. 2. DOX concentrations released from HA in PBS at different pH values

### C. Effect of HA mass variations on DOX concentrations released

The more HA is used, the higher the DOX concentration released because more HA surface areas are in contact with PBS so that more and more are releasing DOX from the HA. The effect of HA mass on DOX concentration can be seen in Fig. 3. The DOX concentration released in PBS solution has a limit value. DOX concentrations do not increase even in prolonged periods. This is due to the longer the concentration in the liquid is getting closer to the saturation concentration in which the concentration gradient gets smaller so that the mass transfer speed gets smaller. Mass transfer speed is determined by the existence of a difference from a state of equilibrium [17]. This fact shows that DOX-loaded HA has a slow, long-term, and stable release rate, which can prevent DOX release explosively and prolong the effect of the drug. Because of that HA can be used as drug delivery because of the long-term continuous drug concentration.

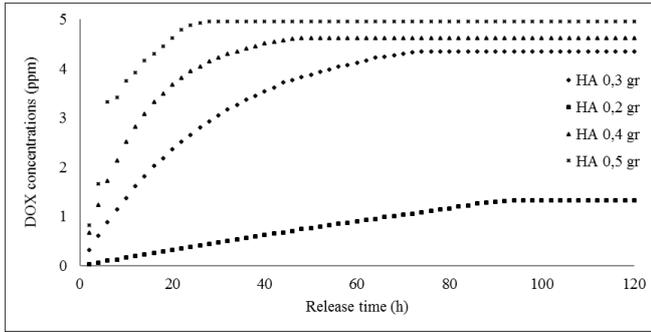


Fig. 3. DOX concentrations released from HA in PBS with HA mass variations

D. SEM-EDX analysis

Fig. 4 shows a comparison of the morphology of HA. In Fig. 4 (a) HA before the addition of DOX, the surface of the HA looks uneven. Whereas in Fig. 4 (b) HA which has been added with DOX, SEM results indicate agglomeration or clumping on the surface of HA, this is due to the addition of DOX adsorbed on the HA surface. In Fig. 4 (c) the particles agglomerated on the surface diminish and the particle size returns unevenly because DOX attached to the HA surface has been released.

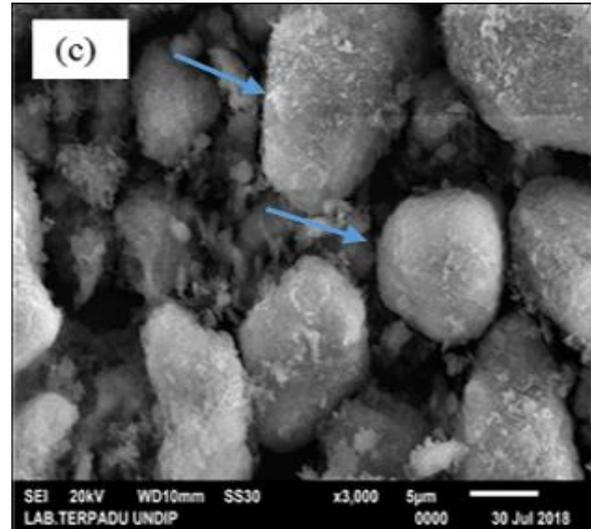
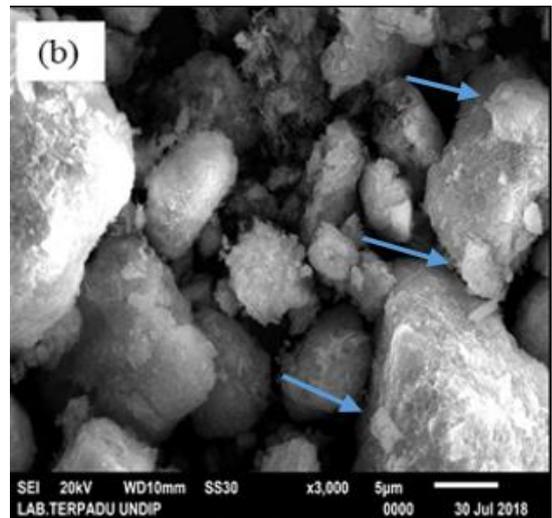
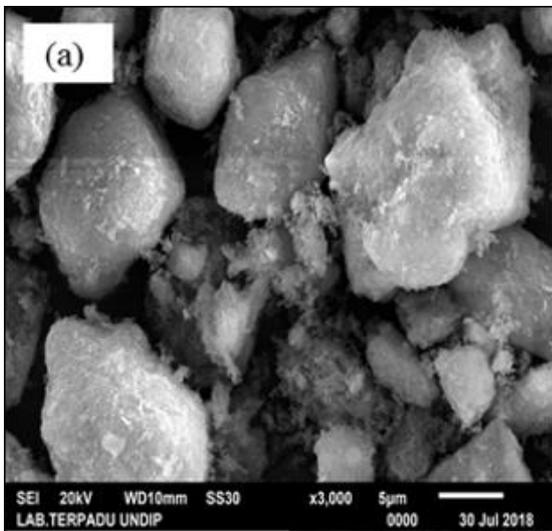
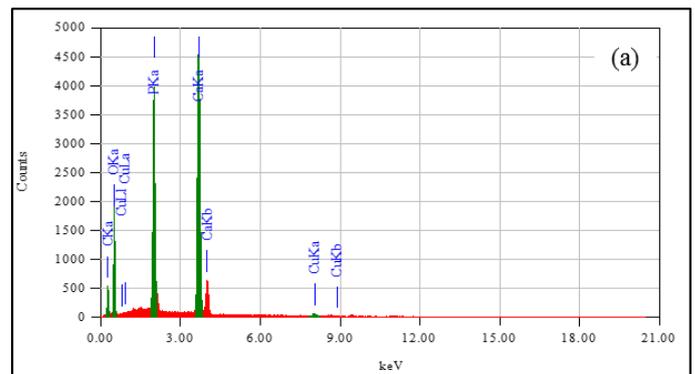


Fig. 4. SEM analysis on (a) initial HA before adsorption (b) HA after DOX adsorption and (c) HA after DOX released in PBS

Fig. 5 (a) is the result of EDX analysis of HA before the addition of DOX. In this picture, there are peaks on certain keV. Peak at 3,690 keV is for element Ca, at 2,013 keV shows the presence of element P and element O can be seen slightly at 1,520 keV. This indicates that there are elements Ca, P, and O in the sample which can be indicated that there are constituent elements of HA in the sample. Fig. 5 (b) shows the results of the EDX analysis of HA after the addition of DOX. In this picture, there are peaks in the keV besides the peaks of the HA constituent elements. The peak at 1,041 keV which is Na element, at 1,253 keV indicates the presence of Mg element, at 2,621 keV shows the presence of Cl element, and Zn element looks slightly at 8,630 keV. This indicates that in the sample there are new elements namely Na, Mg, Cl, and Zn which can be indicated that in addition to containing elements of HA there are also DOX constituent elements in the sample. Then in Fig. 5 (c) DOX is released again in PBS, from the EDX results there is no visible element of DOX composing, there are only HA constituent elements. This proves that DOX was successfully released from the HA surface.



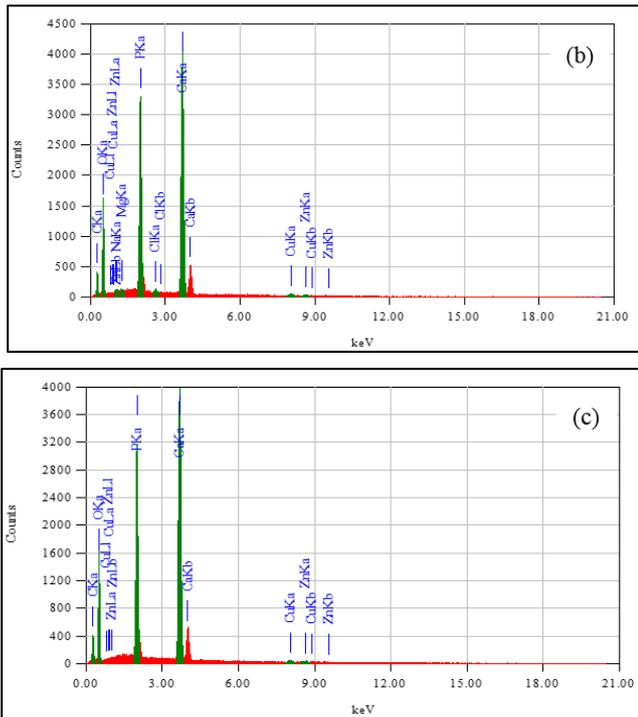


Fig. 5. EDX spectrums of (a) initial HA before adsorption (b) HA after DOX adsorption and (c) HA after DOX released in PBS

In Table 1, it can be seen that the number of constituent components of the sample increases after HA adsorption. there are additions of Na 0.28%; Mg 0.24%; Cl 0.28%, and Zn 1.02%. This is because initially there are only the original compositions of HA exist in the sample, but it increases after the composition of the DOX constituents is added. Then after the DOX release is done, the number of sample constituents is reduced again but still more than the HA component before DOX adsorption, this is because there is still a small portion of DOX components on the HA surface such as Zn compounds as much as 0.86%.

TABLE I. COMPONENT MASS ANALYSIS (%)

Component	Mass (%)		
	HA	HA after DOX adsorption	HA after DOX released in PBS
C	23,77	22,01	21,94
O	31,71	32,18	32,02
Na	-	0,28	-
Mg	-	0,24	-
P	15,84	16,04	15,64
Cl	-	0,28	-
Ca	27,33	26,63	28,34
Cu	1,35	1,30	1,21
Zn	-	1,02	0,86
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>

IV. CONCLUSION

DOX release rate from HA in PBS is influenced by the pH value and DOX/HA ratio. The smaller the pH value, the greater the DOX concentration released. DOX concentration released in PBS at the first 40 hours was maintained at 1.25 and 6.92 ppm at pH 7.4 and 5.6 respectively. The smaller the DOX/HA ratio, the greater the concentration of DOX

released. In the first 40 hours at pH 7.4, DOX concentration released in PBS was maintained at 0.625, 3.541, 4.508 and 4.958 ppm at 0.2, 0.3, 0.4 and 0.5 gram HA.

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