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In-vitro study of antibiotic and strontium release from hydroxyapatite spheres and its PMMA composite

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Chemical Engineering Programme

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1.0. Aim

The purpose of this master thesis project was to investigate the drug release from hydroxyapatite spheres and its polymethylmethacrylate (PMMA) composite in vitro. The drugs that were studied were the antibiotics cephalothin and vancomycin. The hydroxyapatite spheres were to be made containing strontium and the ion release from the spheres would also be measured. The study was to be done with phosphate buffer saline solution (PBS) with differing pH value to see what effect it might have on the release. To simulate blood condition a pH value of 7.4 was desired and a pH of 8 to simulate intestinal fluid. The extracellular compartment where bone resorption occurs tends to be in an acidic environment therefore the release was also to be studied at a pH value of 4[1].

1.1. Introduction

Prosthesis-related infection is a severe problem that can occur when dealing with cemented orthopedic implants and the use of antibiotic-loaded bone cement is just one of many methods applied to prevent and treat this type of infection. Rise of infections can develop through different ways; either through direct contamination of the biomaterial or the surrounding tissue; by blood-bone contamination or by the spread of a superficial infection[2]. Direct contamination is most likely the cause for the greater part of prostheses-related infections. Infected cemented prosthesis lead to a weakened function and pain for the patient. An infection can sometimes require multiple operations and the severity of the problem sometimes lead to amputations in worst cases. Diagnosis of an infection around an implant can be difficult when dealing with less open infections and to replace an old prosthesis with a new one on an infected area without proper antibacterial treatment is obviously dangerous. It has therefore been suggested that every loose prostheses is to be assumed as infected until proven otherwise[3]. However, it has been noted that the risk of infection increases after revision arthroplasty compared to the primary arthroplasty. The cause for prosthesis failure might be due to a combination of mechanical and infectious loosening[4].

To obtain certainty of an infection the causative organism from the implant side is cultured. This can be problematic because a negative culture from the tissue does not necessarily rule out an infection. A negative result can be from failure to get a correct sample but it may also

be due to changed bacterial metabolism. Bacteria in a biomaterial-related infection can be difficult to culture because these bacteria do not grow exponentially; instead they are slow-growing. Furthermore, these bacteria can produce an extracellular matrix that can protect them from inhospitable environment. This allows the bacteria to evade the host immune system and antibiotic treatment, as well as making detection difficult by standard laboratory methods[5].

Treatment of prosthesis-related infection is difficult, and antibiotic treatment generally focuses on removal of the foreign body material combined with surgical removal of infected tissue around the prostheses area. Thereafter antibiotics aimed at the causative bacteria are administrated. To enable a both locally and systematically administrated antibiotic treatment, an antibiotic-loaded bone cement can be implemented. This method aims to maintain a level of local antibiotic concentration which cannot be achieved by using a systemic administration without side effects[6].

Published in vitro studies show that the release of antibiotics from hydroxyapatite and PMMA follows a typical bi-phasic fashion; a burst release gives a peak which is followed by a long, tail of low release that goes on from days up to months. In addition, the amount of antibiotics released increases with increased surface area and also relates with the surface roughness[7].

1.2. Materials used in the study

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HA) is a bioactive bone material that has been widely used in filling bone defects because of its chemical similarities with the inorganic phase of bone. The advantage of using HA as bone material is its formidable osteoconductivity, non-toxicity and ability to form a direct bond with bone[8] . HA is the main mineral component of bones and teeth and has superior adsorption of many molecules making it an excellent choice as an adsorbent[9]. Other advantages are that it does not cause any foreign body reaction or an immunological response. Antibiotic-loaded bone cements have been used in clinical procedures for a long time now and hollow HA has been used as a local drug delivery system because of its porous structure that can allow a high loading capacity[10]. Hydroxyapatite has also shown promise as filler for dental composites not just because of it

being a natural component in teeth but also because it is naturally radio-opaque and highly resistant to moisture[11]. Moreover, hydroxyapatite has the ideal hardness which is used as a standard for composite resin filler particles.

Another, common material for bone substitution and prosthesis fixation is polymethylmethacrylate (PMMA). This bone cement consists of a mixture of liquid methylmethacrylate (MMA) and pre-polymerized PMMA powder which is polymerized in the patient when used in clinic. The mixture of the components gives a viscous paste which gives the advantage of molding and the ability to support prosthesis during insertion. The initial presence of PMMA makes it possible to use less monomer to obtain the same amount of end product. The end result also means less undesirable effects of polymerization. The use of less monomer enables less heat production, reduced volume and increased density because the molecule take up less space in a polymer than their liquid counterpart.

Feng Ye et al. used hydroxyapatite hollow nanoparticles loaded with vancomycin that had a narrow sized distribution, which reportedly gave a high drug loading ratio. These particles were then morphology changed into nanotubes by adding citric acid as a co-surfactant. This would further improve the drug load efficiency and could form a pH-controlled drug release. The effectiveness of hydroxyapatite particles loaded with vancomycin in treatment of Staphylococcus aureus showed impressive results[12]. Uwe Joosten et al. studied the release both in-vitro and in vivo using rabbits for their model. The in vitro study, where hydroxyapatite was mixed with a high concentration vancomycin, showed a high level of vancomycin release over a prolonged period more than sufficient in treating an infection. In the animal study no histological evidence of an infection was seen after 42 days of incubation on the group that been administrated vancomycin loaded hydroxyapatite, nor was any systemic side effects seen.

In vitro release studies of antibiotics from PMMA follows a similar fashion to release from hydroxyapatite with an early burst release followed by a lower release after. Currently, antibiotic loaded PMMA beads are being used clinically and have shown to deliver a high concentration locally and with minor systemic effects[13]. Diefenbeck et al. summarized non-controlled, non-randomized clinical studies of the efficacy of the antibiotic gentamicin

loaded PMMA beads for treating osteomyelitis and showed an infection control rate of almost 90 %. From experimental and clinical data they suggested that the most reliable approach for treatment would be achieved by combining local and systemic antibiotic therapy. The release mechanism for antibiotics from bone cement is not clearly proved; some authors have found that the release depends on the diffusion of antibiotics either through the entire polymer matrix or through capillaries within the matrix. However, most authors have come to the conclusion that the release is dependent on the surface of the bone cement[14]

1.3. Antibiotics and ion of interest in the study

Vancomycin is a glycopeptide antibiotic, applicable against Gram-positive bacteria and are therefore often used clinically against multi-resistant bacteria strains such as Staphylococcus aureus. Staphylococcus aureus is one of the major types of infections that affect bone causing inflammatory destruction of joint and bones. The infections that arise can be acute or chronic and affect vertebrae, native- and prosthetic joints.

Cepalothin is a first generation cephalosporin antibiotic, which is classed as β-Lactam antibiotics. It has a large volume of distribution, a protein binding of nearly 65 % and is mainly eliminated by renal excretion[15].

Strontium increase bone mass and decrease bone resorption, the use of the drug strontium ranelate increase bone calcium which has shown reduction of incidence of fractures in osteoporotic patients. It also enhances bone cell replication and bone formation. Administered strontium is mostly deposited into bone. Although Strontium only makes up a 0.035 of the calcium content in skeleton its behaviors are similar, they both have strong bone-seeking properties and the excretion from the body is similar. Strontium can replace calcium in the hydroxyapatite structure and it is also the only trace metal in bone that was correlated with bone compression strength[16].

2. Materials & Methods

The hydroxyapatite spheres containing strontium were made in the laboratory. The spheres were made by dissolving strontium nitrate (1.0mM) into a solution containing Na^+ (145

mM), K^+ (4.3 mM), Mg^{2+} (0.49 mM), Ca^{2+} (0.91 mM), Cl^- (143 mM) and HPO_4^{2-} (9.6 mM). The solution, made in a glass bottle, was then covered with a lid and put into an oven at 100°C for 24 hours. Thereafter, the solution was centrifuged and the hydroxyapatite spheres were separated from the solution. The vancomycin and cephalothin, both in powder from, were bought from Sigma. Shimadzu 1800 UV-Vis spectrophotometer was used to measure the antibiotic concentration and an inductively coupled plasma atomic emission spectroscopy (ICP-AES) Spectro Ciros CCD was used to quantify the amount of strontium in the samples. The concentrated strontium solution of 1000 ppm that was used for making stock solutions was bought from Analytical Standards AB. The PMMA that was used was the radiopaque bone cement for vertebroplasty Osteopal V from Heaues Medical. PBS was made from tablets that were bought from Sigma and the pH was adjusted with hydrochloric acid and sodium hydroxide.

2.1. Drug loading of hydroxyapatite

The drug loading was carried out at room temperature for 24 hours under stirring. 500 mg of hydroxyapatite was immersed into 20 ml drug solution. For the vancomycin and the cephalothin, the drug solutions were at a concentration of 10 mg/ml. After 24 hours the samples were centrifuged at 3000 rpm. The estimation of amount of antibiotic loaded into the hydroxyapatite spheres was done by an indirect method, where the amount of drug in the solution before and after the loading was found. Percentage of the drug loading was then calculated using the formula[17]: $Percentage\ drug\ loaded\ = \frac{X-Y}{X} \times 100$

Where X is the amount of drug in the solution before loading and Y is the amount of drug in the solution after the loading. The drug solution was analyzed by UV-vis spectrophotometer at a wavelength of 280 nm for the vancomycin and at a wavelength of 237 nm for the cephalothin. Drug content was determined by comparing with the standard curves of vancomycin and cephalothin. The standard curves were made from vancomycin and cephalothin solutions in Milli-Q water with concentration between 10 µg/ml and 125 µg/ml.

2.2. Loading into PMMA

For the PMMA release study four groups were prepared in 6 mm × 12 mm molds:

- Group 1: PMMA + Hydroxyapatite (10 wt %)[18]
- Group 2: PMMA + Vancomycin (2.5 wt %)[19]
- Group 3: PMMA + Vancomycin loaded hydroxyapatite (10 wt %)
- Group 4: PMMA + Hydroxyapatite (10 wt %) + Vancomycin (2.5 wt %)

The groups were prepared by adding respective solid materials into a cup and mixing them for one minute in a cap vibrator. After the mixing the liquid monomer was added and the mixture was mixed for one minute using a spatula. The obtained cement mix was then injected into the molds. The molds were approximately loaded with four gram of obtained mixture.

2.3. Drug release from hydroxyapatite

20 ml PBS with differing pH value (4, 7.4 & 8) was added to 50 mg drug loaded HA particles and the release was carried out at 37°C on an orbital shaker. 3 ml samples of the release medium were collected at specific time intervals, new buffer was added and the concentration of the drug was then measured using the UV-spectrophotometer. These measurements were carried out in triplicate. Collected samples were also measured in ICP-AES at a wavelength of 407,771 nm to determine the amount of strontium released. The strontium samples were determined by comparing them to a standard curve made from strontium in concentrations from 0 ppm to 10 ppm. The stock solutions were prepared by diluting a concentrated solution of strontium (1000 ppm) with the respective PBS solutions.

2.4. Drug release from hydroxyapatite-PMMA composite groups

20 ml PBS of pH value 4, 7.4 and 8 was added to the PMMA groups and the release was carried out at 37^0C on an orbital shaker. Sample collecting and detection was carried out in the same way as the drug release from the hydroxyapatite particles.

2.5. Mechanism study of drug and Sr ion release

To describe the mechanism of the drug release the collected data was implemented to the Korsmeyer Peppas model[20]: $Mt / M\infty = kt^n$

Where $Mt / M\infty$, is the fraction of drug released, t is the release time, k is a constant, and n is the diffusional exponent characteristic of the release. The value of the diffusion exponent n is used to describe the release mechanism as seen on table 1. A drug release that is described as Fickian diffusion requires that the amount of substance goes from regions of

high concentrations to regions of low concentration to an extent that is proportional to the concentration gradient. If the release does not follow this it is said to be non-Fickian diffusion and if the drug release is independent of its concentration it is classified as a zero-order release.

Table 1. Diffusion exponent and drug release mechanism.

Diffusion exponent (n)	Drug release mechanism
0.43	Fickian diffusion
0.43 - 1.00	Anomalous (non-Fickian) transport
1.00	Zero-order release (release is independent of the drug's concentration)

3. Results and discussion

3.1. Release from hydroxyapatite spheres

The UV-vis results from the drug loading combined with the equation on the section about drug loading gave the percentage of loaded antibiotic in the hydroxyapatite spheres. The percentage loaded cephalothin in the hydroxyapatite was 93.3 % and percentage loaded vancomycin was found to be 91.5 %. Thus each milligram of hydroxyapatite could load approximately 0.37mg of antibiotics. Figure 1 shows the in-vitro release of vancomycin from HA particles carried out in 37°C in PBS with different pH values. An initial burst release of the antibiotic appears in the first two hours, and then a slow release continues on the proceeding hours. The results achieved when studying the in-vitro release of cephalothin, carried out the same way as the vancomycin release, can be seen in figure 2. The cephalothin release also has an initial burst release within the first 2 hours, and is slightly continued until after the first 24 hours to then have a continued slow release over the next hours.

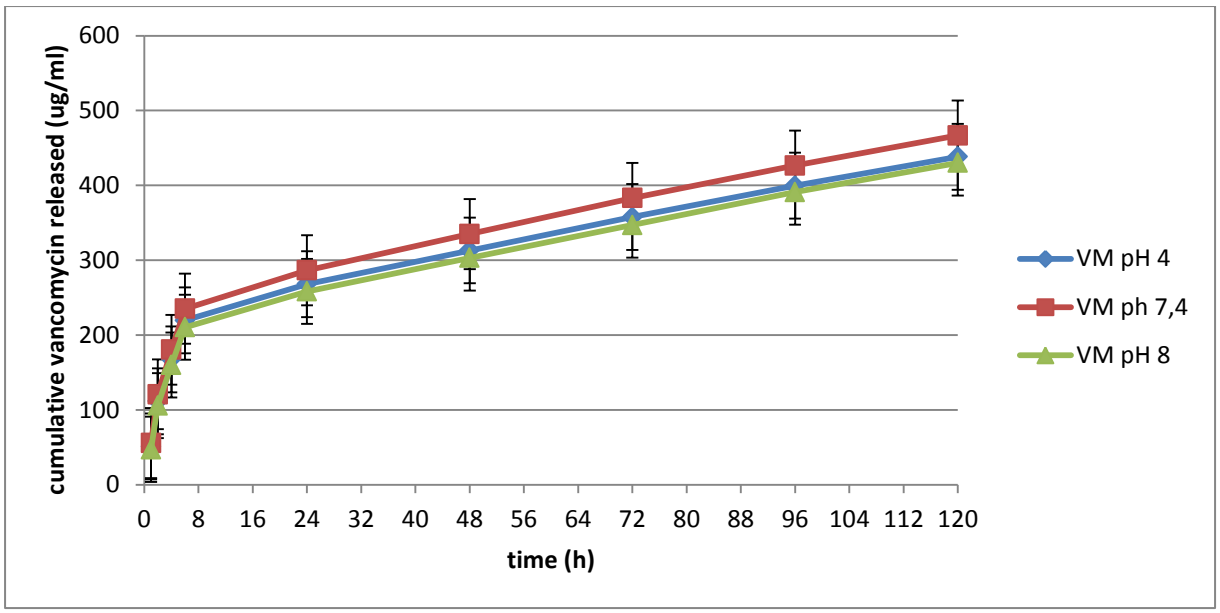

Figure 1. Release of vancomycin from hydroxyapatite in PBS (pH 4, pH 7.4 and pH 8) with standard error bars.

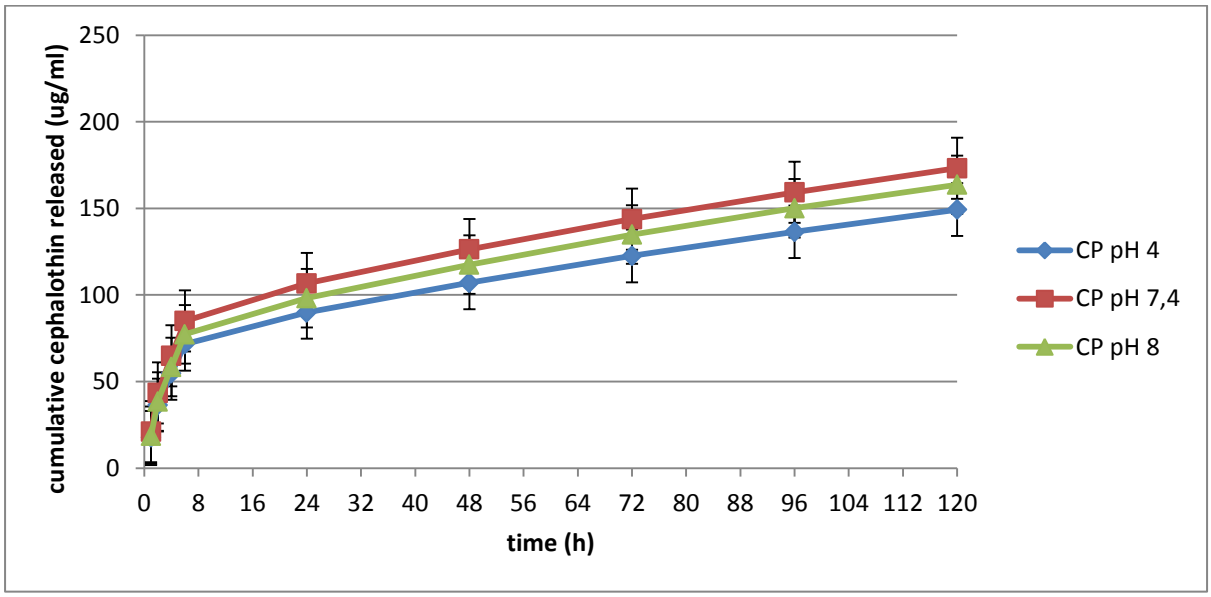

Figure 2. Release of cephalothin from hydroxyapatite particles in PBS (pH 4, 7.4, 8).

Table 2 shows the amount of vancomycin and cephalothin released within the first 24 hours. The burst release within the first 24 hours might be due to the amount of antibiotic on the surface of the hydroxyapatite particles and table 2 gives an indication of how much of the antibiotic was on the surface.

Table 2. Amount of vancomycin and cephalothin released within the first 24 hours.

Vancomycin			**Cephalothin**		
pH 4	pH 7.4	pH 8	pH 4	pH 7.4	pH 8
0.968 mg	1.027 mg	0.959 mg	0.366 mg	0.434 mg	0.416 mg

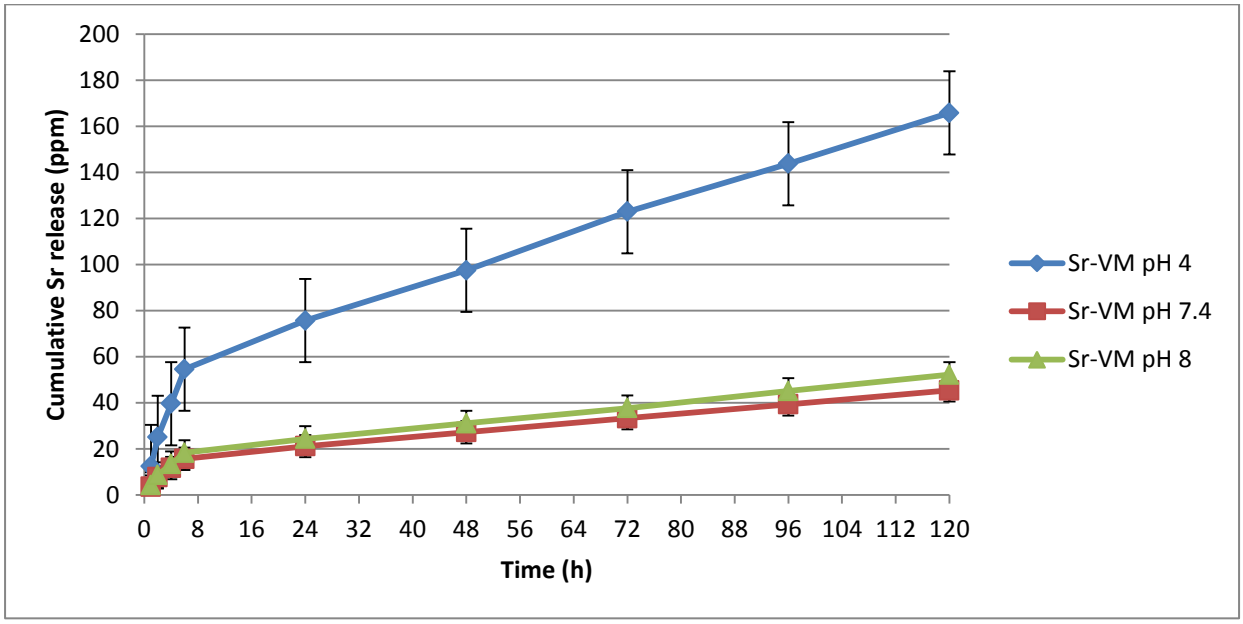

Figure 3. Strontium release from vancomycin loaded hydroxyapatite in PBS (pH 4, 7.4, 8).

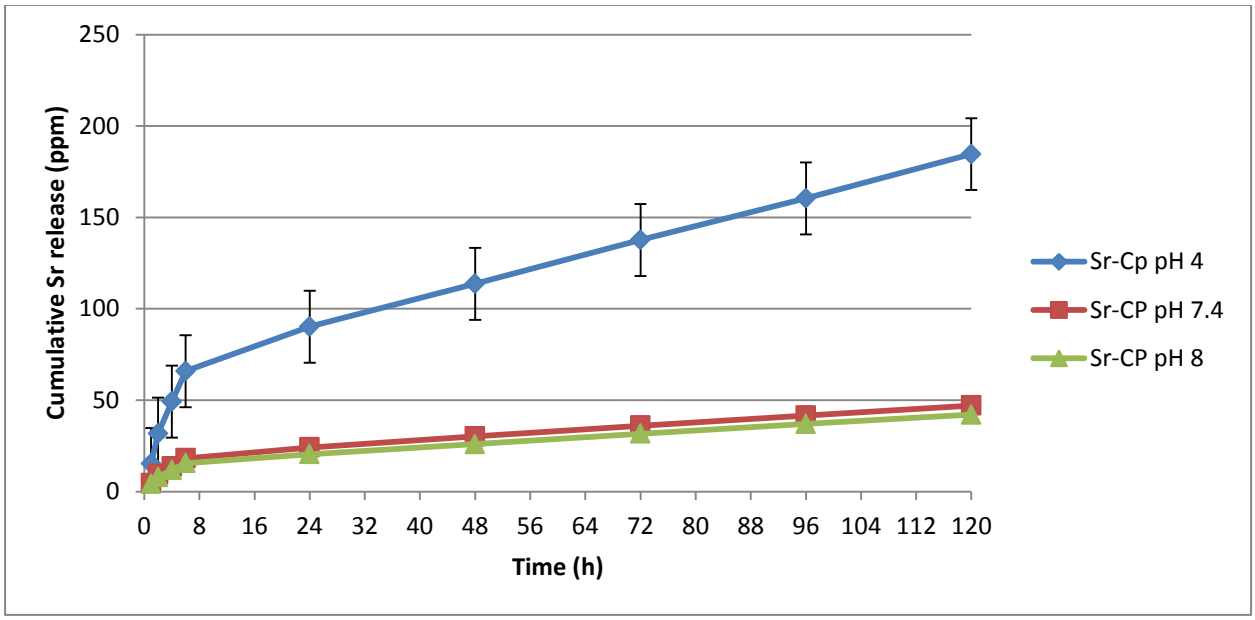

Figure 4. Strontium release from cephalothin loaded hydroxyapatite in PBS (pH 4, 7.4, 8)

In figure 3, the strontium release from the vancomycin loaded hydroxyapatite particles with different pH values is shown. The strontium release for the PBS medium with a pH value of four showed a higher burst release rate than the ones with higher pH value. The pH four release curve also shows a higher release behavior on the following hours compared to the other curves. This could be due to the acid environment making the hydroxyapatite dissolve. The release profiles for the PBS with pH values seven and eight show that the release increases a bit initially the first hours to then come to a slow release. The strontium release profile for the cephalothin loaded hydroxyapatite particles is shown in figure 4. It shows a similar release profile, where the release curve for pH value four has a high release rate and an erratic behavior while the other two release profiles have a small increase of release in the beginning and then have a more slow release.

Figure 5 and figure 6 show Korsmeyer Peppas model for release behavior of vancomycin and cephalothin loaded hydroxyapatite respectively. Regression lines were applied to both the vancomycin and cephalothin releases. Furthermore, both antibiotics gave a diffusion exponent n that is just below explainable release mechanisms but closest to Fickian diffusion, thus it's the closest explanation to the release behavior. The reason for a low diffusion exponent might be due to instability in the sample or that the Korsmeyer Peppas model is not sufficient enough to explain the release behavior. A summary of the release parameter values are shown in table 3.

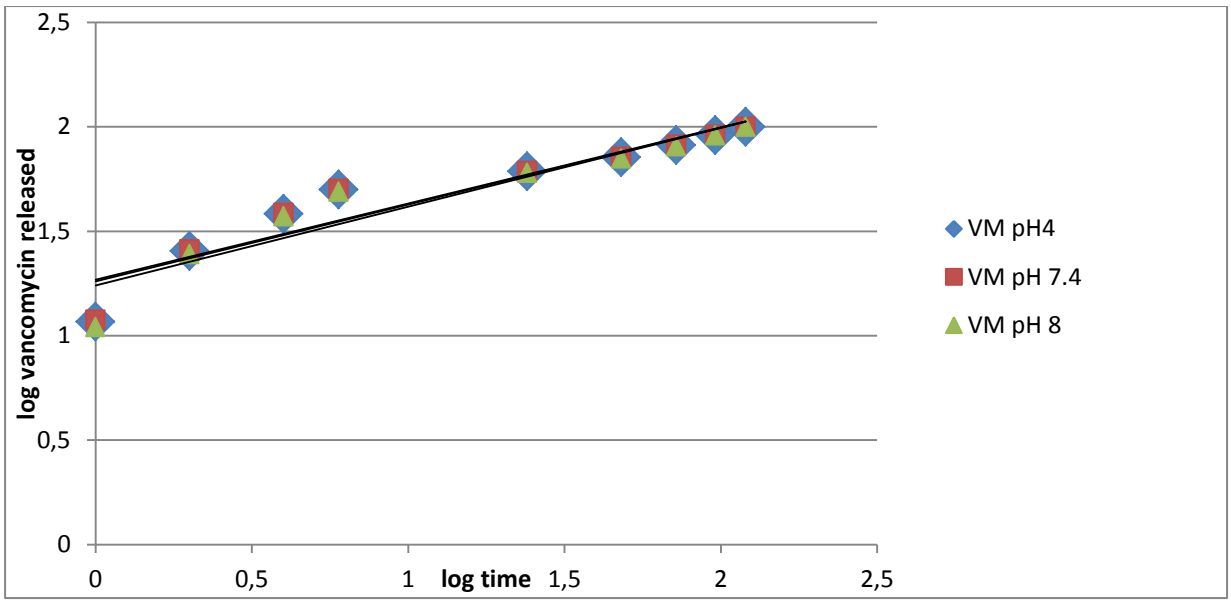

Figure 5. Korsmeyer Peppas model of the vancomycin release from hydroxyapatite in PBS (pH 4, 7.4, 8)

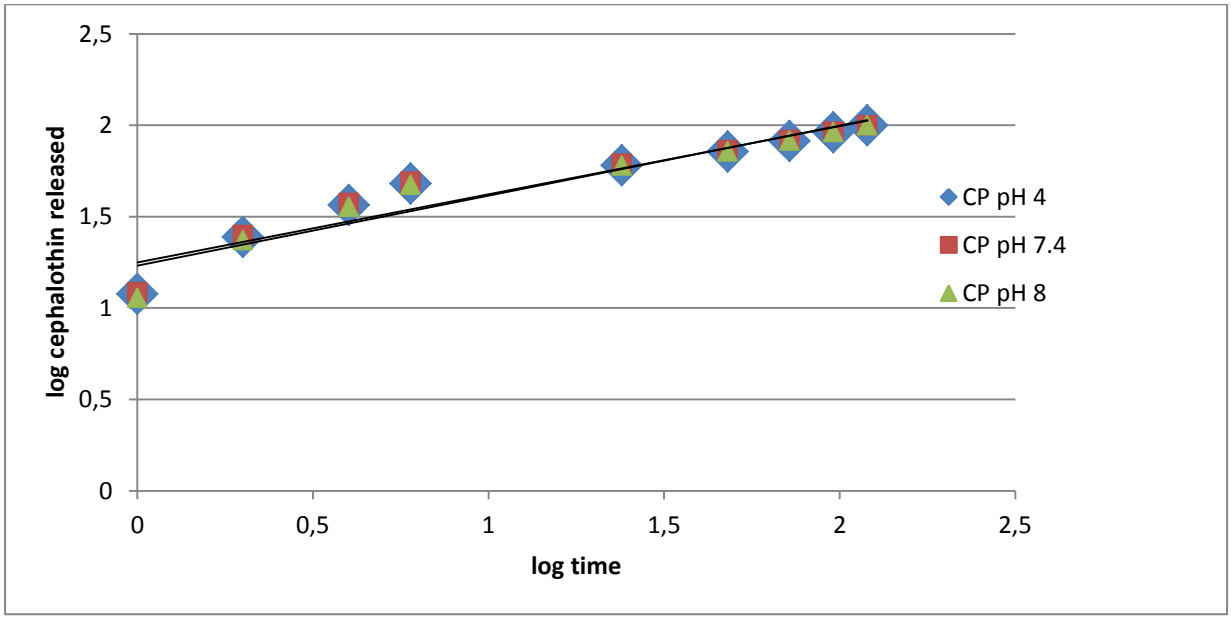

Figure 6. Korsmeyer Peppas model of the cephalothin release from hydroxyapatite in PBS (pH 4, 7.4, 8)

Table 3. Release parameter values for the antibiotics using Korsmeyer Peppas model.

	Vancomycin			**Cephalothin**		
	pH 4	pH 7,4	pH 8	pH 4	pH 7,4	pH 8
R^2	0,8969	0,8972	0,8972	0,9169	0,913	0,9185
n	0,3681	0,3645	0,3775	0,3733	0,3691	0,3833

In figure 7 and figure 8 the Korsmeyer Peppas models for the release of strontium from vancomycin and cephalothin loaded hydroxyapatite particles respectively are shown. Table 4 shows the release parameter values for the Korsmeyer Peppas models of strontium release. Similar to the antibiotic release, the predictable strontium release for both the vancomycin loaded particles as well as the cephalothin loaded particles are improved by using a regression line. The diffusion exponent for all of the releases of strontium from vancomycin loaded particles are just over 0.43, and are therefore described as anomalous release. On the other hand, the diffusion exponent from the strontium release from the cephalothin loaded particles are just below 0.43 with the exception of the release profile at pH 4, which is just over 0.43. It could be assumed that release mechanisms would most accurately be described by Fickian diffusion. The exception being the strontium release from the vancomycin loaded hydroxyapatite spheres at pH 4 which is dependent on the diffusion on the spheres therefore having a higher diffusion exponent.

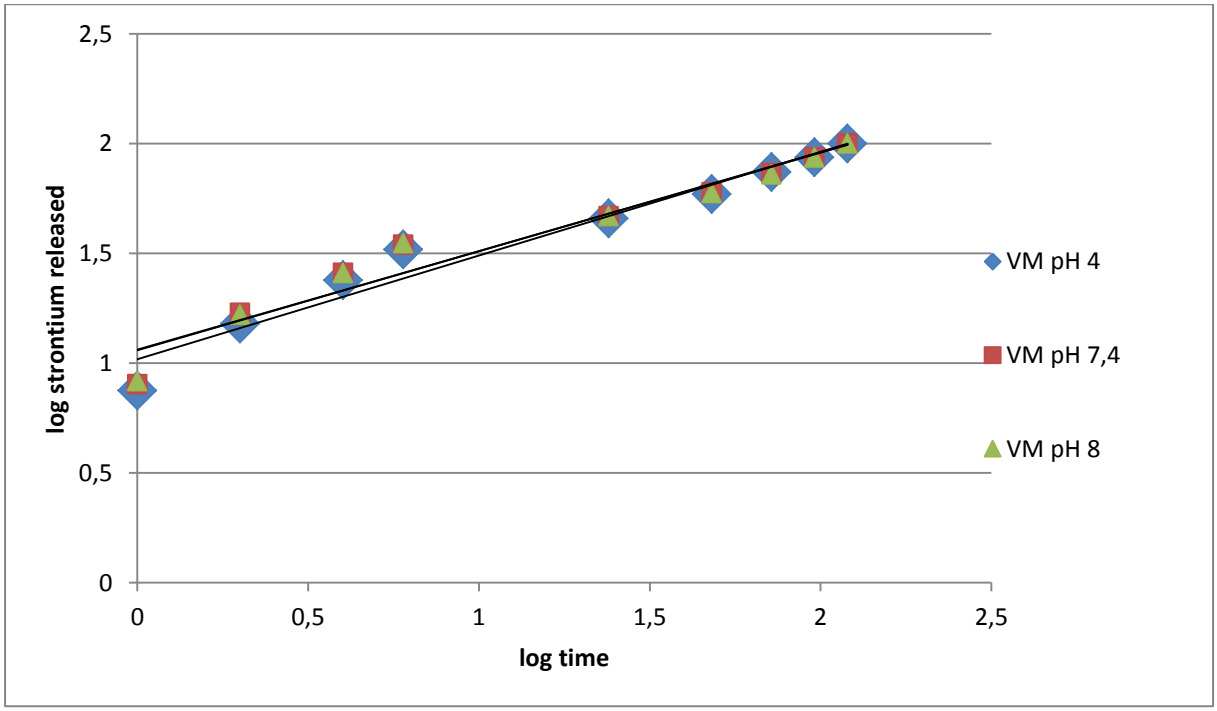

Figure 7. Korsmeyer peppas model of strontium released from vancomycin loaded hydroxyapatite in PBS (pH 4, 7.4, 8).

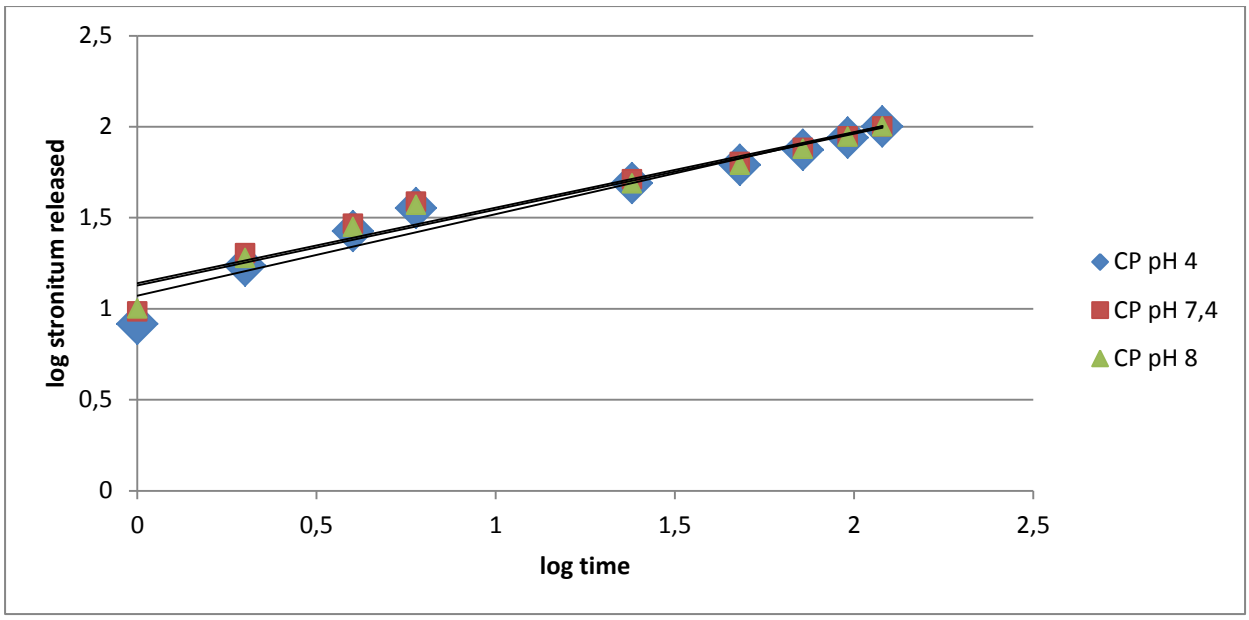

Figure 8. Korsmeyer peppas model of strontium released from cephalothin loaded hydroxyapatite in PBS (pH 4, 7.4, 8).

Table 4. Release parameter values for the Korsmeyer Peppas models of strontium release.

	Sr-Cephalothin			**Sr-Vancomycin**		
	pH 4	pH 7.4	pH 8	pH 4	pH 7.4	pH 8
R^2	0,9488	0,9448	0,9572	0,9588	0,9507	0,9521
n	0,4476	0,4157	0,4165	0,4724	0,4513	0,4481

3.2. Release from PMMA composites

Figure 9 shows the result for the vancomycin release from PMMA composite group 2 (PMMA + Vancomycin (2.5 wt %)). A burst release occurred for the first 24 hours which was a bit lower than the release from the hydroxyapatite spheres (fig 1.). However, the next couple of hours the release increases much more when compared to the spheres.

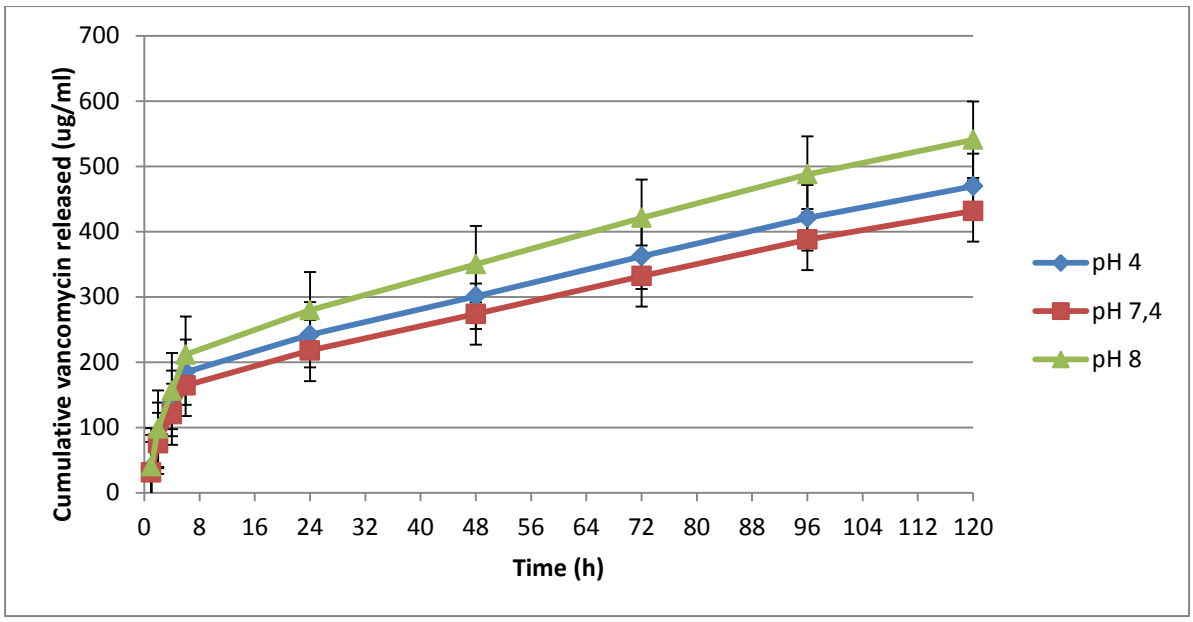

Figure 9. Vancomycin release profiles for PMMA composite group 2 in PBS (pH 4, 7.4 and 8).

The PMMA composite group 3 (PMMA + Vancomycin loaded hydroxyapatite (10 wt %)) had a low burst release and a continued stable slow release the following hours compared to the release profiles of the hydroxyapatite and the other PMMA composite groups. Moreover, the estimated amount of vancomycin in group 3 was about 50 % higher than the other groups which would indicate that loading vancomycin into hydroxyapatite spheres affected the release rate to be slower. The release profiles do not differ much between the different PBS pH values. The release profiles are shown in figure 10.

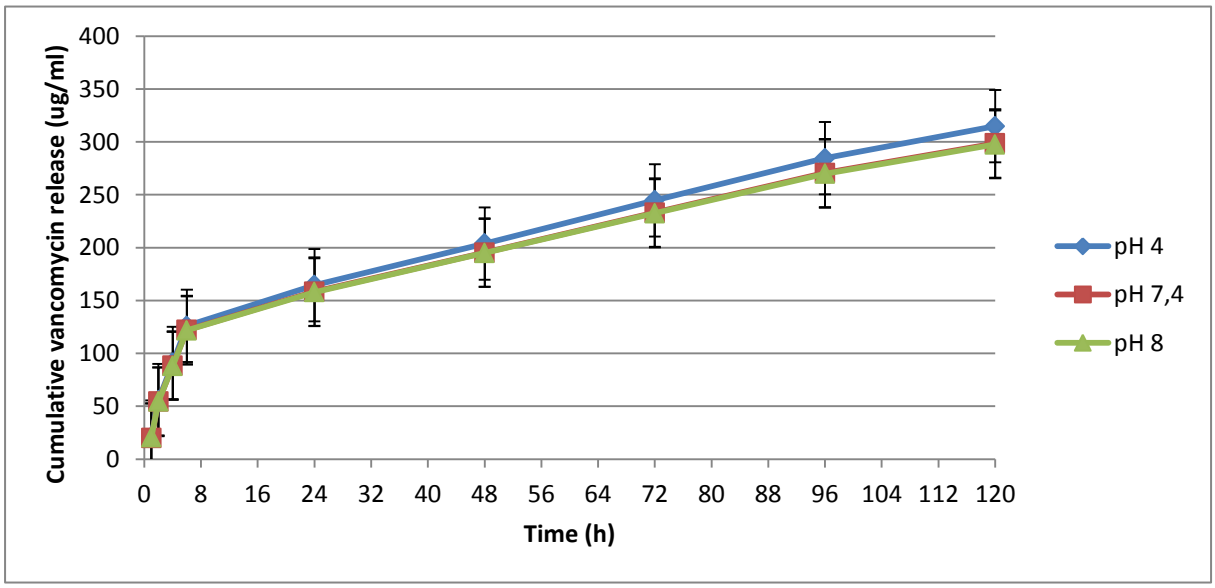

Figure 10. Vancomycin release profiles for PMMA composite group 3 in PBS (pH 4, 7.4 and 8).

The release profiles for PMMA composite group 4 (PMMA + Hydroxyapatite (10 wt %) + Vancomycin (2.5 wt %)), shown in figure 11, are similar to those of PMMA composite group 2. A relative low burst release which then had a high increase of release in the proceeding time. The vancomycin release is slightly higher than the other PMMA groups.

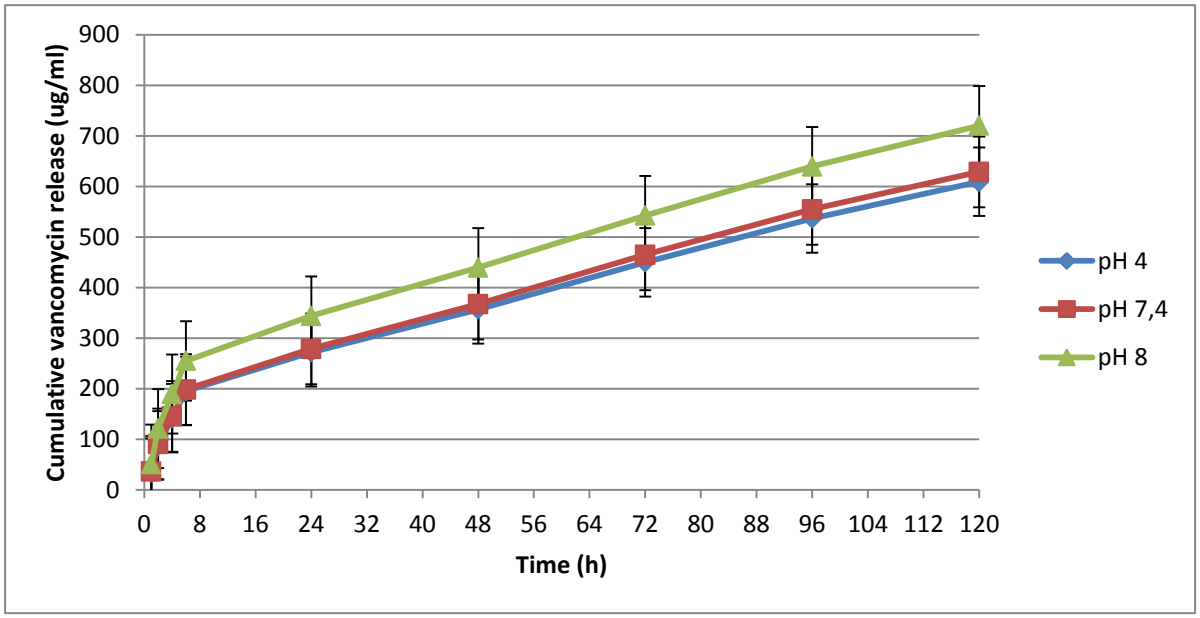

Figure 11. Vancomycin release profiles for PMMA composite group 4 in PBS (pH 4, 7.4 and 8).

In table 5 the amount of vancomycin released within the first 24 hours from PMMA composite group 2, 3 and 4. This amount gives an estimation of much of the antibiotic was on the outer surface of the PMMA.

Table 5. Amount of vancomycin releasedwithin the first 24 hours from PMMA composite groups 2, 3 and 4.

Group 2			**Group 3**			**Group 4**		
pH 4	pH 7.4	pH 8	pH 4	pH 7.4	pH 8	pH 4	pH 7.4	pH 8
1.145 mg	1.073 mg	1.360 mg	0.771 mg	0.724 mg	0.722 mg	1.539 mg	1.608 mg	1.780 mg

The Korsmeyer Peppas model was implemented to describe the release mechanisms for the vancomycin release from the PMMA groups. The release parameter values are summarized in table 6.

Table 6. Release parameter values for the vancomycin release from the PMMA composite groups.

	Group 2			**Group 3**			**Group 4**		
	pH 4	pH 7,4	pH 8	pH 4	pH 7,4	pH 8	pH 4	pH 7,4	pH 8
R^2	0,9284	0,9243	0,922	0,9085	0,9017	0,9048	0,9376	0,9419	0,9364
n	0,4406	0,4625	0,4552	0,4669	0,4637	0,4609	0,5108	0,5086	0,4684

Figure 12, 13 and 14 show the Korsmeyer Peppas model for the PMMA composite groups 2, 3 and 4. All of the profiles have release mechanisms that should be classified as non-Fickian diffusion. However, the diffusion exponents are rather close to the value of 0.43 which indicates Fickian diffusion, especially for the release profile of group 2 at pH 4 while the release profiles for group 4 tend to be more anomalous than the others and should be interpreted that the diffusion is more dependent on the diffusion and degradation of the hydroxyapatite spheres.

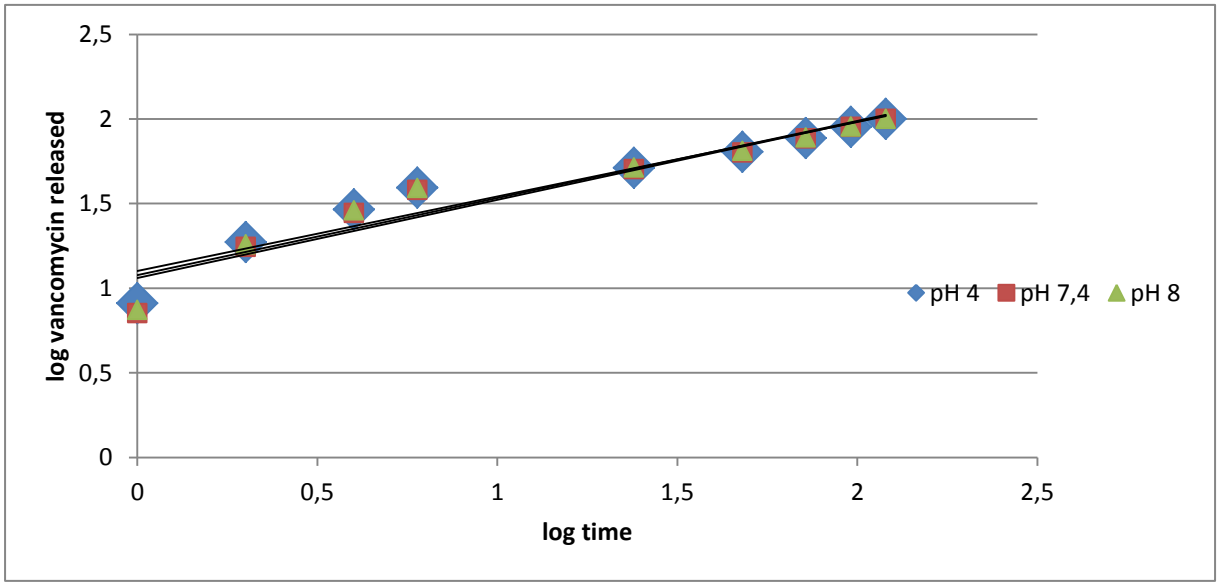

Figure 12. Korsmeyer Peppas profiles for the vancomycin release from PMMA group 2 in PBS (pH 4, 7.4 and 8)

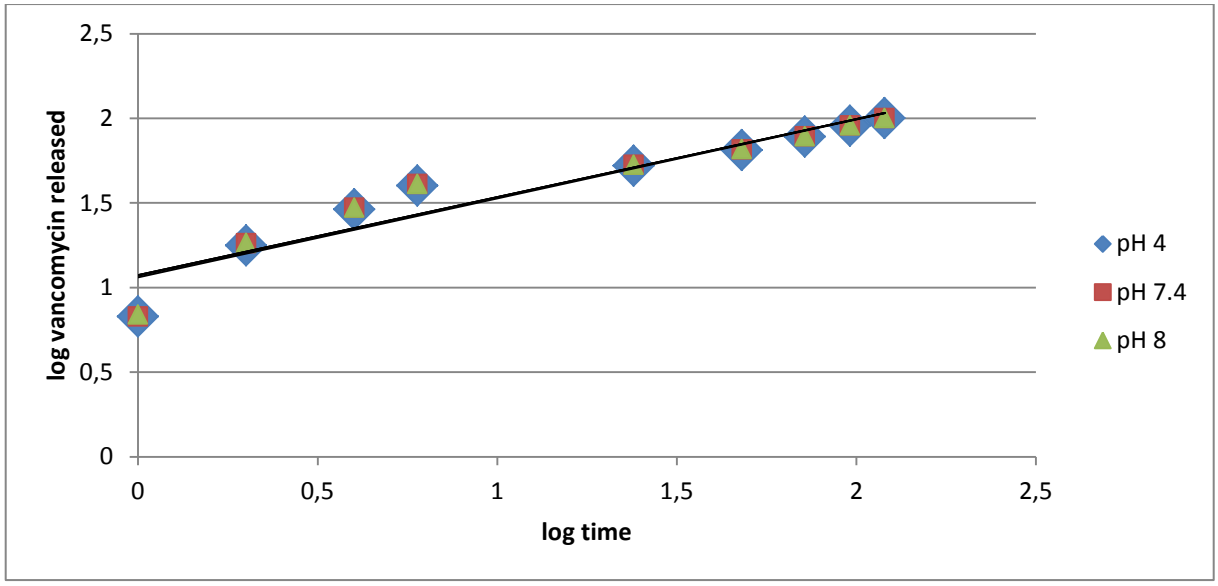

Figure 13. Korsmeyer Peppas profiles for the vancomycin release from PMMA group 3 in PBS (pH 4, 7.4 and 8)

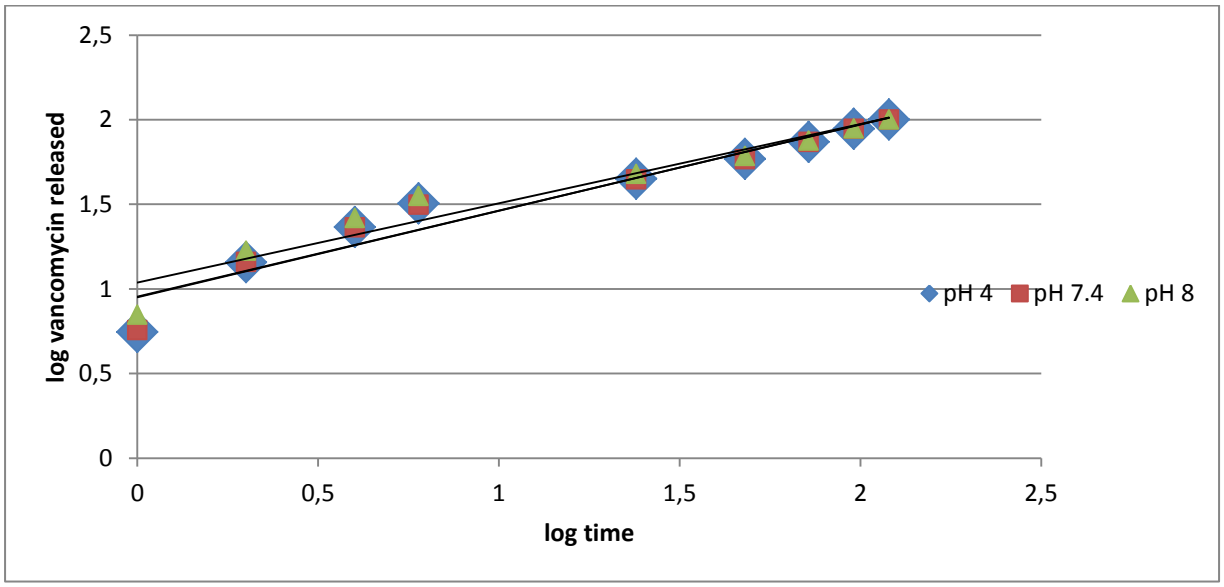

Figure 14. Korsmeyer Peppas profiles for the vancomycin release from PMMA group 4 in PBS (pH 4, 7.4 and 8)

In figure 15 the strontium release from PMMA composite group 1 is shown and the strontium release for group 3 and 4 are shown in figure 16 and 17. The release rate of group 1 is similar to group 4, whereas the release rate for group 3 is lower than the other two which it also had for the vancomycin release when compared to the other PMMA composite groups. The release medium had a similar effect on all the three PMMA composite groups in which the more acidic solution gave a higher release rate. Furthermore, all the three PMMA composite groups had, just as the strontium release

profiles of the hydroxyapatite spheres, an initial burst release the first 24 hours and then a steady release the in the subsequent hours. However, when comparing the release of the composite groups with the release from the hydroxyapatite spheres it can be seen that the rate is much lower for the composite groups. In 96 hours the cumulative strontium release of the vancomycin loaded hydroxyapatite spheres at pH four was over 140 ppm whereas for the composite group 4 it took 21 days in PBS at pH four to have a cumulative release of 140 ppm and the other groups had not released as much after the same time period.

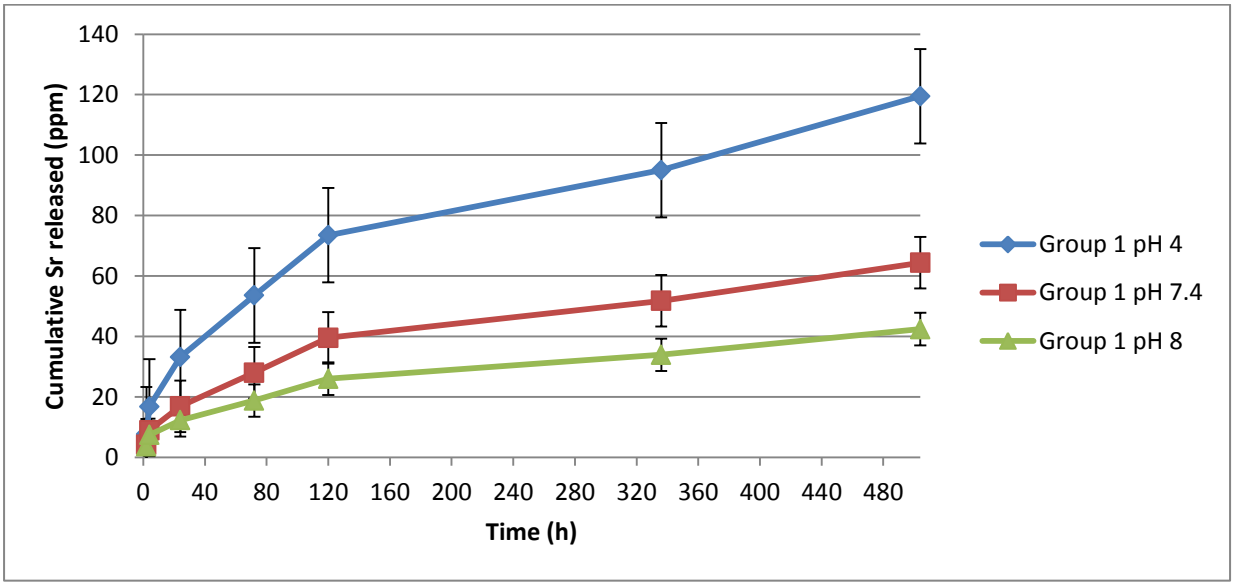

Figure 15. Strontium release from group 1 in PBS (pH 4, 7.4 and 8)

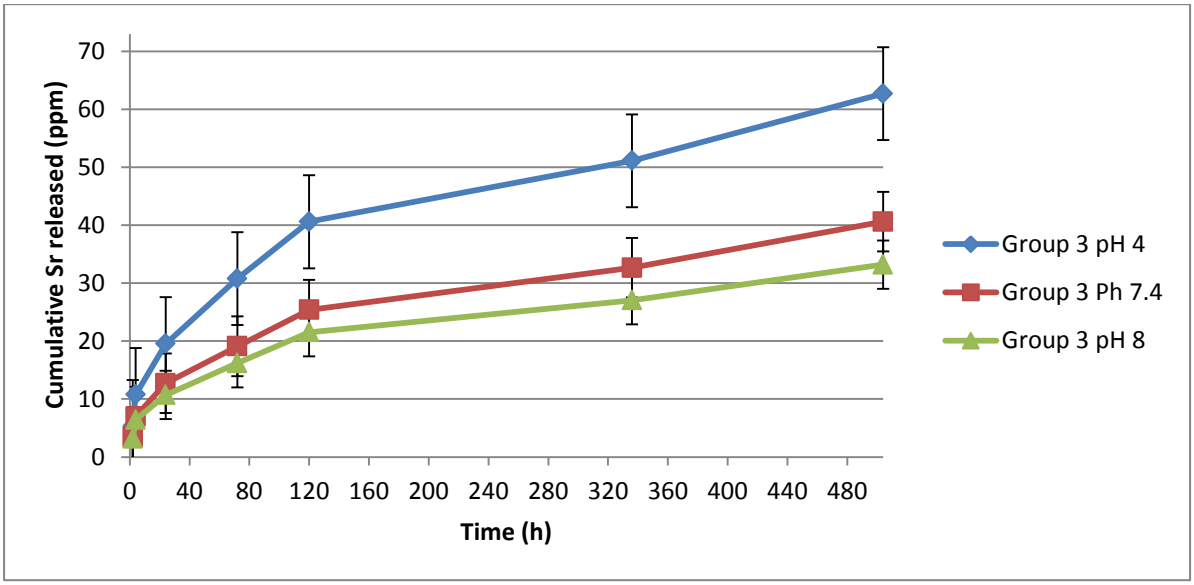

Figure 16. Strontium release from group 3 in PBS (pH 4, 7.4 and 8)

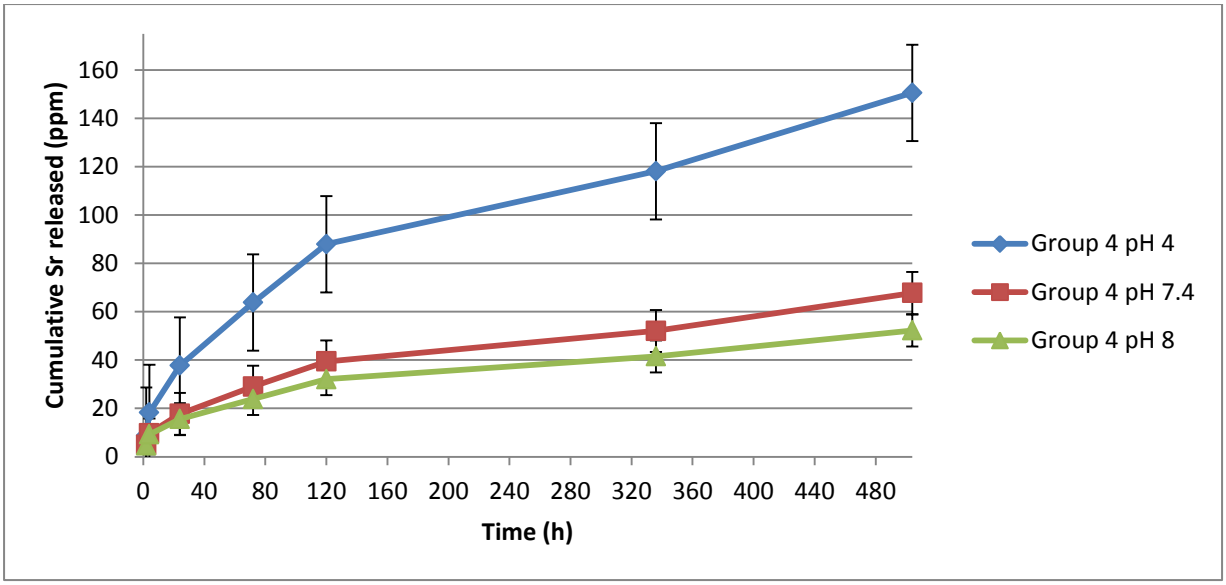

Figure 17. Strontium release from group 4 in PBS (pH 4, 7.4 and 8)

The Korsmeyer Peppas model release parameters for the strontium releases from the PMMA composite groups are shown in table 7. From the diffusion exponents it can be seen that for group 1 and 4 the release mechanism at pH four and 7.4 was controlled by the diffusion of the hydroxyapatite spheres but at pH 8 the release mechanism was controlled by Fickian diffusion. Group 3, which consisted of PMMA and vancomycin loaded hydroxyapatite, has values under 0.43 which would indicate that the release mechanism is best described as Fickian diffusion, whereas the vancomycin release for the same group turned out to be non-Fickian diffusion. The Korsmeyer Peppas profiles for the PMMA composite groups are shown in figure 18, 19 and 20.

Table 7. Release parameter values for the strontium release from PMMA composite group 1, 3 and 4.

	Group 1			**Group 3**			**Group 4**		
	pH 4	pH 7.4	pH 8	pH 4	pH 7.4	pH 8	pH 4	pH 7.4	pH 8
R^2	0.979	0.9758	0.9776	0.9756	0.9767	0.9729	0.9836	0.9837	0.9794
n	0.4648	0.463	0.4092	0.417	0.4128	0.3922	0.485	0.443	0.4032

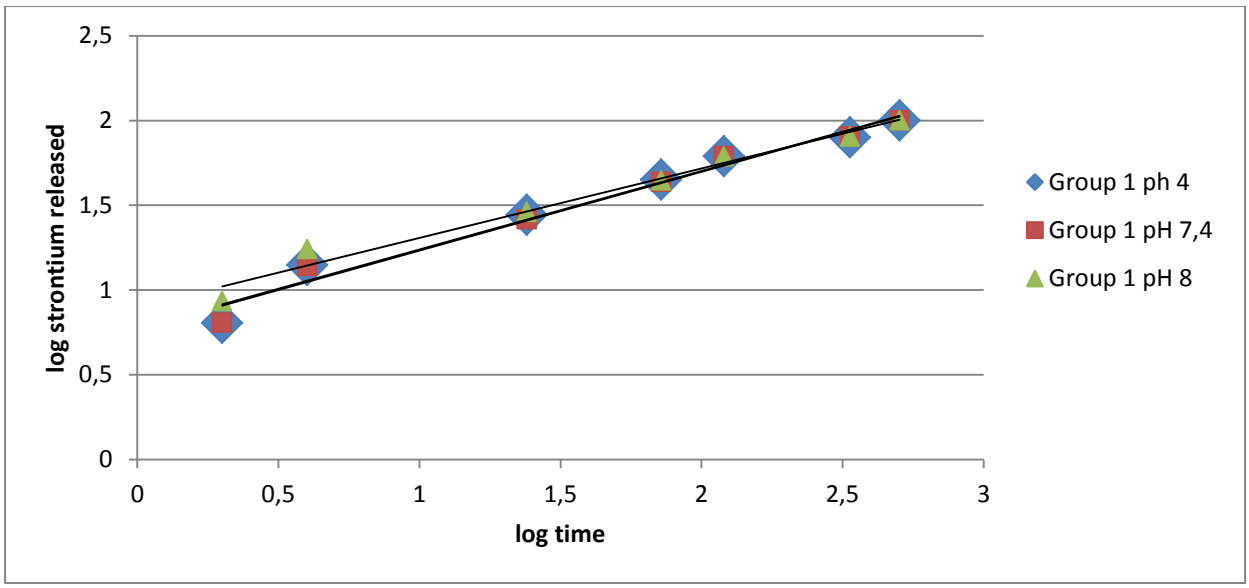

Figure 18. Korsmeyer Peppas model of the strontium release from group 1 in PBS (pH 4, 7.4 and 8)

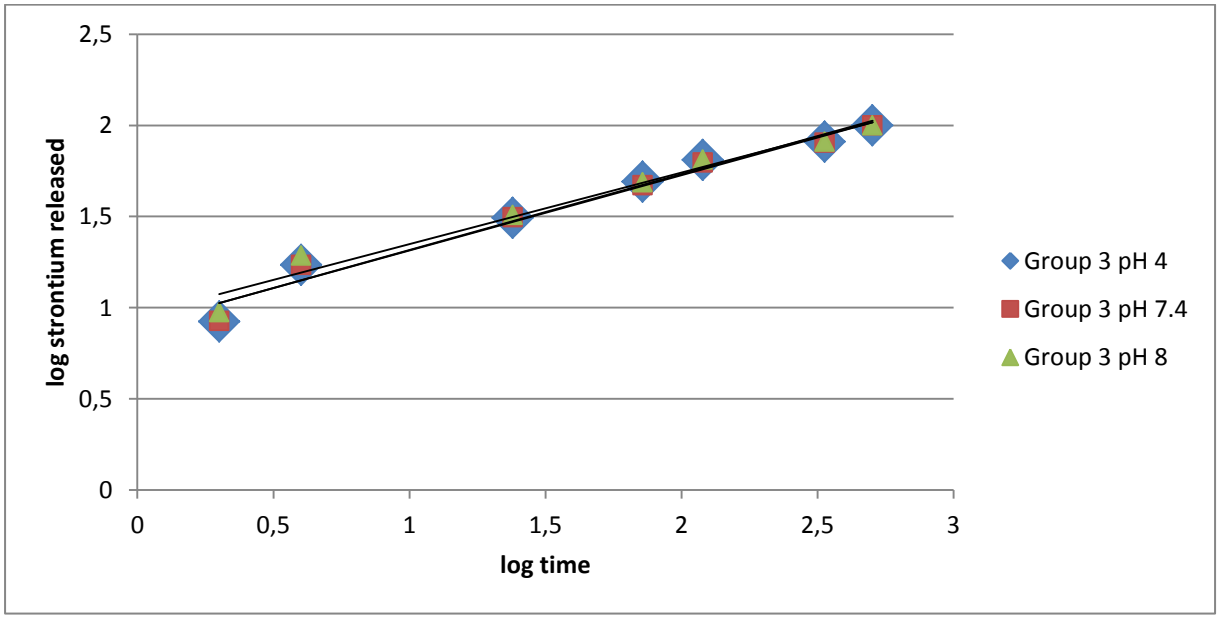

Figure 19. Korsmeyer Peppas model of the strontium release from group 3 in PBS (pH 4, 7.4 and 8)

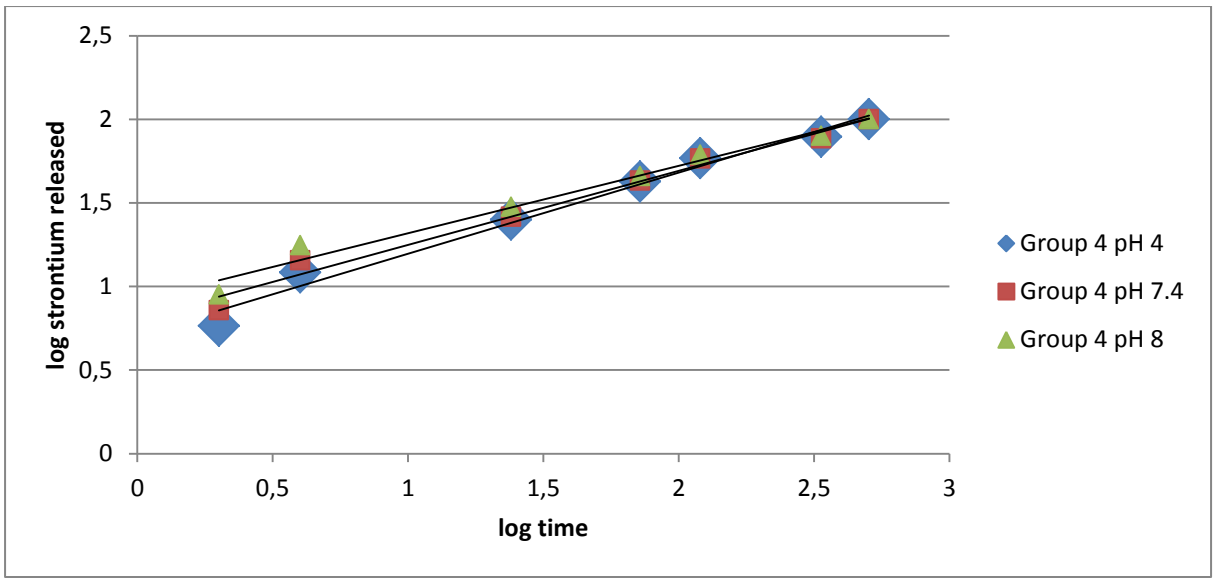

Figure 20. Korsmeyer Peppas model of the strontium release from group 4 in PBS (pH 4, 7.4 and 8)

4. Conclusion

Hydroxyapatite spheres containing strontium were prepared for in vitro release study. The hollow spheres were found to have a high loading capacity for the antibiotics vancomycin and cephalothin. The loading capacity was approximately 0.37 mg antibiotics per mg hydroxyapatite. The antibiotics vancomycin and cephalothin were loaded separately into hydroxyapatite spheres and the in vitro release under different pH values for the release medium PBS was evaluated. All of the releases showed the expected burst release followed by a slow release. The amount of antibiotic released during the burst release was about 5 % of the loaded amount for the vancomycin and 3 % for the cephalothin. The pH value of the release medium had influence on the release rate to some extent for the antibiotic release and the acidic solution had a more significant impact on the strontium release. The cephalothin loaded spheres had higher release rate than the vanomycin loaded spheres in both antibiotic and strontium release rate. The release mechanism for hydroxyapatite spheres was found to be best explained by Fickian diffusion.

Four hydroxyapatite-PMMA composite groups' antibiotic and strontium release were studied. Group 3 consisting of PMMA and vancomycin loaded hydroxyapatite (10 wt %) had the lowest vancomycin release rate and the lowest strontium release rate of all the groups. However, it was also the group that was estimated to be most loaded with vancomycin

suggesting that loading vancomycin into hydroxyapatite spheres affected the release rates. Group 4 (PMMA + Hydroxyapatite (10 wt %) + Vancomycin (2.5 wt %)) had the highest rates both in vancomycin release and strontium release. The influence of the release medium was similar to the influence on the hydroxyapatite spheres which is to say that an acidic environment gave a higher release rate. The release mechanism for the vancomycin release from hydroxyapatite-PMMA composite groups showed signs that could best be explained by the surface phenomenon of the hydroxyapatite. The strontium release showed similar signs with the exception of PMMA composite group 3 which were controlled by Fickian diffusion. All of the composite groups had a much lower strontium release rate than the strontium release from the hydroxyapatite spheres. However, group 2 and group 4 had similar or higher vancomycin release rate than the ones from the hydroxyapatite spheres.

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