Composition influence over delivery ability of hydroxyapatite cements

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Palabras clave: hidroxiapatita, cementos, sistemas de liberación controlada de fármacos, cefazolina, ciprofloxacina.
Key words: hydroxyapatite, cements, drugs controlled delivery systems, cefazolin, ciprofloxacine.

RESUMEN. Se prepararon cementos de hidroxiapatita divididos en dos partes, una sólida compuesta por una mezcla de hidroxiapatita, yeso y dos polímeros, alginato de sodio y poli-*N*-vinil-2-pirrolidona, y una parte líquida conformada en este caso por una mezcla de sulfato y fosfato de potasio en solución acuosa a una concentración específica. Todas las formulaciones fueron dopadas con cefazolina y ciprofloxacina y se estudió la cinética de liberación de los fármacos en regulador fosfato. Los resultados se ajustan al modelo de Higuchi regulado por la difusión en dos etapas. Se determinó la influencia de los contenidos de yeso y de inhibidor sobre la cantidad total de antibiótico liberada que osciló, en dependencia del tipo de fármaco y de la formulación entre un 10 y un 50 % a la semana de iniciado el estudio, alterando de manera inversa la cantidad de yeso y de forma directa la cantidad de inhibidor, sobre la cantidad total de fármaco liberada, en ambos casos, por la competencia de las reacciones de fraguado entre el fosfato de potasio y el yeso y la del alginato de sodio con el segundo. Se discute y demuestra la no existencia de diferencias entre la liberación cuando se habla de ciprofloxacina y lo contrario, si es el caso de la cefazolina. Estos resultados permiten valorar la posibilidad real de la obtención de un cemento de hidroxiapatita de producción nacional, similar a los ofertados hoy día en el mercado internacional y dotar al sistema de salud cubano de una formulación que puede ser un potencial rubro exportable.

ABSTRACT. Hydroxyapatite cements divided in two parts, a solid part composed by a mixture of hydroxyapatite, gypsum and two polymers, sodium alginate and poly-*N*-vinyl-2-pyrrolidone and a liquid part formed by a mixture of salts, potassium sulphate and potassium phosphate in water solution, were prepared. All the formulations were doped with cefazolin and ciprofloxacine. The kinetics of drug delivery in buffer phosphate was studied. The results were adjusted to the Higuchi model regulated by the diffusion in two steps, conditioned by the drug exposed at the sample surface in first place, and a second step oriented by the diffusion through the matrix until to achieve the surface and from these one to the solution. One week after initiate the study, the influence of the gypsum and inhibitors contents, on the liberated antibiotic quantity was determined, oscillating between 10-50 %, depends on drug type and the formulation. The inverse effect of gypsum quantity, and direct effect of the inhibitor quantity, on the liberated drug, occurs in both cases, for the reactions competition that have between the gypsum with potassium phosphate and sodium alginate. In other side, the authors discussed and probed it that no exist differences between drug delivery when studies the accelerator/inhibitor relationship in case of ciprofloxacine and the contrary if it is talking about cefazolin. These results allow to value the real possibility to obtain a hydroxyapatite cement of national production, similar to the offered today in the international market and to endow to Cuban Health System of a formulation that can be a potential exportable item.

INTRODUCTION

Nowadays, it's frequent the use of surgical cements with resins, specifically for the prostheses fixation to the bone structure in surgery and to form substitute elements in losses or cranial bone defects in neurosurgery. This surgical material is presented in two parts, one solid and one liquid part that acquires the necessary properties to achieve the implantation when mixes to room temperature.[1] The ideal material for the bone substitution should imitate to tissue that replaces in size, forms, consistency and operation, it should not cause infections neither to cause rejection answers; should be tolerated permanently by the receiving organism; in other words, it should be biocompatible.[2]

The calcium phosphates cements are great utility in bone restorations. It has been demonstrated that these materials, besides being used as dental cements, it can be used as materials of bone implants. Contrary to the bioceramics or other rigid materials, cements implant can be adjusted to the defect filling cause they don't possess a previous form. As any biomaterial the cements have requirements such as biocompatibility, absence of toxicity or carcinogenic effects of the material or their metabolites, it should be also in intimate contact with the hostage bone as well as an appropriate mechanical resistance. They have to be able to setting and harden in controlled times.[3]

In the last decade, hydroxyapatite composites with antibiotic that liberate it with speed controlled were prepared. They had good mechanical properties.[4] Them, in spite of the kindness before mentioned, they present problems with the heat generation and the monomer residual percent during the polymerization, which it can be, a toxicity focus for the receiving organism of the implants. The hydroxyapatite cements have also been used with these purposes, but some difficulties related with the manipulation and their mechanical properties have restricted their employ and their possible utilization as support matrix of antibiotics.[4,5]

For these reasons, the authors studies the preparation of new hydroxyapatite materials that combined in only one, the best of the composites and the calcium phosphates cements but that they had as significant, those properties that are reported as difficulties in the other ones; that is, toxicity absence for heat generation or monomeric residuals, good and easy manipulation, and ability to antibiotic controlled delivery an antibiotic in the surgical place.[6,7]

The purposes of this work were the formulation of cements based on hydroxyapatite and primes matters of easy obtaining and wide use like sodium alginate, poly-*N*-vinyl-2-pyrrolidone and gypsum. All this for the study of influence of different factors related with composition such as the gypsum and inhibitor concentrations, and the accelerating/inhibitor relationship in the drug liberation over the process of drug delivery.

MATERIALS AND METHODS

Reagents

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and sodium alginate (NaAlg) were obtained in BIOMAT according to own procedures. First case, the HAP with a $R_{Ca/P}$ = 1,68 and a size of particles aggregates below the 160 μm[8,9] and second, the NaAlg, is a natural polymer obtained from the algae arrivals to the Cuban coasts with η = 181,5 cps to pH = 7,5. Gypsum ($CaSO_4 \cdot 2H_2O$) comes from Riedel de Haen. The poly-*N*-vinyl-2-pyrrolidone (PVP) is a synthetic polymer lineal type K90 with average molecular mass 360 000 from Merck. The potassium sulfate and potassium phosphate, came both of Reachim. All the reagents of knowing commercial signatures were used without previous purification. The cement were prepared as shown in figure 1.

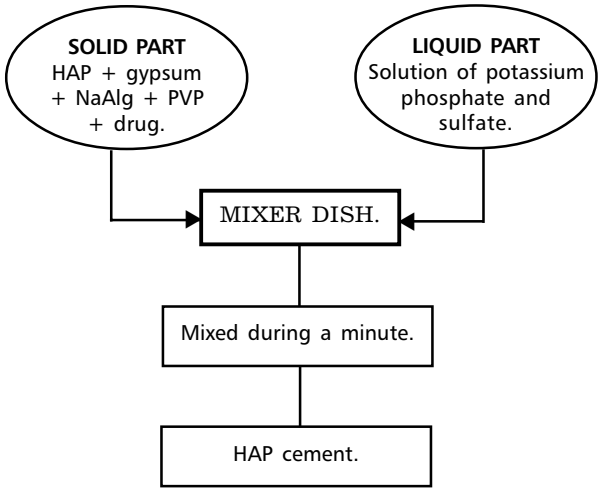

Fig. 1. *Preparation scheme of HAP + gypsum + polymers cements.*

Kinetic studies of drug delivery

Cefazolin and ciprofloxacine were dried off previously for 5 h in P_2O_5. To obtain the calibration curve, a pattern solution of well-known concentration was prepared which should be permanently protected of the light. It took aliquot to obtain patterns of 0, 10, 20, 30, 40, 50 ppm for the cefazolin and 0, 3, 6, 9, 12, 15 ppm for the ciprofloxacine, always completing with phosphate buffer pH = 7,4.

The *in vitro* kinetic studies of the antibiotics liberation from the prepared cements were carried out putting the pill of 1 g with 20 mg of drug in 10 mL of phosphate buffer, in a glass tube with tightly closed cover during the whole kinetic study. The glass tube is introduced in a thermostat to (37 ± 1) °C . They are carried out extractions of the whole added buffer each half hour until the first four hours, later each one hour until 8 h and every day until completing the week. Once extracted the 10 buffer mL containing the dissolved antibiotic, 10 mL of fresh buffer is added and put the tube again in the thermostat.

The calibration curve and the samples were read in quartz recipients of 1 cm optic pass to 262 nm for the cefazolin and 271 nm for the ciprofloxacine in UV-Visible spectrophotometer.[10] The studies of the composition influence of liquid part were made varying from 55-45 up to 70-30 % the relationship K_2SO_4/K_3PO_4. When the R symbol is used, it's refers to the blocks where is varied the percentage of K_3PO_4, in the case of Y, the percentage of gypsum, when it's varied X refers to blocks doped with ciprofloxacine and the Z, with cefazolin. The numbers refer to the percents of the variables that are varied.

RESULTS AND DISCUSSION

It can note with clarity that a marked influence of the inhibitor quantity exists in the drug liberation. In the way that it is increased inhibitor quantity, increase the drug liberation (Fig. 2). This could be due to that, the more inhibitor is in the mixture more it delays this in setting and of course more easier it's to the medication leave the matrix for diffusion through the channels that are believed in the cements.[3]

In the case of the figure 3 as much as minor is the gypsum quantity more drug is liberated. That which is expected if it is analyzed that gypsum is the responsible in a large part of the material setting when contributing the ions Ca^{2+}, necessary for the alginate reticulation (Equations 1 and 2).

$$2\,K_3PO_4 + 3\,CaSO_4 = 3\,K_2SO_4 + Ca_3(PO_4)_2 \quad \textit{Equation 1}$$

$$CaSO_4 + 2\,Na(Alg) = Ca(Alg)_2 + Na_2SO_4 \quad \textit{Equation 2}$$

In a same way, it was carried out a study using the Higuchi equation for the first steps of liberation being that it adjusted perfectly to the equation 3,

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{D \cdot t}{\pi \cdot l^2}}$$

employee when

$$0 < \frac{M_t}{M_\infty} < 0{,}6 \quad \text{(Fig. 4).}$$

For the superior steps the same treatment was also applied, being proven the compliment of this relationship with very good adjustment coefficients the same as in the first 8 h (Fig. 5). These results make us still think of the diffusion like the process that it governs the whole liberation, in the two stages.

This division can be considering logic, if we keep in mind that to the beginning, the drug that is in the blocks surface spreads to the solution. But in second stage, the drug diffuses inside the matrix, first from the center of the cylindrical blocks toward the surface and after this toward the solution buffer. In Table 1 the adjustment results by square least of the samples wrapped in the Higuchi study are presented. It is interesting to remark that the entirety of the adjustments is very appropriate because all the values of the correlation coefficients are bigger than the corresponding $r_{crit.}$ for a = 0.999. In the case of the RX samples, $r_{crit.}(0.999,10) = 0.823$, as long as for the YZ samples, $r_{crit.}(0.999,4) = 0.974$.

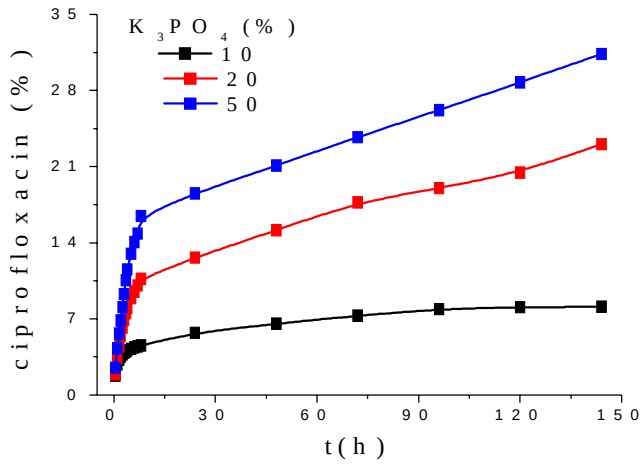

***Fig. 2**. Liberation profile varying inhibitor percentage.*

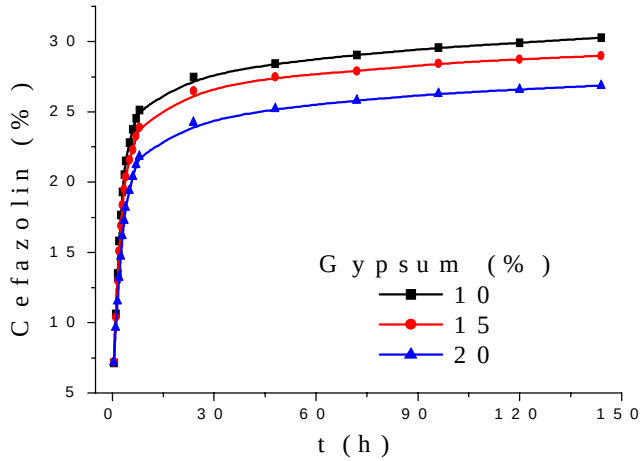

***Fig. 3.** Liberation profile varying gypsum percentage.*

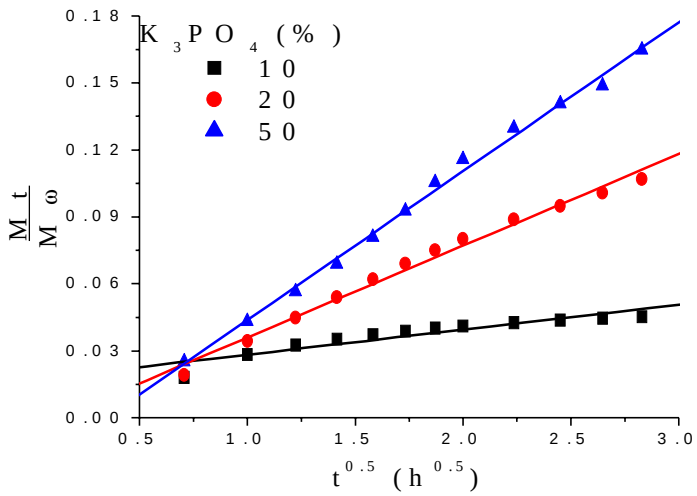

***Fig. 4**. Higuchi treatment for the first 8 h, varying inhibitor percentage.*

Note, taking into account both stages, that the delivery ability of the formulations is more over than the necessary stocking in the surgical place to avoid the bacterial proliferation (0,76 ppm for the case of cefazolin[11] and 0.36 ppm for ciprofloxacine[12]).

For example, the smaller capacity sample is able to liberate 1 ppm every 24 h, bigger quantity than the one reported in the literature, in the ciprofloxacine case. While in the cefazolin case, the difference is still accented, because it ends up reaching between 5 and 40 ppm daily when it becomes smaller the liberation to the 7 d of having begun the study. This is clearly observes in the tables 2 and 3, where the liberation profiles are shown in the last stages of the process that is at least it is liberated.

It is prominent also the marked difference that it exists among the liberation ranges respect to the drug type and varied component, being bigger the differences and the liberation when one works with cefazolin and inhibitor. In other hand, when one works with ciprofloxacine (it structures more compact and less more soluble) and the gypsum percent. This facts could be pointed out the drug solubility as a fundamental factor when it is analyzed the composition influence. The total quantity of liberated drug has also been possible to increase with regard to previous works[12] until 50 %, just as shown in the table 4.

It is exactly to delimit that these systems didn't turn out to be good controlled delivery systems, because only they reach 50 % after the week, but this it is a first system that authors evaluate. In later works the influence of other factors and the possibility of their employment, as controllers of the supply of drugs in bone lesions will be analyzed, where at the same time they are used as reparative of traumas.

CONCLUSIONS

Several formulations of hydroxyapatite, gypsum and polymers

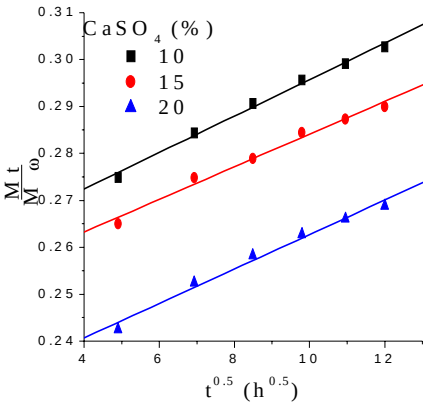

Fig. 5. *Higuchi treatment for the final stage, varying inhibitor percentage.*

cements were obtained and the inverse relationship of the gypsum and direct influence of inhibitor concentration was determined, both cases over drugs controlled liberation. It was proven that the liberation process in these cements fulfills the Higuchi pattern in all its stage what indicates that all is controlled by the diffusion. It was demonstrated that all the formulations maintain an appropriate profile to be used as controlled liberation matrix, cause they liberate bigger drug quantities daily than the necessary ones to maintain the surgical place free of infections. At the end of the study, one week, they liberated an average that oscillates between a 10 and 50 % of the contained active principle in them depending on the formulation type used as matrix and the drug type.

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Table 1. Statisticals results of adjustments by square least method of Higuchi study data.

Sample	A ± ΔA	B ± ΔB	r
RX1	0.017 ± 0.003	0.011 ± 0.001	0.930 20
RX2	–0.005 ± 0.002	0.041 ± 0.001	0.994 44
RX5	–0.023 ± 0003	0.066 ± 0.001	0.997 88
YZ10	0.257 ± 0.001	0.003 9 ± 0.000 2	0.997 00
YZ15	0.249 ± 0.002	0.003 5 ± 0.000 2	0.992 83
YZ20	0.226 ± 0.002	0.003 7 ± 0.000 2	0.993 23

Table 2. Drug liberation (ppm) every 24 h varying inhibitor or gypsum percentage.

Time (h)	RX10	RX25	RX50	YZ10	YZ15	YZ20
24	114.32	252.62	370.57	549.85	530.08	485.10
48	131.53	303.11	422.29	568.80	549.64	504.86
72	145.73	354.65	473.78	581.29	557.79	516.52
96	157.79	380.12	523.44	591.26	568.96	525.49
120	161.41	409.42	575.22	598.21	574.69	532.11
144	162.66	461.14	626.85	605.45	579.94	537.68

Table 3. Drug liberation (ppm) every 24 h varying the K_2SO_4/K_3PO_4 relationship.

Time (h)	X55	X60	X65	X70	Z55	Z60	Z65	Z7
24	189.46	183.04	180.49	195.85	411.57	588.18	625.15	510.42
48	201.79	197.4	193.48	208.47	469.35	687.47	743.31	622.78
72	208.85	204.41	200.44	213.83	509.83	776.42	858.17	726.49
96	215.34	209.92	205.28	219.19	573.49	893.53	972.18	866.12
120	218.31	212.34	207.05	221.61	609.5	954.55	1 027.05	958.50
144	219.65	213.56	208.54	223.48	627.69	987.41	1 059.07	1 011.63

Table 4. Liberated drug at the ends of study for all formulations.

Formulation	Liberated mass (mg)	Liberation (%)
R10	1.65	8.25
R25	4.61	23.05
R50	6.27	31.35
Y10	6.05	30.25
Y15	5.79	28.95
Y20	5.38	26.90
X55	2.20	11.00
X60	2.14	10.70
X65	2.09	10.45
X70	2.23	11.15
Z55	6.28	31.40
Z60	9.87	49.35
Z65	10.59	52.95
Z70	10.11	50.55