

INFLUENCE OF THE COMPOSITION ON SETTING TIME AND POROSITY IN HYDROXYAPATITE CEMENTS WITH ALGINATE AND CHITOSAN

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Abstract— Twenty formulations of synthetic hydroxyapatite cements (HAC) of two different types are presented. The evaluation of the setting time and the porosity has been done through an experimental design. Type A cements consist in a mixture of hydroxyapatite (HA) and gypsum (GS) as a solid part (P), and a potassium phosphate solution (KP) as a liquid part (L). The sodium alginate (SA) powder was dissolved or in the solid part or in the liquid one. The setting times of the Type A cements vary from 8 to 16 min, which are similar values to report in the literature. The porosity values found (41-43 %) were not influenced by the changes evaluated. The best of type A cements was that prepared with 0.5 wt % KP and dissolving the SA in the solid part. The solid part of type Q cements consist in a mixture of HA - non sintered (ns-HA) or sintered (s-HA)-, a calcium generator -calcium oxide (CaO) or β -tricalcium phosphate (β -TCP)-, and an inorganic material which acts as agglutinant - zinc oxide (ZnO) or magnesium oxide (MgO)-. The liquid phase of type Q cements is formed by an acid solution of chitosan, in malic (MH) or succinic (SH) acid. The setting times observed for these cements are ranged between 2 and 24 min. The use of MgO instead of ZnO as agglutinant decreases the setting time of the cements. The same effect was observed when the succinic acid was substituted by malic acid as dissolution medium of the chitosan. The porosity values for type Q cements vary between 50 and 70 %.

Keywords— hydroxyapatite cements, sodium alginate, chitosan, setting time, porosity, polymers.

I. INTRODUCTION

Calcium hydroxiapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] (HA) is well known as the primary constituent of bone and teeth of animal organisms (Jarcho *et al.*, 1977; Katz and Harper, 1986). Many papers have been published about the use of materials based on HA as bone substitutes in medical and dental treatments. Specially, clinical dental applications include the maintenance of periodontal defects (Ogilvie *et al.*, 1987 and Cranin *et al.*, 1987a), the implantation into tooth extraction sockets to conserve alveolar ridge height (Denissen and de Groot, 1979; Scheer and Boyne, 1987), and the augmentation

of a deficient alveolar ridge to improve denture support and stability (Cranin *et al.*, 1987b; Kent *et al.*, 1986; Larsen *et al.*, 1987 and Rothstein *et al.*, 1984). However, when the implantation of HA in the animal organism is performed, the particles are loose and can migrate beyond the intended regions. Moreover, the stabilization of the material seems to require at least one month. The setting occurs in some extent during this time, with the consequent reduction in the implant size (Kawakami *et al.*, 1992).

The hydraulic cements consist of a powdered mixture able to form a plastic paste upon mixing with water or acid solution, commonly phosphoric acid, and hardening with time as a result of the hydration reactions, giving a new form of calcium phosphate (Lemaitre *et al.*, 1992). The results of these reactions are in some cases calcium deficient hydroxyapatite or dehydrated dicalcium phosphate (Mirtchi *et al.*, 1989). However, the exposure of this paste to blood just after mixing results in the decay of the cement, the situation being similar to that observed for the gypsum. The gypsum sets when is mixed with water; however, the gypsum paste also decays gradually when immersed in water just after mixing. The decay is not a problem when the cement paste is used in areas where there is no liquid in contact with the cement, such as in root filling materials. The problem of decay of the cement limits in any case the potential wide applications of this bioactive material (Ishikawa and Asaoka, 1995).

In order to increase the application of this type of materials new formulations have been recently developed, in which different synthesis parameters have been modified. Thus, the addition of sodium alginate to the liquid part in the preparation of a non-decay calcium phosphate cement of fast setting produces a decrease of the setting time value in a factor of eight (Ishikawa *et al.*, 1995). In other way, the solid to liquid ratio and the pressure applied to prepare the cement disc exert an inverse influence on the porosity, *i.e.*, an increase of the pressure produces a decrease of the diametral tensile strength, caused by a low number of pores (Lemaitre *et al.*, 1992). Other factors, such as the solid to liquid ratio and the zinc oxide concentration exert a direct influence upon the setting time of cements containing chitosan.

Two of the most important factors implied in the choosing of determined cement to be used as an implant material are the setting time and the porosity. The principal requirements are a low setting time (in a range of 5-20 min) and a high porosity (more than 50 % if is possible). In the present study, we have prepared several synthetic hydroxyapatite cements of two different types. Twenty formulations have been synthesized by varying some of the components of the mixture with the objective of study the influence of these variables on setting time and porosity. The sodium alginate and the chitosan were synthesized in the lab. A statistical study of the influence of the composition on the setting time has also been evaluated.

II. METHODS

A. Synthesis and characterization of HA

Hydroxyapatite was obtained by the humid method by reaction of a phosphoric acid solution with a calcium hydroxide suspension up to pH = 7. The crystallinity of the ns-HA and s-HA particles was evaluated by X-Ray diffraction (Seiffert TT-3000), using $\text{Cu}_{K\alpha}$ radiation with a secondary monochromator. Infrared spectrum of HA was recorded in a BOMEM DA3 FTIR. The surface area was measured by BET isotherm in Carlo Erba Instrumentazione. The sintering process was carry out to heating speed 10°C/min until to 1200°C by 2 h in normal atmosphere.

B. Synthesis of cements

The gypsum was supplied by Reachim. The different oxides (magnesium, zinc, and calcium) and the acids (malic and succinic) were provided by BDH Chemicals. The sodium alginate was supplied by Aldrich Chemicals and chitosan by Biotechnology Laboratory of UNAM, Mexico. All the reactants were used as purchased. The HA and β -TCP phosphate was obtains by own procedures in our lab (Table 1).

Type A cements consist of the mixture of a solid part formed by hydroxyapatite (HA) and gypsum (GS) with a liquid part formed by potassium phosphate solution (KP). A study through a factorial experimental design 2^2 was made for these cements, by varying the KP concentration (KP variable, see Table 2) and the medium of dissolution (solid or liquid part, M variable) of the sodium alginate (SA). Thus, the concentration of KP used was 0.5 and 1 w/v % and the sodium alginate (SA) powder was dissolved in the solid part or in the liquid one (M variable, see Table 2) in a SA/HA ratio of 0.5 wt%. In all cases, a sample of 1 g of each powder mixture was kneaded into 1 mL of KP solution for 45 s. Therefore, the solid to liquid (P/L) ratio used was 1.0 w/v % (Table 2).

Type Q cements was synthesized by mixing a solid part formed by HA, a calcium generator, and an agglutinant, with a liquid part (acid solution of chitosan). They were prepared using a factorial experimental design 2^4 (Dowdy and Wearden, 1983) where the variables were the HA type (non sintered and sintered, A

Table 1. Composition of solid (P) and liquid (L) parts

A cements		Q cements	
P	L	P	L
HA	KP	HA (A)	Acid soln (D)
GS	M	Ca ²⁺ generator (B)	
M		Agglutinant (C)	

Table 2. Composition of type A cements

Solid part		Liquid part	
HA	0.95 g	K ₃ PO ₄	0.005/0.01 g
Gypsum	0.05 g	H ₂ O	1 mL
NaAlg 0.05 g			

Table 3. Composition of type Q cements

Solid part		Liquid part	
ns-HA/s-HA	0.9200 g	HM/HS	0.0920 g
CaO/ β -TCP	0.0200 g	Quitosana	0.0920 g
ZnO/MgO	0.0600 g	H ₂ O	1.80 mL

variable), the calcium generator (CaO or β -tricalcium phosphate, B variable), the agglutinant (ZnO or MgO, C variable), and the dissolution medium of the chitosan (malic or succinic acid, D variable). The concentration of chitosan in acid was 5 w/v %. Type Q cements was prepared by kneading 1 g of a powder mixture (solid part) into 0.5 g of acid solution of chitosan for 30 s. Therefore, the solid to liquid (P/L) ratio used was 1.0 w/v % (Table 3).

In both types of cements (A and Q), the resulting paste was poured into a cylindrical mould (3 mm high and 10 mm inside diameter). The setting time was measured as the time elapsed from the onset of kneading to the time when the paste did not adhere to the end of a 2-hydroxyethyl methacrylate rod (Ito, 1991). Six assays were conducted with each powder mixture.

All the disks were measured for height (h, in centimetres) and diameter (d, in centimetres), and then weighed (m, in grams). The bulk density was then calculated from the Eq. 1 (Ishikawa and Asaoka, 1995).

$$\rho = \frac{4m}{\pi d^2 h} \quad (1)$$

According with the fact that all reagents except HA are soluble in water, the porosity (P, expressed in %) was then calculated by Eq. 2 (Chang *et al.*, 2000 and Peón *et al.*, 2004),

$$P = 1 - \frac{\rho_{\text{HAC}}}{\rho_{\text{HApure}}} \bullet 100 \quad (2)$$

where ρ_{HAC} is the density of the cements measured after 7 days immersed in distilled water at room temperature and ρ_{HApure} is the density of pure HA (3.155 g/cm³), according to international data (ICDD, 1998). All the statistical studies were made using Statgraphics 2.1 Plus for Windows and MicroCal Origin 6.1.

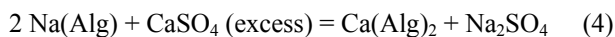
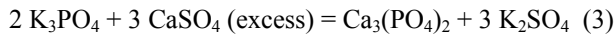
III. RESULTS AND DISCUSSION

A. Synthetic HA

The synthetic HA has a ratio of calcium to phosphorus of 1.68, similar to the stoichiometric one. The XRD and IR results of the synthetic HA used in the synthesis of the cements have been previously reported (Fuentes *et al.*, 2004). The data indicate a high purity degree of the HA and they agree well with literature data (Luo and Nieh, 1996; Fowler, 1974). Both of HA's, ns and s, showed the same behavior related to this characterization. The net parameters and surface area shows in Table 4 comparing with ASTM and literature so called M (Morejón *et al.*, 2003).

B. Type A cements

Figure 1 shows the effect of the KP concentration and the addition medium of SA on the setting time for the type A cements. The reactions occurring between gypsum and other compounds, as KP or SA, are defined by the Eqs. 3 and 4.



These two reactions are usually competitive. The gypsum can react with the KP until a complete consumption, as well as with the SA, a substitution reaction of sodium ions by calcium ions taking over.

Table 4. Characterization of HA

Sample	Net parameters			Surface area m ² /g	Pore volume m ³ /g
	a (Å)	c (Å)	V (Å ³)		
9-432	9.418	6.884	528.8	-	-
Ns-M	9.416	6.893	529.3	47.7	0.0240
Ns-HA	9.412	6.886	528.3	34.2	0.0257
s-M	9.411	6.892	528.7	1.3	0.0026
s-HA	9.411	6.882	527.9	8.6	0.0024

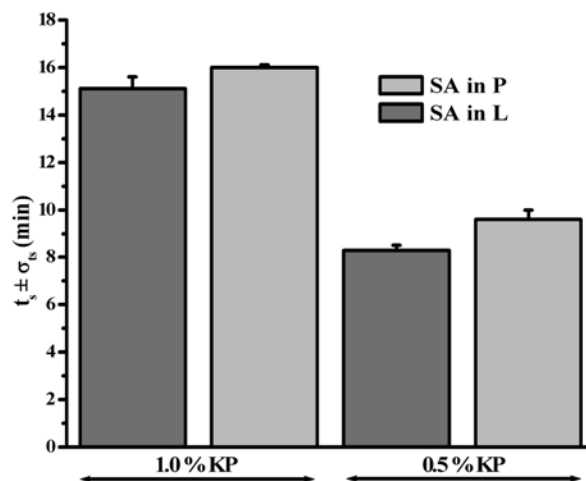


Fig. 1. Setting times obtained for the type A cements.

This last reaction causes the transformation of the alginate from a monovalent into a divalent salt, occur a higher crosslinking of the matrix and provoking the setting of the material (Ishikawa *et al.*, 1995). Therefore, a higher concentration of KP should cause that the reaction of Eq. 4 occurs in a less extent, increasing the setting time.

This fact is consistent with the results shown in Fig. 1, and the setting time values are diminished in a factor of around 1.8 when the KP amount used is decreased to the half. However, there was no significant difference between the setting times of the cements when the SA was added to the liquid part instead of to the solid one. It has been reported (Ishikawa *et al.*, 1995) that the addition of SA to cements of tetracalcium phosphate and dicalcium phosphate diminished the setting time in eight times from 40 to 5 minutes but the different amount of SA added did not affect the setting time value.

The statistical treatment applied to the setting time values provided the polynomial $ts = 12.25 + 3.31 \text{ KP} + 0.56 \text{ M}$ which justified the significant influence of the KP amount on the setting time. However, the dissolution medium (M) of sodium alginate almost does not affect the setting time, as deduced from the polynomial coefficient and as observed in Fig. 1. According to the results obtained, in order to get lower setting times it is necessary to use the smallest percent of KP and to dissolve the SA in the solid part, though this last recommendation is not so significant.

The porosity values obtained for the type A cements are shown in Fig. 2. It can be seen that they are not influenced by the changes evaluated, neither the KP percent nor the dissolution medium of SA. The porosity values found (41-43 %) are comprised between those reported in the literature for samples with P/L ratios ranging from 2.0 to 6.0.

Considering the results shown by Fig. 1 and Fig. 2, the best of the four cements studied is the third bar, which presents the lowest setting time (8 min) and the highest porosity (43 %)

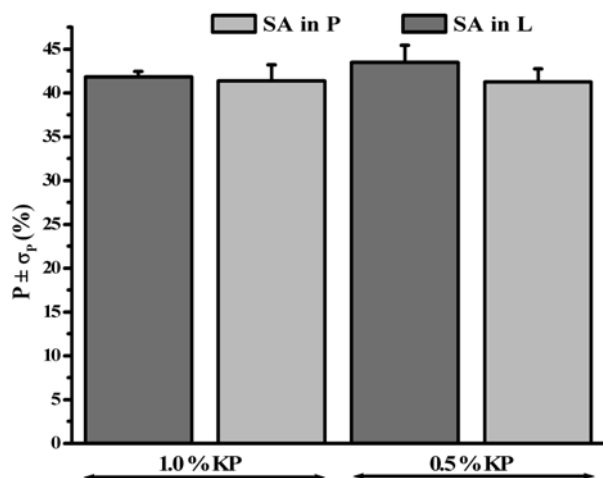


Fig. 2. Porosity values of the type A cements.

B. Type Q cements

The average values of setting times (2 to 24 minutes) obtained for the sixteen type Q cements are shown in Fig 3. Four variables are changed: the type of hydroxiapatite (sintered or not), the calcium generator (CaO or β -TCP), the agglutinant (ZnO or MgO), and the acid medium used to dissolve the chitosan (MH or SH).

The comparison of samples setting times prepared by changing the HA type (A variable), keeping the rest of variables constant, shows there is no a clear influence of that variable on the setting time. However, if is studied the effect of the calcium generator, it can be seen that in six of eight pairs of cements (viewing the pairs 1-3, 2-4, ..., 14-16 according Fig. 3), the setting time is lower for cement prepared using CaO instead of β -TCP. It can be explained in terms of the solubility, which is higher for the oxide, releasing more calcium ions to medium, and provoking that the setting of mixture occurs before (Ito *et al.*, 1995). Furthermore, the CaO produces the dehydration of chitosan sol and the subsequent gelling, because in solution it is transformed into hydroxide, which can form hydrogen bonds, being more reactive than the β -TCP salt. Thus, the gelling of chitosan is faster for CaO than for β -TCP, and subsequently so is the setting process.

Regarding the agglutinant used, the setting times observed for cements prepared with MgO (white and middle grey) are, in most of the cases, lower than for samples containing ZnO (light and dark greys, see Fig. 3). This can be explained in terms of the relative size of cations. Thus, the size of Mg^{2+} is smaller than Zn^{2+} , which allows a better accommodation of the reactants contained in the powder mixture (solid part) in the first case, and facilitates the reaction to occur before. The setting time for the cements prepared with CaO and SH are, however, higher for MgO than for ZnO. In these cases maybe the basic character of the medium favours

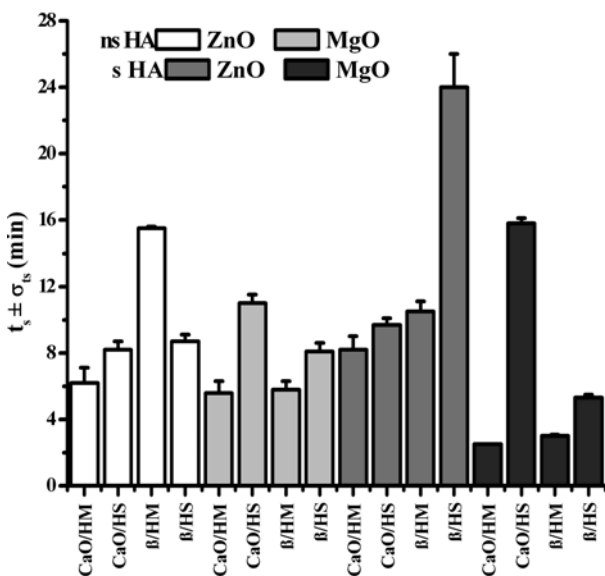


Fig. 3. Setting times obtained for the type Q cements.

the behaviour of ZnO as a base and the formation of $Zn(OH)_4^{2-}$. Then, the setting of cements happens faster than for the MgO under the same conditions (Sanderson, 1964).

The setting times obtained in the present work in most of the cases are significantly lower than those reported for chitosan bone pastes containing CaO, MgO and ZnO, 2-2-4 % to left and 2-4-1 % to right respectively (Ito *et al.*, 1995) (see Fig. 4), which showed values of setting time around 13 min. This fact can be due to the different solubility of the product used as raw in the cement, β -TCP (Ishikawa *et al.*, 1994) and HA in the present work.

On the other hand, the additional OH⁻ group in malic acid ($HOOCCH(OH)CH_2COOH$) as compared to the succinic acid ($HOOC(CH_2)_2COOH$), generates one more point of hydrogen bridge bond, causing a higher degree of mixture of the reagents, the process of setting occurring before (Ito *et al.*, 1995). This explains the decrease observed in general in the setting time when the malic acid is used instead of succinic acid (see Fig. 3).

The statistical treatment applied to the setting times of type Q cements provided a multilinear polynomial, $t_s = 9.29 - 2.15*B - 2.12*D + 1.75*A*D + 2.47*B*C + 1.83*A*B*C*D$ (see explanation of the variables in Table 1). It can be seen that the most influent variables are the type or calcium generator (CaO or β -TCP, B variable) and the acid medium of dissolution of the chitosan (MH or SH, D variable), both parameters appearing three times in the polynomial. On the contrary, the type of HA does not exert an effect by itself on the setting time, as seen above, but combined with other variables. As deduced by the polynomial, the setting time is the result of the combination of different variables, and therefore, in some cases, it could be necessary not to choose the optimum value of a determined variable in order to obtain a lower setting time. As deduced from Fig. 3, the lowest setting times of the study are obtained when the cement composition is formed by sintered HA, MgO, and malic acid.

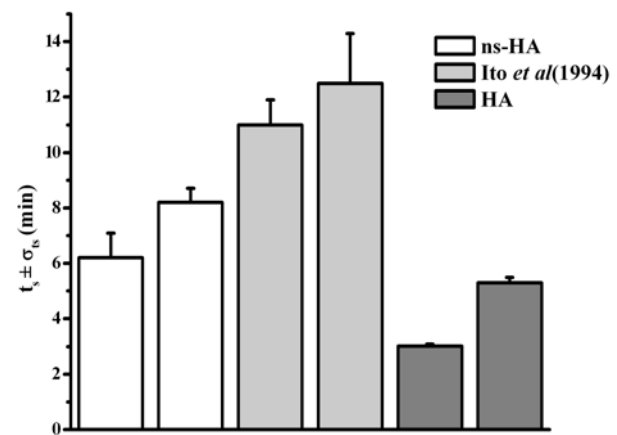


Fig. 4. Setting times of some Q cements (β -TCP/MgO) compared with other reported by Ito *et al.*, 1995

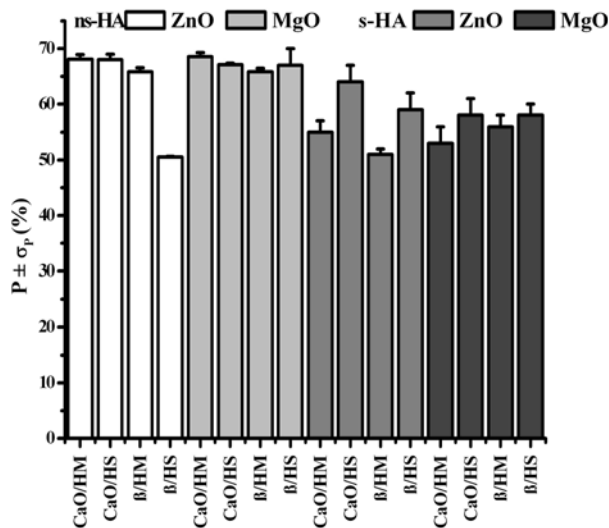


Fig. 5. Porosity values of the type Q cements.

The porosity values (between 50 and 70 %) obtained for the type Q cements are depicted in Fig. 5. In most of cases, the porosity values of cements prepared with not sintered HA are higher than those obtained with sintered HA, as a consequence of the decrease of the pore volume produced during the sintering process. On the other hand, regarding the effect of the calcium generator, it can be seen that the use of CaO generates more porosity than the use of β -TCP, due to the highest solubility of the first one.

In the cements prepared with sintered HA, the porosity values observed for samples prepared with MgO as agglutinant are lower than for samples containing ZnO. The reason could be the same that in case of setting time, including the possible exceptions.

Regarding the data for cements prepared with sintered HA, in all cases the porosity values obtained for those synthesized with SH as acid medium of dissolution of the chitosan, are higher than for cements prepared with MH. It can be explained by the fact that the malic acid has one more point (hydrogen atom) to produce the bond and therefore, the network is more interlocked and less porous than in the case of succinic acid. In any case, the porosity values of the type Q cements prepared in the present work (with P/L = 1.0) vary between 50 and 70 %, and they are similar or even higher than those reported for cements with a higher P/L (41 % for P/L = 2.0, 43 % for P/L = 3.0, and 58% for P/L = 5.0).

IV. CONCLUSIONS

Twenty synthetic hydroxiapatite cements of two different types (A and Q) have been prepared using a synthetic hydroxiapatite as raw material. The influence of synthesis variables on both the setting time and the porosity has been studied.

The formulations of Type A cements have been varied according to a factorial design 2^2 , where the vari-

ables evaluated were the KP concentration and the medium of dissolution of the SA. From the studied carried out, it can be deduced that there is a direct relationship between the first variable and the setting time, whereas the second one seems not to influence on it. The setting times of these cements vary from 8 to 16 min, which are similar values to other reported in the literature. The porosity values found (41-43 %) were not influenced by the changes evaluated. The best of type A cements was that prepared with 0.5 wt % KP and dissolving the SA in the solid part. This cement shows the lowest setting time (8 min) and the highest porosity (43 %) of this series.

Type Q cements were prepared according to a factorial design 2^4 , by varying the HA type, the calcium generator, the agglutinant, and the dissolution medium of the chitosan. The setting times observed for these cements are comprised between 2 and 24 min, which are similar and in some cases lower if compared with other reported in the literature. The type of HA used (sintered or not) does not exert a clear influence on the setting time, but combined with other variables, whereas the cements prepared with CaO as calcium generator show lower setting times than those with β -TCP. The use of MgO instead of ZnO as agglutinant decreases the setting time of the cements. The same effect was observed when the succinic acid was substituted by malic acid as dissolution medium of the chitosan. Regarding the setting times, the best of type Q cements were those prepared with sintered HA, MgO as agglutinant, and malic acid as dissolution medium. The porosity values for type Q cements vary between 50 and 70 %.

The influence of the studied variables on the porosity is less accused than in the case of setting time, and the differences between porosity values are less significant. However, some conclusions can be deduced from the study. In general, the cements prepared with non-sintered HA show higher porosity values than those obtained with sintered HA. The porosity also increases in most of cases when β -TCP is substituted by CaO as calcium generator. Conversely, the substitution of ZnO by MgO as agglutinant produces a decrease of the porosity. The same effect was observed when MH is used instead of succinic acid, as dissolution medium of the chitosan.

The cements prepared in the present work show adequate values of setting time and porosity to be applied in processes of bone regeneration. However, additional studies related to the mechanical properties must be done, in order to design the most appropriate formulation.

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