Elaboration of hydroxyapatite / polymers / cefuroxime systems for sensitivity study of *escherichia coli* atcc 25922 strain in agar medium.

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Summary:

Hydroxyapatite ceramic biomaterial, whose mineral composition is identical to that of bones and teeth is the most used osteoconductive material for the release of active principles. In vitro studies have shown its usefulness for controlled release of various active agents (Drug Delivery System) such as antibiotics. Because of its properties, we set a goal to make the hydroxyapatite-based matrices mixed antibiotics able to lead to inhibition of a bacterial culture in agar medium.

Materials and methods

The study of sensitivity was carried out on a strain of *Escherichia coli* ATCC 25922. The antibiotic used is a beta-lactam, cefuroxime. Inhibition diameters on MH medium were measured after 24 hours of incubation with the hydroxyapatite disc base loaded with different concentrations of the antibiotic (0.75 to 4.5 mg).

Results

The results showed very satisfactory diameters of inhibition greater or equal to the control disc loaded with 300 mg of cefuroxime. These inhibition diameters varied according to the initial load of antibiotic.

Conclusion

The use of hydroxyapatite-based ceramic can be a true alternative to blotter paper discs for the in vitro determination of the sensitivity of bacteria to antibiotics.
Moreover, the load is small compared to the blotter paper disk impregnated with antibiotics.

**Keywords:** hydroxyapatite - cefuroxime - *Escherichia coli* - sensitivity.

**INTRODUCTION**

The hydroxyapatite ceramic biomaterial, whose mineral composition is identical to that of bones and teeth is the osteoconductive material most used for the release of active ingredients [1].

Allowing it to meet the definition in the Chester agreement which stipulates that the biomaterials are materials intended to come into contact with living tissue and / or biological fluids to assess, treat, modify or replace any tissue forms, organ or function of the body [2]

In vitro studies have shown its usefulness for the controlled release of various active agents (Drug Delivery System), such as antibiotics, anti-inflammatory and analgesic [3].

But the potential activity of an antibiotic is not dependent on that of its intrinsic in vitro activity on isolated bacteria but also its pharmacokinetic characteristics. Indeed, he is also active in vitro, an antibiotic that does not reach the site of infection due to inadequate pharmacokinetic will have little therapeutic effect in vivo [4].

Because of the properties of hydroxyapatite, we set a goal to make the hydroxyapatite-based matrices mixed with antibiotics can lead to inhibition of a bacterial culture in agar medium.

**MATERIALS AND METHODS**

**MATERIALS**

Cefuroxime Ph.Eur (Lot No. wsdx / 09 Directorate of Pharmacy and Laboratories Dakar) is the active ingredient used in this study.
Hydroxyapatite, the carrier prepared in the laboratory was obtained by precipitation reaction between calcium hydroxide and orthophosphoric acid with a ratio \( \text{Ca} / \text{P} = 1.66 \), between 80 and 95 °C under stirring [5].

The annealed form at 800 °C of hydroxyapatite powder was used and is formed of 100 to 200 nm grain with a surface area of 75 \( \text{m}^2 / \text{g} \). Esterified copolymers of acrylic acid and methacrylic containing a low content of quaternary ammonium groups (Eudragit® RS 100 Lot No. 8390808118 and RS PM Lot No. 0400938137 of Röhm GmbH - Chemische Fabrik Kirschenallee D-64293 Darmstadt, Germany) serve as binders.

The strain used for the production of standard susceptibility testing was supplied to us by the Laboratory of Bacteriology - Virology of the Aristide Le Dantec hospital (HALD). This is a reference strain of \textit{Escherichia coli} ATCC 25922.

**METHODS**

**MANUFACTURING SYSTEMS**

We developed 50 matrices of different antibiotic loads with 0.92 mm thickness and 13 mm diameter.

Hydroxyapatite, binders in internal phase and the antibiotic for a total weight of 150 mg are mixed in a porcelain mortar until a homogeneous mass, then we added the wetting liquid containing the Eudragit® RS 100 (binder external phase).

After trituration for 10 minutes with spatula, we fill the mold pellet and apply a pressure of 103 Pa 68.98757 using an hydraulic press. Systems thus obtained are dried at 30° C for 24 hours.

**Systems characterization**

**Mass uniformity test**

The mass, diameter and thickness of the systems were taken and compared with each other and to standard antibiotic discs commercially available.

**Study of bacterial inhibition**
The antibiotic release studies (cefadroxil) were conducted on a pure culture of reference strain of *Escherichia coli* ATCC 25922 inoculated agar Muller Hinton (MH) cast on petri dish.

For each batch, the study of bacterial inhibition was performed on four mass units plus a control disk (blotting paper impregnated with antibiotic cefadroxil 300 mg) six experiments.

The manipulation is performed on bacteriology bench in sterile area under the flame. Inhibition diameters’ reading was performed 18 hours after incubation.

**RESULTS**

Figure 1 shows images of inhibition diameters obtained with the different loads of antibiotics in Experiment 1 to 6.

![Image](image1.png)

**Figure 1:** Images of inhibition diameters obtained with the different loads of antibiotics in experiments 1 to 6.
The diameters shown in Table I and Figure 2 below are medium diameters of inhibition obtained for each initial antibiotic charge from experience 1 to 6.

**Table I:** Results of the sensitivity of *Escherichia coli* ATCC 25922 strain according to cefadroxil charges.

<table>
<thead>
<tr>
<th>Load (mg)</th>
<th>0,75</th>
<th>1,50</th>
<th>2,25</th>
<th>3,00</th>
<th>3,75</th>
<th>4,5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium diameters (mm)</td>
<td>36±0,021</td>
<td>27±0,019</td>
<td>28±0,030</td>
<td>28±0,025</td>
<td>32±0,020</td>
<td>30±0,024</td>
</tr>
</tbody>
</table>

**Figure 2:** Medium diameters (mm) of inhibition in function of the load in Experiments 1 to 6.

**DISCUSSION**

These results indicate that the antibiotic was continuously released from the device and conveyed through the culture medium by diffusion due to concentration gradients.

Generally, the diameters of inhibition obtained from different batches are comparable.

Drug release from the non-functionalized hydroxyapatite matrix may be controlled by the penetration of the release medium in the system, the drug
dissolution and diffusion of the dissolved drug through the "pores" filled with water of the matrix.

Indeed, Hoang et al, showed unlike non-functional implants, the release of active ingredients from functionalized implants, is further controlled by the dissociation of the active ingredient from the complex in the release medium [6].

A number of unsatisfactory pore matrices influence the porosity and specifically on the ability of matrices to release the antibiotic in the agar. It is more likely that materials having different macropores (pore size and total porosity) will behave differently as regards the penetration of biological fluids [7].

Nature of matrix pore (diameter and shape) may be conditioned by pressure applied during matrix’s manufacturing. However, some authors have demonstrated that some calcium phosphate materials, more resistant, have a lower macroporosity rates, but there is no direct relationship between porosity and the compressive strength [8].

A non-homogeneous distribution of the antibiotic in the matrix due to the size of the powders negatively influences the diffusion of the antibiotic. Indeed in the case of solid, uniformity is never perfect; it is proportional to the fineness of the particle size of powders. It then seeks to have a random grain distribution of the two components, the particle size to be substantially the same for the two powders [9].

Poor distribution of the antibiotic linked to agar’s limit degree of wetting and to incubation temperature (too high or too low) can lead to poor distribution of the antibiotic in the agar [10].

These different physical phenomena may explain the discrepancies observed with inhibition diameters according to disks batches tested in this study.

Clearly, when time progressed, the amount of drug released from the matrix increased, leading to promote the diffusion and an increase in the concentration of the antibiotic in the different areas of the agar.
For the small loaded matrix (0.75 mg) as well as those high loaded one (3.75 mg and 4 mg) in antibiotic, very satisfactory most important inhibition diameters are observed varying from 33 to 36 mm.

The very satisfactory inhibition diameters observed with antibiotic low charged matrix is explained by the fact that they are emptying quickly within 24 hours.

Our results are similar to those observed by Mbaye et al. which showed that the matrices based on hydroxyapatite charged small amounts of active ingredient in vitro study in a liquid solution, emptied much faster than those charged in significant quantities. Indeed this drug release kinetics depends on the initial charge and the residual amount [11].

By comparing our results to those obtained in another study that used ofloxacin, we find that quinolone causes most important inhibition diameters. This difference in inhibition diameter can be explained by the mechanism of action involved by the antibiotic. Thus quinolones would act more effectively on the *Escherichia coli* [12].

**Conclusion**

The use of hydroxyapatite based ceramic can be a true alternative to blotter paper discs for the in vitro determination of bacteria’s sensibility to antibiotics. Moreover, the load is small compared to the blotter paper disk impregnated with antibiotics.
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