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Effect of Heteropolyanions of Dawson Type on the Development of the Bacteria (*Staphylococcus Aureus* and *Echerichia Coli*)

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Abstract: The objective of this study is to test the inhibiting activity of new molecules of heteropolyanions synthesized such as($K_6P_2W_{18}$, $H_6P_2W_{18}$, $K_6P_2W_{12}$ M_{O6}, $H_6P_2W_{12}$ M_{O6}) with respect to two bacterial strains (Staphylococcus aureus and Echerichia coli). The sensitivity of these two strains to the molecules of heteropolyanions is evaluated by the inhibiting minimal concentration (IMC) in liquid medium by the technique of the macro method. The tests of the latter proved a remarkable effectiveness. *Copyright © 2014 IFSA Publishing, S. L.*

Keywords: Heteropolyanions, Bacteria, Antibioresistance, Antibiotic, Inhibiting activity.

1. Introduction

Heteropolyanions (HPA) of Dawson type are multipurpose materials, they have very particular characteristics (large sizes, solubility in polar and no polar solvents, high capacity to transfer electrons and the protons, heat resistance, etc) which allow them to be used in various fields such as catalysis [1-4], chemical analysis [5-7], environment as electrochemical sensors [8], waters treatments [9], science of materials [10], biochemistry and medicine [11, 12]. A great number of heteropolyanions was employed as chemical reagents in the detection, the separation and the complexation of elementary, pharmaceutical and biological materials. Some of these substances such as phosphorus (P), silicon (Si), uric acid and protein were efficiently analyzed using heteropolyanions [13]. Many salts of polyoxometallates are biologically active, especially the molybdates and vanadates. They are very selective with respect to certain enzymes (phosphatase, deshydrogenase, isomerase) [14]. The active work of research is currently carried out on the medical applications of the HPA. A great number of polytungstates was tested as antitumor, antivireux agents [15-19] and antiAIDS [20]. In medicine the antibioresistance is a worrying and complex phenomenon which requires, in the same time, a revision of the practices of the infections care and the development of new tools to thwart the capacity of the astonishing reactions of the bacteria. Currently, promising molecules are under development for the treatment of infectious pathologies. Among the newly synthesized anti-infectious agents appear the heteropolyanions (HPA). Some HPA of the Dawson type, saturated and lacunar synergically **i**ncreased the antibacterial activity of antibiotics of β-lactams against the methicilline [21].

In this prospect, four molecules of HPA ($K_6P_2W_{18}$, $H_6P_2W_{18}$, $K_6P_2W_{12}Mo_6$, $H_6P_2W_{12}Mo_6$) were prepared and used to test their inhibiting activities with respect to two bacterial strains Staphylococcus aureus and Echerichia coli (E.colis and S.aureus). The sensitivity of the two strains to the four molecules of HPA is evaluated by the inhibiting minimal concentration or (IMC) by using the technique of the macro method. The tests of the latter gave an encouraging result with a remarkable efficiency.

2. Experimental

2.1. Preparation and Structures of Heteropolyanions

The mixed compound saturated ($K_6P_2W_{12}$ Mo_6 O_{62}, $nH_2O)^{6-}$, abbreviated to ($P_2W_{12}Mo_6$), is obtained by a series of reactions starting from the substance mother ($K_6P_2W_{18}$ O_{62}, $nH_2O)^{6-}$ abbreviated to(P_2W_{18}) according to the mode described by the literature [22, 23] (see Fig. 1). The acid forms ($H_6P_2W_{18}$, $H_6P_2W_{12}Mo_6$) were prepared by extraction with ether in hydrochloric acid medium [24, 25].

2.2. Preparation of the Various Inhibiting Mother Solutions

A series of (04) numbered sterile tubes from 01 to 04 is taken.

A weighed quantity (1 g) of each HPA is dissolved in distilled water (10 ml), and then introduced into the test tubes.

After dissolution in distilled water, the various heteropolyanions gave different colors. The various solutions are mixed with the bubble glucose with the indicating crimson of bromocrézol (BCP), other colors are obtained (Table 1).

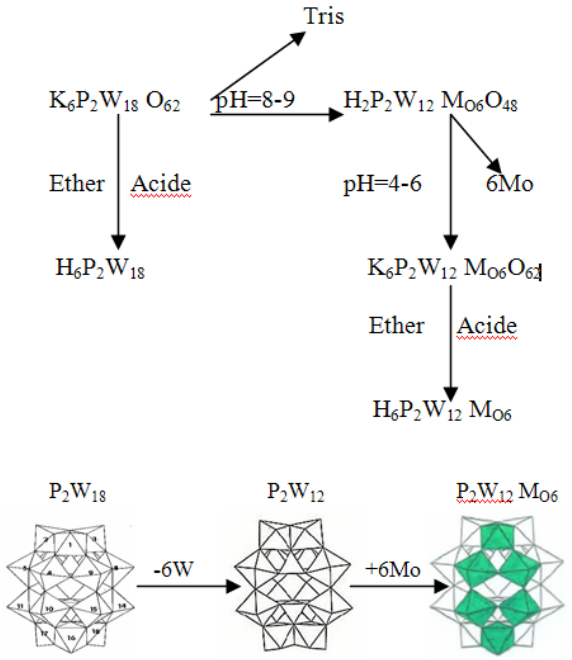


Fig. 1. Structures of heteropolyanions.

Table 1. Various concentrations of HPA and their change of color after their solubilization in distilled water and bubble.

Tubes	HPA	Concentrations of solutions (g/ml)	Colors after dissolution	Colors after mixture of bubble(BCP)
01	$K_6P_2W_{18}$	0.1	Blue very Claire	Purple
02	$H_6P_2W_{18}$	0.1	Blue sky	Purplished dark green
03	$K_6P_2W_{12}$ Mo_6	0.1	Green bleaches on grass	Purple
04	$H_6P_2W_{12}$ Mo_6	0.1	Dark green	Purplished dark green

2.3. Preparation of the Bacterial Strains to be Tested

Two strains test (E.coli, S.aureus) of nosocomiales type isolated on patients hospitalized in the hospital of Annaba (Algeria). Their identification was carried out by the microbiology laboratory of this hospital.

2.3.1. Reactivation

Starting from conservation media (nutritive Gelose), the strains are sown on a nutritive bubble, and incubated during 24hours to a temperature of 37 °C. This culture is intended to revivify the stocks. The reactivation which is a bacterial growth translated by a disorder of the nutritive bubble.

2.3.2. Stock Purity Check

Using a sterile platinum handle, few drops of the nutritive bubble (rich in germ) are sown in the form of ridges on the surface of the gelose Mac ConKey for E.coli and on the Chapman medium for S.aureus. The boxes are turned over and incubated with 37 °C during 24 hours. The purity of the stock is checked by the colonies aspect on the solid media and the microscopic observation after Gram coloring.

2.4. Microscopic Examination

The microscopic examination is an important step for the study of a bacterium. It is done after Gram coloring; this one makes it possible to better know the morphology of the bacteria, their regrouping and their Gram. The coloring of Gram was developed in a fortuitous way which differentiates the Gram bacteria (+) from the Gram bacteria (-).

2.5. Determination of the Inhibition Minimal Concentration (IMC)

The IMC is the smallest concentration of antibiotic able to inhibit a bacterial growth visible to the naked eye. This technique mainly makes it possible to define the spectrum of a new antibiotic and to study the sensitivity of a bacterial species with respect to one or several antibiotics. The IMC of a given inhibitor can be measured by various processes:

- The IMC in liquid medium by the technique of the macromethod or the micromethod.
- The IMC in solid medium or method by diffusion of disc (Antibiogramme).

In this work the sensitivity of the two bacterial strains E.coli and S.aureus to the molecules of heteropolyanions is measured by the IMC in liquid medium by the technique of macromethod [26].

2.5.1. Technique

- Take a series of (11) numbered sterile empty tubes from 0 to 10. The tube 0 is a witness.
- A volume of 9 ml of (BCP) is put in the tube 1 on the other hand; the other tubes are filled with a volume of 5ml of the same bubble.
- Take 1ml from mother solution of the heteropolyanion and add it to the tube 1 (1/10 of dilution).
- Transfer 5ml from the tube 1, and add it to the tube 2, we obtain a diluted solution of half compared to that of the tube 1.

To repeat the same operation to the tube 10. The tube 0 is the witness does not receive antibiotic.

All the tubes receive 100 µl of a bacterial suspension of E.coli or S.aureus containing a microbial load ranging between 10^5 - 10^6 germ/ml. The two bacterial strains consume glucose as energy source. Their culture is done in bubble glucose with the colored BCP. In absence of antibiotic or insufficient IMC, the bacteria develop while multiplying on the medium. The bacteria consume glucose and transform it into acid. The acidification of the nutritive bubble involves the turn of the BCP contained in the medium of crimson- from purple to orange yellow.

In case of a bacterial inhibition there is neither consumption of glucose nor production of acid consequently, the color of the nutritive bubble does not change (remains purple). (See Fig. 2).

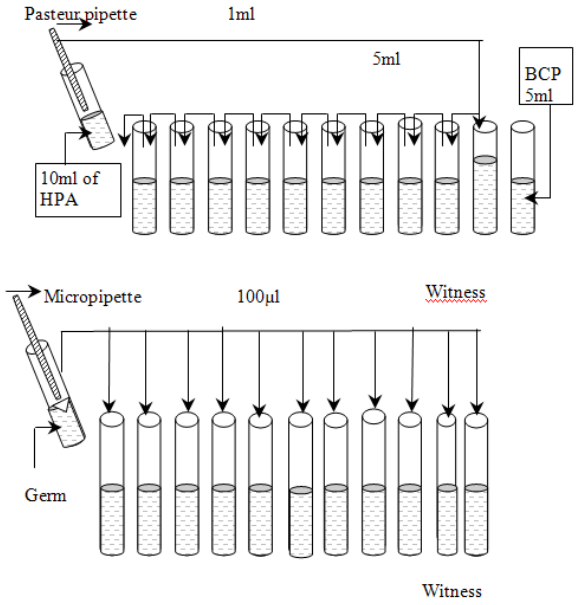


Fig. 2. Technique of determination of the IMC in liquid medium.

2.5.2. Reading

The IMC of the inhibitor for the studied stock are given by the first tube in which there is not a color turning, which proves that there is not a consumption of glucose from where absence of a bacterial growth.

3. Results and Discussion

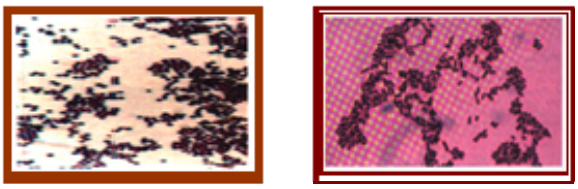
3.1. Macroscopic Examination of the Cultures

- Growth of S.aureus on the medium of chapman: The colonies are golden yellow surrounded by a yellow halo due to the mannitol attack.
- Growth of E.coli on the medium of Mac ConKey: On this medium the colonies are of big size, red brick surrounded by an opaque halation due to the precipitation of biliary salts. For lack of this medium the strains are mended on liquid medium BLVB (Bubble lactose with vertbrillant).

3.2. Microscopic Examination After Coloring of Gram

After coloring, E.coli are presented to the microscope in the form of sticks with rounded ends (coccobacilles) colored pink. They are bacilli of the negative Gram types (See Fig. 3 b). After coloring of S.aureus, the colonies are presented in spherical

forms (cocci) colored purple. They are the bacilli of positive Gram, gathered in bunch of grapes, diplocope or tetrad Gram (See Fig. 3).



(a) S.aureus (b) E.coli

Fig. 3. Coloration of Gram for the two types of colonies. (a) Cocci Gram (+). S.aureus under objective x100, (b) Coccobacille (-). E.coli under objective x100.

3.3. Determination of the IMC

For each bacterial strain a test of sensitivity was carried out by the method of dilution in liquid medium. The results of the antibacterial activity of E.coli and S.aureus to the various forms of heteropolyanions tested are mentioned in Tables 2, 3, 4 and 5.

Table 2. Result of the inhibiting activity of $K_6P_2W_{18}$ on E.coli and S.aureus.

[C]mg/ml	10	5	2,5	1.25	0.625
E.coli	-	-	-	IMC	+
S.aureus	+	+	+	+	+

Table 2 (cont.). Result of the inhibiting activity of $K_6P_2W_{18}$ onE.coli and S.aureus.

[C]mg/ml	0.31	0.156	0.078	0.039	0.019
E.coli	+	+	+	+	+
S.aureus	+	+	+	+	+

Table 3. Result of the inhibiting activity of $H_6P_2W_{18}$ on E.coli and S.aureus.

[C]mg/ml	10	5	2.5	1.25	0.625
E.coli	-	-	-	-	IMC
S.aureus	-	-	IMC	+	+

Table 3 (cont.). Result of the inhibiting activity of $H_6P_2W_{18}$ on E.coli and S.aureus.

[C]mg/ml	0.31	0.156	0.078	0.039	0.019
E.coli	+	+	+	+	+
S.aureus	+	+	+	+	+

Table 4. Result of the inhibiting activity of $K_6P_2W_{12}Mo_6$ on E.coli and S.aureus.

[C]mg/ml	10	5	2,5	1.25	0.625
E.coli	-	-	-	-	-
S.aureus	-	-	-	-	-

Table 4 (cont.). Result of the inhibiting activity of $K_6P_2W_{12}Mo_6$ on E.coli and S.aureus.

[C]mg/ml	0.31	0.156	0.078	0.039	0.019
E.coli	IMC	+	+	+	+
S.aureus	IMC	+	+	+	+

Table 5. Result of the inhibiting activity of $H_6P_2W_{12}Mo_6$ on E.coli and S.aureus.

[C]mg/ml	10	5	2.5	1.25	0.625
E.coli	-	-	-	-	-
S.aureus	-	-	IMC	+	+

Table 5 (cont.). Result of the inhibiting activity of $H_6P_2W_{12}Mo_6$ on E.coli and S.aureus

[C]mg/ml	0.31	0.156	0.078	0.039	0.019
E.coli	-	IMC	+	+	+
S.aureus	+	+	+	+	+

- Effect of $K_6P_2W_{18}$:

It inhibits E .coli with the concentration of 1,25 g/l whereas it is inactive on S.aureus.

- Effect of $H_6P_2W_{18}$:

E.coli is sensitive to this molecule starting from the concentration 0,625 g/l. On the other hand, the CMI for S. aureus is of 2,5 g/l. (The concentration is 4 times higher than that of E coli).

- Effect of $K_6P_2W_{12}Mo_6$:

The two stocks are inhibited with the same concentration which is of 0,31 g/l.

- Effect of $H_6P_2W_{12}Mo_6$:

This form of HPA has the highest inhibiting capacity with respect to E.coli. This stock is inhibited with the concentration of 0,156 mg/ml. However, the concentration necessary to the inactivation of S .aureus is much higher since it is of 2,5 mg/ml. (16 times more important than that of E. coli).

With the examination of these results, it appears clearly that:

- heteropolyanions tested exert an inhibiting effect with respect to the two stocks.
- the mixed forms saturated with HPA ($H_6P_2W_{12}Mo_6$, $K_6P_2W_{12}Mo_6$) are more inhibiting than the saturated forms ($K_6P_2W_{18}$, $H_6P_2W_{18}$).
- E. coli is sensitive to all the forms of HPA and is particularly with the acid form. $H_6P_2W_{12}Mo_6$.
- S. aureus is nonsensitive to molecule $K_6P_2W_{18}$, but, more sensitive to the form $K_6P_2W_{12}Mo_6$.

This difference in sensitivity between the two bacteria could be attributed to their walls. Indeed, the two stocks are different primarily by the nature of

their wall. E.coli is a bacterium with negative Gram whereas S. aureus is a bacterium with positive Gram. The wall of the bacteria with negative Gram is made up of a not very thick layer of peptidoglycane, surmounted of an external membrane very rich in lipids. Whereas, the wall of the bacteria with positive Gram has only one layer of peptidoglycane but five times thicker and very low in lipids. The wall does not constitute a selective barrier like the membrane but, it is permeable in the bacteria with negative Gram. Consequently, the wall of E.coli is crossed easily by the HPA which inside the bacterial cell can inhibit its metabolic activities. It is also noted that the saturated salt form of the HPA ($K_6P_2W_{18}$, $K_6P_2W_{12}Mo_6$) is less inhibiting than their acid form ($H_6P_2W_{18}$, $H_6P_2W_{12}Mo_6$) with respect to the two stocks. Thus the presence of proton (H^+) in these heteropolyanions increases their antibacterial activity. It is also noticed the presence of molybdenum in the salt form or acid of the HPA makes these molecules more inhibiting, from where it is deduced that molybdenum (Mo) constitutes an active site for these HPA.

4. Conclusion

Through this study, we highlighted the inhibiting activity of four synthesized molecules of HPA ($K_6P_2W_{18}$, $H_6P_2W_{18}$, $K_6P_2W_{12}$ M_{O6}, $H_6P_2W_{12}$ M_{O6}) with respect to two strains tested (Staphylococcus aureus and Echerichia coli). The results obtained showed that:

E.coli is more sensitive than S.aureus to the synthesized HPA.

The mixed forms of HPA are more effective than the saturated forms.

The molecule of saturated heteropolyanion $K_6P_2W_{18}$ does not exert any bacteriostatic effect on the strains of S.aureus.

The results are encouraging and would deserve to be refined and followed by a study using more elaborated processes such as the microplate technique or the method of disc and on a more significant number and varied micro-organisms (bacteria, yeasts and moulds).

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