ISSN: 0975-766X
CODEN: IJPTFI
Available Online through **Research Article**
www.ijptonline.com

IMPROVEMENT OF BACTERICIDAL PROPERTIES OF BENTONITE BY MEANS OF SODIUM CARBONATE

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Received on 15-10-2016 *Accepted on 18-11-2016*

Abstract.

Ecological expediency conditions the necessity to make research of raw materials base and to use for medical purposes those mineral resources which were not subjected to modifications due to aggressive chemical action. Development and sale of pharmaceutical forms with optimal pharmacokinetic characteristics which would not induce occurrence of resistant bacterial population and sensitization of a body is a perspective trend in pharmaceutical engineering.

The article contains comparative characteristics of the effect of native and sodium carbonate-activated bentonite on biocenosis of the subgingival space. It was established that burning at the temperature of 200^0C improves bactericidal activity of bentonite however burning at the temperature over 400^0C has an inverted effect.

Key words: paradontium, bentonite, bactericidal activity, sodium carbonate.

Introduction.

Intensive development of industrial production results in increase of pollutant emission to the environment which in its turn affects the ecological situation [1,2]. Pharmacological component has significant impact on environmental pressure on a human body. During the second half of the XXth century the term of "pharmacological safety" came into general use. The WHO understands this term as "scientific investigations and activities related to disclosure, assessment, comprehension and prevention of adverse effects or any other problems attributed to medicines". Adverse reactions to medicines represent significant problem in all branches of medicine [3].

Taking into consideration the growing environmental pressure pharmacologists pay increased attention to natural resources inclusive of clay minerals which include bentonites among others. The clay minerals component reaches 10-12% of the Earth crust volume.

Significant amount and variety of bentonites condition the interest in investigation of their properties and possibilities of use in different branches of medicine. The problem with the target properties of bentonites is that they can be modified by various additives [4-6].

Research objective: To assess the effect of sodium carbonate on bactericidal activity of bentonite affecting microflora of the subgingival space.

Methods. The research involved 30 patients suffering from chronic periodontitis of medium severity. The microbiological content of crevicular fluids of the patiens was subjected to study. Crevicular fluids withdrawal was performed by standard strips of blotting paper. Before the study the teeth and the gum contiguous to them were isolated from saliva by means of cotton rolls and dried. A pointed end of the blotting paper strip was introduced into the subgingival space entry preventing it from coming to the bottom in order not to cause mechanical irritation of the tissues and further fluids flow increase. The blotting paper strips were introduced into the space in the area of interdental gingival papillae [7]. Identification of isolated colonies and counting of the same was made with use of slant Brain-Heart Agar or AC semisolid culture medium. Bentonite from the Korochanskoye field which refers to the group of high swelling clays was used for the research. Native and uniformly activated samples were taken for investigation. Activation of the sub-samples was performed with two percent solution of sodium carbonate [8].

Activated bentonite wad dried at the temperature of 60^0 C. Activated and native sub-samples with the weight of 4 grams were burned in an electrical muffle at the temperature of 200^0C, 400^0C, 600^0C, 800^0C. The temperature increase rate at time of the samples burning within the interval from 20 to 400 ^{0}C made 100 ^{0}C/h, and over 400 ^{0}C made 200 ^{0}C /h. Isotermal exposure of the samples at the final burning temperature made 1 hour.

The bactericidal properties of bentonite altered by activation were determined by means of treatment of the isolated colonies plated on the Petri dishes. The control group of the Petri dishes was plated with native bentonite. The seedings were incubated at the temperature of 30.0 ± 1.0^0C within 72 hours under the aerobic conditions. After exposition the colonies density (colony forming unit (CFU)/ml) and the difference in the microorganisms growth dynamics were determined.

Main part.

The study of the bactericidal effect of bentonite which was not exposed to thermal treatment allowed to establish that introduction of native clay in the Petri dishes resulted in decrease of the colonies density by 35% - 46%. In its turn use of activated sodium carbonate allows to reduce the colonies density by 66% - 74%.

Burning of clay at the temperature of 200^0C improves its bactericidal efficiency. Adding of native bentonite in the Petri dishes resulted in decrease of CFU/ml index by 39 - 49%. The activated medicine demonstrated more significant bactericidal effect. The colonies density decreased by 70% - 76% (Table 1).

Table 1. Decrease of the number of colony forming units (%) after adding of untreated bentonite and bentonite after burning at the temperature of 200^0C to the Petri dishes (n=30)

	Before treatment by clay	*Native clay not treated by burning		*Activated clay not treated by burning		*Native clay after burning at 200^0C		*Activated clay after burning at 200^0C	
Specie	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml
Streptococcus intermedius	9.6 ± 0.23	40	5.76± 0.2	72	2.69± 0.07	44	5.38± 0.2	75	2.4± 0.07
Streptococcus sanguis	9.4 ± 0.23	37	5.92± 0.2	66	3.20± 0.08	41	5.55± 0.2	71	2.76± 0.07
Streptococcus milleri	6.9 ± 0.21	42	4.00± 0.21	74	1.79± 0.06	44	3.86± 0.21	76	1.65± 0.06
Streptococcus mitis	7.0 ± 0.21	46	3.78± 0.18	72	1.96± 0.06	49	3.57± 0.18	76	1.68± 0.06
Peptosrtreptococcus spp.	9.8 ± 0.15	38	6.08± 0.12	67	3.23± 0.08	42	5.68± 0.12	70	2.94± 0.07
Actinomyces naeslundii	8.3 ± 0.2	35	5.40± 0.2	71	2.41± 0.07	39	5.06± 0.2	74	2.15± 0.07
Prevotella intermedia	8.3 ± 0.1	41	4.90± 0.09	69	2.57± 0.07	45	4.57± 0.09	73	2.24± 0.07
Actinobacillus actinomycetemcomitans	8.4 ± 0.18	36	5.38± 0.07	68	2.69± 0.06	40	5.04± 0.07	73	2.26± 0.06
Fusobacterium nucleatum	8.6 ± 0.23	36	5.50± 0.22	70	2.58± 0.07	41	5.07± 0.2	74	2.23± 0.06
Staphylococcus	7.4 ± 0.2	42	4.29± 0.1	71	2.15± 0.07	47	3.92± 0.09	74	1.92± 0.05

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Staphylococcus aureus	7.0 ± 0.14	39	4.27± 0.09	66	2.38± 0.06	45	3.85± 0.09	70	2.1± 0.06
Staphylococcus saprophyticus	7.2 ± 0.15	44	4.03± 0.09	72	2.02± 0.06	48	3.74± 0.09	75	1.8± 0.05
Candida albicans	3.5 ± 0.21	37	2.21± 0.15	67	1.16± 0.05	44	1.96± 0.13	71	1.15± 0.04

*Differences between the bactericidal activity of native and activated clays are significant at p ≤0.05.

The further increase of the burning temperature reduces the bactericidal effect of bentonite. After exposition at the temperature of 400^0C the colonies density reduced in the Petri dishes treated by native clay by 17% - 20%, in the Petri dishes treated by activated clay by 31 - 38%. Burning of the sub-samples at the temperature of 600^0C reduces the bactericidal effect of native and activated clay. Treatment of the Petri dishes by such clays results in decrease of CFU/ml index by 6-9%. After sintering of bentonite at the temperature of 800^0C the bactericidal effect is determined at the level of statistical error (Table 2).

Table 2. Decrease of the number of colony forming units (%) after adding of bentonite burned at the temperature of 400^0C, 600^0C and 800^0C to the Petri dishes (n=30).

Burning temperature ^{0} C		400^0C				600^0C				800^0C	
	Before treatment	*Native clay		*Activated clay		*Native clay		*Activated clay		Native and activated clay	
Specie	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml	Decrease (%)	CFU/ml
Streptococcus intermedius	9.6 ± 0.23	19	7.78± 0.21	33	6.43± 0.15	6	9.02± 0.23	8	8.83± 0.21	0	9.6 ± 0.29
Streptococcus sanguis	9.4 ± 0.23	18	7.71± 0.21	35	6.11± 0.15	7	8.74± 0.23	7	8.74± 0.21	0	9.4 ± 0.37
Streptococcus	6.9 ±	1	5.59±	38	4.28±	6	6.49±	7	6.42±	0	6.9 ±

milleri	0.21	9	0.21		0.12		0.2		0.2		0.58
Streptococcus mitis	7.0 ± 0.21	20	5.60± 0.21	37	4.41± 0.12	7	6.51± 0.2	9	6.37± 0.2	0	7.0 ± 0.44
Peptosrtreptococcus spp.	9.8 ± 0.15	20	7.84± 0.14	31	6.76± 0.13	6	9.21± 0.15	7	9.11± 0.15	0	9.8 ± 0.83
Actinomyces naeslundii	8.3 ± 0.2	18	6.81± 0.18	34	5.48± 0.13	6	7.80± 0.20	8	7.64± 0.15	0	8.3 ± 0.4
Prevotella intermedia	8.3 ± 0.1	20	6.64± 0.1	35	5.40± 0.9	7	7.72± 0.1	7	7.72± 0.1	0	8.3 ± 0.6
Actinobacillus actinomycetemcomitans	8.4 ± 0.18	17	6.97± 0.12	33	5.63± 0.08	7	7.81± 0.16	9	7.64± 0.15	0	8.4 ± 0.48
Fusobacterium nucleatum	8.6 ± 0.23	18	7.05± 0.23	32	5.85± 0.15	7	8.00± 0.21	9	7.83± 0.2	0	8.6 ± 0.29
Staphylococcus spp	7.4 ± 0.2	18	6.07± 0.15	35	4.81± 0.13	8	6.81± 0.19	8	6.81± 0.2	0	7.4 ± 0.2
Staphylococcus aureus	7.0 ± 0.14	17	5.81± 0.12	33	4.69± 0.1	6	6.58± 0.14	8	6.44± 0.14	0	7.0 ± 0.23
Staphylococcus saprophyticus	7.2 ± 0.15	20	5.76± 0.12	36	4.61± 0.1	6	6.77± 0.15	9	6.55± 0.13	0	7.2 ± 0.7
Candida albicans	3.5 ± 0.21	19	2.84± 0.17	35	2.28± 0.1	6	3.29± 0.21	9	3.19± 0.21	0	3.5 ± 0.29

* Differences between the bactericidal activity of native and activated clays are significant at $p \leq 0.05$.

Discussion. Clay minerals refer to the group of sheet and sheet-band silicates able to swell [9]. Their microcrystals have platelet shape the size of which does not exceed 20—30 microns [10] which determines the bactericidal potential of bentonites to a large extent. With their high adsorptive capacity bentonites are not only ingredients of a pharmaceutical preparation but act themselves as a medicinal preparation.

Depending of a solution pH it is possible to recharge the frontal areas of crystals. Such effect is connected with amphoteric character of the lateral chipping of the octahedral lattice. In acidic medium ion-exchange process which involves replacement of cathions of metals by hydrogen ions (H^+) first takes place. In addition the degree of dissociation and negative charge of the surface become lower due to possible presence of the fragments of anions of weak acids on the crystals surface. With strongly acidic media destruction of aluminum-hydroxide octahedrons is possible:

$$[Al(OH)_6]^{3-}+H^+ \rightarrow [Al(OH)_5H_2O]^{2-}$$

In alkaline medium destruction of silicon-oxygen tetrahedrons and aluminum-hydroxide layer due to disaggregation and dissolution is possible.

$$[Al_m(OH)_n]^{(3m-n)+} + 3OH^- \rightarrow [Al_{m-1}(OH)_{n-3}]^{(3m-n)+} + [Al(OH)_6]^{3-}$$

As a result of this process the lateral chipping of a clay particle is charged positively in acidic and neutral media and negatively in alkaline medium. The dynamics of electrochemical characteristics under the changing conditions is one of the main factors regulating the processes of structure formation and formation of bactericidal properties of bentonites. The data obtained by us are congruent with the opinion of the authors who studied the properties of bentonite clays modified by ions Na+ Mg2+, Zn2+, Li+, Ag+, K+ and proved their activity in respect of Gram-negative and Gram-positive flora [11,12]. These findings are the basis for selection of an optimal physical-chemical model of preparatory treatment of bentonites in order to create drug formulations having bactericidal effect.

Conclusions

1. The bactericidal potential of bentonite from the Korochanskoye field may be improved by activation of sub-samples with 2% sodium carbonate.
2. Burning at the temperature of up to 200^0C increases the bactericidal properties of native bentonite by 39% - 49% and of activated bentonite by 70%-76%.
3. Heating of bentonite up to 400^0C and higher results in impairment of the bactericidal potential both of native and of activated bentonite.

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