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EFFECT OF SILVER BASED PRODUCTS ON SIMULATED GASTRIC FLUID

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Abstract. Silver Zeolite A, Silver clinoptilolite, and Silver bentonite are called Silver Based Products (SBPs) .They have been used for treating some types of infectious diseases as antibacterial agents. Gastric fluid is vital fluids in body.. The first aims of this study is to study the effect of SBPs on the pH changes of gastric fluids in different concentrations, however, studying the effects of simulated gastric fluid (SGF) as model of gastric fluid on the structures of SBPs was the second aim. Zeolites and clay were applied as the carrier materials which silver ion (Ag+) could attach to them and produce SBPs as antibacterial agents. SBPs are analyzed by EDX and FTIR spectroscopy. The effects of SBPs on (SGF) are studied in different concentrations. The chemical framework variations of SBPs are analyzed by FTIR spectroscopy after contact with SGF in different concentrations and the results shown, the most important structural peaks which are same as those parents. SBPs had some insignificant structural variations after contacted with SGF. With increasing concentration of SBPs to SGF, more changes are occurred on their structures. At last the pepsin activity in SGF is studied

Keywords ZeoliteA, Clinoptilolite, Bentonite , Silver, Pepsin

1.0 INTRODUCTION

Antibiotic is a type of medications that functions as an antibacterial. Sometimes, antibiotics may fail to prevent from the growth of bacteria. Therefore, it is essential to find a new method that could replace the antibiotics for treating bacterial infections. To solve this problem, many researchers recently have attempted to find effective antimicrobial agents free of resistance and low cost, which has led to the use of Ag-based antibacterial agents .

Silver (signified chemically as Ag) that is commonly known as a heavy metal is widely used in many products in our daily life. In the medical field, silver could be employed as an antimicrobial agents .In the current study, silver nitrate (AgNo3) has been used in the preparation of silver based products (SBPs). Today, many well-known SBPs are globally produced, including silver nanoparticles, silver sulfadiazine, silver zeolites, and silver clays.

One of the supporting material for silver ion and other cations is zeolite that has a porous structure and consists of hydrated aluminosilicates. Zeolites do not have antibacterial activity but it can be induced with adding the silver ions and surfactant molecules[1]. Zeolites that are structurely modified with silver are capable to function as an antibacterial agent.

Another supporting material is natural clay minerals. Clays like bentonite same as zeolites provide a physically stable surface for silver nanoparticles and silver ions to be a supporting material for them[2]. There are very few studies on the effects of them on human organs and fluids such as gastric fluids.

Simulated gastric fluid (SGF) with a pH of below 2, during fasting state as model of gastric fluid is studied. It is noticeable that its pH can be ranged from 1 up to 7. 5 after food intaking.[3] The simulated gastric fluid was initially developed for estimating the effect of drug on the human gastric fluid and providing a situation similar to the real human stomach. It should be performed in order to evaluate the reciprocal impacts of SGF with the structures of the drugs or materials.

Here, to study the antibacterial impacts of SBPs, *Escherichia coli* (*E. coli)* was used as a microorganism model. The pepsin activity in simulated gastric fluid is studied. It is essential for human health to find out the interactions between SBPs and human gastric fluid, and the effects on these fluids resulted from the mentioned interaction are significant. Due to each kind of changes in chemical, biological, and physical features of human gastric fluid which is important for human health, we conducted the current study to investigate interactions between SBPs and this fluid and their effects on each other.

In this study, SBPs are added to gastric fluid in different concentrations. Among them, those that showed less pH changes in the body fluids and more stability in SBP structure could be used in treating infectious gastric fluids or other human stomach infection diseases in a way to have the least unwanted effect. Thus, these results can help the pharmaceutical and medical industry to produce proper medicine to treat the infectious diseases.

2.0 EXPERIMENTAL

2.1 Materials

Materials that used in this study for preparation of silver based products are Silver nitrate, zeolite A, clinoptilite, bentonite. For preparation of simulated gastric fluid, sodium chloride (Merck) and pepsin (Emory) with activity of 800 units per mg of protein and distilled water are used. Luria-Bertani broth (LB) culture media. Nutrient agar (NA) is used as a solid medium for culturing bacteria. Normal saline 0.9%for preparation 0.5 McFarland standard turbidity .devices are include FTIR,EDX, whatman paper, vacuumed filter, incubator ,Petri dish, bench top pressure device and soap.

2.2 Preparation of Materials

The first step in the project was the preparation a solution of silver nitrate 1000 ppm that should reacted with zeolites and clays to obtain silver-zeolites and silver-clays. 1.0 g of AgNO3 powder which is purchased from Merck, was accurately weighed and dissolved in 0.9 L of distilled water in a beaker. Using magnetic stirrer, the suspension is stirred to be completely dissolved. The solution was then transferred to volumetric flask 1 L and top up with distilled water to1 liter in order to prepare 1000 ppm of AgNO3 solution.

2.2.1 Preparation of Silver-Zeolites and Silver-Clay

Silver-zeolite and silver-clay are prepared by mixing AgNO3 (1000 ppm) stock solution to natural and synthetic zeolite and mineral clay samples. 10 g of each synthetic zeolites A and clinoptilolite as a natural zeolites also bentonite as mineral clays samples are mixed with 500 ml of AgNO3 (1000 ppm) solution. The mixtures are stirred using magnetic stirrer in the beaker that is covered with aluminum foil. Solutions are stirred for 24 hours. The homogenized mixture is then filtered by using vacuum pump and then dried in an incubator at 70 ºC overnight. The dried solid products are crushed into powder using mortar, they were then kept in plastic tubes and labeled according to the type of silver zeolites and silver mineral clay for further applications.

2.2.2 Preparation of Simulated Gastric Fluids

Simulated Gastric fluid (SGF) is prepared according to the British pharmacopoeia specific standard instructions. 2 g of sodium chloride (Merck) and 3.2 g of pepsin (Emory) with activity of 800 unit per mg of protein are added in a volumetric bottle which contains 700 ml of distilled water, then the mixture is stirred using magnetic stirrer for few minutes until the pepsin and sodium chloride were dissolved completely, in the next stage, 7.0 ml of hydrochloric acid 1 molar (Merck) is added into the solution and the pH is adjusted to 1.2. Subsequently, distilled water (DW) is added gradually until the total volume of the solution is 1 litre. Solution is kept in refrigerator with 5-8 ˚C. Figure 2.4 illustrates the steps for preparation of SGF.

2.3 Characterization of Materials

In order to determine the successful preparation of silver modified zeolites and clays, it is important to characterize the studied materials. The structure of zeolites and clay are analyzed using Fourier Transform Infrared Spectroscopy (FTIR) before and after modification. Energy Dispersive X-Ray (EDX) analyzer is used for the elemental analysis especially to detect Ag on silver zeolites and silver clays.

2.3.1 Infrared Spectroscopy (FTIR)

Infrared (IR) Spectroscopy is used for the structural analysis of zeolites and clay. FTIR model Nicolet iS5 is used for the structural crystal analysis. This instrument is applied for solid sample using ATR (Attenuated Total Reflectance) technique. About 1 mg of each sample is placed on sample area. FTIR spectrum was then recorded using OMNIC software in the range of 4000 to 400 cm-1, in duration of 3 to 4 minutes.

2.3.2 Energy Dispersive X-ray (EDX) Analyzer

Zeolites A, clinoptilolite, bentonite and their silver-modified forms are characterized by (EDX) Analyzer. EDX coupled FESEM (Energy Dispersive X-Ray (EDX) (JED 2300 analysis station) analyzer equipped together with CARL ZEISS 35 VP Supra Field Emission-Scanning Electron Microscopy (FESEM)) is used to analyse the element in the samples.

2.4 Antibacterial Assay

The antibacterial effects of SBP on E. coli are tested with Disk Diffusion Technique. Testing steps are explained below.

2.4.1 Preparation of Antibacterial Pellet

small pellets (disk) of uniform size (9 mm diameter) containing SBPs are prepared including silver-zeolite A, silver-clinoptilolite and silve-bentonite disk. Zeolites and clays disk were prepared as a negative control disk and for positive one, silver nitrate disk is used. They were punched out by benchtop hydraulic press device and under 3000 Pa pressure and, then, stored in plastic bag, in room temperature [4]

2.4.2 Antibacterial Assay by Disk Diffusion Technique

The antibacterial activities of silver-zeolites and silver-clays is evaluated against E. coli which is taken from the collection of bacterial culture in Microbiology Laboratory of Universiti Teknologi Malaysia (UTM), in form of the stock culture in Luria-Bertani broth (LB). Nutrient agar (NA) is used as a solid medium for culturing bacteria.

2.4.3 Preparation of Inoculums

Sterile loop is used to touch colonies of the E. coli that should be tested, then the organism is suspend in 5 ml of disinfected normal saline 0.9 % (NaCl). After that, normal saline pipe vortexes to produce suspension in 0.5 McFarland standard turbidity. One more time, these steps are repeated but by taking bacterial colonies from pure culture palate of the E. coli which is prepared by contaminated simulated body fluid in order to investigate the antibacterial activity of SBPs on infectious SBF .

2.4.4 Inoculation on the NA Plate

The hygienic swab is dipped into the inoculums tube of bacteria with 0.5 McFarland turbidity. The surface of NA agar plate is inoculated by streaking the swab four times on the entire agar surface. Eight plates are prepared by this method to be used, then 3 disks of silver products disk were prepared including silver-zeolite A, silver- clinoptilolite and silver-bentonite pellet. Zeolites and clays disks are considered as negative control disks, while silver nitrate disk as positive control disk. They are pressed down on the palates with forceps to ensure complete contact with the agar surface. Next, all plates are sealed by parafilm and kept in 37 °C for 24 hours, and subsequently, the diameter of inhibition zones for each disk is measured.

2.5 Different Concentrations of SBP in SGF

Different concentrations of SGF combinations with silver-modified zeolites and silver-modified clay (SBPs) are prepared to examine the effects of SGF on SBPs framework. Another goal is to study pH variations in simulated fluid after adding SBPs as an effect of SBPs in simulated gastric fluids. For this purpose, seven different concentrations of SBP that was mixed by SGF are prepared. The selected percentage of concentrations are 0.5%, 1%, 2%, 3%, 5%, 7%, and 10%. Each mixture solution contained a consistent volume of SGF equal to 50 ml, but the amount of SBPs were varied.

2.5.1 Preparation of Different Concentrations of SBP in SGF

To make 0.5% concentration of silver-zeolite A combined with SGF, 0.25 g of silver-zeolite A which weighted by electrical weight is added to shaker flask that contained 50 ml of SGF. The mixture is inserted in incubator and the temperature is set to 37 ºC (Same as human body temperature). The mixture has been shaken gently in 170 rpm for 15 minutes .

In the next step, the solution was filtered using Whatman filter paper in filter funnel, then filter paper containing samples is dried in an incubator with 70 ºC for 24 hours to get silver-zeolite A that was affected by simulated body fluids. Solids are crushed to powder by mortar. Their structural changes are analyzed by FTIR. Residual liquid of SGF is kept in the test tube to measure the pH by pH meter in room temperature. To prepare the other concentrations of silver-zeolite A and SGF, the same SGF volume of 50 ml is applied for all samples, but variable amounts of silver-zeolite A are selected . To avoid repetition, it is noticeable that the above-mentioned process has been taken place for adding SGF to other SBPs including Ag-clinoptilolite and Ag-bentonite, with similar concentrations

3.0 RESULT AND DISCUSSION

3.1 Elemental Studies of Samples by EDX

The elements of the materials are analyzed for two times. First, before modification with silver ion, and second, it was analyzed after silver loading on parent materials. It is done to analyze the existence of silver ions (Ag) in Ag-modified zeolites and clay by Energy Dispersive X-Ray (EDX) Analyzer. There was the silver ion in all Ag-modified samples.

The common elements available in zeolites are silica (Si), oxygen (O), aluminum (AI), and sodium (Na). The existence of (Ag) in the EDX spectra of modified zeolites verifies the successful loading and attachment of Ag on the parent zeolite during the preparation of SBPs.

On the other hand, typical elements existing in bentonite are silica (Si) oxygen (O), aluminum (Al), sodium (Na), and iron (Fe). The present of silver ions (Ag) in the EDX spectra of silver-modified clay proves the successful loading of Ag on the parent clay after treating silver-based mineral clay.The presence of Ag ions in the EDX spectra confirms the loading of Ag onto zeolites and clays in the Ag-modified samples.

3.1.1 Characterization of Ag-Modified Zeolites and Clay by FTIR

FTIR was used to study the molecular structure of zeolites and clay before and after modification with silver ions. FTIR is able to detect the changes which happen in crystalline composition of chemical materials. Since the most important elements in clays and zeolites are aluminum and silica, back bone bands in both of them were Si-O-Si and Si-O-Al. These main band's peaks in FTIR are generally situated in average spectrum from 1300 cm^{-1} to 500 cm^{-1}. As demonstrated by FTIR , modification of zeolite A, clinoptilolte and bentonite, can not create any changes in their spectra specially in typical peaks which are related to Si-O-Si and Si-O-Al bonds.

3.2 Characterization of Samples after Contact with SGF

The structural changes in SBPs caused after contacted with SGF in different concentrations are analyzed by FTIR spectroscopy. On the other hand, pH variations in SGF after contacted with SBPs in different concentrations were also studied.

3.2.1 FTIR and pH Variations after Contact with SGF

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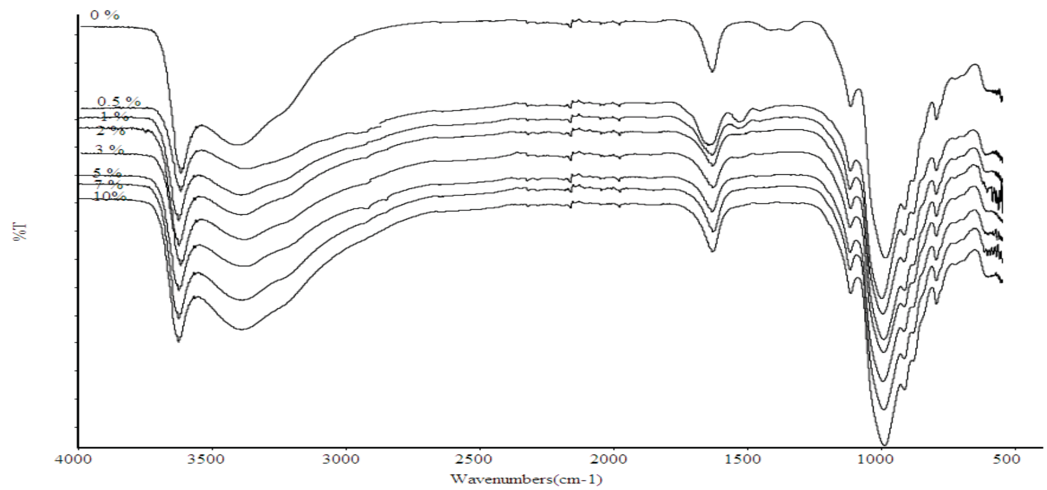

Figure.3.1 The FTIR spectra of Ag-bentonite and their spectrum after combination with SGF in different concentration from 0.5% to 10%, and spectrum of silver-bentonite which was not added to SGF (0%).

After contact the Ag-bentonite with SGF, more variation was observed at 550 cm^{-1} to 570 cm^{-1} which signifies Si-O and Si-O-Al bonds in main framework. These peaks had little variations that were not significant, and it shows that these bonds in acidic attack were weaker than others. Generally, the FTIR spectrum of Ag-modified bentonite after contact with SGF (Figure3.1) did not show significant changes in comparison with its parent. Table 3.1 gives the variation of pH of SGF after contact to Ag-modified bentonite samples in different concentrations. As results show, pH variation increases by the increasing contact percentage. .

Table 3.1: The pH changes of SGF after mixing with Ag-bentonite samples in varied concentration.

Concentration level	0%	0.5%	1%	2%	3%	5%	7%	10%

pH	1.2	1.24	1.26	1.37	1.46	2.03	2.78	3.06

The Si/Al ratio in bentonite construction, as well as Si-O-Si and Si-O-Al bonds in bentonite framework make the bentonite partly hard and firm structure that adsorbs water solution better than other mineral clays. Therefore, the bentonite stability in SGF acidic situation is weak[5]. Because of its layered structure, high Si/Al ratio and abundance of strong bands such as Si-O, this mineral clay has stable building the same as natural zeolite, but more stable than synthesized zeolites

FTIR spectrum of Ag-zeolite A showed some peaks from 1000 to 500 cm^{-1} which are related to aluminosilicate structure of Ag-modified zeolite A. As the concentrations of SGF mixing with zeolite A rise, the FTIR spectrum shows some changes in three important peaks such as 1000, 670, and 550 cm^{-1} that are related to asymmetric Si-O-T, symmetric Si-O-T band, and double rings vibrations, respectively.

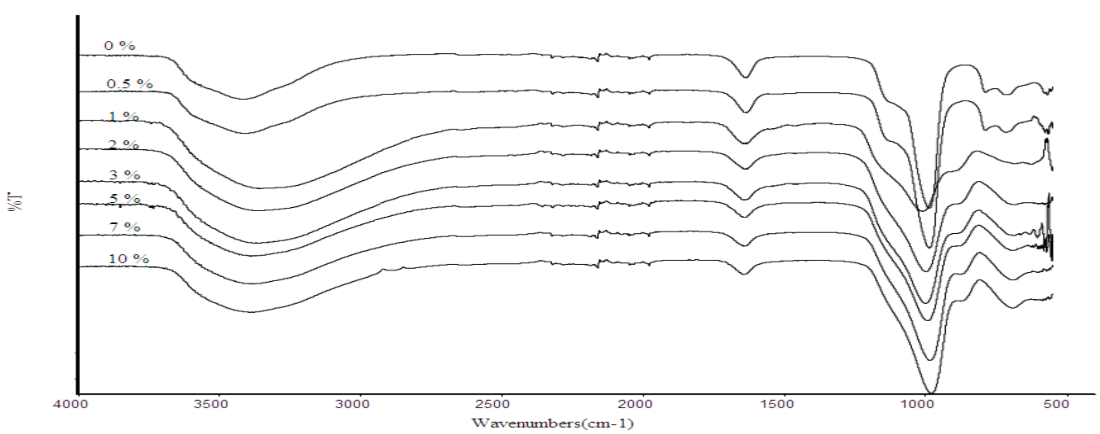

Figure 3.2 The FTIR spectra of Ag-zeolite A and their range after mixing with SGF in different concentrations from 0.5% to 10%, and spectrum of silver-zeolite A which was not added to SGF (0 %).

These changes correlate with elemental changes in silver-zeolite A composition and show a low stability of this zeolite configuration against acidic pH of SGF.
FTIR spectrum of silver clinoptilolite contains particular peaks from 1000 to 500 cm^{-1} which are correlated with main structure of Ag-modified clinoptilolite. FTIR

spectrum shows some changes in three important peaks during intensifying the concentrations of SGF mixing with this material .Important peaks such as 1010, 790 and 520 cm^{-1}show a little changes, these peaks indicate asymmetric Si-O-T, symmetric Si-O-T band, and double rings vibrations separately. Table 3.2 demonstrates the pH of SGF samples after mixing with Ag-zeolite A in different concentrations.

Table 3.2: The contrast between changes in pH of SGF after mixing with different concentrations of synthesized zeolite A and Y

Concentration percentage %	**pH of SGF after mixed by silver-zeolite A**
0	1.20
0.5	1.80
1	3.03
2	3.27
3	3.67
5	3.86
7	4.10
10	4.19

Table 3.3 illustrates the pH of SGF after combining with Ag-clinoptilolite in different concentrations. As it is observable, pH rises by increasing the concentration combination.

Table 3.3: The pH changes of SGF after adding to Ag-clinoptilolite in different concentrations.

Concentration %	0	0.5	1	2	3	5	7	10
pH	1.20	1.26	1.27	1.30	1.32	2.39	2.73	3.11

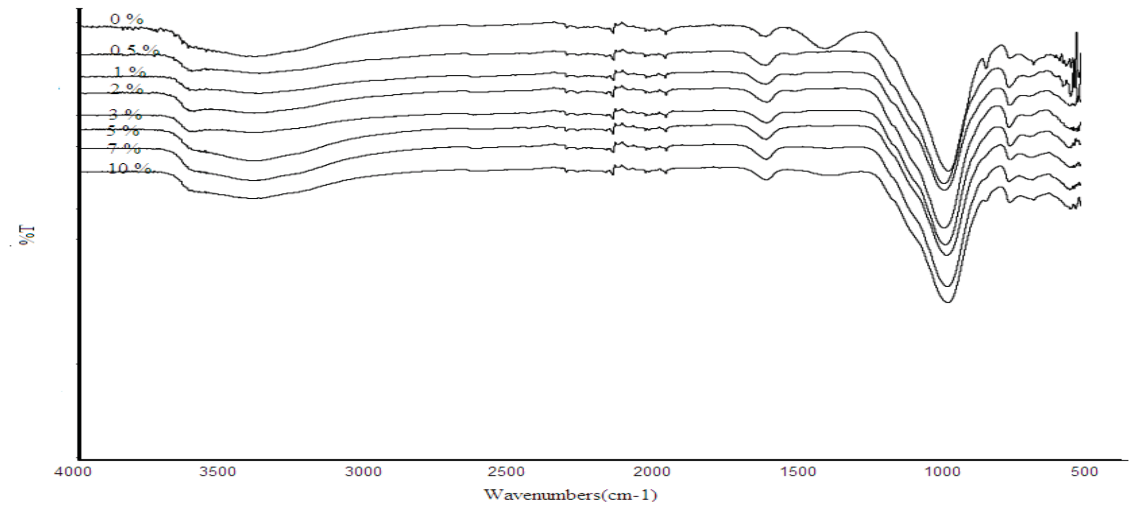

Figure 3.3 The FTIR spectra of Ag-clinoptilolite and their range after mixing with SGF in different concentrations from 0.5% to 10%, and spectrum of silver-clinoptilolite which was not added to SGF (0 %).

FTIR spectrum of silver clinoptilolite contains particular peaks from 1000 to 500 cm^{-1} which are correlated with main structure of Ag-modified clinoptilolite. FTIR spectrum shows some changes in three important peaks during intensifying the concentrations of SGF mixing with this material. Important peaks such as 1010, 790 and 520 cm^{-1}show a little changes, these peaks indicate asymmetric Si-O-T, symmetric Si-O-T band, and double rings vibrations separately.

Table 3.4 illustrates the pH of SGF after combining with Ag-clinoptilolite in different concentrations. As it is observable, pH rises by increasing the concentration combination, however this rising, similar to mineral clays, was not very significant. Due to structural stability of Ag-modified natural zeolites, the pH changes in SGF which were mixed with these materials were noticeably less than Ag-modified synthesized zeolite A.

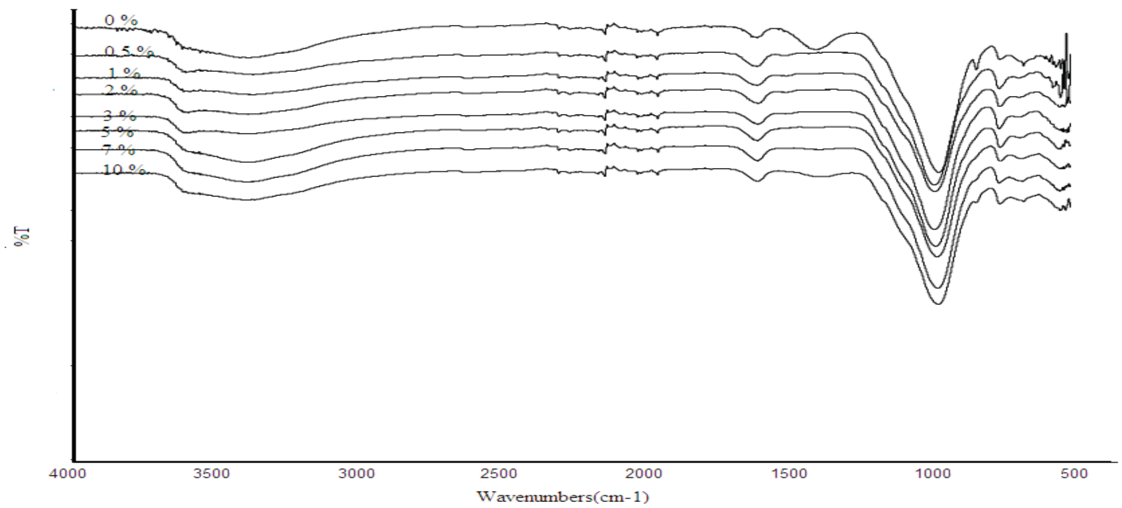

Figure 3.4 The FTIR spectra of Ag-clinoptilolite and their range after mixing with SGF in different concentrations from 0.5% to 10%, and spectrum of silver-clinoptilolite which was not added to SGF (0 %).

Table 3.4: The pH changes of SGF after adding to Ag-clinoptilolite in different concentrations.

Concentration %	0	0.5	1	2	3	5	7	10
pH	1.20	1.26	1.27	1.30	1.32	2.39	2.73	3.11

Generally, FTIR spectrum of samples shows the structural stability level of SBPs in acidic condition of SGF in an order from those of higher level to lower ones as follows bentonite > clinoptilolite > zeolite A. The current study showed that, compared to zeolites, mineral clay have stronger structural frameworks in acidic states. Additionally, clinoptilolite composition is more stable than zeolite A .

When SBPs get combined with SGF, their alkaline properties cause an increase of the pH of SGF, and by increasing the concentration of mixture, the pH increases more and more. This growing was showed remarkably in case of zeolite A. On the other word, in case of increasing the level of pH, we can rank the materials in an order from the highest influence to the least as follow: zeolite A > clinoptilolite > bentonite.

3.3 The Pepsin Activity Assay

Pepsin enzyme is active in pH ranged from 1.2 to 4, and in pH more than 4, it will be reversible denature, and in pH more than 6, it will be irreversible denature and does not have any activity. The present study shows that by increasing the concentration of Ag-zeolite A in SGF, the pH will have the higher rising rate than Ag-clinoptilolite. Increasing rate of pH. Based on the results obtained from studies conducted on the ability of SBP in increasing the pH of SGF after adding together, the materials are ordered from the higher ability to lower ability as follow: Ag-zeolite A > clinoptilolite > bentonite. All of SBPs that are added to SGF with concentrations ranged from 0.5% to 10% do not have any effect on pepsin enzyme activity because they could not increase the pH of SGF more than 4, except for zeolite A which had pH 4.19 in 10% concentration.

3.4 Antibacterial Activity of SBP on *E. coli*

In order to investigate the antibacterial activity of SBPs on *E. coli,* Disk Diffusion technique (Kirby-Bauer) was used. Bacterial concentration Are cultured in nutrient agar plates. The pellets prepared from SBPs were placed on the NA palates and all dishes were kept in the same temperature (37˚C) for 24 h, the silver nitrate pellet was used as positive control and zeolite A pellets also were used as negative controls. After one night NA palates Are studied in other to investigate the antibacterial activity of SBPs on *E. coli*. Inhibition zone around each pellet was measured using a ruler. Results are tabulated in Tables3.5.

Table 3.5 The inhibition zone on direct bacterial sample of *E. coli* pure culture.

Antibacterial Agent	Diameter of inhibitory zone (mm)
Silver-zeolite A	21
Silver-clinoptilolite	18
Silver-bentonite	19

E. coli was sensitive to all of considered silver zeolites and silver clay because the diameter of standard inhibitory zone for this bacteria is around 8mm, and all of studied sample show inhibitory zone diameter more than 8mm [6] Positive control was a silver nitrate pellet with a diameter of 25 mm in the inhibition zone. The negative controls did not show any inhibition zone. These results can be interpreted based on the power of different kinds of SBPs to release silver ions in the bacterial culture media. In order to use Kirby-Bauer method, SBPs must be in a pellet form with an invariable weight and diameter. Therefore, all antibacterial pellets had 0.9 cm diameter and 0.5 g weight.

4.0 CONCLUSION

This study has focused on the usage of silver-modified zeolites and silver-modified clays as antimicrobial agents and investigated the effects of these materials on simulated gastric fluid (SGF). In this study, synthetic zeolite (zeolites A), clinoptilolite as a natural zeolite, and mineral clays (bentonite) were used as supported materials for silver. They are modified by silver ions (Ag) and SBPs were provided which are used as antibacterial agents. Using EDX and FTIR spectroscopy. Results obtained from EDX confirmed that Ag ions were successfully loaded on zeolites and clays. Comparing the FTIR spectra of parent zeolites and clay with their modified spectra revealed that silver ions can not change the crystal structure of studied materials. The second part of this research was examining the simulated gastric fluid in order to find out the effects of SBPs (silver zeolites and silver clay) on these fluids in different concentrations. After adding Ag-zeolites and Ag-clay to SGF with various concentrations, structural changes in SBPs were examined using FTIR spectroscopy. Results showed that these combinations had a number of effects on the framework of studied materials, The final part of the study was investigating the antibacterial activity of silver-zeolite A, silver-clinoptilolite and silver-bentonite against *E. coli*. Disk diffusion technique was used to investigate the antibacterial activity of SBPs. The amount of inhibition zones were measured around the SBPs pellets on the bacterial cultured of contaminated SBF by *E. coli*. This measuring also was conducted on direct bacterial sample of *E. coli* pure culture. Antibacterial activity assay showed that all of these products had strong inhibitory effects on *E. coli* growing, especially silver-zeolite A by the largest inhibition zone (21mm). FTIR spectroscopy of SBPs, after adding to SGF, showed significant changes in their structures especially in Si-O-Si and Si-O-Al bands. The pH of SGF increased by rising the concentration of SBPs. Results indicated that SGF had the most pH increasing after combination with silver zeolite A. the pepsin enzyme activity on it has decreased, so zeolite A with 7% and more concentration can be destructive for SGF and pepsin activity. As a result, the silver modified zeolites and clays can be used as substitute antibacterial agents on contaminated gastric fluids with certain concentration which do not increase the pH of these fluids detrimentally. The use of SBPs which have more stable structure is more suggested in order to have less interaction with SGF , and fewer structural changes will happen, and also they have less damaging effects on gastric fluid.

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