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FULL PAPER

Study of some physical changes of a binary combination of a drug Amphotericin B with Cetyl Trimethylammonium bromide (CTAB)

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The research included studying the effectiveness of Amphotericin B with cactus aloe vera extract for its therapeutic properties by studying the physical changes with the surfactant active substance which has the ability to form thread-like micelles, and with the drug Amphotericin B in which the mixture of the surface-active substance CTAB with the drug Amphotericin B at temperatures (283.15, 293.15, 310.15, and 323.15 K) and it was found that the highest value of viscosity was at (4:6), at 6 mL of CTAB surfactant and 4 mL of Amphotericin B, from 3% wt of CTAB and 0.02 g of Amphotericin B indicating the formation of filamentous micelles. The research also included studying the effect of the plant extract of aloe vera in different sizes (1, 2, 3, and 4) mL and at different temperatures. The thermodynamic functions were calculated and the results were clarified. The formation of worm-like micelles was increasing by maximizing the size of the plant extract of aloe vera as a result of interference with the composition of micelles and the formation of hydrogen bonds with the polar aggregates of the surfactant molecule. The biological effectiveness of the mixture was confirmed, The results showed that the sample mixture (A) was effective for both killing and inhibiting the growth of (Basillus pumilus) bacteria with inhibitory diameters (12.3, 12.5 and 14.7 mm), which appeared in the form of halos around the drilling area and were distinct from the culture medium inside the dish, while the inhibitory diameters were biological (Nystatin) (12.3, 12.3, and 12.8 mm), as it is noted that the sample mixture (A) has an effectiveness in killing Basillus pumilus more than the effectiveness of the antibiotic (Nystatin) itself, as these results are significant and excellent.

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KEYWORDS

Amphotericin B, Aloe vera, viscosity, micelles.

Introduction

Amphotericin B [1] is a drug used to treat life-threatening infections caused by fungi. It is an anti-fungal approved by the United States Food and Drug Administration (FDA) to treat several types of fungal infections, such as histoplasmosis and cryptococcus. Amphotericin B is also FDA-approved for the treatment of two forms of leishmaniasis as a parasitic infection. The uses of amphotericin B approved by the Food and Drug Administration vary, depending on the drug formulation. Amphotericin B is used to treat infections caused by certain parasites, such as: Leishmania of the skin tissue, brain inflammation, and its membranes caused by amoeba. The mechanism of the drug's effectiveness is to kill the fungi and prevent its reproduction. Amphotericin is given

mainly by intravenous injection as a composition containing sulfate or lipids, or as a particulate lipid composition (Liposomal) [2].

The goal of developing these drug combinations is to reduce their toxicity. Oral amphotericin B preparations are available and used in the treatment of candidiasis of the oral cavity. The form of this oral preparation is not considered toxic compared to formulations of amphotericin for intravenous administration. Amphotericin B [3] was also used to treat black fungus in the early stages of the disease. It was used in India to treat cases of black fungus and was considered as the main treatment against black fungus infection. Aloe vera extract [4] has been used for thousands of years. Despite the emergence of many medicinal herbs used for medicine, aloe vera is still one of the few ancient plants that are still used in the manufacture of medicines and cosmetics for various reasons. The use of aloe vera is not new, and its usage has a long history. The drawing of the cactus plant is engraved on the walls of the ancient Egyptian temples, as it was one of their sacred plants. The importance of "Aloe Vera" is due to its multiple therapeutic benefits, as well as its effective role in obtaining soft and supple skin. Aloe vera [5] is likely used in traditional medicine to treat colic pain, constipation and infections, in addition to healing wounds as well. To date, aloe vera is one of the few plants still used in the pharmaceutical, cosmetic, and food industries. The German Gesundheit website reviewed five reasons for utilizing this plant as for wound healing: The ancient medical encyclopedias talked often about the use of aloe vera to soothe burns and promote wound healing, which was recently confirmed by many recent studies, as it was found that aloe vera accelerates wound healing and promotes the growth of new cells. Aloe vera also contains an antibacterial component. A recent report indicated that using aloe vera after surgical intervention helps heal wounds faster.

Digestion: Aloe vera [6] is characterized by containing a large number of amino acids, enzymes, minerals and vitamins similar in composition to those found in the human body than any other plant. These substances enhance the ability of aloe vera to rid the intestines of food waste. For those who suffer from constipation problems, health experts advise them to drink aloe vera juice on a regular basis. Also, eating aloe vera reduces symptoms of colitis and stomach inflammation.

Arthritis: Aloe vera stimulates the immune system because it is a powerful anti-inflammatory and pain-relieving. Rheumatoid arthritis is a chronic disease, and when infected, the immune system attacks the joints, which leads to stiffness, swelling and severe pain. To treat this pain, cortisone injections and non-steroidal anti-inflammatory drugs are often used, which can reduce the symptoms of arthritis but cannot repair damaged tissue, as well as its side effects, while aloe vera is characterized by its effective ability to regenerate cells, which makes it a great remedy for those who suffer from Painful arthritis. Aloe vera can be taken in the form of capsules or applied topically.

Cancer: Each year, more than 12 million people die of cancer. However, a new study conducted on mice confirmed that the mixture of honey and aloe vera helps a lot to prevent cancer. Aloe vera contains sugars which can reduce tumor mass, while honey prevents the growth of cancer cells. A recent Italian study confirmed that the use of aloe vera during chemotherapy for cancer patients is more effective than chemotherapy alone, as it improves the patient's quality of life and has fewer side effects.

To strengthen the immune system: Many people suffer from the problem of the "stressed" immune system, and this is due to the unhealthy eating style as well as the life pressures, which negatively affects the

immune system. Besides, the sugars in aloe vera juice stimulate white blood cells and improve their performance to fight viruses. Aloe vera also contains antioxidants which help the immune system expel free radicals. Figure 1 displays the stages of the cactus plant.

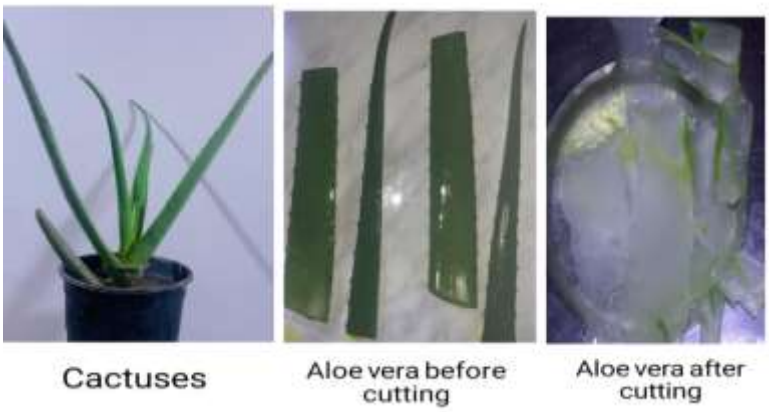

FIGURE 1 the stages of the cactus plant

Experimental part

It included the solutions preparation selected for this scrutiny, the effect of studying the viscosity and proportions of the drug mixture (Amphotericin B) and the surfactant (CTAB) at temperatures (283.15, 293.15, 310.15, and 323.15 K) and using water as a solvent. In addition , the effect of the plant aloe vera extract was examined by volumes (1, 2, 3, and 4) and temperatures (283.15, 293.15, 310.15, and 323.15 K) on the formation of thread-like micelles for the mixture of materials at the ratio (4-6) for the drug and the active-surface substance.

TABLE 1 The used devices

Origin	Workplace	Model	name The device	No.
German	Samarra University – Iraq	**Sartorius**	Sensitive scale (four decimal places)	**1**
German	Samarra University – Iraq	**Termaks**	drying oven	**2**
Malaysia	Samarra University – Iraq	**Casio**	Electronic watch to calculate the time	**3**
English	Samarra University – Iraq	**Carbolite**	burning oven	**4**
Chinese	Samarra University – Iraq	**capillary Dia 0.7 mm**	Viscometer (Ostwald)	**5**

Chemicals used

Viscosity measurement solutions

Preparation of the CTAB surfactant solution

(100 mL) at a concentration of (3% wt) was prepared as a stock solution of CTAB by dissolving (3.0928 g) in an amount of distilled water in a volumetric vial (100 mL), and then completing the volume to the mark with distilled water.

Preparation of the drug solution (Amphotericin B)

Prepare (100 mL) at a concentration of 5×10^{4} M as a stock solution of Amphotericin B by dissolving (0.02 g) in an amount of distilled water in a volumetric flask of (100 mL) capacity, then completing the volume up to the mark with distilled water.

Mixture of a solution of the surfactant active substance CTAB with the drug Amphotericin B

Different solutions were prepared, which are in the form of a mixture with a volume of (10 mL) by drawing certain volumes of the stock solution of CTAB and mixing them with certain volumes of the stock solution of Amphotericin B, and thus we get solutions with different mixing, but the final

concentration of each mixture remains constant, the amount of volumes withdrawn from the stock solutions of both substances (CTAB) and the drug (Amphotericin B) are indicated in Table 1.

Viscosity measurements

The water bath is set to the temperature required for measurement and left until it reaches the desired temperature. After that, the viscosity measuring instrument (Ostwald) containing the model to be measured is placed in the water bath so that it is almost completely immersed and left for 5 minutes with stirring from time to time. Then, we measure the descent time of the model from the starting point to the ending point which was determined on the measuring instrument, and the process is repeated within 3-5 times to get an average of several readings, and this measurement is done with four temperatures, which are (283.15, 293.15, 310.15, and 323.15 K). The experiment is repeated for all the solutions referred to in Table 1 above, and the viscosity of the model is calculated from the following Equation 1:

$$\eta_1\eta_2 = \sigma_1 t_1 \sigma_2 t_2 \quad (1)$$

In which σ1, t1, and η1 represent the density, descent time and viscosity of water, respectively.

Whereas, σ2, t2, and η2 represent the density, descent time and viscosity of the surfactant solutions to be measured, respectively.

The effect of the presence of aloe vera extract on the formation of worm-like micelles at the ratio (4-6) of CTAB and Amphotericin B.

Preparation of aloe vera extract

Aloe vera samples at 3 years old were obtained from plant nurseries in the city of Samarra, where the aloe vera leaves were cleaned and washed well with distilled water several times to clean them from dirt and dust, and then left to air dry, after that the leaves were cut and the outer part of the leaves was removed and the sticky substance inside the aloe vera leaves was extracted. It was collected and then, placed in a blender, filtered, and the certain sizes were taken.

Preparation of a mixture of a solution of the surfactant active substance (CTAB) with the drug Amphotericin B in different volumes from aloe vera extract

Four different solutions of (CTAB) and (Amphotericin B), which represent the volumes (1, 2, 3, and 4), were prepared, then mixed the similar concentrations in the ratio (4:6) of (CTAB) and (Amphotericin B), respectively to form a mixture with a volume of 10 mL for each volume, and the amount of volumes withdrawn from the prepared solutions for both substances are indicated in the Table 1.

Biological efficacy test

The biological activity test was carried out by studying the mixture ability of the samples to kill pathogenic bacteria Klebsiellapneumonia, Micrococcus roseus, Staphylococcus aereus, Escherichia coli as well as Candida albicans which was compared with the antibiotic inhibitory ability (Nystatin) (7).

Method for testing the ability of a mixture of samples to kill staphylococcus bacteria and Candida fungus (Candida albicans)

Biological activity was carried out by using agar well diffusion method, and bacteria of (Klebsiella pneumonia, Micrococcus roseus, Staphylococcus aereus, and Escherichia coli) were activated in a nutrient broth, and isolates of Candida albicans were activated in a sugar solution of dextrose (8).

(Dextros) Pits were made in the medium of (Mueller Hinton agar) with a diameter of (8 mm), then (50 µL) of the sample mixture, and (75 µL) of the antibiotic (Nystatin) for fungi

and (Neomycin) for bacteria were transferred at a concentration of (0.002 M) dissolved in dimethylformamide.

Dimethyl formamide-DMF was applied to the pits and left for an hour, then it was incubated at a temperature of (310.15 K) for (24) hours, after which the diameters of the inhibition zones were measured using a zone reader.

Statistical analysis

The statistical program (SPSS) was applied by T-test to compare samples and (Nystatin) for bacteria of the type (Klebsiella pneumonia, Micrococcus roseus, Staphylococcus aereus, Escherichia coli, and Candida albicans), and the significant differences were calculated at Perform statistical analysis of all data at the probability level ($p \leq 0.05$).

Results and discussion

Viscosity and thermodynamic measurements of micelles formation

Molecular assemblies lead to a marked increase in any solution when thread-like or worm-like micelles are formed (9). Therefore, the viscosity presence can be considered as a clear evidence for the presence of worm-like micelles in which in the proposed research, the ability to form worm-like micelles in the mixture of the surfactant (CTAB) and the drug (Amphotericin B) was studied on the ability to form worm-like micelles by measuring the solutions viscosity with different mixing ratios and at different temperatures. Table 1 indicates the viscosity of a 3 wt% mixture of (CTAB) and the drug (Amphotericin B) at different mixing ratios and temperatures. The results indicated that the highest viscosity was within the ratio of (4/6), because CTAB possesses a hydrophobic hydrocarbon chain whose length exceeds (four CH_2 groups) more than that of Amphotericin B. We note that the figure displays the relationship between viscosity and percentages of the CTAB mixture and Amphotericin B at different temperatures (10). As can be seen in Table 2, increase temperature leads to a decrease in viscosity due to an increase in the kinetic energy of the particles by dismantling the forces of molecular interactions, that is a cause for a decrease in interaction forces between water molecules with each other, on the one hand, and between water molecules with molecules of the surface active substance, on the other hand. The results also indicate that the CTAB viscosity alone is greater than the viscosity. For Amphotericin B alone, the reason for this is the increase in the length of CTAB hydrocarbon chain compared to Amphotericin B, and these values are consistent with the values obtained from previous studies. In general, the results demonstrate the formation of worm-like micelles, where an increase in viscosity values is observed with different mixing ratios of CTAB/Amphotericin B, as the highest viscosity was obtained at (K283.15) temperature at a mixing ratio of (4/6).The unique property of Supramolecular gel or molecular assemblies as thermodynamically controlled live polymers [11] gives it the special important applications in many fields. Therefore, this study should be carried out at different temperatures in order to calculate the thermodynamic functions. The thermodynamic variables were calculated for the micelle formation process in a range of temperatures ranging (283.15, 293.15, 310.15, and 323.15K) by using the following equations: The enthalpy of the formation of micelles (ΔH) was calculated using equation No. (0) (Vant Hoff) from drawing the relationship between (ln η) and the reciprocal of temperature (1/T), as it was calculated from the slope values of linear relationships (R \ HΔ- by substituting for the value of R) (in the amount of 8.314 $J.mol^{-1}.K^{-1}$, and the free energy values) (ΔG^0) were calculated using Equation 1. As for the entropy values (ΔS^0), they were calculated

from the relationship between (ΔG^o), (ΔH), and (ΔS^o) as in Equation 2 as well as the activation energy for thread-like micelles can be calculated from the following relationship [11]:

$$\eta \propto e^{Ea/RT} \quad (2)$$

As indicated in Table 2, the ΔG^o values increase generally with maximizing temperature, which is accompanied by a decrease in viscosity and thus, the lack of formation of worm-like micelles is resulted for the mixture of ionic surfactants (CTAB/Amphotericin B). The results also indicate that at a temperature K for the ratio (6) and (4) for the mixture CTAB/Amphotericin B, the ΔG^o value is as low as possible (kJ/mole), respectively.

TABLE 2 Viscosity (η) values and other related thermodynamic functions for the CTAB/ Amphotericin B Mixed system at different temperatures

CTAB 3%wt	Amphotericin B 5×10^{-4} M	η (Pa.s) $\times10^2$ (ΔGº kJ.mol^{-1}) {ΔSº J.mol^{-1}.K^{-1}} 283.15K	293.15K	310.15K	323.15K	ΔHº kJ.mol^{-1} SE[a] r^2[b]	Ea kJ.mol^{-1} SE[c] r^2[d]
10	0	0.0037587 (9.353) {-5.513}	0.003226 (10.054) {-5.514}	0.00328 (10.591) {-5.514}	0.002583 (11.687) {-5.516}	-5.484 ±12.516 {0.8529}	0.0078 ±0.0003 {0.855}
9	1	0.0037095 (9.381) {-81.29}	0.003113 (10.143) {-81.66}	0.003294 (10.582) {-82.98}	0.002754 (11.516) {-82.11}	-3.903 ±11.885 {0.7375}	0.00069 ±0.0002 {0.870}
8	2	0.0036531 (9.420) {-101.62}	0.003088 (9.959) {-102.38}	0.002961 (10.869) {-104.60}	0.002607 (11.666) {-102.76}	-5.018 ±12.342 {0.8941}	0.00157 ±0.0005 {0.822}
7	3	0.0041764 (9.101) {-75.12}	0.003266 (10.023) {-74.57}	0.002855 (10.951) {-75.58}	0.002637 (11.623) {-75.71}	-7.050 ±13.132 {0.8536}	0.00066 ±0.0002 {0.937}
6	4	0.004035 (9.181) {-116.53}	0.003272 (10.022) {-116.27}	0.002949 (10.871) {-119.16}	0.002589 (11.676) {-117.54}	-13.814 ±14.638 {0.0788}	0.00194 ±0.0006 {0.835}
5	5	0.0031662 (9.750) {-91.300}	0.002920 (10.304) {-90.204}	0.002723 (11.071) {-90.977}	0.002367 (11.910) {-92.172}	-4.620 ±12.316 {0.9882}	0.00214 ±0.0004 {0.965}
4	6	0.002946 (9.921) {-83.218}	0.002685 (10.503) {-86.604}	0.002630 (11.163) {-105.19}	0.002286 (12.011) {-85.155}	-3.777 ±12.033 {0.9322}	0.00062 ±0.0001 {0.940}
3	7	0.0038906 (9.273) {-82.183}	0.002954 (10.279) {-85.586}	0.002750 (11.052) {-85.111}	0.002286 (12.011) {-84.061}	-7.861 ±13.541 {0.8898}	0.00041 ±0.0001 {0.922}
2	8	0.0029435 (9.935) {-80.781}	0.002654 (10.539) {-83.727}	0.002631 (11.161) {-83.221}	0.002162 (12.163) {-82.545}	-4.673 ±12.404 {0.9043}	0.00041 ±0.0001 {0.952}
1	9	0.003358 (9.261) {-77.806}	0.002903 (10.313) {-80.666}	0.002882 (10.932) {-79.903}	0.002487 (11.789) {-79.548}	-4.371 ±12.174 {0.8896}	0.00023 ±0.0001 {0.983}
0	10	0.0031654 (9.760) {-78.464}	0.002779 (10.421) {-81.945}	0.002711 (11.096) {-80.681}	0.002325 (11.961) {-80.475}	-4.444 ±12.258 {0.9133}	0.00035 ±0.0001 {0.999}

The negative enthalpy HΔ values, as depicted in Table 2, indicate that the mixing process of (CTAB/ Amphotericin B) is an exothermic process [12], and by drawing a relationship between the reciprocal of temperature (1/T) and the logarithm of viscosity (ln η), as it gives the slope, we calculate from the value of enthalpy ΔH^o, and note that it gave linear relationships with a good correlation coefficient. The relationship

between ln η and 1/T is applied to calculate the enthalpy value ΔH at different temperatures for the mixture. At the ratio, as for the ΔS^0 values for the formation of micelles, which are indicated in Table 2, they have a negative sign. The reason for this is attributed to the composition of the surface active substances containing two groups [9,10], namely; a hydrophilic head group and a hydrophobic tail group, as the hydrocarbon tail group is not soluble in water which leads to an energy unfavorable distortion in the composition of the water molecules, as they are arranged in a way that increases the number of bonds between the water molecules surrounding the organic aggregates, causing a significant increase in the overall randomness of the system. Although the process of forming micelles will lead to a more regularity of the surfactants molecules, and this reduces the randomness of the system, however this decrease is not effective compared to the increase in randomness due to the release of water molecules which are of high order around the tail group of surfactants before the process of the micelles formation, which consequently causes an increase in the overall randomness of the system, the presence of a number of free conjugate ions in the solution, and an increase in the freedom degrees of the micelle, and these results are similar to the values obtained in the literature [13].

The values of activation energy Ea were calculated for the micelle formation process of the mixture (CTAB/Amphotericin B), and the highest value for the ratio was 4/6 (J/mole) and the lowest value was for the ratio was (J/mole) as shown in Table 3. And from drawing the relationship between the reciprocal of temperature and viscosity, it gave linear relationships and correlation coefficients. The effect of the volume of aloe vera extract (1, 2, 3, and 4) on the viscosity of the mixture CTAB/Amphotericin B at temperatures (283.15, 293.15, 310.15, and 323.15 K) was studied, and the results in Table 3 indicate a significant increase in the viscosity values of the mixture in the presence of aloe vera extract, and it is also noted from the Table 3 that the viscosity values of the mixture increase irregularly with the increase in the volume of the plant extract [14].

TABLE 3 Viscosity (η) values and other related thermodynamic functions for the CTAB/ Amphotericin B and Ginger Mixed system at different temperatures

sample code	CTAB 3wt%	Amphotericin B 5×10^{-4}M	Ginger (mL)	η (Pa.s) $\times10^2$ (ΔG^o kJ.mol^{-1}) {ΔS^o J.mol^{-1}.K^{-1}} 283.15K	293.15K	310.15K	323.15K	ΔH^o kJ.mol^{-1} SE[a] r ^{2}[b]	Ea kJ.mol^{-1} SE[c] r^{2}[d]
A	6	4	1	0.002916 (9.959) {-139.52}	0.002615 (10.578) {-144.243}	0.002768 (11.031) {-139.713}	0.002516 (11.754) {-143.950}	-1.821 ±11.238 {0.5341}	0.00069 ±0.0003 {0.931}
B	6	4	2	0.003145 (9,777) {-125.222}	0.002773 (10.42) {-128.856}	0.002962 (10.866) {-127.395}	0.002598 (11.666) {-128.446}	-2.360 ±11.388 {0.5718}	0.0016 ±0.0005 {0.951}
C	6	4	3	0.003620 (9.445) {-139.726}	0.003092 (10.16) {-142.97}	0.003106 (10.735) {-142.383}	0.002507 (11.764) {-142.933}	-5.365 ±12.506 {0.8681}	0.0017 ±0.0007 {0.951}
D	6	4	4	0.003535 (9.505) {-144.595}	0.003092 (10.161) {-144.144}	0.002925 (10.896) {-146.249}	0.002715 (11.555) {-146.555}	-3.826 ±11.933 {0.8951}	0.0068 ±0.0015 {0.885}

The ability of the sample mixture (A) to kill staphylococcus Klebsiellapneumonia, Micrococcus roseus, Escherichia coli and Candida albicans

The effect of the sample mixture (A) was studied on a type of staphylococcus bacteria. The effect of the sample mixture (A) on a type of Candida albicans, which causes many fungal infections in humans, was studied. The inhibitory activity of the sample mixture (A) was compared with the inhibitory activity of the antibiotic, bio-fungal (Nystatin), and bacteria (Neomycin) towards the same microorganisms.

The pure isolates of microorganisms were obtained from the biological efficacy laboratories of the General Company for Drugs Industry and Medical Appliances Samarra -SDI, and the examination protocol was according to the United States Pharmacopeia - USP (15).

The results indicated that the sample mixture (A) was effective in killing and inhibiting the growth of (Basillus pumilus) bacteria with inhibitory diameters (12.3, 12.5, and 14.7 mm), which appeared in the form of halos around the drilling area and were distinct from the culture medium inside the dish, while the inhibitory diameters were the biological (Nystatin) (12.3, 12.3, and 12.8 mm), as it is noted that the sample mixture (A) has an effectiveness in killing Basillus pumilus more than the effectiveness of the antibiotic (Nystatin) itself, as these results are significant and excellent, which is illustrated in Table 3.

The results illustrated that the sample mixture (A) was effective in killing and inhibiting the growth of Candida albicans with inhibitory diameters (14.2, 13.4, 12.8, and 11.7 mm), while the inhibitory diameters of the antibiotic were (19, 20, 19, and 19.5 mm), although the effectiveness of the antibiotic (Nystatin) on killing Candida albicans is stronger than that of the sample mixture (A), however the killing results of the sample mixture (A) are significant and considerable, which is illustrated in Table 4.
Table 4 shows the inhibition diameters of Basillus pumilus and Candida albicans treated with the sample mixture (A) and the antibiotic (Nystatin).

TABLE 4 Diameters of inhibition in millimeters for Staphylococcus Klebsiellapneumonia, Micrococcus roseus, Escherichia coli and treatment with the mixture of samples and antibiotic (Neomycin)

The Smaple	Staphylococcus	Klebsiellapneumonia	Micrococcus roseus	Escherichia coli
Neomycin	19 mm	20 mm	19 mm	19.5 mm
A	14.2 mm	13.4	12.8	11.7

TABLE 5 Diameters of inhibition in millimeters for Candida adlicans and treatment with the mixture of samples and antibiotic (Nystatin)

Candida adlicans	The Smple
20 mm	Nystatin
13.7 mm	A

Conclusion

1- The highest viscosity can be obtained at the mixing ratio (4:6)

2- The viscosity of the binary mixture (formation of helminth or filamentous micelles) from the surfactants increases as the temperature decreases.

3- The process of formation of worm or filamentous micelles for most of the studied systems is spontaneous (ΔG°) negative), exothermic (ΔH) negative), random and

irregular (ΔS°) negative), which was shown by the values of Calculations of thermodynamic functions

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