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Preparation of Titanium Dioxide Nanoparticles and PolyVinyl Pyrrolidone Polymer Films as Antibacterial, Antibiofilm against Pathogenic Bacteria on Different Surfaces

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ABSTRACT In this paper, the structural properties that included X-ray diffraction (XRD) of Polyvinyl Pyrrolidone (PVP) and PVP with titanium dioxide nanoparticles have studied. PVP still appeared amorphous structure in spite of presence of titanium dioxide nanoparticle with crystalline form. The films of pure PVP and PVP with titanium dioxide nanoparticle have been prepared by casting method and used in covering glass plates and plastic to study the effect of these films on growth of bacteria. The antibacterial effect against *Staphylococcus aureus* and *Escherichia coli* has been tested. In this study the prepared films had antibacterial effect on plastic and glass plates, the reduction of bacterial growth percentage for PVP films on plastic and glass plates reached to 60.89% and 62.5% against *S. aureus* and 49%, 51% against *E. coli*, respectively. Whereas the percentage for prepared films from a mixture of PVP with titanium dioxide nanoparticle on plastic and glass plates reached 89.42% and 86%, respectively against *S. aureus* and 100% , 69% against *E.coli*. Antibiofilm effect of PVP with titanium dioxide nanoparticle against pathogenic bacteria on catheters was studied for (4) weeks. PVP with titanium dioxide nanoparticle films had the ability to inhibit biofilm formation for pathogenic bacteria on catheters, the inhibition of biofilm percentage reached to 83.97% against *S.aureus* and 65.3% against *E.coli* after fourth week of storage.

Keywords: (PVP) Polymer; TiO_2 nanoparticles; Antibacterial Activity; Antibiofilm; *Staphylococcus aureus* and *Escherichia coli*; catheters.

INTRODUCTION

Polyvinyl Pyrrolidone (PVP) is a polymer with different grades according to its molecular weight. It applicable to use as a binder in the tablet formulations. The wet granulation for PVP with a molecular weights of 25,000 to 90,000 compared to other binders, generally gives harder granulates with very good flow ability, higher binding and low friability (Chowhan, et al., 1992). Also, to enhance these properties, PVP

increases the dissolution of the active ingredient. The tablets of Acetaminophen (paracetamol) that formulate with ratio 4% of PVP with molecular weight 90,000 used as binder released the drug more quickly than tablets with gelatin or hydroxypropyl cellulose as binder, this mean the povidone tablets were harder (Jun et al., 1989).The same results were obtained with ratio 0.6 or 1.0% of PVP (Mw 90,000) or hydroxypropyl cellulose (Sinchalpanid, 1993). A lot of the active substances have poor aqueous solubility because they have limited bioavailability. The easy way to enhance the bioavailability of an active substance by improving its dissolution by adding solubilizing agents, such as the soluble PVP grades. These water-soluble complexes with many active substances and increase the bio availability and a large number of organic solvents; such as alcohols, amines, acids, chlorinated hydrocarbons, lactones and amides. In addition to, polymer is insoluble in common Esters, hydrocarbons, ethers and ketones (Basf, 2009). All grades of povidone can be used as hydrophilic polymers which physically stabilize suspensions. The protective colloids is considered themost important and primary function in all suspensions,that hydrophilize the individual solid particles and sterically separate them. This led to increase the volume of any sediment and makes it is very easy to redisperse by shaking. Also Povidone prevents dissolved portion of active substance from crystallizing out by forming soluble complexes with it (Kadajji& Mitrevej, 2011).

Titanium dioxide nanoparticles have become a new generation of advanced materials because their novel and interesting optical, dielectric, and photo-catalytic properties from size quantization (Alivisatos, 1996). Titanium dioxide (TiO_2) is a photo catalyst and widely utilize as a self-disinfecting and self-cleaning material for surface coating used in many applications. Titanium dioxide has a more helpful role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect (Fujishima& Honda, 1972).These properties have used to remove bacteria and harmful organic materials from water and air. Also in self-cleaning or self-sterilizing surfaces for places such as medical centers (Wong et al., 2008). The aim of this work, used the TiO_2 nanoparticles/PVP films inhibit bacterial growth and biofilm formation in different surfaces like glass and plastic and catheters.

MATERIAL AND METHODS

TiO_2 nanoparticles/PVP Films

Pure PVP and TiO_2nanoparticles doped PVP films have been prepared by employing solution-casting method (Al-Kadhemy, 2012; Nawaf, 2016). Hot distilled water (~55°C) (10 ml) was used to dissolve (0.5 g) from PVP) PVP is a

granular powder with molecular weight (M_w=40000 g/mole) obtained from (Ourchem for Laboratory Use Only) this solution was magnetically stirred continuously for (30 min) until mixture became homogeneous viscous solution. Then it poured into glass and plastic petri dish with diameter (10 cm), keeps under room temperature (~ 30°C) for (5 days) to evaporate all solvent slowly, and obtained PVP thin film with thickness about (0.00091 µm).In order to prepare TiO_2nanoparticles/PVP composite films with two particle sizes for TiO_2nanoparticles (15.7 and 45.7) nm; the amount of powder for each particle sizes as used (0.01 g) with (10 ml) hot distilled water. (6 ml) of this TiO_2nanoparticles solution was added to PVP solution to get TiO_2 (15.7 and 45.7) nm/PVP films. X-Ray Diffraction instrument used with type (SHIMADZU XRD – 6000) made in Japan to check XRD pattern. The instrument has the following specifications; Target is CuK_α, wavelength is 1.5406˚A, Current is 30 (mA) and Voltage is (40 KV).

Coated of Catheters by TiO_2 Nanoparticles with (PVP) Polymer

The catheter pieces with length (2 cm) had coated in solution consisting of mixture (PVP) polymer with volume (10 ml) and TiO_2 with particle size (15.7 nm) with volume (6 ml). Then put the catheter pieces in solution and left to dry for (7 days) and these were stored for (4) weeks to study the effect of storage on antibiofilm.

Antibacterial Effect of (PVP), TiO_2 Nanoparticles/PVP Films

Antibacterial activity of PVP films (40000 g/mole) doped with TiO_2 nanoparticles was studied against *Staphylococcus aureus* and *Escherichia coli* (Department of Biology / College of science / Mustansiriyah University/ Baghdad /Iraq). (PVP) and TiO_2 nanoparticles/PVP were coated on plastic and glass plates, dried for (5) days. After drying the suspensions of bacterial isolates 10^8 cell/ml are poured onto the film of plastic and glass plates, the control included plates with bacterial suspensions without (PVP) and TiO_2 nanoparticles/ PVP films. All plates were incubated at 37°C for 24 h. After the incubation 0.1 ml of each dilution was taking, spread on Nutrient agar (Hi Media), incubated at 37°C for 24h (Salman et al., 2014). The colonies were counted and the reduction of bacterial growth percentage was calculated using the following equation described by (Ghosh et al., 2010):

$$R(\%) = \frac{(A - B)}{A} \times 100 \qquad (1)$$

R = the reduction rate of bacterial growth, A = the number of colonies from control,
B= the number of colonies from coated plates with (PVP) or TiO_2nanoparticles /PVP films.

Antibiofilm effect of TiO_2 Nanoparticles/PVP in Catheters

The effect of (PVP) on biofilm formation of pathogenic bacteria in catheters was examined according to method of (Namasivayam et al., 2013) with some modification. Briefly, the coated pieces were immersed in 10 ml of nutrient broth that inoculated with *S. aureus* and *E. coli* separately, incubated at 37^{o}C for 24 h. After incubation, the broth was decanted then all coated and uncoated catheter pieces (without any coated treatment) were stain for 30 min at room temperature with (0.1 ml) crystal violet solution. Catheter pieces were washed with distilled water to remove the addition stain and washed three times with (95%) ethanol, then ethanol was collected for measuring the absorbance of each piece at wavelength (570 nm) using spectrophotometer and inhibition of biofilm formation percentage was calculated as equation described as (Namasivayamet al., 2013):

%Inhibition of biofilm formation=

$$\frac{\text{ODincontrol}-\text{ODintreatment}}{\text{ODincontrol}} \times 100 \qquad (2)$$

Control: uncoated catheters, treatment: coated catheters.

Measurement (LD50) of Polymer PolyVinylPyrrolidone (PVP) and TiO_2 Nanoparticles

Six group of male Swiss mice (4 Weeks old), Weight approximately 20 g, obtained from National Centre for Drug Control and Research (NCDCR). For each of (PVP) and TiO_2 nanoparticles solution were daily administered orally for 10 days with (0.1 ml) with a dose of (5000, 10000, 15000,20000,25000,30000) mg/kg and with a dose of (0.05-5.00) mg/kg, respectively. Additional group of mice received normal saline (0.1 ml) as a control group. At the end of dosing, all mice in all groups were examined, and the concentration which was killed half of animals was determined and considered LD50.

RESULTS AND DISCUSSIONS

The X-ray diffraction pattern of pure PVP powder is shown in fig. (1). The pure PVP scan shows very a broad diffraction peak around 2θ = 20.9402º corresponding d-spacing 4.23888 Aº with intensity (62) and peak with 2θ = 11.4695° corresponding d-spacing 7.70892Aº with intensity (36). That it confirms the amorphous nature of the prepared polymer film. That is conformity with either reported in literature (Rawat et al., 2012; Abdelghany et al., 2015). Table (1) illustrated some structural properties for pure PVP powder. Fig. (2- A, B) show the X-ray diffraction of pure TiO_2 nanoparticles powder with two particles sizes, respectively. Strong diffraction peaks at 25º, 48º and 37º indicating TiO_2 in the Anatase phase, the intensities of XRD peaks of the sample reflects that the formed nanoparticles are crystalline (Saleh et al., 2014; Shehap et al.,2016). The intensity is increased with decreasing the Particle size, the particle size is nearly (15.7 nm) for fig. (2- A)

and (45.7 nm) for fig. (2- B). Table (2 A, B) illustrated some structural properties for pure TiO_2 nanoparticles with two particle sizes, respectively. When adding (6ml) of TiO_2 with two particles sizes ((15.7 nm) and (45.7 nm)) to PVP polymer are shown in figs. (3- A, B), respectively. From these figs., the effect of TiO_2 nanoparticles increased on PVP polymer become semi-crystalline with decreasing the particle size, the intensity is increase with increasing of particle size of TiO_2 nanoparticles and the particle size is nearly (8.296, 1.71554) with increasing with particle size. An average grain size of all samples was estimate from the X-ray line broadening analysis by formula of Scherer's (Nawaf, 2016; Al-Kadhemy& Nawaf, 2017):

$$D = \frac{0.9\lambda}{\beta Cos\theta} \quad (3)$$

Where: λ is the wavelength of X-ray, β is the value of FWHM and θ is Bragg's angle.

nm for TiO_2 (15.7 ,45.7) nm/PVP films, respectively. The peak 25.3424° of TiO_2 (15.7 nm) appear in TiO_2 (15.7) nm/PVP film. The table (3- A, B) illustrated the magnitude of (2θ) for three high peaks with their intensities, FWHM, d-spacing and particle size. Then the particle size (D) from eq. (3), specific surface area (S) calculated from eq. (4) and dislocation density (δ) from eq. (5). It can be conclude from table (2- A, B) the specific surface area (S) and dislocation density (δ) are decreasing

$$S = \frac{6 \times 10^3}{D \cdot \rho} \quad (4)$$

Where: S are the specific surface area and ρ is the density of TiO_2 (3.9 g/cm^3) and TiO_2 (4.23 g/cm^{3}for particle sizes (15.7 and 45.7) nm, respectively.

$$\delta = \frac{1}{D^2} \quad (5)$$

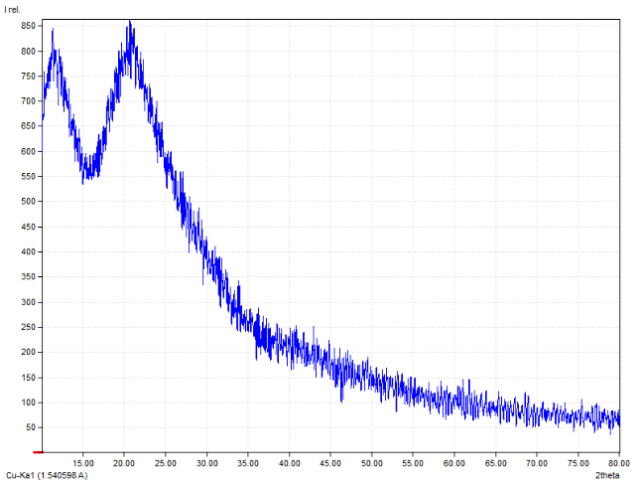

Fig. 1: XRD Pattern for pure PVP powder

Table 1: XRD Parameters for Pure PVP Powder

2θ (degree)	Intensity (counts)	D(°A)
11.4695	36	7.70892
20.0826	52	4.41792
20.3418	52	4.36221
20.9402	63	4.23888

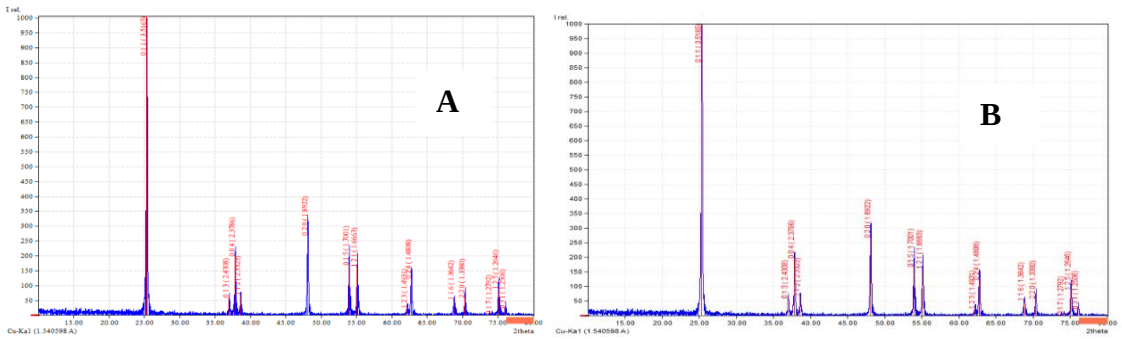

Fig. (2) XRD Pattern for Pure TiO_2 nanoparticles powder with two particles sizes A-(15.7 nm) B-(45.7 nm)

Table 2- A: XRD Parameters for Pure TiO_2 (15.7 nm) Powder

2θ(degree)	FWHM (degree)	Intensity (counts)	d (Å)	hkl	D(nm)	Sx10^6 (m^2.g^{-1})	δx10^6 (m^{-2})
25.3424	0.54100	285	3.51165	011	15.1	0.1018	4.385
36.9362	0.28000	16	2.43168	013	30.4	0.0505	1.082
37.8804	0.67000	50	2.37321	004	12.6	0.1220	6.298
38.7148	0.50000	12	2.32396	112	16.9	0.0910	3.501
48.0716	0.59500	77	1.89120	020	14.7	0.1046	4.627
53.9815	0.75000	39	1.69727	015	11.9	0.1292	7.061
55.0311	0.73000	39	1.66735	121	12.2	0.1260	6.718
62.0836	0.40000	12	1.49380	123	23.4	0.0657	1.826
62.7034	0.76000	28	1.48052	024	12.3	0.1250	6.609
68.8616	0.76000	10	1.36237	116	12.7	0.1211	6.200
70.3212	0.64000	12	1.33763	220	15.2	0.1011	4.328
75.0799	0.84000	16	1.26421	125	11.9	0.1292	7.061

Table 2- B: XRD Parameters for Pure TiO_2 (45.7 nm) Powder

2θ (degree)	FWHM (degree)	Intensity (counts)	D (Å)	hkl	D (nm)	$Sx10^6$ ($m^2.g^{-1}$)	$\delta x10^5$ (m^{-2})
25.3712	0.21100	759	3.50773	011	39.5	0.0358	6.409
37.0077	0.19670	46	2.42715	013	43.0	0.0329	5.408
37.8515	0.2010□0	172	2.37496	004	41.9	0.0338	5.696
38.6286	0.18750	48	2.32895	112	45.9	0.0308	4.746
48.0967	0.19930	253	1.89027	020	44.6	0.0317	5.0272
53.9434	0.20180	159	1.69838	015	44.4	0.0319	5.0726
55.1201	0.21570	147	1.66487	121	42.2	0.0336	5.6153
62.1691	0.17900	25	1.49196	123	52.2	0.0271	3.6699
62.7443	0.20810	119	1.47965	024	45.1	0.0314	4.9163
68.8009	0.21860	45	1.36343	116	44.2	0.0320	5.1186
70.3422	0.19800	55	1.33728	220	49.9	0.0284	4.0160
75.0910	0.214200	85	1.26405	125	47.3	0.0299	4.4696

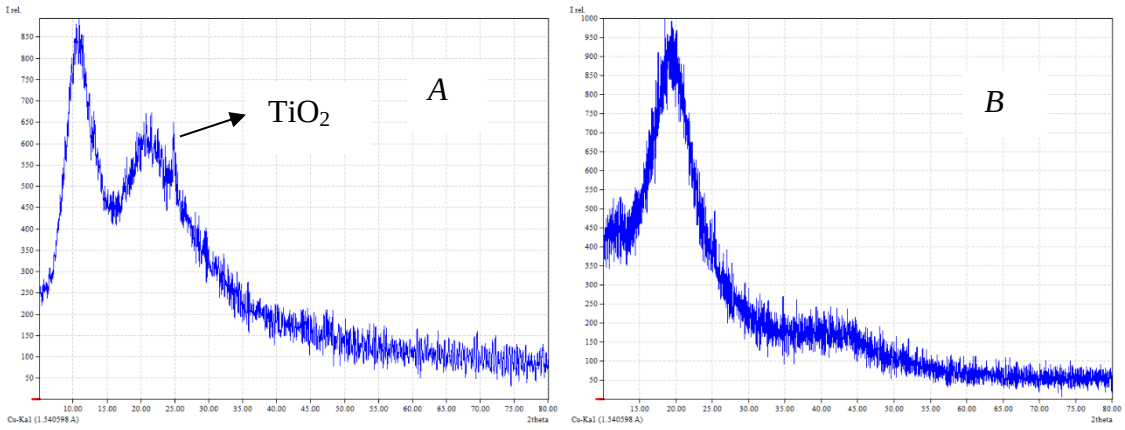

Fig. 3: XRD Pattern for A- TiO_2 (15.7 nm)/PVP and B- TiO_2 (45.7nm)/PVP Films

Table 3- A: XRD Parameters for TiO_2 (15.7 nm)/PVP Film

2θ(deg)	FWHM(deg)	Intensity(counts)	d (Å)	D(nm)
8.9490	1.36	35	9.87369	5.862
10.4163	2.76	72	8.48589	2.891
10.9527	3.18	69	8.07147	2.510
24.9112	0.64	21	3.57145	12.838

Table 3- B: XRD Parameters for TiO_2 (45.7 nm)/PVP Film

2θ(deg)	FWHM(deg)	Intensity(counts)	d (Å)	D(nm)
19.3648	4.7	261	4.58003	1.71554
20.5811	0.000	220	4.31203	0.00000
21.8777	0.000	150	4.05931	0.00000

Reduction of *S. aureus* and *E. coli* growth were tested by using PVP (Pure) and TiO_2 nanoparticle (45.7nm)/PVP, TiO_2 nanoparticle (15.7nm)/PVP films on plastic and glass plates. The best results about reduction growth of *S. aureus* were obtained by PVP (Pure) film on glass plate reached to (62.5%) and TiO_2 (15.7nm)/PVP film on plastic reached to (89.42%). And the best results for reduction growth of *E.coli* were observed for TiO_2 (45.7nm)/PVP film reached to (59%) and TiO_2 (15.7nm)/PVP film reached to (100%) on plastic, while the best reduction for PVP(Pure) film on glass reached to (51%) (Table4).

Table 4: Reduction of bacterial growth for PVP and TiO_2 nanoparticles/PVP films

Bacterial isolate	Treatment	Type of plates	Reduction of growth (%)
Staphylococcus aureus	**PVP (Pure)**	**Plastic**	**60.89**
	PVP+TiO_2(45.7nm)		**55.1**
	PVP+TiO_2(15.7nm)		**89.42**
	PVP (Pure)	**Glass**	**62.5**
	PVP+TiO_2(45.7nm)		**69.55**
	PVP+TiO_2(15.7nm)		**86**
Escherichia coli	**PVP (Pure)**	**Plastic**	**49**
	PVP+TiO_2(45.7nm)		**59**
	PVP+TiO_2(15.7nm)		**100**
	PVP (Pure)	**Glass**	**51**
	PVP+TiO_2(45.7nm)		**49**
	PVP+TiO_2(15.7nm)		**69**

Table 5 illustrated the results for antibiofilm effect of TiO_2 (15.7 nm)/PVP nanocomposite was studied against *S. aureus* and *E. coli* on catheters for different storage time. Results showed that one week of storage of coated catheters the biofilm inhibition ratio was (29%), after two weeks of storage the inhibition ratio (42.6%), and after the three weeks the inhibition ratio reached to (56.21%). The best inhibitory effect obtained after four weeks of storage with inhibition ratio (83.97%).The results for inhibition of biofilm for *E. coli* reached to (22%) after one week, while two weeks of storage of catheters the inhibition ratio (30.9%), and after the three week of storage reached to (39.87%) and (65.3%) after three and four weeks, respectively.

Table 5: Inhibition of Biofilm Formation of TiO_2 (15.7 nm)/PVP against *S.aureus* and *E.coli* on Catheter after different times

Bacterial isolate	Times	Optical Density (O.D) Control	Coated Catheter	Inhibition biofilm formation (%)
Staphylococcus aureus	**(1) Week**	**0.234**	**0.167**	**29**
	(2) Week	**1.511**	**0.867**	**42.6**
	(3) Week	**1.158**	**0.507**	**56.21**
	(4) Week	**1.479**	**0.237**	**83.97**
Escherichia coli	**(1) Week**	**0.157**	**0.122**	**22**
	(2) Week	**0.440**	**0.304**	**30.9**
	(3) Week	**1.134**	**0.455**	**39.87**
	(4) Week	**1.222**	**0.424**	**65.3**

The antibacterial activity is increase with decreasing the particle size of TiO_2 nanoparticles with increasing the specific surface area and dislocation density**.** PVP is use as stabilizers; it has optical purity that authorizes the exploration of nanoparticle formation. PVP acts as a copping agent and the antimicrobial activity of PVP caused modification of nanoparticles, the polymer is most effective agent in the particles stabilization against aggregation (Jayaprakash et al., 2015). The metal oxides carry the positive charge while the bacteria carry negative charges; this causing electromagnetic attraction between bacterial surface and the metal oxides that caused oxidization and death of bacteria (Zhand& Chen, 2009). They cause holes in the cell walls of bacteria, increasing permeability and death of cell (Holt & Bard, 2005). The opposite charges of nanoparticles and bacteria are attributing to their bioactivity and adhesion due to electrostatic forces. Nanoparticles have larger surface area, which enhances bactericidal activity than the large size particles; they realize cytotoxicity to the bacteria (Bhupendra et al., 2009). Fungicidal and bactericidal effects of TiO_2 on *Pseudomonas aeruginosa, E. coli, Salmonella choleraesuis, Vibrio parahaemolyticus, Listeriamonocytogenes,S.aureus,Diaport heactinidiae and Penicillium*expansum have reported. The development of TiO_2-coated or incorporated packaging of food and equipment of food preparing has also interest. (Chaweng kijwanich & Hayata, 2008), concluded that the TiO_2 coated film could reduce the bacterial contamination on the surface of food products and reduce the risks of bacterial

growth on fresh-cut products. Inhibition activity of metallic nanoparticles on biofilm formation of bacteria has been importance, as the device-related infections that cause of morbidity and mortality in hospitalized patients (Del-pozo et al., 2009). TiO_2 nanoparticle had inhibitory effect on biofilm formation of multidrug resistant bacteria (Ibrahem et al., 2014). (Haghighiet al.2013) showed that TiO_2 nanoparticles could kill *Candida albicans* and inhibit the formation of biofilm. Different shape and size of TiO_2 nanoparticles can be used for the photo catalytic treatment of aqueous biofilm, pathogenic bacteria and multi drug resistant bacteria. Maurer-Jones et al. (2013) observed, significant changes in bacterial biofilm after treatment with TiO_2nanoparticles, nanoparticles caused altered gene expression relating to growth and biofilm formation. TiO_2 nanoparticles leads to larger reduction of bacterial biofilm formation in the glass surface (Chorianopoulos et al., 2010). The TiO_2 nanoparticles efficiently inhibited bacterial adhesion to acrylic surfaces as well as have strong antibacterial effect in the planktonic stage and biofilm formation (Bahador et al., 2014).

Measurement (LD50) of (PVP) Polymer and TiO_2 Nanoparticles.

Toxicity of (PVP) and TiO_2 nanoparticles was detect by determination the dose that cause death of 50% of laboratory animals. Results showed that no effect of (PVP) and TiO_2 nanoparticles on the laboratory animals, LD50 was (> 2000 and> 5) mg\Kg, respectively.

CONCLUSION

The effect of TiO_2 nanoparticles on crystal structure of PVP polymer has investigated by x-ray diffraction. There is some peaks fromTiO_2 appeared into structure of polymer. The TiO_2 nanoparticles/ PVP films have antibacterial effect against bacteria in plastic and glass plates. In addition, it has antibiofilm effect in catheters.

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