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Research Article

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Toxicity study of silver nanoparticles synthesized using aqueous and alcoholic extract of seaweed *Sargassum angustifolium* in *Barbus sharpeyi*

Seraj Bita[1], A. J. Keikha[1] and M. Y. Abdollahzadeh Jamalabadi[2*]

[1]*Chabahar Maritime University, Chabahar 99717-56499, Iran Department of Mechanical,*
[2]*Robotics and Energy Engineering, Dongguk University-Seoul Seoul, 04620, Korea*

ABSTRACT

In this study, the lethal concentration and acute toxicity of silver nanoparticles synthesized using aqueous and alcoholic extract of seaweed Sargassum angustifolium that is produced by biological methods were investigated at static renewal condition during 96 hours in common carp in accordance with standard methods (1998) OECD. TEM analysis showed that the average size of the bionanoparticles extracted from alcoholic solution was finer and their toxicity is higher than the particles acquired from aqueous solution.

Keywords: toxicity, silver nanoparticles, seaweed, *Barbus sharpeyi*

INTRODUCTION

Silver nanoparticles (AgNps) are widely investigated because of wide range applications such as antibacterial, catalyst, medical devices, photonics, optoelectronics and biosensors. In recent years, advancement of technology from micro [1] to nanoscales [2], many theoretical phenomena [3] have found their importance in emerging applications in chemical engineering [4]. One of these phenomena is the using nanoparticles [5] that could be controllable by MHD forces [6-10] in microchannel [11] and micro polar fluids [12]. Many applications are open to this area such as power plant industry [13-20], heat conversion [21-25] and management [26-28] ,and tribology [29-35]. Membrane biofouling has a negative impact on the membrane treatment performance. Silver nanoparticles (AgNPs) are well-known antimicrobial agent. Herein, AgNPs with approximately 15 nm in diameter were effectively attached to the surface of polyamide (PA) thin-film composite (TFC) membrane via covalent bonding, with cysteamine as a bridging agent. Scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and cross-sectional transmission electron microscopy (TEM) studies all showed the immobilization of AgNPs [36-49]. Compared with the pristine TFC membrane, thiol-terminated membrane (TFC–SH) and AgNPs grafted membrane (TFC-S–AgNPs) both showed a higher water flux with slightly lower salt rejection. At a constant transmembrane pressure of 300 psi, the water permeability of TFC–SH, TFC-S–AgNPs, and control TFC membranes was 70.6±0.5, 69.4±0.3, and 49.8±1.7 L/m2h, respectively, while NaCl rejection was 93.4±0.1%, 93.6±0.2%, and 95.9±0.6%, respectively. TFC-S–AgNPs had an improved antibacterial ability to inhibit E. coli growth. The silver leaching from the TFC-S–AgNPs membrane surfaces was minimal, as tested by both batch and flow-through methods. The results successfully demonstrated that AgNPs could be grafted onto TFC via chemical bonding, leading towards the development of an advanced functional TFC membrane with anti-biofouling properties [50-66].

The binni, or Mesopotamic Barbel, Mesopotamichthys sharpeyi, is a species of cyprinid fish endemic to the Tigris-Euphrates river system. This barbel is the only member in its genus, but was included in the "wastebasket genus" Barbus by earlier authors. Not evaluated by the IUCN, it is probably not rare or endangered, though it may have declined notably in recent times due to habitat destruction.

The binni is a deep-bodied dark silvery barbel. The large scales bear parallel stripes; the fins are comparatively small. Full-grown specimens may reach a length of almost half a meter and weigh 800 grams or more, but as most individuals encountered are by far smaller, it seems to take some time to grow to such good size. Sexes are probably almost identical, though it may be notable that a male was among the largest recorded specimens (in many cyprinids females are larger than males). Not much is known about its ecology, but like similar relatives it is believed to be benthopelagic and feed on small animals and water plants [1]. Known in the local Iraqi Arabic dialect as binni or bunni, this fish is valued highly by the Marsh Arabs. Their fishermen traditionally employ an unusual technique of combined spearfishing and Datura poison-fishing to catch it; until recently net-fishing was mostly restricted to the Berbera tribe and held in low esteem. Since the 1960s however, large-scale fisheries have also been developed; once of prime importance throughout Iraq, the marshland fish stocks presumably declined notably following the draining of the Mesopotamian Marshes [2][3] .

Umm al Binni lake in Maysan Governorate, Iraq, was named after this species. Now mostly dried-up following the draining of the Central Marshes, its name attests to the former abundance of this fish and possible use as spawning ground (Umm is Arabic for "mother", but does not necessarily imply procreation). The lake is of interest as a possible impact crater mentioned in the Epic of Gilgamesh.

EXPERIMENTAL SECTION

Seaweeds were collected from the intertidal region of Bushehr coast, Iran. The samples were brought to laboratory, cleaned and washed thrice with fresh water followed twice by distilled water to remove the adhering salts and other associated contaminants. Then the shade-dried was used for 5 days and powdered using mixer grinder [46]. Later, aqueous extract was prepared by dissolving 10g of powdered seaweed in 100ml of sterile distilled water. The mixture was heated at 60°C for 10mins, centrifuged twice at 4,000 rpm for 25mins and filtered through Whatmann no. 1 filter paper. For the biosynthesis of silver nanoparticles, 90ml of 1mM silver nitrate ($AgNO3$) was added to 10ml of seaweed extract [47]. After that a color change from yellowish brown to reddish or blackish brown, visually confirms the formation of AgNPs [47]. Also the sample have been characterized for the construction of silver nanoparticles by using Ultra Violet –Visible Spectrum (Perkin-Elmer *Uv*-Vis, Lambda 12) , Transmission Electron Microscopy (LEO 906E).

Fig. 1. Barbus sharpeyi

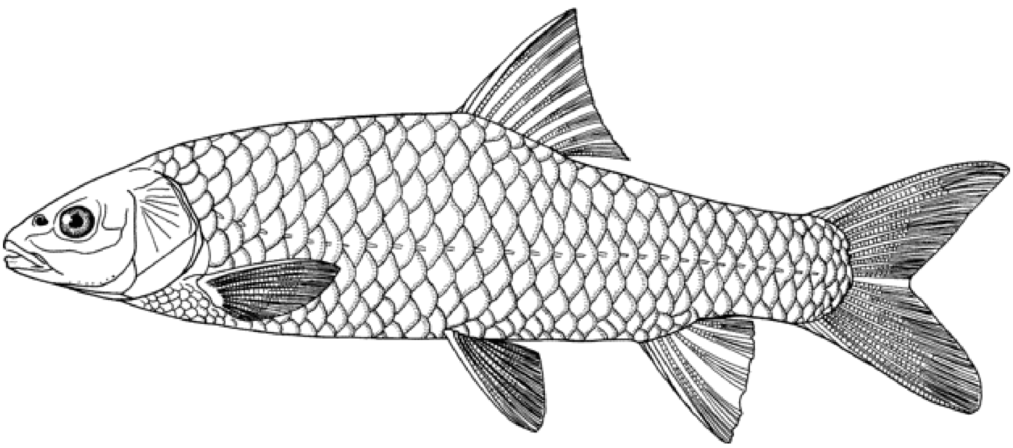

Fig. 2. Composite drawing, Barbus sharpeyi

RESULTS AND DISCUSSION

After adding silver nitrate to *Sargassum anustifolium* extract, the brownish-yellow color in mixture turned in to dark brown color after 110 min (Fig 1). To make sure the synthesis of silver nanoparticles, nanoparticle absorption peak was measured using UV-visible spectrometer (UV-Vis) in the wavelength range of 200-700 nm. The best peak was observed after 2h of reaction time in the range of 406 nm, which corresponds to plasmon excitation of the AgNPs.

Table 1. Lethal Concentrations (LC_{10-99}) of synthesis silver nanoparticles using aqueous extract of sargassum (mean ± Standard Error) depending on time (24-96h) for Barbus sharpeyi

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	22/64 ± 0/009	7/74 ± 0/010	1/15 ± 0/012	0/64 ± 0/024
LC_{30}	36/47 ± 0/009	17/72 ± 0/010	9/42 ± 0/012	2/26 ± 0/024
LC_{50}	**46/04 ± 0/009**	**24/63± 0/010**	**15/16 ± 0/012**	**5/65 ± 0/024**
LC_{70}	55/62 ± 0/009	31/54 ± 0/010	20/90 ± 0/012	9/04 ± 0/024
LC_{90}	69/44 ±0/009	41/52 ± 0/010	29/18 ± 0/012	13/94 ± 0/024
LC_{99}	88/52 ± 0/009	55/29 ± 0/010	40/60 ± 0/012	20/70 ± 0/024

Table 2. Lethal Concentrations (LC_{10-99}) of synthesis silver nanoparticles using alcohlic extract of sargassum (mean ± Standard Error) depending on time (24-96h) for Barbus sharpeyi

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	1/44 ± 0/122	0/63 ± 0/108	0/06 ± 0/139	0/01 ± 0/280
LC_{30}	2/47 ± 0/122	1/49 ± 0/108	0/75 ± 0/139	0/17 ± 0/280
LC_{50}	**3/18 ± 0/122**	**2/08± 0/108**	**1/22 ± 0/139**	**0/45 ± 0/280**
LC_{70}	3/89 ± 0/122	2/68 ± 0/108	1/70 ± 0/139	0/73 ± 0/280
LC_{90}	4/92 ±0/122	3/54 ± 0/108	2/39 ± 0/139	1/14 ± 0/280
LC_{99}	6/33 ± 0/122	4/72 ± 0/108	3/34 ± 0/139	1/69 ± 0/280

Table 3. Mortality rate in Barbus sharpeyi exposed to different concentrations of silver nanoparticles using aqueous extract of sargassum at different times

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
1.5	0	0	0	16/67
3	0	0	16/67	53/33
6	0	13/33	20	70
12	0	20	63/33	83/33
24	26/67	63/33	80	96/67
48	46/67	90	96/67	100

Table 4. Mortality rate in Barbus sharpeyi exposed to different concentrations of silver nanoparticles using alcoholic extract of sargassum at different times

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
0/1	0	0	0	20
0/2	0	0	13/33	46/67
0/4	0	6/67	26/67	60
0/8	0	20	46/67	80
1/6	33/33	53/33	73/33	93/33
3/2	40	73/33	93/33	100

The maximum acceptable toxicant concentration (MATC) of silver nanoparticles for common carp at intervals of 24, 48, 72 and 96 hours was determined 7.95, 5.22, 3.06 and 1.13mg/L respectively. The lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) of silver nanoparticles with maximum allowable toxicant concentration *(*MATC*)* of LC50 values were calculated and compared to the results of a study of the factors in the LC50 as follows:

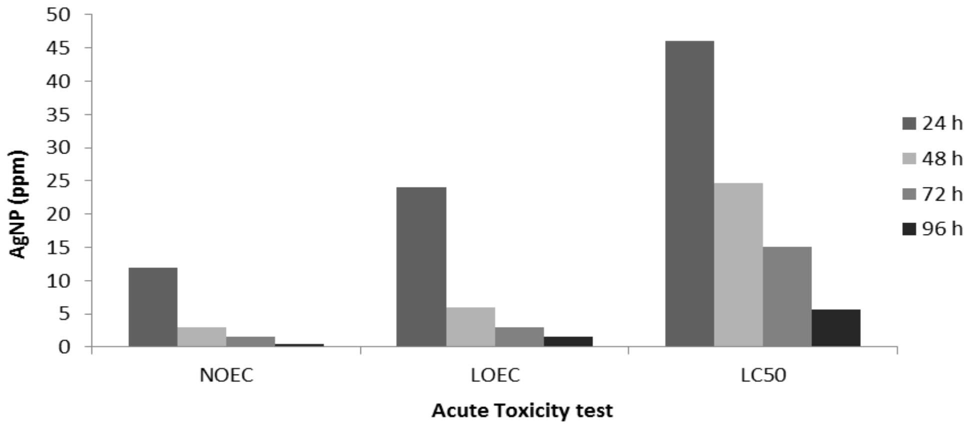

Fig. 3. Acute toxicity testing statistical endpoints of AgNP silver nanoparticles using aqueous extract

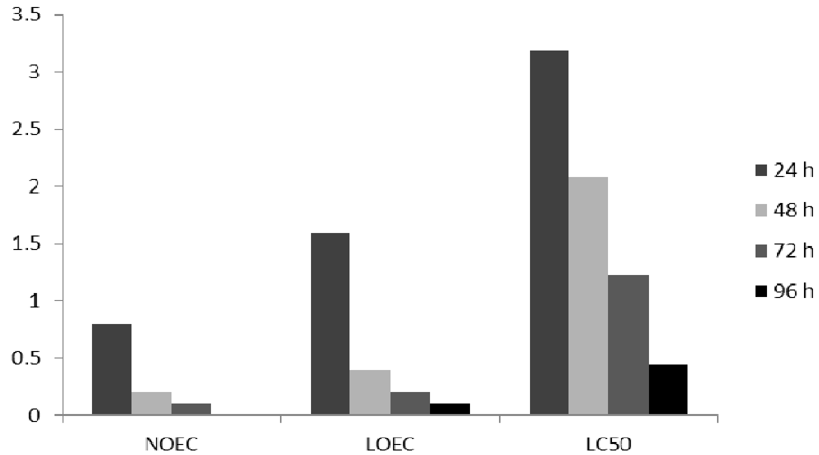

Fig. 4. Acute toxicity testing statistical endpoints of AgNP silver nanoparticles using alcoholic extract

Table 5. Values LOEC, NOEC and MATC of silver nanoparticles using aqueous extract synthesized from seaweed *Sargassum angustifolium* in Barbus sharpeyi

LOEC (mg/L)	NOEC (mg/L)	MATC (mg/L)
1.5	0.5	**0.56**

Table 6. Values LOEC, NOEC and MATC of silver nanoparticles using alcoholic extract synthesized from seaweed *Sargassum angustifolium* in Barbus sharpeyi

LOEC (mg/L)	NOEC (mg/L)	MATC (mg/L)
0.1	0.01	**0.045**

CONCLUSION

Toxicity results in this study compared with other researchers on Barbus sharpeyi [42, 59] have been shown that silver nanoparticles synthesized using biological methods are less toxic than chemically synthesized nanoparticles. One of the other reason for the differences in toxicity, in addition to differences in particle size, condition and size of the fish species, can be due to greater stability of biologically synthesized silver nanoparticles that have many biomolecules compounds and resulting in less release of silver ions in water. Also for biological synthesized nanoparticles toxicity is generally lower. In some studies higher toxicity of silver nanoparticles compared with silver ions in addition to the physicochemical properties of the particles have been reported resulting the release of silver ions from silver nanoparticles [61-62]. Comparison of mortality in fish exposed to silver nanoparticles treatments with a control group that did not cause any mortality. It shows that the only cause of fish mortality in the different treatments was the addition of silver nanoparticles in water. TEM analysis showed that the average size of the bionanoparticles extracted from alcoholic solution was finer and their toxicity is higher than the particles acquired from aqueous solution.

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