Effect of Ferrofluids on Microbes

Lubna Sheikh[1,2,4], Abinash Dutta[3], Roshan Chandra[1] and Suprabha Nayar[1]

[1] CSIR-National Metallurgical Laboratory, Jamshedpur, India
[2]CSIR-IICB, Kolkata, India
[3] Department of Biotechnology, Utkal University, Bhubaneswar, Odisha, India
[4]AcSIR-IICB, Kolkata, India

E-mail: suprabha.nayar@gmail.com

Abstract: The chance finding of aqueous ferrofluids (FF) as antibacterial leaves one wondering whether it is the metallic part which has anti-bacterial properties or is it the nano size and structure which is responsible. Compared to silver, the known antimicrobial agent, there could be potential advantages that iron has to offer. In order to study the effects of ferrofluids containing iron oxide nanoparticles coated with surfactant such as polyvinyl alcohol (PVA), synthesized by a novel biomimetic process via matrix mediated process at different concentrations. These ferrofluids were tested against bacteria, fungus and algae. FF was characterized by MFM, TEM and DLS.

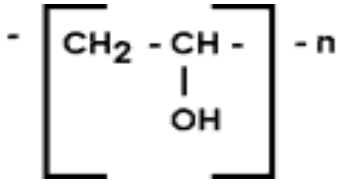
Key Words- Antibacterial, Ferrofluids, Inhibition zone, Iron oxide nanoparticles, TEM

I. INTRODUCTION

Ferrofluid has been widely used in biomedical research because of its biocompatibility and magnetic properties [5, 6]. Ferrofluid consists of iron oxide nanoparticles of size 5-20 nm coated with a surfactant dispersed in a carrier fluid such as distilled water. The influence of iron ions on the bacterial metabolism was underlined in various scientific reports developed considering that iron is the fourth element in the Earth crust and represents an essential substance for most organisms species. Though, nano-silver is now been put to practical use in commonly used items, such as, clothes, electric home appliances, and electronic industry, it has yet not been widely applied in the medical or pharmacological fields. In humans, nano-silver is found to have a significant cytotoxic effect on PBMCs-Silver has been used to treat infections for centuries, but with the advent of nanotechnology, the use of silver in nanoparticle form has opened new treatment avenues [1-3]. The cost incurred in producing these nanoparticles is huge. Super Paramagnetic Iron Oxide Nanoparticles (SPIONS) are now the new novel nanomaterials which are being widely implemented in various fields such as drug delivery, magnetic hyperthermia and as contrast agents in MRI owing to its low cost and easy availability [4].The exact mechanism by which these iron oxide nanoparticles attain an antibacterial nature is still not understood [1,7-10]. This paper deals with the synthesis

of ferrofluids and testing them against pathogenic strains of bacteria (*E.coli*) which infect the gastrointestinal tract.

The polymer (PVA) structure [Fig 1] is the coating agent which prevents the agglomeration of magnetite nanoparticles in water.



[Fig 1] PVA structure

Escherichia coli or *E. coli* [Fig 2] is a large group of bacteria that exists in many forms. *E. coli* can exist as pathogenic or non-pathogenic – that is, harmful or non-harmful. E.coli is a gram negative rod shaped bacteria. It is also known to possess an ability to resist antibiotics such as pencilin.

[Fig 2] The Bacterial (*E.coli*) Culture

Aspergillus niger [Fig 3] is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil. There are useful effects of several species of *Aspergillus* as some of them are employed in cheese manufacturing. This specie in particular is used bio-assay of metals as it can detect copper even in traces.

[Fig 3] The Fungal (*A. niger)* culture

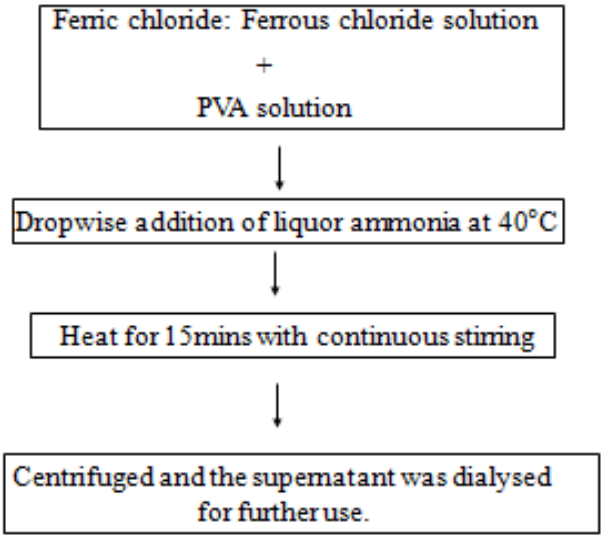
Spirogyra granulata [Fig 4] is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas, and there are more than 400 species of *Spirogyra* in the world.

[Fig 4] The Algal (*S. granulata*) culture

II. Experimental Materials and methods

Iron oxide nanoparticles were synthesized in-situ using different polymers and proteins by a biomimetic process. The aqueous solution of polymer such as polyvinyl alcohol (PVA) was mixed with equal volumes of ferrous/ferric (1:2) aqueous solutions under ambient conditions.

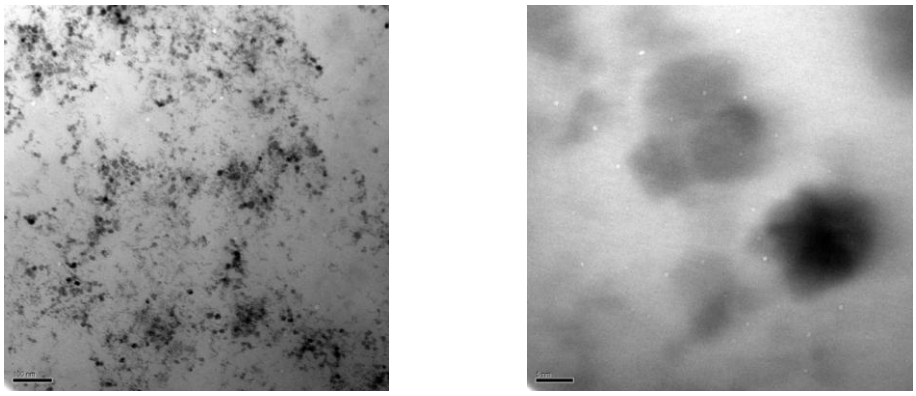
FLOWCHART FOR FERROFLUID SYNTHESIS



III. RESULTS AND DISCUSSION

Characterization of ferrofluid:

Transmission Electron Microscpoe (TEM) confirmed the formation of well dispersed iron oxide nanoparticles (Fe_3O_4) in PVA matrix ranging from 5-10 nm, depicted in [Fig 5] and [Fig 6].



[Fig 5, 6] Well dispersed iron oxide nanoparticles (Fe_3O_4) in PVA matrix ranging from 5-10 nm

The effect of ferrofluids was tested against *E. coli*, *A. niger* and *S. granulata*. Iron stock solution and ferrofluids with different concentrations of surfactants were used.

Effect of ferrofluid on *E. coli*

For antibacterial activity, the samples (25µl) poured into the wells which had been punched out in and nutrient agar medium (Himedia). The pH of the nutrient agar medium was 7.0 ± 0.2 and it contained peptone, beef extract, yeast extract, sodium chloride and agar. The aforementioned species was plated onto dishes and incubated at 37 º C for 24 hours. A zone of inhibition was observed after 24 hours (Fig-7, 8, 9). The well diffusion test was conducted as a qualitative test only. What was interesting was that the zone of inhibition of iron salt solution used to prepare our ferrofluid is less antibacterial than the ferrofluid. Giving a scope as a new antibacterial agent of many biomedical applications.

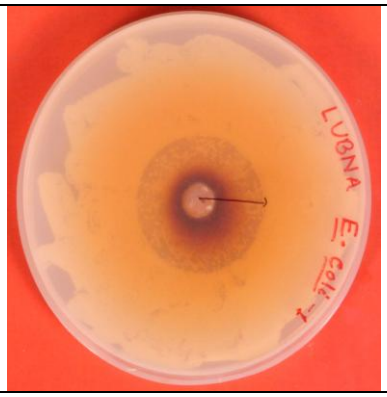
For algal activity on ferrofluid, BG 11 medium was prepared for the culture of *Spirogyra*. Ferrofluid was swabbed onto the dishes and algae were put on that. The dishes were kept under 18 hours of light per day at 23 ºC for a week.

E.coli is known to possess an ability to resist antibiotics (such as Penicillin). Thus it is necessary to find an alternative treatment (perhaps without the use of antibiotics) for *E. coli* infection that is directed to the site of infection, localized and difficult for the bacteria to mutate and to formulate resistance.

Along this line, some have hypothesized that Reactive Oxygen Species (ROS) generated by Fe_3O_4 nanoparticles could kill bacteria. The cellular death process can be attributed to the fact that these nanoparticles are capable of entering the cellular membrane resulting in oxidative stress [11,12].

[Fig 7] Zone of inhibition (44mm) in presence of ferrofluid (2.5% PVA FF)

[Fig 8] Zone of inhibition in presence of Iron stock (26mm)

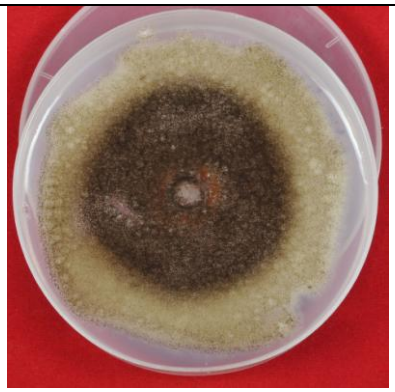


[Fig 9] Zone of inhibition (36 mm) in presence of ferrofluid (1.75% PVA FF)

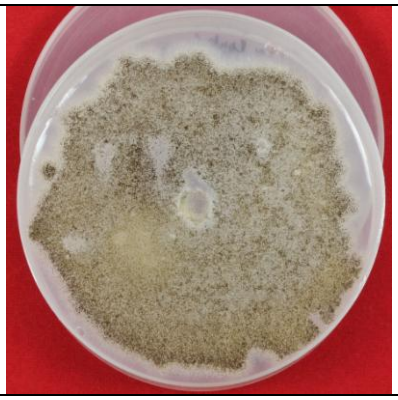
SERIAL NUMBER	SAMPLES	INHIBITION ZONE DIAMETER (mm) ON *Escherichia coli*
1.	IRON STOCK	26
2.	2.5% PVA_FF	44
3.	2.25% PVA_FF	40
4.	2% PVA_FF	38
5.	1.75% PVA_FF	37
6.	1.5% PVA_FF	32
7.	0.5% PVA_FF	20

Activity of ferrofluid on *A. niger*

For fungal activity of ferrofluid, the sample (25 µl) poured into the wells of CZAPCKDOX AGAR medium. The fungus was plated on to the dishes and incubated at 37º C for 24 hours. The fungus was grown on CZAPAKDOX AGAR as a growth medium containing Sucrose as a prime Carbon source. The plates were incubated for 48 hours and after comparing with the control, it was observed that the plate having Ferrofluid showed an enhanced growth in a specific area near ferrofluid as compared to the control which was without ferrofluid. The black region [Fig-10] denotes the area of enhanced fungal activity in presence of ferrofluid.
As aforesaid that this specie is used in bio-assay and if this ferrofluid has the potential to enhance its growth it could be a easy and cost effective way to do so.



[Fig 10] The growth of fungus in presence of ferrofluid



[Fig 11] The growth of fungus in control (i.e. without ferrofluid)

Activity of ferrofluid on *Spirogyra*

The algae were grown on BG-11 agar medium with ferrofluid. The plates were observed after one week, which were kept in 18 hours of light per day and at 23º C. As it is known that this alga can be used as a source of organic matter, food, chemicals and in soil colonization it could be grown rapidly with the aid of ferrofliud.

	
[Fig 12] The growth of algae in presence of ferrofluid	**[Fig 13] The growth of algae (without ferrofluid)**

IV.CONCLUSION

One step Biomimetic Process of producing Magnetic Nanofluid leads to high dispersion yields and small particle size. From the observations cited above confirmed that smaller size of the constituent nanoparticles exhibit higher degree of antibacterial activity.
In the [Fig 7 and 9], it was observed that the higher concentration of the surfactant renders small sized nanoparticles which shows greater inhibition zone. The denser matrix restricts the nucleation of particles and hence the smaller size.
The iron stock solution [Fig 9] did not show any appreciable antimicrobial activity indicating the salt does not contribute much as compared to iron oxide nanoparticles.
And on the other hand the iron oxide nanoparticles in ferrofluid acted as the fertilizer in the enhanced growth of fungus and algae compared to the colonies which were grown without ferrofluids.

ACKNOWLEDGEMENTS

The authors acknowledge CSIR, Ministry of Science and Technology, Govt. of India for providing financial assistance from the network project “ESC-0103” and Lubna

acknowledges Department of Science & Technology (DST), Govt. of India for providing INSPIRE fellowship.

REFERENCES

[1] L. Babes, B. Denizot, G. Tanguy, J.J. Le Jeune, and P. Jallet, "Synthesis of iron oxide nanoparticles used as MRI contrast agents: A parametric study," J Colloid Interface Sci, 212(2), 474–482, 1999.

[2] R.G.H. Beets-Tan, J.M.A. Van Engelshoven, and J.W.M. Greve, "Hepatic adenoma and focal nodular hyperplasia: MR findings with superparamagnetic iron oxide- enhanced MRI. Clin Imaging," 22(3), 211–215,1998.

[3] C.C. Berry, and A.S.G. Curtis, "Functionalisation of magnetic nanoparticles for applications in biomedicine," J Phys D Appl Phys, 36, 198–206, 2003.

[4] D.C.F. Chan, D.B. Kirpotin, and P.A. Bunn, "Synthesis and evaluation of colloidal magnetic iron oxides for the site-specific radiofrequencyinduced hyperthermia of cancer," J Magn Mag Mat, 122(1–3), 374–378, 1993.

[5] B. Chertok, B.A. Moffat, and A.E. David, "Iron oxide nanoparticles as a drug delivery vehicle for MRI monitored magnetic targeting of brain tumors," Biomaterials, 29(4), 487–496, 2008.

[6] M. Gonzales-Weimuller, M. Zeisberger, and K.M. Krishnan, "Size-dependent heating rates of iron oxide nanoparticles for magnetic fluid hyperthermia," J Magn Mag Mat, 321(13), 1947–1950, 2009.

[7] M. Grinholc, B. Szramka, J. Kurlenda, A.Graczyk, and K.P. Bielawski, "Bactericidal effect of photodynamic inactivation against methicillinresistant and methicillin-susceptible Staphylococcus aureus is strain-dependent," J Photochem Photobiol A Chem, 90(1), 57–63, 2008.

[8] A.K. Gupta, and M. Gupta, "Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications," Biomaterials 26(18), 3995–4021, 2005.

[9] J.S. Kim, E.K. Kyeong N. Yu and J.H Kim, "Antimicrobial effects of silver nanoparticles," Nanomedicine: Nanotechnology, Biology, and Medicine, 3, 95– 101, 2007.

[10] N. Kohler, C. Sun, J. Wang, and M. Zhang, "Methotrexate-modified superparamagnetic nanoparticles and their intracellular uptake into human cancer cells," Langmuir, 21(19), 8858–8864, 2005.

[11] O. Mahapatra, S. Ramaswamy, S.V.N. Kumar, Y. Tejabhiram, and C. Gopalakrishnan, "Corn flake-like morphology of iron nanoparticles and its antibacterial property," The Journal of General and Applied Microbiology, 57**,** 59-62, 2011.

[12] R.A. Pareta, E. Taylor, T.J. Webster, "Increased osteoblast density in the presence of novel calcium phosphate coated magnetic nanoparticles," Nanotechnology, 19(26), 265101, 2008.