Use of tools of NLD in analysis and calibration of Cu-doped ZnO nanoparticles (NPs) for biological applications

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Abstract

ZnO based nanoparticles find a wide range of applications starting from biosensors and drug-delivery systems to solar cells. Keeping an eye on the prospect for an application in the field of biotechnology, we analyze Cu-doped ZnO nanoparticles after the fabrication and necessary characterization of the XRD data obtained, by using the tools of Nonlinear Dynamical Theory (NLD). One of the tools used called Lyapunov Exponent, bears the signature of the dynamical evolution of the particles as well as structure formation. This is calculated in order to quantify the underlying strange attractor present in the nanosystems, which happens to be the driving force behind the structure formation. The changes in the values of this parameter with the variation of the physical and chemical conditions, would pave the way for an efficient calibration for meaningful biological applications, which happens to be the focus of the present work. Differently sized ZnO nano particles are obtained by changing dopant percentage for inhibiting human pathogenic bacteria. Calibrations are made between the Zone of Inhibition (ZOI) and Lyapunov Exponent to obtain the required dopant percentage for a given ZOI vis-à-vis the same anti-bacterial effectiveness in terms of ZOI of a ZnO doped nano particle. Copyright © 2016 VBRI Press.

Keywords: Cu-doped ZnO NPs, XRD, lyapunov exponent, antibacterial activity, ZOI.

Introduction

Non-linear dynamics is the study of systems which respond disproportionately to initial conditions or perturbing stimuli [**1**]. Nonlinear systems may exhibit "chaos" and its characteristics are- It is a system which is highly sensitive to initial condition; unpredictable; deterministic and gives different attitude toward the concept of variability. The study of nonlinear dynamics has been an active area of research since 1980s [**2, 3**]. Proper understanding of nonlinear dynamical systems in terms of phase space geometry, bifurcation, strange attractor, entropy, and theory of chaos in general lead to path-breaking discoveries from astrophysics and cosmology [**4**] to Nanoscience and Nanotechnology [**2, 5**]. Tools of Non-linear Dynamical (NLD) Theory, such as, Lyapunov Exponent, Correlation Dimension, Hurst Exponent, Continuous Wavelet Transform etc. are apt candidates for not only analyzing the non-linear time series obtained in the form of XRD or i-V data for any apparently hidden correlations, but also for detecting subtle changes in the optical or electro-magnetic responses of a nano-sensor. Though it is at its nascent stage, these tools are used for analyzing systems of particles in material science too as it can be seen in the work of Melendez et al. for dynamical study of current fluctuations of nanostructured films [**2**]. The dynamical nature of system of particles during their formation can be understood from the physical parameters of the system resulting in various dimensions, entropies, indices, coefficients, and exponent, such as, the Lyapunov Exponent [**1**], as the system evolves dynamically. This motivates us to employ the tools of Non-linear Dynamical Theory (NLD), particularly Lyapunov Exponent (λ) in order to quantify the inherent nonlinearity in the mechanism.

ZnO nanoparticles have unique physical and chemical pronounced properties than their bulk equivalents. They show higher activity particularly when the size of the particles is less than 100 nm [**6**]. Due to low cost of production of ZnO nanoparticles and their high effectiveness in antibacterial activities, they find wide applications in medicine, therapeutics and industries [**7, 8, 9**]. Moreover, larger their surface-volume ratio, more enhanced is the activity [**10**]. Treatment of Nano-ZnO at higher temperature shows comparatively lower activity. Nano-ZnO has high anti-bactericidal properties than nano-CuO and nano-Fe_2O_3 [**11**], they have shown strong antibacterial and good antifungal activities [**12**]. ZnO

improves the antibacterial activity of chitosan in a system of CS/ZnO/Ag nanoparticles. ZnO nanoparticles are found to be good ultraviolet B absorber and therefore they can be used in sunscreen, also they can be used as antibacterial agents in ointments and lotions to cure skin diseases [**13**]. They have been used also to protect food stuffs against food borne pathogens [**14**]. Nano-ZnO can inhibit the gram-positive and gram-negative bacteria, the gram negative found to be more resistive than the gram-negative [**15, 16**]. We too have found this behaviour though with a non-linear correlation. Similarly, the antimicrobial activities for *Escherichia coli* and *Staphylococcus aureus* showed higher zone of inhibition(ZOI) for pectin capping ZnO NPs than chemically synthesized ZnO NPs. These properties depend on its particle size, surface area, concentration, structure formation, and morphology [**17**]. The activities increase with decrease in the sizes [**8, 6**], and with increase in the concentration of the nanoparticles. The size of nanopaticles can be controlled with capping agents [**18**], also it depends on the dopant percentage [**19**]. Surfactant assisted combustion synthesis of ZnO exhibited strong antimicrobial activity against the bacterial species [**20**]. Apart from analyzing these antimicrobial activities and verifying the reported results, in this current study, it has been studied the Nonlinear Dynamical behaviour in order to quantify the correlation between the geometrical structure formation in the samples and their activity with the human pathogenic bacteria- *Staphylococcus aureus* and *Escherichia coli*.

A time series is a sequence of data points, measured typically at successive points in time spaced at either uniform or non-uniform time intervals. Even a single time series contains a relatively complete historical record of the dynamics, as it is affected by all the relevant dynamical variables. Hence it is possible to study the dynamics from a single time series without reference to other physical variables. The approach is similar to the one followed by Singh *et al.* [**21, 22**] and Saikia *et al.* [**23**].

In mathematics, the Lyapunov exponent or Lyapunov characteristic exponent of a dynamical system is a quantity that characterizes the rate of separation of infinitesimally close trajectories [**24**]. Quantitatively, two trajectories in phase space with initial separation δZ_0 diverge at a rate given by $|\delta Z(t)| \approx e^{\lambda t} |\delta Z_0|$ where λ is the Lyapunov Exponent. The rate of separation can be different for different orientations of initial separation vector. Thus, there is a spectrum of Lyapunov exponents-equal in number to the dimensionality of the phase space. It is common to refer to the largest one as the Maximal Lyapunov Exponent (MLE), because it determines a notion of predictability for a dynamical system. A positive MLE is usually taken as an indication that the system is chaotic the pattern formation.

Four different ZnO samples doped with 2.5%, 5%, 7.5% and 10% weightage of copper are prepared under the same synthesis conditions, and the XRD time series obtained from the doped samples. Lyapunov Exponents are calculated from the XRD data for the four samples. Also the antibacterial susceptibility test of human pathogenic bacteria: gram-positive (*S aureus*) and gram-negative (*E coli*) are carried out in Meuller Hinton Agar medium by well diffusion method. The diameters of inhibitory zones of clearance of both the organisms are measured for all the differently doped ZnO:Cu samples. Some calibrations among dopant percentage, Lyapunov Exponents and ZOI are obtained with a prospect for finding dopant percentage of given ZOI of unknown samples of same kind.

The significance of the current study is to provide an estimate for the optimum dopant percentage of a ZnO doped nanoparticle to be used for anti-bacterial applications. If the required rate and extent are known in terms of the Zone of Inhibition (ZOI), the controlling physical parameters, such as, the dopant percentage, temperature, molarity, pH value etc. may be optimized using parameters from the non-linear dynamical analysis obtained from the XRD data alone.

Experimental

Materials and Methods

Materials for the work were bought from commercial distributors with highest purity (99.90%). Zinc nitrate hexahydrate [$Zn(NO3)_2.6H_2O$] and ammonium hydroxide (NH_4OH) are the base materials, polyvinylpyrrolidone (PVP) were used as the capping agent, Copper Chloride Dihydrate [$CuCl_2.2H_2O$] is taken as the dopant material and distilled water as the solvent. These materials were used to prepare the Cu-doped ZnO nanoparticles and they have been manufactured by Merck Specialities Private Limited, Mumbai, India. All the culture media were purchased from and manufactured by HiMedia Pvt. Ltd., Mumbai, India.

Materials Synthesis

Samples are prepared by chemical wet method [**25**] due to its low cost and simple procedures. At the very outset, 75ml of 0.1 M solution of $Zn(NO_3)_2.6H_2O$ was taken in a beaker, stirred constantly for 3 hrs at 60^0. Then it is allowed to cool and NH_4OH was added drop by drop into the stirring solution at room temperature till the pH value of the solution became 7.5, whence the white solution of ZnO (say, soln A) was formed. In a separate beaker, 3 wt. % of Poly Vinyle Pyrollidone (PVP) solution (say, solution B) was stirred constantly at 60^0C for 3hrs. For the dopant, 75ml of $Cucl_2.2H_2O$ of 0.01 M solution [soln C], taken in another beaker was stirred constantly for 30min at 60^0C. The final mixture (solnA + solnB + solnC) was stirred constantly for 1.5 hrs at 60^0C and allowed to cool down at room temperature till the white precipitate of ZnO is formed [**26**]. The particles of the solution in the container were allowed to settle down overnight in a dark chamber. Finally, the precipitate of the solution is filtered and dried at 60^0C in an oven for about 12 hrs. As far the number of moles in the $Cucl_2.2H_2O$ solution (dopant) is concerned with respect to that in the solution A, the sample ZnO prepared is 10% Cu- doped [**25**]. In the

similar manner, when 75ml of $Cucl_2.2H_2O$ solution of 0.0025 M, 0.005 M and 0.0075 M when taken as solution C in the above procedure, then 2.5%, 5% and 7.5% Cu-doped ZnO sample was prepared.

The formation of ZnO according to the precursor reactions [**25**] is shown:

$$Zn(NO_3)_2.6H_2O \longleftrightarrow Zn^{2+} + 2NO_3^{2-} + 6H_2O$$
$$NH_4OH \longleftrightarrow NH_4^+ + OH^- \longleftrightarrow NH_3 + H_2O$$
$$Zn^{2+} + 4NH_3 \longleftrightarrow Zn(NH_3)_4^{2+}$$
$$Zn(NH_3)_4^{2+} + 2OH^- \longleftrightarrow ZnO + 4NH_3 + H_2O$$
$$Zn^{2+} + 4OH^- \longleftrightarrow Zn(OH)_4^{2-}$$
$$Zn(OH)_4^{2-} \longleftrightarrow ZnO + H_2O + 2OH^-$$

Data

For analyzing a nanosystem, the data points for the system can be the Intensities (I) versus 2θ (angles) which are obtained from XRD. As in a continuous diffraction process, the angles are monotonically increasing functions of time (t), therefore time series from the data obtained from XRD are used as our time series. To ensure adequate data size, the step size of angles of 0.02^0/step are taken.

Characterization

Powder X-ray diffraction (XRD) data of the prepared Cu-doped ZnO nanoparticle have been recorded by Philips X-ray Diffractrometer (X'Pert Pro) with Cu $K_{\alpha 1}$ radiation (λ = 1.5406 Å).

Preparation of inoculums and culture media

The test organisms, *Escherichia coli* and *Staphylococcus aureus*, were grown in 5ml Luria Bertani Broth at 37°C and 0.5 McFarland's standard was used during antibacterial susceptibility test. Mueller Hinton Agar medium was the choice of media used in the antibacterial tests.

Antibacterial activity assay

The test compounds were dissolved in autoclaved double distilled water to make up a final concentration of 100mg/ml, that is, 100 mg of the compound was dissolved in 1ml of water. The antibacterial susceptibility test was done by well-diffusion method. After overnight incubation of the bacterial isolates in Luria Bertani (LB) Broth, were diluted in LB broth to obtain turbidity corresponding to 0.5 McFarland densities. This suspension was then swabbed onto MH agar plates and wells of 5mm each were made. For the assessment of antibacterial activity, 30 µL of the test compound was added to each well and incubated overnight. The test compounds were serially diluted two-fold such that the concentrations of the samples were now – 100, 50, 25, 12.5 µg/mL. On a separate inoculated agar plate the serially diluted test compounds were added and incubated. Upon 18 hours incubation, the plates were examined for zone of clearance around the wells containing the test compound and diameter of the zone was measured. Triplicates were maintained and the experiments were repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded [**27**]. The results for the inhibition were taken only for the concentration of 50 µg/mL.

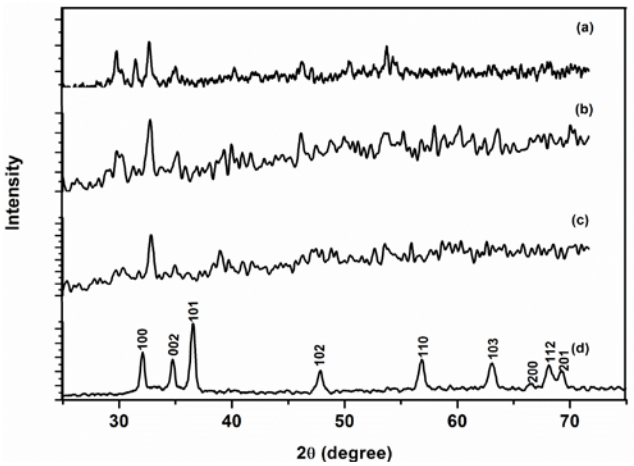

Fig. 1. XRD Time Series for (a) 2.5%, (b) 5%, (c) 7.5% and (d) 10%.

Results and discussion

Irregular XRD time series as may be seen in **Fig. 1** of the samples, their Power Spectra and other related calculations based on Fourier Transformation, suggest the presence of low-dimensional chaotic attractor in the dynamically evolving systems as portrayed by the samples considered. This justifies the need of application of the tools of NLD and hence we have calculated the Lyapunov Exponent of the time series in order to measure the rate at which the information is lost from the system as the size transformation and structure formation of the particles take place. Since the information content of a dynamically evolving system may be linked with its geometrical structures, Lyapunov Exponent can also be treated as a sensitive indicator for structure variation. In order to estimate the Lyapunov Exponent, the trajectories of a dynamically evolving system in the phase space are allowed to evolve for a certain time duration, say EVOLV, with a definite difference in initial conditions; and the variation in the ratios of the successive instantaneous differences from the initial conditions are noted down while the projection of the multidimensional dynamical system is embedded incorporating a time delay, say TAU, into a 2-dimensional configuration space. **Fig. 2** shows Lyapunov Exponent versus no. of iterations the systems undergo in the corresponding geometrical phase spaces, for varying values of either Time Delay (TAU) or Evolution Time (EVOLV), giving the Largest Lyapunov Exponent (LLE) or Maximal Lyapunov Exponent (MLE).

To compute the largest Lyapunov Exponent λ_1, definition of Wolf [**28**] is used here. It may be seen from the **Table 1** that as the dopant percentage increases, the Lyapunov Exponent decreases, indicating that the rate of loss of information from the system is decreasing, and

hence predicting an increase in the biological activity of in the form of enhanced anti-bacterial or anti-fungal response due to more regular structure formation.

Table 1. Lyapunov Exponents Estimated.

ZnO:Cu(2.5%)	0.5
ZnO:Cu(5%)	0.42
ZnO:Cu (7.5%)	0.3
ZnO:Cu (10%)	0.16

Table 1 shows Lyapunov Exponent of the samples are constructed for analysis and study of the nanosystems. Physically, it may be interpreted that when this value is calculated to be 0.3, it tells us that if the initial state of the physical system was known with an accuracy of 30 bits, the future of the system is unpredictable after about 100 s (= 30bits/0.3 bits/s). Hence, this must be the rate of structure formation. The spectrum of Lyapunov exponent is plotted for around 3500 iterations of each of the time series, varying one of the two most important parameters influencing the estimation, namely, the time delay required for the iterations to get folded within the embedding dimension of the phase space to retain most of the information, and the optimum evolution time necessary for the letting the trajectory evolve before calculating the fractional increment of the initial difference of any two adjacent trajectories. The average value of the exponent obtained from the plateau region of each of the curves is considered as the Maximal Lyapunov Exponent or Largest Lyapunov Exponent, and it is clear from **Fig. 2** that the plateau region for each of the four samples is well established and sustained.

Then, we proceed to determine the correlation between the dopant percentage and the biological activity of the four samples prepared. **Fig. 3** shows the Zone of Inhibition (ZOI) of the samples for gram-negative (*E coli*) and gram-positive pathogens (*S aureus*) after 18 hrs of incubation, confirming the antimicrobial activity of the samples under the same synthesis conditions.

The average diameters of the zones of inhibition for both the pathogens and for all the samples are measured, and are shown in **Table 2**. It shows maximum ZOI of the ZnO:Cu(10%) for both the pathogens and almost same trend for varying dopant percentage. This suggested that variation of Lyapunov exponent with dopant and that of ZOI with dopant can be used for obtaining a calibration between the ZOI and Lyapunov Exponent.

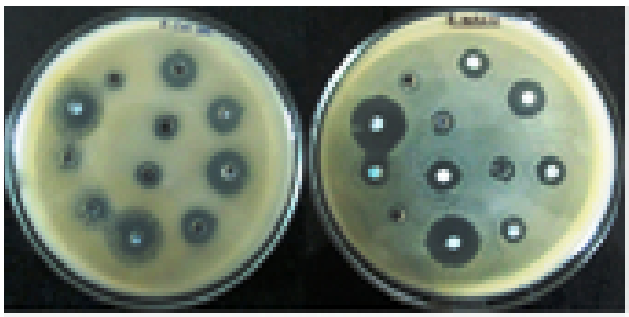

Fig. 3. Antibacterial activity of the ZnO nanoparticles.

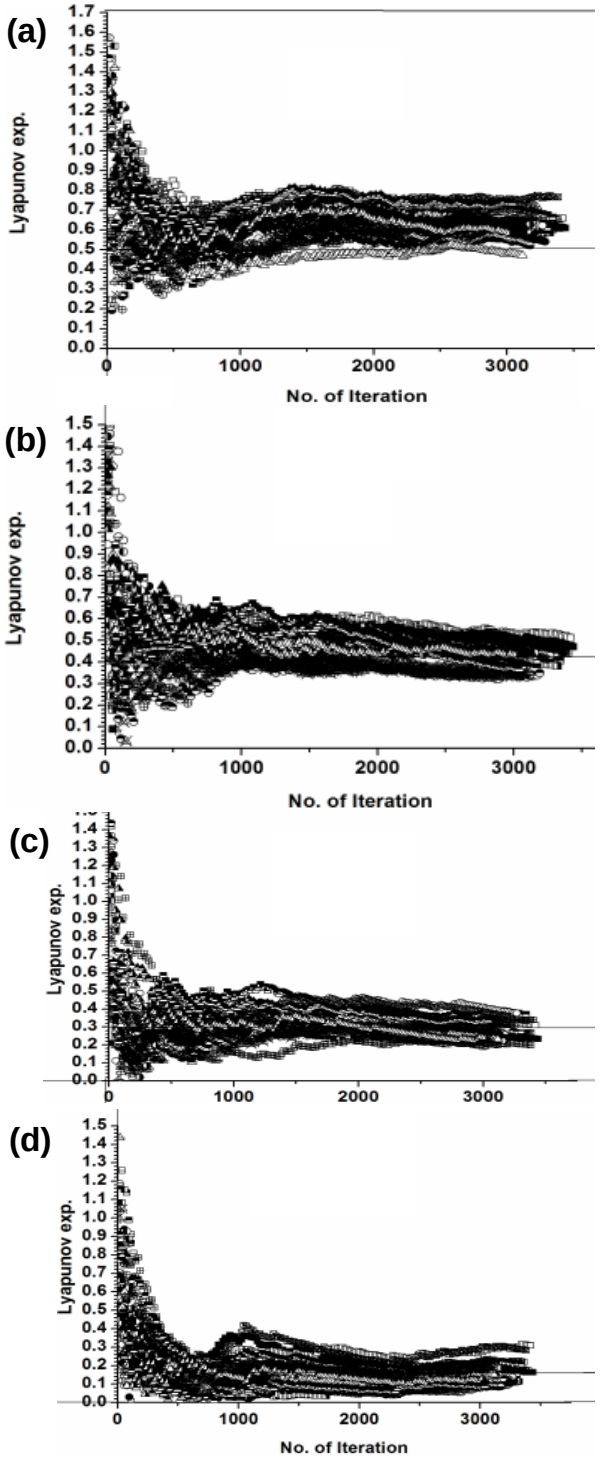

Fig. 2. Maximal Lyapunov Exponent versus the iteration number for different values of *TAU* (with *EVOLV* fixed at 19)

Table. 2. Zone of Inhibition / Clearance in case of *E.coli* and *S.aureus*.

Test Compound	Diameter of ZOI (in mm) E. coli	S. aureus
ZnO:Cu (2.5%)	10	14
ZnO:Cu (5%)	15	16
ZnO:Cu (7.5%)	10	11
ZnO:Cu (10%)	20	25

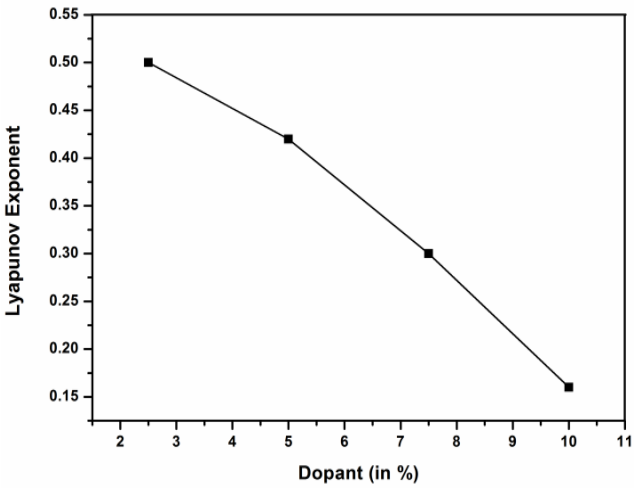

Fig. 4. Lyapunov Exponent Vs Dopant Percentage

Fig. 4 shows the correlation between Lyapunov Exponent and Dopant Percentage for the samples considered and it is evident that it requires non-linear treatment.

Fig. 5(a) and **Fig. 5(b)** show the variation between ZOI for pathogens E coli and S aureus respectively with Dopant percentage of the samples. **Fig. 5(c)** and **Fig. 5(d)** show the variation between ZOI for pathogens *E coli* and *S aureus* respectively with Lyapunov Exponent of the samples. It is clear here too that the variations are nonlinear in nature and simple linear regression or interpolation will not work. Instead, adequate treatment is required for using the variation between the ZOI and the Lyapunov Exponent as the calibration graph. The results also show prominent zone of clearance which confirms the ability of our test compound to show bacterial growth inhibition even at a lowest concentration of 50mg/ml (MIC). The bacterial isolates were identified to be antibiotic resistant to a wide group of third generation cephalosporins, carbapenems and monobactams. It was observed that the ZnO:Cu (10%) showed a greater zone of clearance for both the pathogens. This higher antibacterial activity of the nano-ZnO:Cu is now understood as a consequence of the best structure formation, when the λ_1 and hence the rate of information loss is the smallest one, in comparison to the other samples with lower dopant percentage, resulting in poorer structure formation as confirmed by the higher numerical value of λ_1 or higher rate of information loss.

Conclusion

The inherent nonlinearity in the dynamically evolving ZnO:Cu nanoparticles is proved by the existence of positive Lyapunov exponent, not only characterizing the strange attractor in the system, but also quantifying the information loss occurred as a result of structure formation. While trying to relate this structure formation to biological activity of these particles, we have come up with a calibration graph which may be used to set the initial conditions needed to provide the optimum environment for creating such nanoparticles that result in a required ZOI. We plan to take the present work further to check the validity of the results in case of other nanoparticles formed under different physical, chemical, and/or biological conditions that would lead us to the formulation of a predictive tool based on the theory of NLD, for anticipated anti-bacterial, or other biological activities.

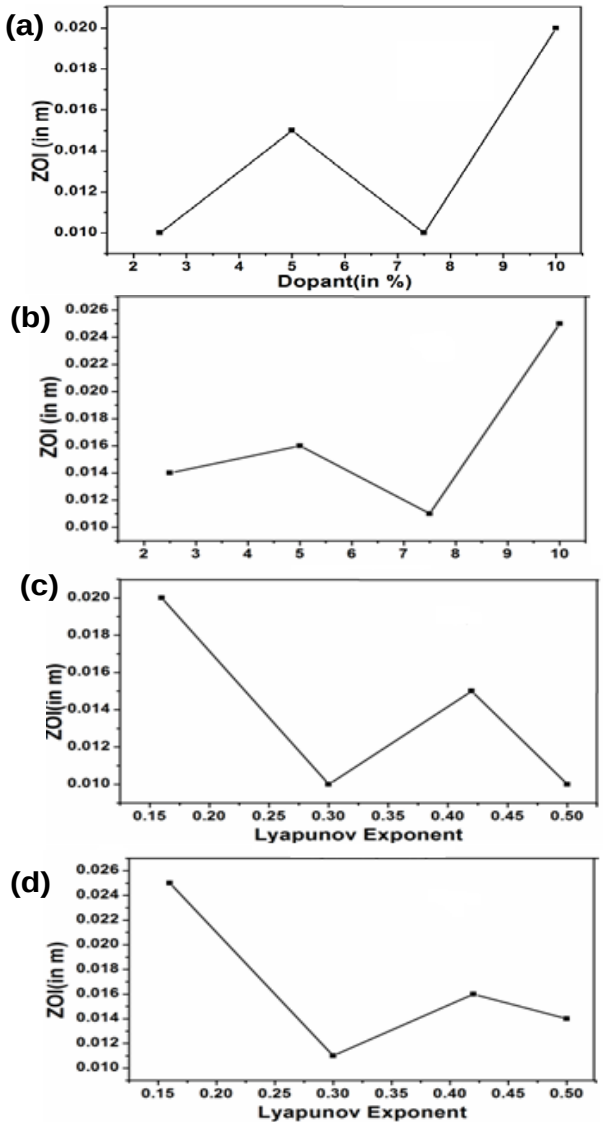

Fig. 5. ZOI Vs Dopant for (a) *E coli* and (b) *S aureus*; ZOI Vs Lyapunov Exponent for (c) *E coli* and (d) *S aureus*

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Author's contributions

Conceived the work: RKS, ES, MKD, SMB, VVB, KKS; Executed the expeirments: MKD, KKS; Data analysis: RKS, ES, KKS; Paper written: RKS.

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