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Effect of phosphate affinity of the Lanthanum oxide nanoparticles on the anti-bacterial activity

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ABSTRACT

Among the rare earth oxide, the importance of Lanthanum oxide nanoparticles on antibacterial activity had been realized within few years. Lanthanum oxide also had great ability to bond the complex ions such as hydroxide, carbonate, organic ligands and phosphate. Here the affinity of Lanthanum oxide nanoparticle on phosphate and the effect of such affinity on anti-bacterial activity were studied. For that Lanthanum oxide nanoparticle was prepared using lanthanum chloride and ammonium carbonate as starting materials with DNA and STRACH as capping agents. Hence three different sources of phosphorous rich solutions such as Sugar cane Solution (SS), Fertilizer Solution (FS) and Fish Tank Solution (TS) were taken. The phosphate affinity of the prepared Lanthanum oxide nanoparticles was studied by adding it to the different phosphate rich media that were taken for the phosphate identification test and was determined using different techniques like phosphate estimation kit and UV-Visible spectrophotometer. To understand the effect of this phosphate affinity on antibacterial activity, we choose the same solutions and selected bacteria like E-coli and S-aureus were cultured in the solutions with and without LOD I and LOS I in Agar well diffusion method.

Keywords: ***Lanthanum oxide nanoparticles, DNA, STARCH, SS, FS, TS, Phosphate affinity, Phosphate estimation kit, UV-Visible spectrophotometer, Antibacterial activity***

1. INTRODUCTION

Nanotechnology is the developing branch of science that opens the way to explore in clinical field, material science, bioscience, and so on. The formation of new nanoparticles is responsible for this rapid development in nanotechnology. New nanomaterial based antibacterial agents are developed widely due to their unique physical, chemical and biological properties based on their small size and large surface to volume ratio. So, the formation of new nanoparticles based on their antimicrobial properties is very essential to develop new antimicrobial agents to inhibit bacterial action [1]. The size effect properties of nanoparticles are more significant for their interaction with the biological system. Since the nanoparticles possess a high surface to volume ratio, a large number of atoms are interacting with its environment. Due to their small size, it can easily penetrate the inner cell through the cell membrane and cause damage to cell DNA and the respiratory system. Lanthanum oxide is one of the pertinent uncommon earth oxides. The primary attributes highlight of such uncommon earth components is its high density, high melting point, high warm conductance and exceptional physical and chemical properties because of its 4f electron [2]. This make it is a good agent for electronics, medical, biomedical and agronomic fields.

2. SYNTHESIS METHOD

Here we synthesised Lanthanum Oxide nanoparticles with varying particle sizes using Lanthanum Chloride and Ammonium Carbonate. 0.1 M solution of Lanthanum Chloride and 0.1 M solution of Ammonium Carbonate were added to the prepared 0.01weight percentage of DNA/ STARCH and stirred continuously for 5 hours. The wet precipitate obtained after filtration was then allowed to dry naturally before being finely ground to obtain the carbonate precursor powder. Further, the lanthanum carbonate was annealed at 600^{o}C temperature obtained from TGA/DTA for 3 hours to get the required lanthanum oxide nanoparticle. To study the effect of temperature on different properties, the obtained oxide samples are further annealed at 700°C and 900°C. The samples used for this study are labelled as LOD & LOS represents La_2O_3 nanoparticles synthesized using DNA and STARCH as capping

agents; while the Roman letters I, II and III represent the annealing temperatures at 600°C, 700°C and 900°C respectively.

3. EVALUATION TECHNIQUES

Antibacterial activity

The agar well diffusion method is used here to evaluate the antibacterial activity against the bacteria. The samples having antibacterial activity are allowed to diffuse out into the medium containing the test organisms. After the interaction between them, a zone of inhibition with uniformly circular shape having millimeter range diameter is formed around the test region. The diameter of the inhibition zone formed around the well is measured to assay the antibacterial activity of the samples. Streptomycin having 10mg/mL is used as a standard antibacterial agent for the study.

Phosphate removal activity

Phosphorus is the eleventh most abundant element essential for the growth of plants and animals. It is commonly seen as phosphate which is the main building block for nucleic acids, proteins, and energy carries and is very essential to all the organisms for its proper growth. It is very necessary for microbial growth in the aqueous medium [3]. The abundance of phosphate in the surface water caused heavy algal growth due to eutrophication [4]. So, it is very necessary to solve this undesired condition by using various phosphate removal strategies [5]. There are commonly three types of phosphorous removing techniques – chemical co-precipitation, biological remediation and adsorption [6]. In this study we use the adsorption technique to remove phosphate ion from the taken solutions. For this study we choose three different sources of phosphorous rich solutions.

i) Sugarcane Solution (SS :- For the preparation, 5 ml of sugar cane juice is taken and prepared it into 100 ml using distilled water.
ii) Fertilizer Solution (FS) :- 5 gm of fertilizer is taken and prepared it into 50 ml using distilled water. Then it is filtered through Whatmann-41 filter paper to avoid the unwanted impurities and preparedit into 100 ml using distilled water.
iii) Fish Tank Solution (TS) :- 100 ml of fish tank solutions is taken from the fish pond.

Phosphate identification tests

For the identification of phosphate in the solutions we use two methods.

Water testing kit for phosphate

This is a qualitative analysis method. In this identification test, the blue colour formed in each solution after the addition of 5 drops of reagent1 and 1 drop of reagent 2 is taken as the evidence for the presence of phosphate in the solution. After the addition of reagents, the solution is shaken well and a blue colour is developed. The darkness of blue colour gives the concentration of phosphate present in the solution.

UV-Visible spectrophotometer

The inorganic phosphate is determined through the molybdenum blue method in which the reduction of 12-molybdophosphoric heteropoly acid results the blue color. Here the acidified phosphate solution is mixed with ammonium molybdate and thus formed hetero poly acid is then reduced to phosphomolybdenum blue by using hydrazine hydrate [7]. The absorbance of thus formed phosphomolybdenum blue was measured around 830nm spectrophotometrically and λmax, the wavelength at which maximum absorbance observed, was determined. On plotting absorbance against wavelength, the wavelength of maximum absorption (λ max) was found around 830 nm for the given phosphate solutions and the corresponding calibration curve is drawn.

4. RESULTS AND DISCUSSION

Antibacterial activity of Lanthanum oxide nanoparticles

The antibacterial activity of lanthanum oxide nanoparticles against E.coli and S.aureus is studied. Lanthanum oxide nanoparticles synthesized using DNA and STARCH as capping agents at different temperatures proved to be good antibacterial agents against the selected microorganisms. The prepared sample shows high antibacterial activity than that of the annealed samples due to the enhancement of oxygen deficiency [8]. The antibacterial activity of Lanthanum oxide nanoparticles using DNA and STARCH as capping agents at different temperatures with E-coli are given in the table 1.

Table 1: Results obtained for the antibacterial activity of Lanthanum oxide nanoparticle at different temperature

Organism	Concentration(µg/mL)	Zone of inhibition					
		LOD I	LOD II	LOD III	LOS I	LOS II	LOS III

E-Coli	**Streptomycin (100µg)**	**25**	**23**	**25**	**25**	**23**	**25**
	250	**Nil**	**Nil**	**Nil**	**10**	**Nil**	**Nil**
	500	**11**	**Nil**	**Nil**	**12**	**Nil**	**Nil**
	1000	**12**	**10**	**Nil**	**13**	**13**	**Nil**
S-aureus	**Streptomycin (100µg)**	**26**	**28**	**26**	**26**	**28**	**26**
	250	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
	500	**10**	**Nil**	**Nil**	**11**	**Nil**	**Nil**
	1000	**11**	**Nil**	**Nil**	**12**	**10**	**Nil**

From the table it is clear that Lanthanum oxide nanoparticles synthesized using STARCH as capping agent show more active than lanthanum oxide nanoparticle synthesized using DNA as capping agent. It also reveals that the antibacterial activity decreases with an increase in temperature. This result also agrees with the size effect of the synthesized nanoparticles through its reactivity due to its large surface to volume ratio.

Since Lanthanum oxide nanoparticles is non-soluble one, the antibacterial activity of the nanoparticle is not due to the direct physical contact with the microorganisms but due to its high affinity to phosphate present in the medium. Thus occurs a severe competition between the nanoparticle and bacteria for the available phosphate in the medium. But the antibacterial activity of the samples against E-coli is slightly greater than that of S-aureus [9]. This is because, E-coli bacteria have the ability to produce phosphate binding protein within the cell and transported it to the periplasm for enhancing the phosphate uptake while phosphate deficiency occurs. But in the presence of Lanthanum oxide nanoparticles, the bacteria cannot assimilate essential phosphate. This also increases the antibacterial activity of the samples by capturing the internal phosphate of the bacteria. So, the extreme completion of phosphate between the nano lanthana and the bacteria leads in the death of bacteria in the medium. Thus, the toxicity of the lanthanum oxide nanoparticles against bacteria is controlled by the addition of phosphate. For that, we conduct more experiments to identify the phosphate affinity of the lanthanum oxide nanoparticles synthesized using DNA and STARCH as capping agents

Phosphate affinity of Lanthanum oxide nanoparticles

Lanthanides have a great ability to bondthe complex ions such as hydroxide, carbonate, organic ligands and phosphate[10]. Most of the lanthanum-containing materials are used to remove and recover phosphate from different phosphate rich media for last few years [11]. Likewise, Lanthanum oxide nanoparticles have a great affinity to phosphate and are checked by adding lanthanum oxide nanoparticle to the different phosphate rich media that are taken for the phosphate identification test.

Using Phosphate estimation kit

In this test 0.01g of LOD I and LOS I are added to each 100 ml solution of SS, TS and FS and the corresponding colour change is noted. The result obtained from phosphate estimating kit is shown in the Figure1.

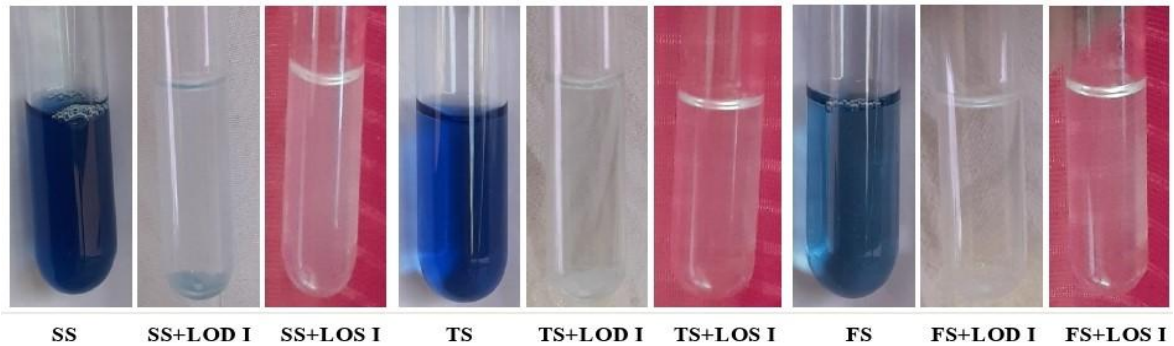

Figure1. Identification Phosphate using water detecting kit for phosphate

The blue colour formed after the addition of reagents in the water detecting kit shows the presence of phosphate in the solutions. The dark blue seen in SS indicates that the phosphate concentration is more for SS than other solutions. Add 0.01g of LOD I and LOS I separately into 100 ml of SS, TS and FS taken in different beakers and rest it for 24 hours. Then the solutions are filtered using Whatmann-41 filter paper and take5 ml of each filtered

solutions in different test tubes for conducting phosphate identification test. The colour disappearance in the solution reveals the absence of phosphate in the solution.

Using UV-Visible spectrophotometer

In this method, the absorbance is measured around 830 nm wavelength using UV-Vis light. As in the earlier method, 0.01g of LOD I and LOS I separately added into 100 ml solutions of SS, TS and FS taken in different beakers and rest it for 24 hours. After filtering by Whatmann-41 filter paper, the solution is given for UV-Vis spectrum analysis. The absorbance measured around 830 nm wavelength using UV-Vis light for different solution with and without the samples (LOD I and LOS I) are given in the Figure2.

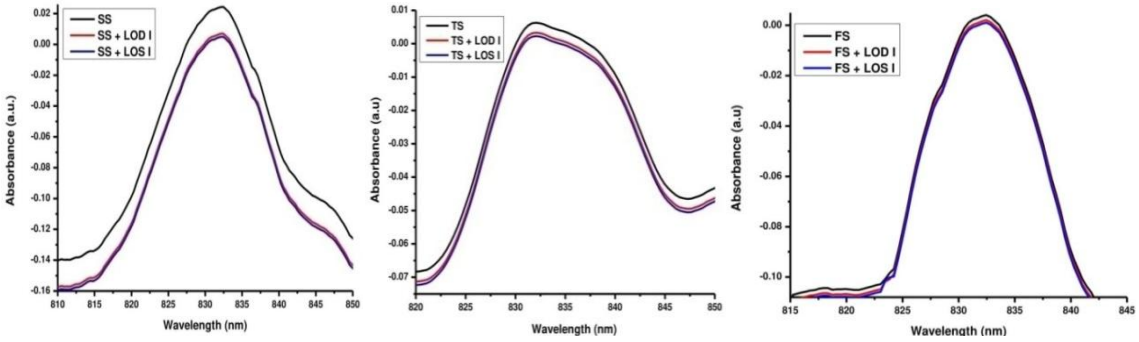

Figure 2. Identification of phosphate using UV-Vis spectrophotometer

From the Figure2, it is very clear that the absorbance of the solution decreases with the addition of the samples. This indicates that the phosphate concentration in the solutions after the addition of LOD I and LOS I decrease and correspondingly the absorbance of phosphate decreases. This also gives the evidence to the phosphate adsorption ability of the prepared samples.

Calibration curve

Calibration curve is very essential to explain the relation between absorbance and the phosphate concentration for spectrophotometric analysis. The curve is obtained by plotting the absorbance against the concentration of phosphate in µg at 830 nm wavelength. The linear plot obtained from the observation obeys Lambert-Beer's law. The calibration curve for the determination of phosphate is shown in the Figure3.

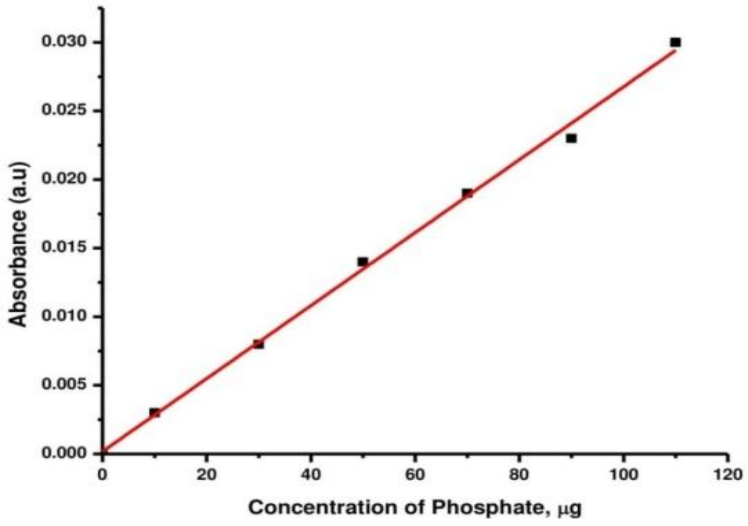

Figure 3. Calibration curve for the determination of phosphate

Table 2 : Amount of phosphate in different solutions

Sample	Absorbance	Phosphate in µg/ml
SS	0.024	4.1
SS+LOD I	0.007	1.3
SS+LOS I	0.005	0.9
TS	0.006	1.1

TS+LOD I	**0.003**	**0.5**
TS+LOS I	**0.002**	**0.3**
FS	**0.004**	**0.7**
FS+LOD I	**0.002**	**0.3**
FS+LOS I	**0.001**	**0.2**

Determination of Phosphate in various solutions

The amount of phosphate in various solutions SS, TS and FS with and without prepared samples is determined spectrophotometrically using molybdenum blue method. For the measurement 20 ml of each solution is taken and adding the reagents under optimized condition. For developing maximum blue colour, each solution is left at room temperature for about 35 minutes and measured the absorbance spectrophotometrically. The amount of phosphate in the solution is calculated using the equation given below and the result of the analysis is given in the table 2.

$$\text{Amount of phosphate in the solution} = \frac{\text{Concentration of phospahte corresponding to each absorbance measured}}{\text{Solution volume taken}}$$

From the table it is very clear that the amount of phosphate in the solution decreases with the addition of LOD I and LOS I to the solution. This shows the phosphate affinity of the samples towards different phosphate rich media. The amount of phosphate in the solutions added with LOS I is less than the solutions with LOD I.

5. EFFECT OF PHOSPHATE AFFINITY ON ANTIBACTERIAL ACTIVITY

The high affinity of lanthanum oxide nanoparticle towards phosphate is applied here to control the phosphate in the entire system. To understand the effect of this phosphate affinity on antibacterial activity, we choose the same solutions taken for the phosphate identification test. In this study the selected bacteria are cultured in the solutions with and without LOD I and LOS I. The antibacterial activity of the solutions with and without the lanthanum oxide nanoparticles against E-coli and S-aureus is shown in the Figure 5 and Figure 6 respectively.

From the Figure 5 and 6, it is very clear that there exists no zone of inhibition around the well. This indicates the inactivity of all the solutions with and without LOD I and LOS I towards the E-coli and S-aureus bacteria. This is because in different phosphate rich media competition occurs between the nanoparticles and the bacteria for phosphate to survive in that media. This also means that the bacteria incubated with these nanoparticles grew similarly as in the absence of any lanthanum oxide nanoparticles. This is another evidence for the antibacterial inactivity of lanthanum oxide nanoparticles.

2925 – SS
2926 - TS
2927 – FS

2928 – LOD I +SS
2929 – LOD I + TS
2930 – LOD I + FS

2931 – LOS I + SS
2932 – LOS I + TS
2933 – LOS I + FS

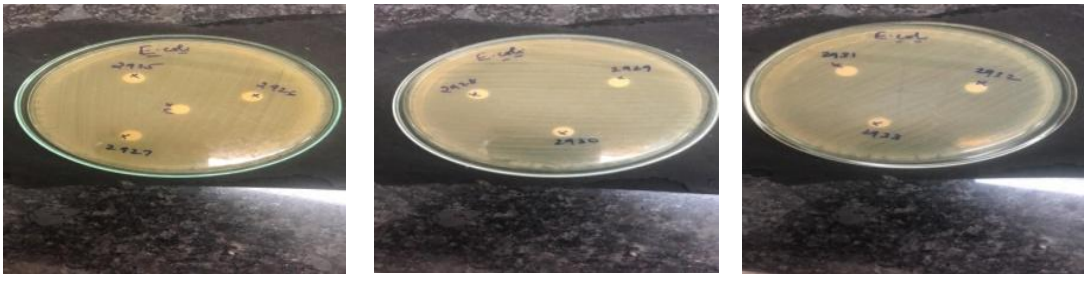

Figure 5. Antibacterial activity of different solutions against E-coli

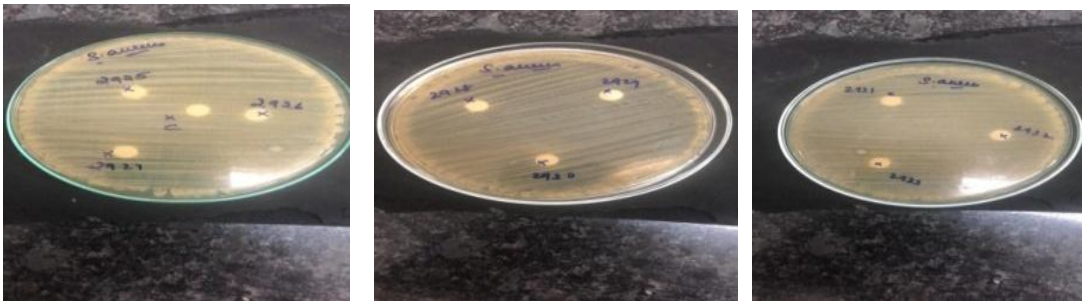

Figure 6. Antibacterial activity of different solutions against S-aureus

In another way it can be explained as the toxicity of the nanoparticles depend not only the concentration of the sample taken for the study but also the amount of phosphate seen in the medium [12]. i.e., if the binding capacity of the lanthanum oxide nanoparticle is insufficient to bind all the phosphate present in the solution, then it shows no toxicity to the bacteria. Similarly, the amount of phosphate is less than the binding capacity of the nanoparticle results the complete cell death in the medium.

CONCLUSION

The high affinity of the nanoparticle towards phosphate is investigated through phosphate estimation kit test and UV-Visible spectrophotometer and that leads to apply the lanthanum oxide nanoparticles in phosphate removal applications. The disappearance of blue colour of the phosphate rich solution after the addition of Lanthanum oxide nanoparticles estimated through the phosphate estimation kit reveals the phosphate adsorption ability of the nanoparticles from the solutions. The UV-Vis spectrum also reveals the phosphate affinity of the nanoparticle in such a way that the absorbance of the solution decreases with the addition of nanoparticles into the phosphate rich medium. From the calibration curve the amount of phosphate present in different solutions with and without the nanoparticle is calculated and the addition of nanoparticle to different phosphate rich media results the reduction of phosphate concentration present in the solution. But in different phosphate rich media, the high affinity of nanoparticle towards phosphate shows the antibacterial inactivity of the nanoparticles against E-coli and S-aureus bacteria. Thus, it can be concluded that in the absence of sufficient phosphate in the medium, the nanoparticles had produced severe antibacterial activity against E-coli and S-aureus, while in phosphate rich medium no toxic effect is produced against the bacteria.

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