Synthesis, characterization, antioxidant and antibacterial studies of praseodymium complex with glutathione

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Reduced glutathione (GSH) is a biologically important component that plays a critical role in antioxidant activity. Praseodymium(III) complex with GSH was synthesized and the complex formed was characterized by FTIR, XRD, TGA and SEM analysis. Infrared studies provided information on the mode of complexation between the lanthanide metal ion and GSH. XRD and TEM analysis showed the nanocrystalline phase and irregular morphology of the complex. TGA thermogram indicated good thermal stability of the complex. The *in vitro* antioxidant and antibacterial properties of the complex were studied. The results suggest that the praseodymium(III) complex possesses antioxidant and antibacterial activity.

Keywords: Antibacterial and antioxidant activity, glutathione, lanthanide, praseodymium complex.

THE field of lanthanide (Ln) chemistry has seen a resurgence in interest as well as increased research over the last few decades[1,2]. A significant portion of these endeavours revolves around the development of complexes with novel structural features for the generation of advanced materials and utilization of their special spectroscopic properties in the development of biological probes and sensors in the areas of molecular biology and clinical chemistry[3]. Due to the wide range of uses of lanthanide complexes in biology and medicine, lanthanide coordination chemistry has become increasingly important, playing a major role in modern chemistry[4,5]. Lanthanide complexes are also known for their anticancer, antiallergic, anti-inflammatory and antibacterial properties[6]. Lanthanide research on polydentate ligands has been the subject of interest for scientists due to their potent biological properties. In this context, Ln(III) ion complexes can be exploited as bioactive compounds because of their high possible coordination numbers and good flexibility of the metal ion coordination sphere, which accounts for easy ligand changes[7].

We report the synthesis and characterization of the praseodymium(III) complex with glutathione. Glutathione is a sulphur compound made up of three amino acids that occur in two different forms, viz. the active form, reduced glutathione (GSH) and the inactive form, oxidized glutathione (GSSG) (Figure 1)[8,9]. GSH is well-known for its key role in oxidative stress management. The *in vitro* antibacterial activities of the synthesized compound against pathogenic bacteria (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Bacillus subtilis*) were examined. We have also evaluated the *in vitro* antioxidant activities of the complex using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays.

Experimental details

Materials and methods

L-Glutathione reduced ($C_{10}H_{17}N_3O_6S$, ≥98.0%) and praseodymium trinitrate hexahydrate ($Pr(NO_3)_3 \cdot 6H_2O$, 99.99%) were procured from Sigma-Aldrich and used as received. Nutrient agar, streptomycin, DPPH and Trolox were purchased from HiMedia and Merck, India respectively. Deionized water was used to make the aqueous solutions.

Infrared (IR) spectra of the sample were obtained using a Perkin Elmer FT-IR spectrometer (Spectrum-Two) in the

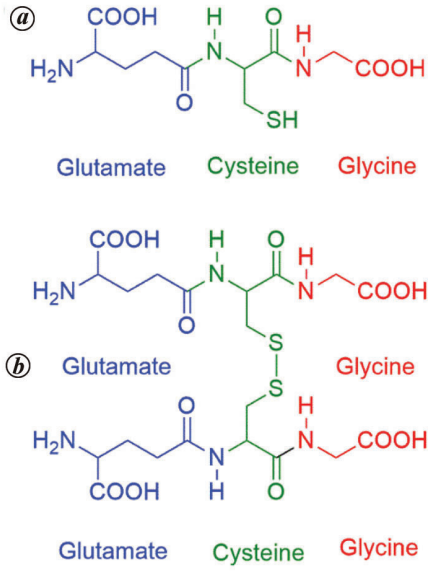

Figure 1. Chemical structures of (***a***) glutathione (GSH) and (***b***) glutathione disulphide (GSSG).

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400–4000 cm^{-1} range. The morphology of the sample was examined using field emission scanning electron microscopy (FE-SEM; Sigma, Carl Zeiss, UK). X-ray powder diffraction (XRD) analysis was done (Rigaku Ultima IV X-ray diffractometer) with Cu K_α radiation ($\lambda = 1.540$ Å) from 10° to 80° (2θ) at room temperature. Thermal analysis was carried out (SDT Q600 V20.9 Build 20 thermal analyzer) with a heating rate of 20°C/min using a nitrogen atmosphere.

Preparation of the complex

To an aqueous solution of $Pr(NO_3)_3 \cdot 6H_2O$ (0.01 mol), an aqueous solution of GSH (0.05 mol) was added dropwise with continuous stirring. The solution was stirred thoroughly and refluxed for about 4 h. The resulting solution was concentrated and kept overnight. The obtained solid product was collected by filtration and washed with acetone and distilled water before being dried in a vacuum oven.

In vitro antioxidant assays

The antioxidant activity of the sample was evaluated utilizing two separate assays: DPPH and FRAP[10]. The antioxidant activity of the sample was tested using the two assays and compared with the standard Trolox. The experiments were carried out in triplicate and the results were averaged. The IC_{50} values for the standard and the sample were derived for the DPPH assay. The DPPH free-radical scavenging percentage was calculated using the measured absorbance as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100,$$

where A_{control} is the absorbance of the control (DPPH + methanol) and A_{sample} is the absorbance of the sample. The IC_{50} values were used to assess the antioxidant activity.

For the FRAP assay, the absorbance of the reaction mixture was measured at 700 nm using a UV/Vis spectrophotometer. Greater absorbance indicated greater reducing power.

In vitro antibacterial activity

The *in vitro* bactericidal activity of the Pr(III)–glutathione complex was compared against four different strains of Gram-negative bacteria (*E. coli* and *K. pneumonia*) and Gram-positive bacteria (*S. aureus* and *B. subtilis*) using the well-diffusion method[11]. The antimicrobial property of the synthesized compound was evaluated by determining the zone of inhibition (mm) and compared with the inhibition diameter of positive control streptomycin. The minimum inhibitory concentration (MIC) of the sample was determined by a twofold serial broth dilution technique and compared with the standard drug streptomycin. All the tests were carried out in triplicate.

Results and discussion

Infrared spectra

IR analysis was used to study the mode of complexation between praseodymium and GSH. FTIR analysis helped in elucidating the chemical composition and environment of the complex formed. Figure 2 shows the IR spectra of free glutathione and the praseodymium complex. The IR spectra of GSH and the Pr(III)–GSH complex exhibit several vibrations. The appearance and disappearance of certain bands in the lanthanide complex and the free GSH, provide information on the bonding mechanism of the complex and determine the binding mode in the complex formed. The presence of water molecules can be attributed to the broad band around 3000–3300 cm^{-1}. From the FTIR spectra of free glutathione, the notable vibrations observed are at 2525, 1713 and 1599 cm^{-1}, which correspond to the vibrations of the sulphydryl (–SH) group and carbonyl group (C=O) of carboxylic and amide group respectively (Figure 2). The bands from N–H vibrations can be seen at 3348 and 3251 cm^{-1} (refs 12, 13). These bands are characteristic of the functional moieties of GSH.

Several important changes were observed in the IR spectrum of the praseodymium complex, which indicates the coordination of Pr(III) with GSH. The formation of the complex is supported by the disappearance of the -SH band in the IR spectrum of the praseodymium complex. The disappearance of the thiol band of glutathione from the praseodymium complex can be attributed to the interaction of GSH with the lanthanide ion through the sulphur atom of the thiol group from the cysteine moiety of GSH. This could suggest the deprotonation of –SH and its subsequent participation in coordinating with the lanthanide ion. Additionally,

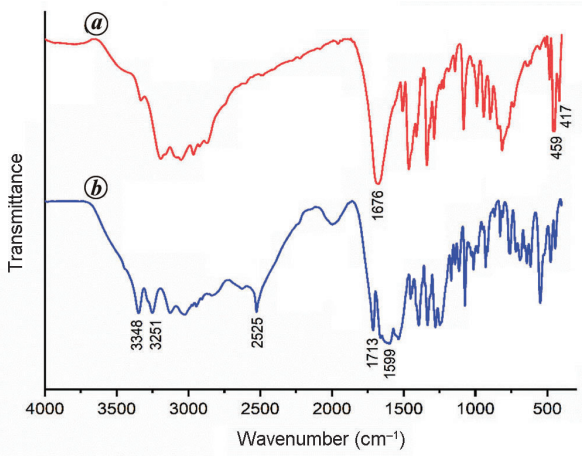

Figure 2. FTIR spectra of (***a***) praseodymium(III)–GSH complex and (***b***) glutathione.

the C=O band at 1713 cm^{-1} observed in the free GSH appears at a lower frequency in the lanthanide complex. This also indicates the involvement of the –COOH group of glycine in the coordination of glutathione with praseodymium. The shift of the N–H band to a lower frequency indicates that nitrogen is involved in bonding. Apart from these, the IR spectrum of the praseodymium complex also exhibits a new peak at 417 cm^{-1}, which can be attributed to the stretch vibration of the M–O bond[13]. This clearly demonstrates the formation of the bond between GSH and praseodymium in the complex. Figure 3 presents the possible structure of the complex.

XRD analysis

XRD analysis of the praseodymium(III)–GSH complex was done in the 2θ angles range 5°–80° at a wavelength of 1.54 Å. Figure 4 shows the XRD pattern of the praseodymium(III) complex. Table 1 shows the resulting interplanar spacing (d) for various values of 2θ, as well as the associated (h k l) indices. The GSAS II software was used to index the XRD pattern of the praseodymium(III)–glutathione complex in terms of major peaks with relative intensities greater than 10%. The lattice parameters calculated for the unit cell value of the Pr(III)–GSH complex were: a = 20.20867 Å, b = 5.51476 Å, c = 10.69533 Å and unit cell volume V = 1191.95 $(Å)^3$. As a result, an orthorhombic crystal system has been suggested for the praseodymium(III) complex with GSH. The crystallite size was calculated using Scherrer's equation, where the full width at half maximum (FWHM) of the most intense peak was considered.

$$d = \frac{k\lambda}{\beta \cos\theta},$$

where k is the Scherer constant (~0.9), λ the X-ray wavelength of Cu k_α radiation, β the FWHM of the diffraction peak and θ is the Bragg angle. The average crystallite size of the praseodymium(III)–GSH complex was found to be 39.66 nm, suggesting the nanocrystalline phase of the system.

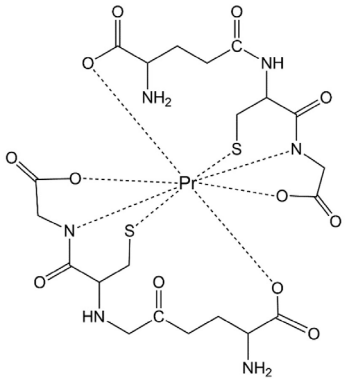

Figure 3. Probable structure of praseodymium(III)–GSH complex.

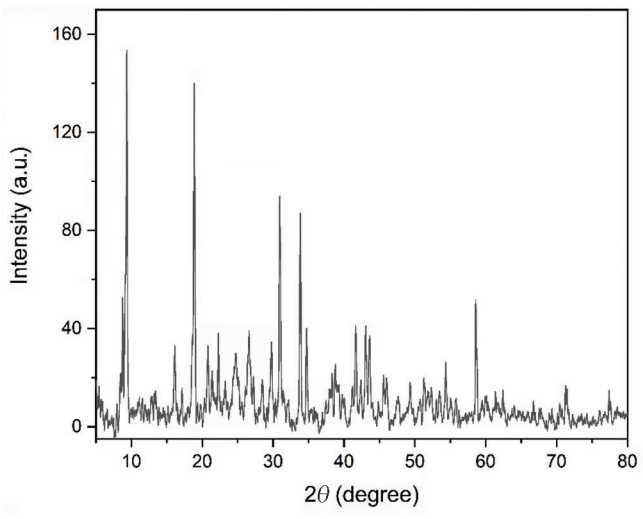

Figure 4. XRD pattern of praseodymium(III)–GSH complex.

SEM

Figure 5 shows SEM images of the praseodymium(III)–GSH complex. According to SEM observations, the powder obtained after precipitation comprises large aggregates. These images emphasize the formation of micron-sized agglomerates with no discernible morphology. The sample is devoid of a defined morphology, and is irregular in shape and size. Smaller particles can also be seen between the grains and on their surfaces.

TGA

Figure 6 shows the thermogravimetric analysis (TGA) curve of the praseodymium–glutathione complex. The thermogram indicates that the complex is stable in air at room temperature and starts to decompose when the temperature rises to 100°C.

Table 1. Powder XRD data of praseodymium(III) complex with glutathione

Position	*d*-obs	*d*-calc	*hkl*
8.757	10.088	10.104	200
9.337	9.463	9.453	101
16.103	5.499	5.514	010
18.852	4.703	4.726	202
22.250	3.991	3.963	311
24.691	3.602	3.588	212
26.574	3.351	3.368	600
30.932	2.888	2.874	610
33.804	2.649	2.647	121
34.7101	2.582	2.584	204
41.623	2.167	2.168	123
43.018	2.100	2.094	604
58.588	1.574	1.575	606

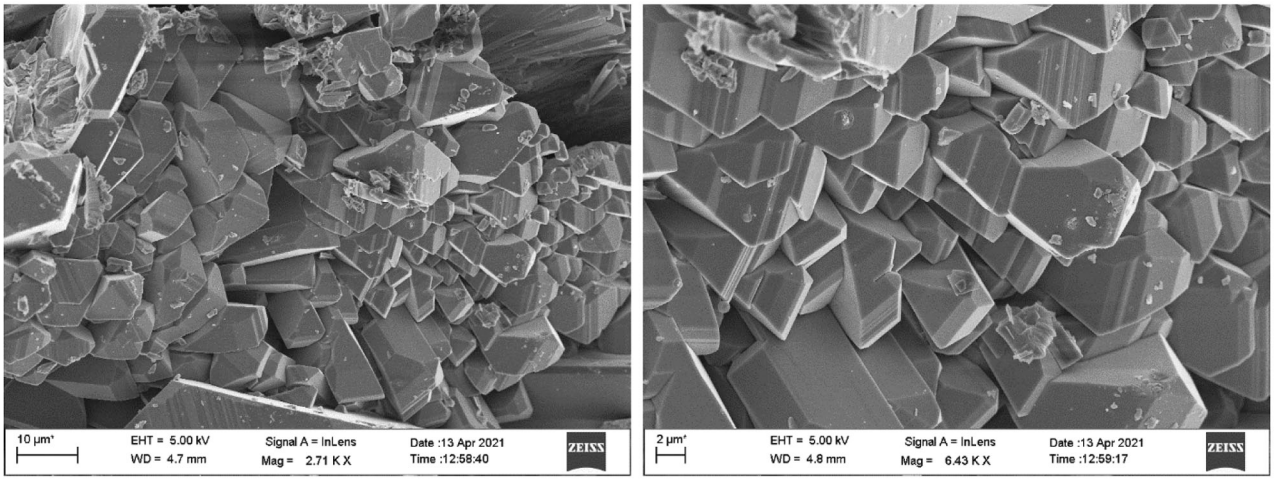

Figure 5. SEM images of the praseodymium(III) complex.

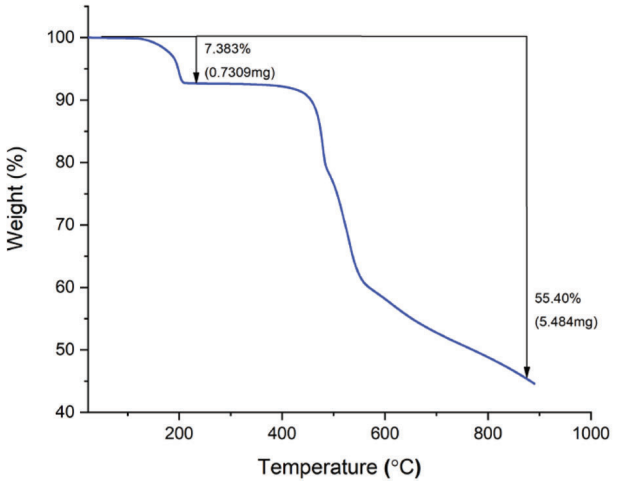

Figure 6. TGA curve of the Pr(III)–GSH complex.

The degradation of the complex starts around 100°C; it takes place slowly at the initial stage and is incomplete until 900°C with a total weight loss of about 55.4%. The thermal decomposition of the complex takes place in two stages. Figure 6 shows that the first decomposition occurs between 100°C and 298°C, resulting in a loss of 7.38% weight. This weight loss contributes to the loss of free and coordinated water, and indicates the presence of free or coordinated water in the complex. This result is in accordance with the FTIR data. At a temperature of 330–890°C, the second stage of decomposition (48.01% weight loss) is observed, which could be due to the loss of organic moiety from the praseodymium–GSH complex. The char yield of the sample was recorded to be approximately 44.6 wt% at 890°C. No stable intermediate was observed in the decomposition curve, possibly suggesting that no other ligand is attached to Pr(III). The slow decomposition of the complex may be a possible indication of the multidentate behaviour of the ligand with varying thermal stability that corresponds to the different binding sites of GSH[14]. TGA analysis could provide important information regarding the thermal stability of the complex. The results indicate good thermal stability of the Pr(III)–GSH complex with high decomposition temperature. The high thermal stability of the complex could be attributed to the highly polarized M–O bond.

In vitro antioxidant activity

Radical scavenging assay (DPPH): Reactive oxygen species (ROS) are highly reactive chemicals produced during aerobic metabolism in organisms. Excess production of ROS can cause oxidative stress in the body. Oxidative stress is the imbalance between the production of ROS and the ability of the body to counteract them with antioxidants. ROS like the hydroxy radical ($OH^{\bullet}$), peroxide ($ROO^{\bullet}$) and superoxide radical ($O_2^{\bullet-}$) can cause extensive damage to the nucleic acids, lipids and proteins. Oxidative stress has been linked to ageing and development of diseases like cancer, diabetes and cardiovascular diseases[15,16]. The primary objective of antioxidants administration is the elimination of these radicals. Consequently, the antioxidant activity of the Pr(III)–GSH complex has been analysed in this study by determining its radical scavenging and reducing potentials using DPPH and FRAP respectively.

FRAP and DPPH are the most widely used assays for determining antioxidant activity. They provide rapid analysis, are easily standardizable and relatively simple. DPPH is an N-centred free radical, stable at room temperature with an odd electron. It gives off a purple colour in methanol solutions and exhibits a strong absorption at 517 nm. The purple colour of DPPH changes to yellow with a pairing of its odd electron by donating a hydrogen or alkyl radical in the presence of a radical scavenger. This results in the formation of the reduced DPPH-H (Figure 7)[17]. The change in colour

from purple to yellow upon scavenging also results in a subsequent lowering of absorbance at 517 nm. Such a low absorbance, in this case, indicates a higher antioxidant activity. Antioxidant analysis using the DPPH free-radical method works by quantifying the ability of antioxidants to quench the DPPH radical. We have used Trolox as the standard for the assay.

The free-radical scavenging effects of the sample and Trolox in various dilutions (20, 40, 60, 80 and 100 µg/ml) on the DPPH radical are exhibited in Figure 8 by the graph of % scavenging versus concentration. Table 2 gives the IC_{50} values of the standard Trolox and the Pr(III)–GSH complex. The antioxidant activity was determined using a calibration curve based on the percentage of antioxidant activity and expressed as the half-maximal inhibitory concentration (IC_{50}). IC_{50} represents the concentration of a substance that produces 50% of the maximal effect. In this case, the concentration of the sample is effective in reducing 50% absorbance of the DPPH radical or inhibiting 50% of the DPPH radical. The lower the IC_{50} value, the greater is antioxidant activity of the sample. The Pr(III)–GSH complex showed $IC_{50} = 83.40$ compared to the standard Trolox, which possessed $IC_{50} = 40.29$. Trolox showed a higher DPPH scavenging activity than the sample providing 50% inhibition (IC_{50}) at a concentration of 40.29 µg/ml, while the Pr(III)–GSH complex exhibited the same at a concentration of 83.40 µg/ml. The results demonstrate that the praseodymium(III)–glutathione complex has a free-radical scavenging ability. The inhibition activity was found to be dose-dependent, with a proportionate increase of DPPH radical scavenging with concentration. Maximum scavenging of 54.19% for the sample was observed at a concentration of 100 µg/ml.

Reducing power assay: Another popular method for measuring the antioxidant capacity is the FRAP assay. It is fast, simple and cheap. It is a nonradical method that depends on reducing ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+})[18]. This assay primarily assesses the reducing capacity of an antioxidant when it reacts with Fe^{3+} ($K_3Fe(CN)_6$) to produce a coloured Fe^{2+} ($K_3Fe(CN)_6$) complex. Although tripyridyltriazine (TPTZ) was used in the original FRAP assay, other iron-binding ligands have been used in recent years, with potassium ferricyanide being the most popular ferric-binding reagent. In this case, the end-product is a blue-coloured ferrous complex which was spectrophotometrically evaluated, revealing the reducing power of the antioxidant. Increased absorbance at 700 nm indicates antioxidant activity. The ability of any compound to donate an electron or hydrogen atom to a metal atom accounts for its reducing power. The sample compound was used as a reducing agent in a colorimetric reaction in the FRAP assay.

The test solution, which was initially yellow in colour changed to various shades of green and blue depending on the reducing potential of the test compound. Consequently, there was an increase in absorption at 700 nm. The antioxidant activity was evaluated by measuring the change of absorbance at 700 nm. Hence, the higher absorbance value, the higher is antioxidant activity. The resultant blue colour of the Fe^{2+} complex can be produced in two ways, both of which yield the same result[19]. The two possible routes are:

(i) Reduction of Fe^{3+} to Fe^{2+}, which binds to ferricyanide and produces a blue colour.
(ii) Ferricyanide reduction to ferrocyanide, which binds to free Fe^{3+} to yield blue colour[20].

Following are the simplified schemes for the two reactions[20]:

$$\text{Antioxidant} + Fe^{3+} \rightleftarrows Fe^{2+} + \text{oxidized antioxidant}.$$

$$Fe^{2+} + Fe(CN)_6^{3-} \rightleftarrows Fe[Fe(CN)_6]^-,$$

or

$$\text{Antioxidant} + Fe(CN)_6^{3-} \rightleftarrows Fe(CN)_6^{4-} + \text{oxidized antioxidant}.$$

$$Fe(CN)_6^{4-} + Fe^{3+} \rightleftarrows Fe[Fe(CN)_6]^-.$$

Figure 9 shows the results obtained using FRAP assay. It presents a concentration versus absorbance graph of the sample and Trolox. Absorbance values obtained at 700 nm were plotted against their corresponding concentrations (µg/ml). The sample had a lower absorbance than the standard Trolox indicating reduced antioxidant activity. The FRAP assay followed a similar trend shown by the DPPH assay. With increasing concentration, the reducing power of the sample also increased (Figure 10). The findings of this study demonstrate that the Pr(III)–GSH complex possesses antioxidant activity.

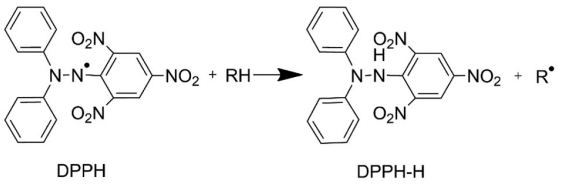

Figure 7. Mechanism of DPPH scavenging.

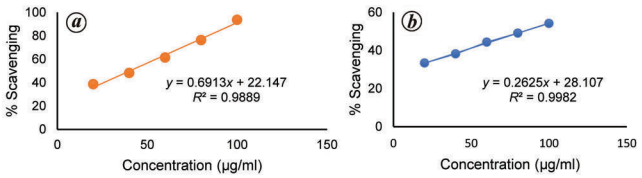

Figure 8. DPPH free-radical scavenging activity in the presence of different concentrations of (***a***) Trolox and (***b***) the sample.

Table 2. DPPH radical scavenging activity and IC_{50} values of the standard Trolox and the sample

	Trolox		Sample	
Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
20	38.6		33.41	
40	48.15		38.18	
60	61.4	40.29	44.38	83.40
80	76.32		49.11	
100	93.64		54.19	

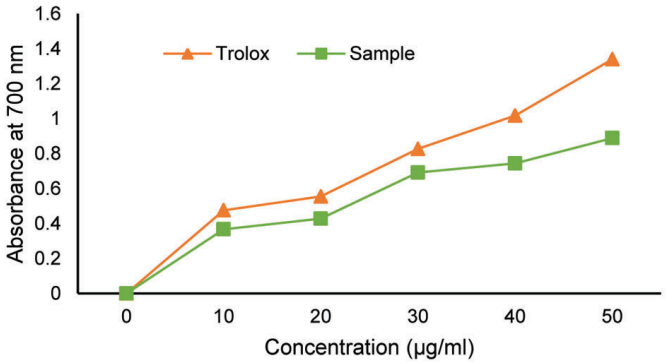

Figure 9. Antioxidant activity of standard (Trolox) and the sample using FRAP assay.

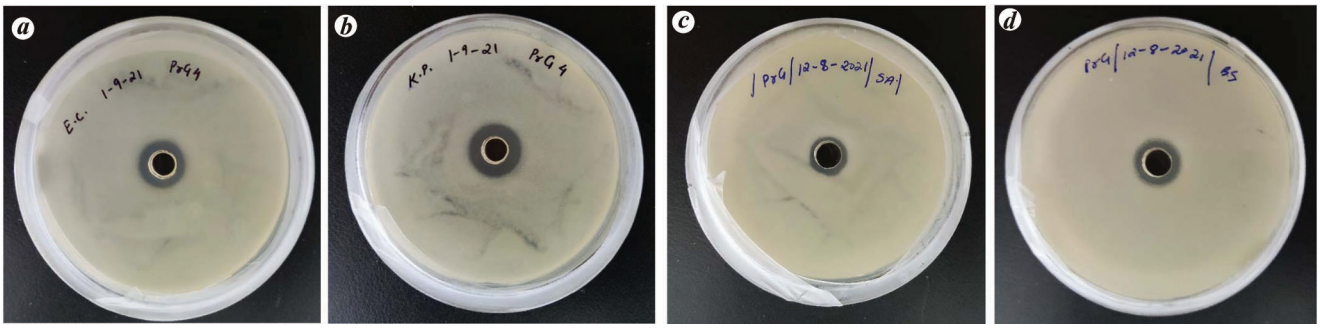

Figure 10. Estimation of antimicrobial activity by agar plate diffusion experiment: ***a***, *Escherichia coli*; ***b***, *Bacillus subtilis*; ***c***, *Staphylococcus aureus*; ***d***, *Klebsiella pneumonia*.

In vitro antibacterial activity

Tables 3 and 4 summarize the *in vitro* antibacterial activities of the Pr(III)-GSH complex against *E. coli*, *B. subtilis*, *K. pneumonia* and *S. aureus*. The well-diffusion method was used for the antibacterial analysis of the sample and streptomycin. Both the sample and reference were taken at concentrations of 10 mg/ml in sterilized water. The antibacterial efficacy was assessed by measuring the diameter of the zone of inhibition. The zone of inhibition is the clear circular area around the test compound where microbial growth is inhibited. Table 3 presents the zone of inhibition (mm) of the praseodymium complex against different microbes. The results show that the Pr(III) complex exhibits potent antimicrobial activity against all the selected bacterial strains. The *in vitro* assay results show that the praseodymium complex has the highest activity against the Gram-negative bacterium *K. pneumonia*, with an inhibition zone of 14 mm. Figure 10 shows images of the antibacterial activity of the praseodymium complex against Gram-positive and Gram-negative bacteria.

The MIC of the compound was calculated against the four bacterial strains to determine its bactericidal potency. MIC is the lowest concentration of an antimicrobial agent that completely inhibits visible microorganism growth. Compounds with lower MIC scores are more effective antimicrobial agents because fewer compounds are required to inhibit the growth of the organisms. Table 4 shows the MIC results of the sample and streptomycin. The MIC values of the complex ranged from 0.7 to 1.4 mg/ml. These values were

Table 3. Zone of inhibition (mm) of Pr(III)–GSH in comparison with the standard streptomycin

Bacterium	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*
Sample	11	12	14	10
Streptomycin	30	32	30	31

Table 4. Minimum inhibitory concentration of Pr(III)–GSH (mg/ml) in comparison with the standard streptomycin

Bacterium	*B. subtilis*	*E. coli*	*K. pneumonia*	*S. aureus*
Sample	0.703	0.703	1.406	1.406
Streptomycin	0.0072	0.0029	0.0072	0.0058

used to determine the lowest concentration of the sample that inhibited visible growth of the bacteria under the given assay conditions. Hence, the MIC values represent the minimum concentration of the sample that effectively promotes antibacterial activity in the different bacterial strains tested. The MIC of the sample was found to be lowest against *B. subtilis* (0.7 mg/ml) and *E. coli* (0.7 mg/ml).

Since biological systems are complex, determining the exact mechanism of antimicrobial activity of metal complexes is not feasible. However, the antibacterial behaviour of metal complexes can be explained using the Overton concept[21] and Tweedy's chelation theory[22]. According to the lipid membrane model of cells proposed by Overton, the cell membrane is composed of a thin layer of lipids. This layer only allows lipid-soluble substances to pass through the cell membrane. As a result, liposolubility is an important determinant of antimicrobial activity. In this context, the correlation between liposolubility and antibacterial activity of metal complexes may be explained by the Tweedy chelation theory. According to this theory, on chelation of a metal ion with its corresponding ligand, the polarity of the metal is reduced. The main reason for this is the overlap of the ligand orbital and partial sharing of its positive charge with the donor groups. Consequently, the delocalization of π-electrons is increased across the entire chelate ring, enhancing the lipophilicity of the metal complex. This would suggest that chelation aids in permeating the metal complex through the bacterial cell membrane by increasing its lipophilicity. Penetration of the lanthanide complex could result in cell-wall disruption leading to cell lysis and subsequent cell death. The passage of the lanthanide complex through the cellular membrane could also result in the blockage of metal-binding sites in the bacterial enzymes, obstructing normal cell functions such as metabolism, respiration and ATP production. This inhibits protein synthesis and cell processes, limiting the growth of the organism and eventually leading to bacterial cell death.

Conclusion

Here we present a method of synthesizing praseodymium complex with glutathione. The synthesized complex was characterized by FTIR, XRD, SEM and TGA. FTIR studies indicated the interaction of Pr and GSH through the thiol, amine and carboxylate groups of the GSH ligand. The formation of a metal–O bond was also observed. XRD studies revealed the nanocrystalline phase and orthorhombic system of the praseodymium complex. TGA analysis showed that the complex was thermally stable. Antioxidant studies, as analysed through DPPH and FRAP assays, confirmed that the complex possessed antioxidant activity. The antioxidant capacity of the complex, even though lower than that of the standard Trolox, was potent. *In vitro* antibacterial studies also demonstrated the inevitable antibacterial potential of the complex. The complex showed antibacterial activity against all the tested Gram-positive and Gram-negative bacteria. The findings of the present study could suggest potential applications of the praseodymium–GSM complex in the clinical field.

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