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Evaluation of Heavy Metal Concentrations in Enzyme Embedded Poly Lactic Acid (EPLA) by ICP-OES: Lipase from Candida Rugosa.

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Abstract: ***Enzymes like lipases are essential for catalyzing important reactions in the fields of biotechnology and industrial operations. For quality control and safety evaluations, however, the quantification of these contaminants is crucial because the presence of heavy metals in enzyme preparations can negatively affect their activity and stability. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), a key component of this investigation, is used to measure the levels of heavy metals in lipase enzyme preparations embedded in polylactic acid (PLA). The lipase enzyme being studied comes from Candida rugosa, which is well-known for its considerable industrial applications. According to preliminary findings, the lipase enzyme is successfully protected from external heavy metal pollutants by the encapsulating technique, maintaining its enzymatic activity and stability. The lipase-PLA composite had only minimal quantities of heavy metals, according to the ICP-OES study. The research's conclusions show great potential for the creation of reliable and contaminant-free lipase enzyme preparations, which will increase their suitability for usage in a variety of industrial processes and encourage the use of biodegradable polymers, supporting environmental sustainability. By highlighting the significance of quality control and safety assessment through the determination of heavy metal concentrations, this research contributes to the larger field of enzyme biotechnology. It also highlights the potential of Candida Rugosa lipase implanted in PLA matrices for eco-friendly and sustainable biocatalytic applications in sectors ranging from food and pharmaceuticals to biofuel generation and other biodegradable polymers.***
Keywords: Enzymes, heavy metals, lipase, polymer, polylactic acid

I. MATERIALS AND FILM PREPARATION

Because of its capacity to produce films and its biocompatibility and biodegradability, polylactic acid (PLA) has been selected as the matrix material. In particular, Nature Tech Pvt Ltd's PLA 2003D was chosen as the PLA grade for the film preparation. Following known techniques, the Lipase from Candida rugosa (Product Number: 89444, Company: Sigma-Aldrich Chemical Pvt Limited) was immobilized on Immobead 150. Lipase was attached to the surface of Immobead 150, an immobilization procedure that gives the enzyme a stable support matrix. Using a twin screw extruder thermo electric company having L/D ratio 25:1 (PTW 16) [1] thermal electron corp, Lipase-embedded EPLA films were produced. PLA 2003D pellets with 2% were melted and homogenized during the extrusion process, and then [2% lipase (immobilized on Immobead 150)] was added. The substance extruded and blown to obtain EPLA film[2] [3] as per the parameter of screw speed 150 RPM, torque 66 Nm and barrel temperature 165^{o}C to 175^{o}C.

II. EXPERIMENTAL

A. Instrumentation

Agilent 5800 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [4]is the instrument used for heavy metal analysis. It was equipped with a nebulizer, an Advanced Valve System (AVS), and a Multimode Sample Introduction System (MSIS) for the analysis of heavy metals in the sample. In general, the light wavelength approach is used to do the analysis. When a sample is run through an analyser in plasma form, multiple light waves of varying wavelengths are released, designating the presence of various metals as well as their concentration. The wavelength's intensity is directly proportionate to the concentration. The simultaneous measurement of hydrides and non-hydride elements is made possible by the MSIS Thus, by the determination of the wavelength of the light emitted from the sample we can arrest the element present in the sample [5]. The operation parameter is given in Table -I.

Table 1. Instrument operating parameter for Agilent 5800 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Condition Set Title	Condition Set
Nebulizer Flow (L/Min)	0.7
Aux Flow (L/min)	1
Oxygen Percent (%)	0
RF Power (KW)	1.2
Plasma Flow (L/min)	12
Makeup Flow (L/min)	0
Stabilization Time (s)	15
Read Time (s)	5
Viewing Mode	Axial

B. Micro Wave Digestion

With the help of a microwave digestion system sample preparation was carried out for the analysis of ICP-OES. By MARS 6 Microwave Digestion System sample digestion was processed. MARS 6 Wave gives the flexibility to select the method programming as per the need of digestion of EPLA sample. Preparation by automatically determining the digestion parameters and digestion for the EPLA sample performed [6].

C. Microwave Digestion Process

Weigh out 50 mg of the EPLA sample, then move it into a microwave-safe digesting vessel.

Fill the sample vial with 10 millilitres of a mixed acid solution made up of 6 parts hydrofluoric acid (HF), 3 parts nitric acid (HNO_3), and 1 part hydrochloric acid (HCl).

Fill the sample vessel with 0.5 ml of a 10 mg/ml rhodium (Rh) internal standard solution.

Heating Chamber Setup

Follow the operating procedure described in Table II for setting up the heating chamber of the MARS 6 Microwave Digestion System.

Verify that the sample vessels are firmly shut before inserting them into the heating chamber.

D. Heating Process

Begin the microwave digestion process begins by following Table II's guidelines for the various stages and parameters.

In Stage 1, apply 100% power, ramp the temperature to 100°C over ten minutes, and keep the maximum pressure control at 100 Kpa. For two minutes, maintain this temperature.

In Stage 2, maintained a maximum pressure control of 130 Kpa while ramping the temperature to 180°b over the course of 15 minutes while running at 100% power. For two minutes, maintain this temperature.

In Stage 3, apply 100% power, ramp the temperature to 200°C over the course of 15 minutes, and keep the maximum pressure control at 200 Kpa. For two minutes, maintain this temperature.

Table 2. Operating Condition of MARS 6 Microwave Digitation System

Stage	Ramp Temp.	Power(%)	Max(W)	Press Control	Hold Time	Max Temp.
01	10 min	100	300	0100 Kpa	2min	150^{o}C
02	15min	100	600	0130 Kpa	2min	180^{o}C
03	15min	100	1200	0200 Kpa	2min	200^{o}C

1) Cooling and Transfer: a. Let the vessel cool to room temperature when the heating procedure is finished.
2) To relieve any residual pressure, carefully vent the vessel under a fume hood.
3) Carefully move the digested sample into a Teflon beaker, being cautious to avoid contamination or spillage.
4) Fill each beaker with one millilitre (ml) of HNO_3, or nitric acid, that was collected.

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E. Reconstitution and Evaporation

In the sample beaker, the solution until I evaporated.

Use 250 millilitres of a 1:1 HNO_3 (nitric acid) solution to dissolve the residue**.**

The digested sample can now be used for additional analysis or heavy metal concentration quantification using the suitable analytical method. All safety precautions are taken, such as wearing personal protective equipment and operating in an area with enough ventilation, throughout the microwave digestion process.

III. STANDARD SOLUTION PREPARATION

The standard solution is prepared from the multielement standard solution (ICP Calibration Standard 23 analytes[7] having Item: ICM-103, Lot No.:000664034). The reagent is used to prepare a standard solution sample & blank for the analysis. Distilled deionized water from Millipore (Elix 10) HNO_3 is used. The standard solution of different concentrations is prepared by adding the reagent [8].

A. Calibration by Certified Reference Material

Using a certified material supplied by M/s. Agilent, a multielement standard solution (ICP Calibration Standard 23 analytes, Item: ICM-103, Lot No.: 000664034), the calibration procedure was carried out times. For the calibration, this standard solution was diluted to various parts per million (PPM) levels. The calibration was carried out prior to starting the sample analysis in order to certify the ICP-OES equipment using a certified reference material (CRM). Preparing and measuring various PPM levels of the standard CRM was part of the calibration process. The concentrations of blank (0 PPM), 1 PPM, 2 PPM, 3 PPM, 4 PPM, 5 PPM and 6 PPM were among the calibration points.

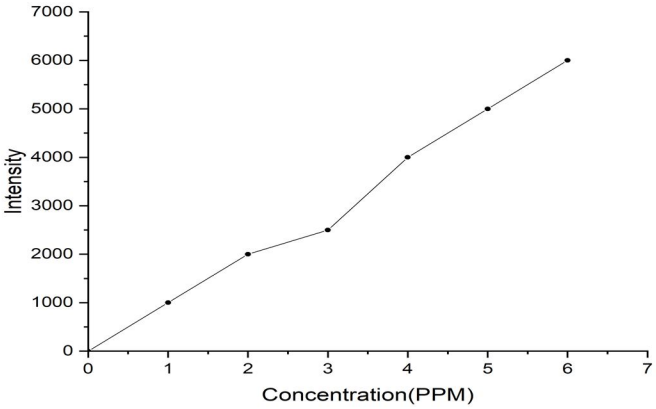

Figure. 1. The intensity verses concentration

Plotting the observed values of the standard CRM at each concentration against the corresponding concentrations was done throughout the calibration process. After the calibration curve was created, a straight-line relationship was anticipated, which signifying a strong correlation between the apparatus and the reference solution. There was confidence in the ICP-OES instrument's precision and dependability because to the straight-line calibration curve.

Following a successful calibration, the calibrated instrument was used to analyse the prepared digested samples. The calibration curve and the observed intensities of the sample solutions were used to calculate the heavy metal contents in the enzyme-embedded poly lactic acid (PLA) samples. The technique made it possible to measure the levels of heavy metals in the PLA samples that had been implanted with enzymes. Following a successful calibration, the calibrated instrument was used to analyse the prepared digested samples. The calibration curve and the observed intensities of the sample solutions were used to calculate the heavy metal contents in the enzyme-embedded polylactic acid (PLA) samples. Through this technique, the amounts of heavy metals in the PLA samples including enzymes were quantified. The ICP-OES instrument was calibrated using approved reference material, which guaranteed the device's accuracy and dependability. The heavy metal concentrations in the digested samples were ascertained using the calibration curve as a guide, which gave important details regarding the heavy metal content of the PLA that was embedded with enzymes[9].

IV. RESULTS AND ANALYSIS

The digested sample are analysed by was Agilent 5800 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The presence of different elements & its concentration was recognised by the plasma ray of ICP.

Table. 3. Wave used for analysis in Agilent 5800 ICP-OES

Element	Wavelength (nm)	Label
Cd	214.439	Cd (214.439 nm)
Co	238.892	Co (238.892 nm)
Cr	267.716	Cr (267.716 nm)
Cu	327.395	Cu (327.395 nm)
Ni	231.604	Ni (231.604 nm)
Pb	220.353	Pb (220.353 nm)
Zn	213.857	Zn (213.857 nm)
Hg	184.887	Hg (184.887 nm)
As	188.980	As (188.980 nm)

V. SAMPLE ANALYSIS

The analysis was carried out by the developed method of ICP-OES[10] for digested sample. The elements & its concentration is given as below.

Table 4.

Element Label(nm)	Units	First run	Second run	Third run	Standard deviation
Cd	PPM	0.03	0.03	0.02	0.006
Co	PPM	2.36	2.53	2.42	0.086
Cr	PPM	1.17	1.35	1.28	0.091
Cu	PPM	0.82	0.95	0.88	0.065
Ni	PPM	0.76	0.86	0.84	0.053
Pb	PPM	1.73	1.89	1.78	0.082
Zn	PPM	0.98	0.86	1.02	0.083
Hg	PPB	0.00	0.02	0.00	0.012
As	PPB	0.02	0.01	0.02	0.006

The measurements show which elements are present in the EPLA samples and at what concentrations [11]. Throughout the three runs, cadmium (Cd) values were constantly around 0.03 ppm, with a standard deviation of 0.006. The values of cobalt (Co) ranged from 2.36 ppm to 2.53 ppm, with a 0.086 standard deviation. The amounts of chromium (Cr) ranged from 1.17 ppm to 1.35 ppm, with a 0.091 standard deviation. The values of copper (Cu) ranged from 0.82 ppm to 0.95 ppm, with a 0.065 standard deviation. The concentrations of nickel (Ni) ranged from 0.76 ppm to 0.86 ppm, with a 0.053 standard deviation. The amounts of lead (Pb) ranged from 1.73 ppm to 1.89 ppm, with a 0.082 standard deviation. The amounts of zinc (Zn) ranged from 0.86 ppm to 1.02 ppm, with a 0.083 standard deviation. Mercury (Hg) readings ranged from 0.00 ppb to 0.02 ppb, with a standard deviation of 0.012. These extremely low concentrations are either close to or below the detection limit. The quantities of arsenic (As) ranged from 0.01 ppb to 0.02 ppb, with a 0.006 standard deviation. The precision and unpredictability of the measurements made from each run are revealed by the standard deviation values. They show how widely distributed the data are around the mean concentrations [12].

These findings provide important details on the relative quantities of different elements in the PLA samples that have been implanted with enzymes[13]. The information can be used to determine the components' possible effects on the environment or hazards, analyse the composition of the samples[14], and direct future studies or applications employing PLA materials with embedded enzymes[15].

VI. CONCLUSION

In conclusion, the purpose of this work was to use inductively coupled plasma optical emission spectrometry (ICP-OES) to measure the amounts of heavy metals in enzyme-embedded polylactic acid (PLA). The lipase that came from Candida rugosa [16]was the main focus. The research results provided significant new information on the possible heavy metal contamination in PLA that has been implanted with enzymes, thanks to a meticulous experimental design and analysis [17]. The outcomes showed that heavy metal concentrations could be accurately and consistently measured using the ICP-OES technique. The quality and safety of PLA-based products [18]should be carefully considered, as evidenced by the detection of heavy metals in enzyme-embedded PLA. The study emphasized how crucial it is to keep an eye out for heavy metal contamination in biodegradable materials, especially those meant for use in food packaging, medicine, and other applications[19]. Moreover, this study advances the fields of polymer chemistry and enzyme immobilization. Through the successful integration of Candida rugosa lipase into PLA, the research broadens our knowledge of enzyme-based materials and their possible uses. This information can be applied to the creation of creative, long-lasting solutions for a range of businesses. This study includes limitations, just like any other scientific investigation. The results of the study, which concentrated on the lipase from Candida rugosa, might not apply directly to other enzymes or microbes[20]. Furthermore, the study only looked at heavy metal analysis; it did not examine any potential effects on the functionality or performance of PLA that has enzymes incorporated in it. In summary, the analysis of lipase from Candida rugosa using ICP-OES to measure the amounts of heavy metals in enzyme-embedded PLA offers important new information on the possible dangers of heavy metal pollution in biodegradable materials. This work lays the groundwork for future research that will hopefully lead to the creation of safer and more sustainable enzyme-based materials in the future.

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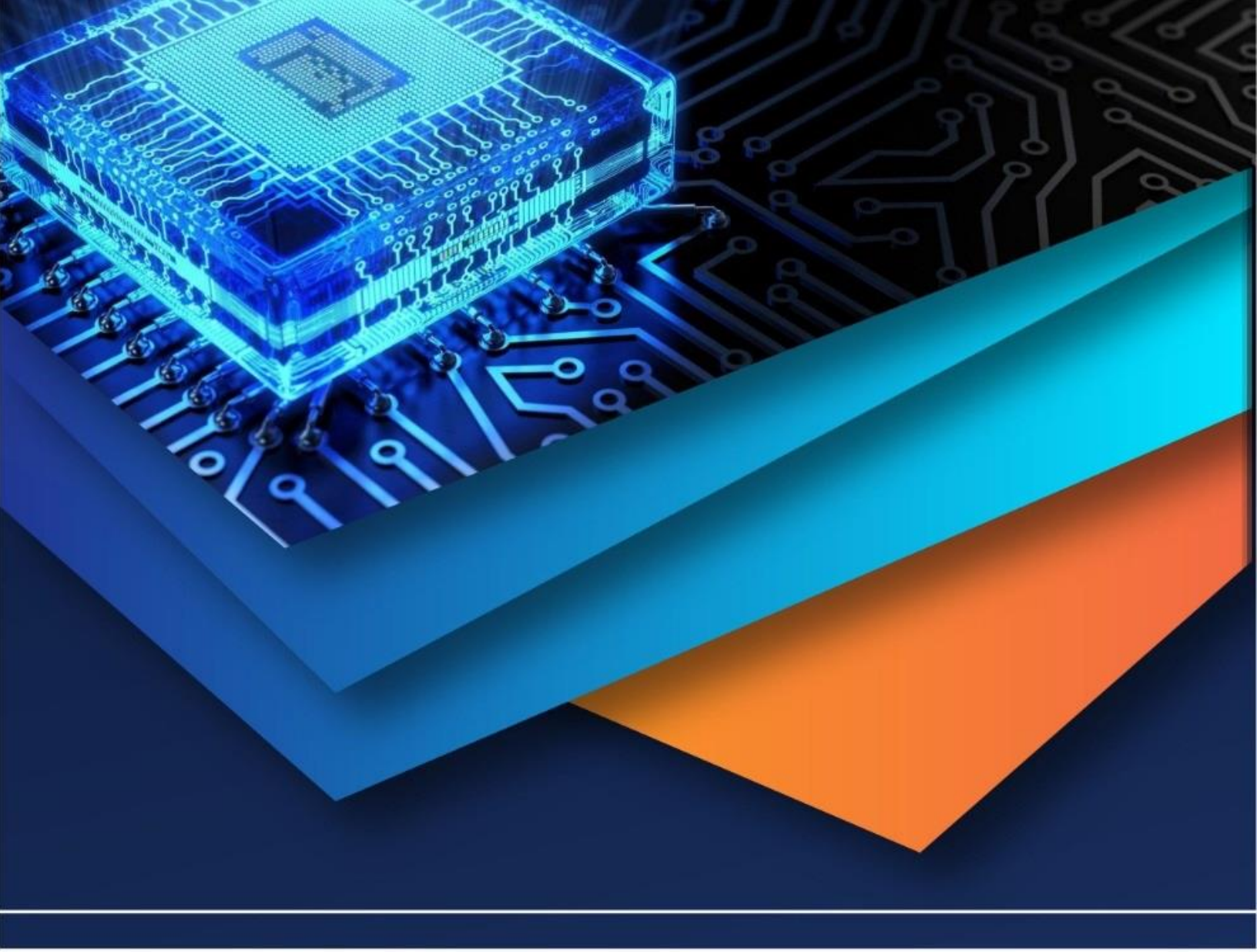

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