

Original Research

Boosting Biocatalytic Redox Cycles: One-Step B–Se Co-doped g-C₃N₄/Eosin B for Solar 1,4-NADH Recycling

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Received: February 18, 2026; Accepted: June 9, 2026

Abstract: The efficient and selective regeneration of nicotinamide adenine dinucleotide (NADH) remains a significant challenge in biocatalysis due to the limited availability of sustainable and metal-free photocatalytic systems. Herein, we report a novel metal-free photocatalytic strategy that integrates boron- and selenium-doped graphitic carbon nitride (B–Se–g-C₃N₄) with Eosin B (EB) as a synergistic photosensitizer for highly selective NADH regeneration. The novelty lies in the rational combination of heteroatom co-doping (B and Se) with an organic dye to enhance charge separation and selectively promote 1,4-NADH under irradiation. The developed system exhibits a high photo-regeneration efficiency of 95.83% with excellent selectivity toward the biologically active 1,4-NADH isomer. This approach not only expands the scope of dye-assisted, metal-free photocatalysis but also establishes a sustainable and efficient platform for cofactor regeneration, advancing green chemistry and biocatalytic applications.

Keywords: visible light, EB, B–Se–g-C₃N₄, EB–B–Se–g-C₃N₄, biomaterial (1,4-NADH), amide bond

1. Introduction

Photosynthesis is a process by which photoautotrophs store energy in chemical bonds. It is a most significant biological process on Earth that fixes atmospheric carbon dioxide (CO₂), thereby serving as a natural energy source for living organisms [1,2]. In this context, an artificial photocatalytic pathway was constructed to satisfy the extraordinary challenges of the chemical society. In biochemical society, the cofactor cycling to reduced nicotinamide adenine dinucleotide (NADH) has a significant role in assisting redox reactions involving oxidoreductase enzymes [3–5]. The in-situ photo-regeneration of NADH is crucial due to the optimal quantity of NADH-dependent enzymes required in the biocatalytic procedure [6,7]. Previous studies have reported that the three NADH-dependent enzymes were used to fix atmospheric CO₂ into respective useful fuels, i.e. hydrogenases like formate, formaldehyde, and alcohol [8,9]. While enzymatic conversion of CO₂ into biofuel is environmentally beneficial, the high cost of NADH makes the process unrealistic, highlighting the need for an efficient regeneration system [10].

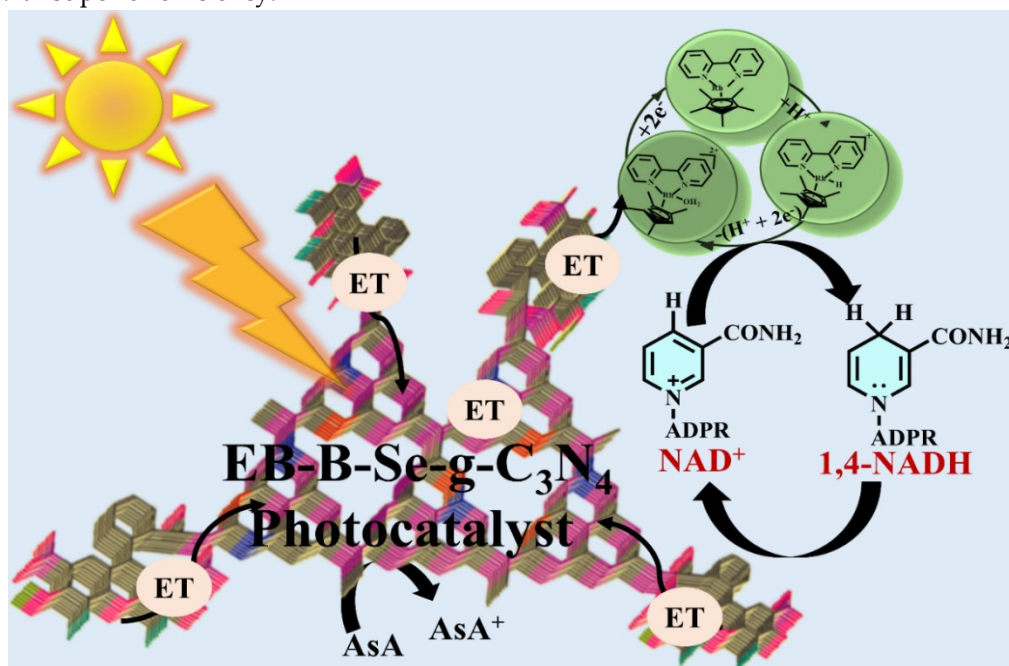
Visible-light-driven 1,4-NADH regeneration has emerged as a green and energy-efficient strategy for

sustaining biocatalytic redox cycles. In artificial photosynthesis, coupling photocatalysts with enzymatic

systems enables efficient solar-to-chemical energy conversion, where continuous NADH regeneration is crucial for driving downstream transformations [11,12]. Among the range of photocatalysts, graphitic carbon nitride (g-C₃N₄) has gained significant interest due to its acceptable band structure, high stability, and visible-light activity. However, its realistic application is limited by poor light absorption, rapid carrier recombination, and a limited number of active sites. To overcome these limitations, heterostructure engineering and doping strategies have been widely used to modulate its electronic properties and enhance charge separation. In particular, constructing heterojunctions enhances interfacial electron transfer, while introducing transition heteroatom–nitrogen (Se/B–N) sites, creates active centres and facilitates charge delocalization [13]. Additionally, defect engineering and heteroatom incorporation further improve the visible-light absorption and charge migration efficiency.

Efficient NADH regeneration is essential for maintaining stable NAD⁺/NADH cycling in enzymatic reactions. Earlier studies, including those by Galarneau and co-workers, demonstrated that integrating enzymatic regeneration systems significantly enhances product yields in multi-enzyme processes [14,15]. NADH can be regenerated via chemical [16] photochemical [17–19] enzymatic [20–22] or electrochemical routes [23,24]. Among these methods, photocatalysis has gained attention due to its compatibility with solar energy [25]. In this regard, light-harvesting components such as Eosin B and g-C₃N₄ have been widely explored for solar-driven production of NADH and NADPH [26,27]. Although metal-based photocatalysts exhibit high activity, their limited response to ultraviolet light restricts practical applications [28]. However, semiconductor-based systems with engineered band structures have been developed to enhance visible-light utilization [29]. Beyond cofactor regeneration, photocatalysis has also shown strong potential in green organic transformations, including C(sp³)–O bond activation, due to its efficiency and environmentally benign nature [30].

Despite these advances, achieving highly selective and efficient 1,4-NADH regeneration remains a significant challenge due to limited control over charge transfer pathways and product selectivity. In the present study, we introduce a novel strategy built on the synergistic integration of boron and selenium co-doping in g-C₃N₄ with Eosin B as an organic photosensitizer. The novelty of this work lies in (i) the rational design of a dual-modified photocatalytic system combining heteroatom co-doping and dye sensitization, (ii) the enhancement of visible-light absorption and charge carrier separation, and (iii) the achievement of highly selective 1,4-NADH regeneration with superior efficiency.



Scheme 1. An artificial photosynthetic pathway showing photo-regeneration of 1,4-NADH.

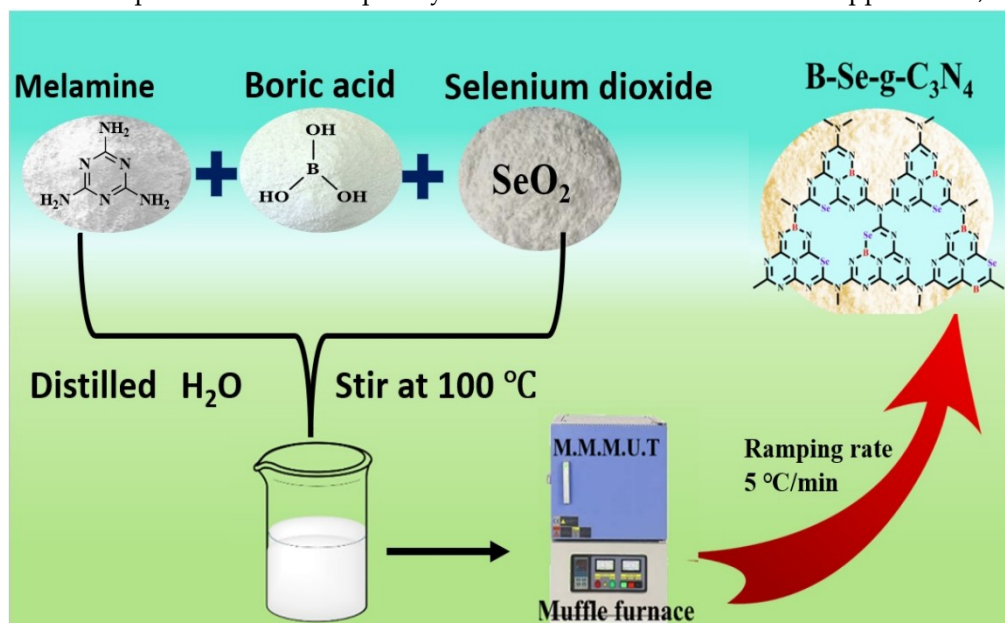
2. Materials and methods

The materials were purchased from TCI India, BLD Pharma, and Sigma Aldrich. Boric acid (purity= 99.5%), melamine (purity= 97.5%), selenium dioxide (purity= 99.5%), Eosin-B/EB (purity= 90%), 1,2-Dichlorobenzene (ODCB) (purity= 99%), Hexafluorophosphate Azabenzotriazole tetramethyluranium (HATU) (purity= 98%), ascorbic acid (AsA) (purity= 99.7%), sodium/potassium mono/dibasic salts (extra pure) and nicotinamide adenine dinucleotide (NAD⁺) co-factor (purity= 98%), N, N' dimethylformamide (DMF) (purity= 99.5%). The phosphate buffer solution (PBS) was prepared via sodium/potassium mono/dibasic salts. PentamethylcyclopentadienylRhodium (III) dichloride dimer (purity=96%) was utilized for preparing rhodium complex (Rh-C). Deionised water was used throughout the experiment.

A Shimadzu UV-1900 and 800 IR spectrophotometer was used to obtain absorption and infrared spectra. A CH1608E, 220 V instrument was used for analyzing electrochemical characterizations. However, PXRD and morphological analysis were performed via D8 Advance Eco diffractometer and JEOL SEM IT 300, respectively.

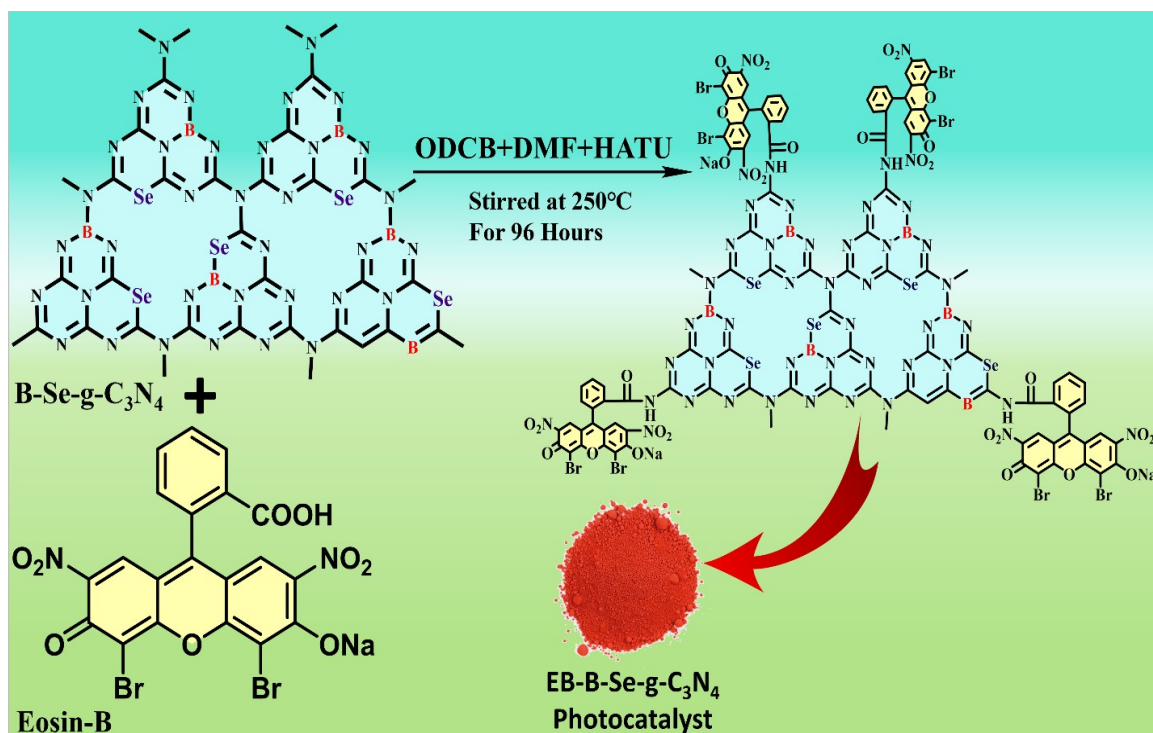
2.1. Synthesis of B-Se-g-C₃N₄

Synthesis of B-Se-g-C₃N₄ followed the standard method [31]. 500 mg of boric acid, 500 mg of selenium dioxide, and 1g of melamine were allowed to be mixed in distilled water. The dissolved mixture was subsequently evaporated at 100°C using a magnetic stirrer. The obtained white precipitate was dried and kept in the ceramic-based crucible, which was further calcined at 500°C with a heating rate of 5 °C/min for 4 hrs. The obtained yellow-coloured crude product was subsequently washed for characteriations and applications, Scheme 2.

**Scheme 2.** Schematic representation of grafting B-Se-g-C₃N₄.

2.2. Synthesis of EB-B-Se-g-C₃N₄ photocatalyst

The EB-B-Se-g-C₃N₄ photocatalyst was demonstrated through a polycondensation mechanism [32]. 300 mg of B-Se-g-C₃N₄, 500 mg of EB, 10 mL ODCB, 5mL DMF, and a catalytic amount of HATU were taken in a double-necked round-bottom flask. The flask was then refluxed for 96 hours at 250 °C. After 96 hours, the red-coloured reaction mixture was filtered to obtain red-coloured precipitate, which was further washed with distilled water, Scheme 3 [33].



Scheme 3. Schematic representation of the synthesis of EB-B-Se-g-C₃N₄ photocatalyst.

3. Results & discussion

3.1. Photocatalytic regeneration of 1,4-NADH

The photo-regeneration of 1,4-NADH was performed via the reported literature. [9, 37] In a quartz glass vial configured with, a magnetic stirrer was placed below a blue LED light. The glass vial was composed of AsA, Rh-C (prepared using the standard method) [34], NAD^+ co-factor, EB-B-Se-g- C_3N_4 photocatalyst dispersed in a PBS at pH-7. The mixture was purged with N_2 to maintain inertness. The photo-regeneration of 1,4-NADH was tracked through a spectrophotometer at regular intervals. The resultant spectra show an increment in absorbance at 340 nm at every successive interval, which implies 1,4-NADH regeneration with an excellent yield of 95.83%, implying better ability to give photocatalytic activity than their precursors [85.33 %] (Figure 1a and b) [31].

Moreover, irradiation ($\lambda \geq 420$ nm), the photocatalyst generates charge carriers, with electrons promoted to the conduction band (-3.335 V) and holes left in the valence band (-5.995 V). The photogenerated electrons are sequentially transferred from the conduction band to the Rh catalytic center (-3.96 V) and subsequently to NAD^+ (-4.20 V), facilitating its reduction to NADH. Concurrently, the valence band holes are effectively scavenged by ascorbic acid (AsA), which undergoes oxidation, thereby suppressing charge recombination and maintaining continuous electron flow. This cascade electron transfer pathway promotes efficient charge separation and enables selective photocatalytic regeneration of NADH, Scheme 4 [35].

Additionally, the reusability of the EB-B-Se-g- C_3N_4 photocatalyst was evaluated via five consecutive catalytic cycles. The photocatalyst retained its activity with negligible loss in NADH regeneration efficiency over multiple cycles, confirming its excellent structural stability and recyclability under visible-light irradiation (Figure 1c).

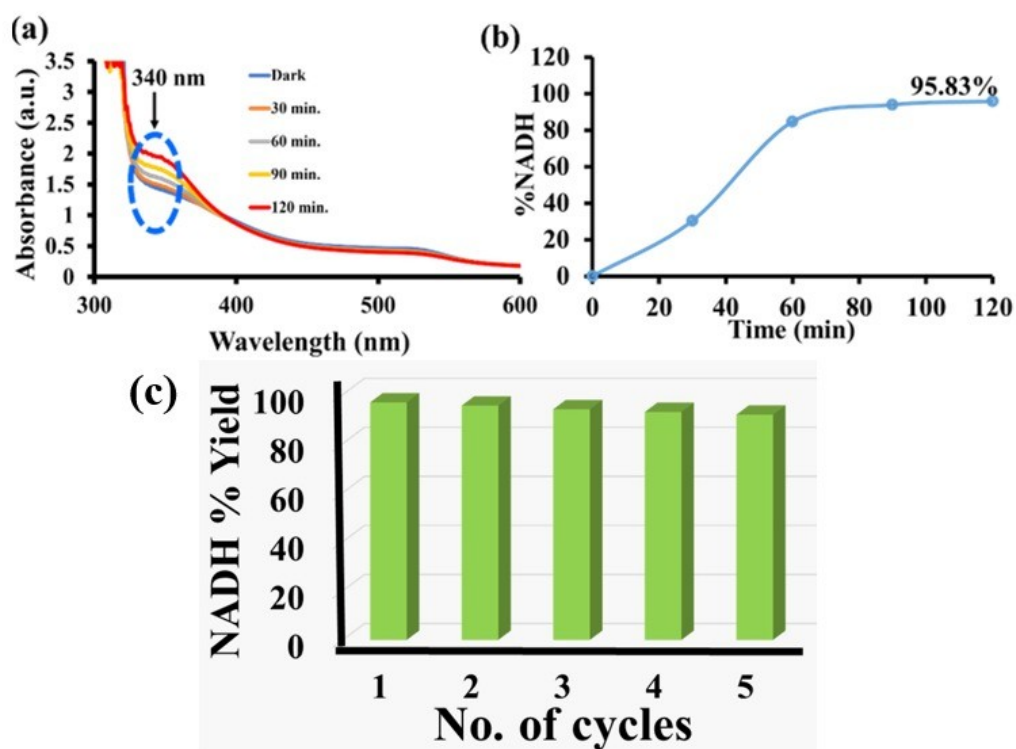
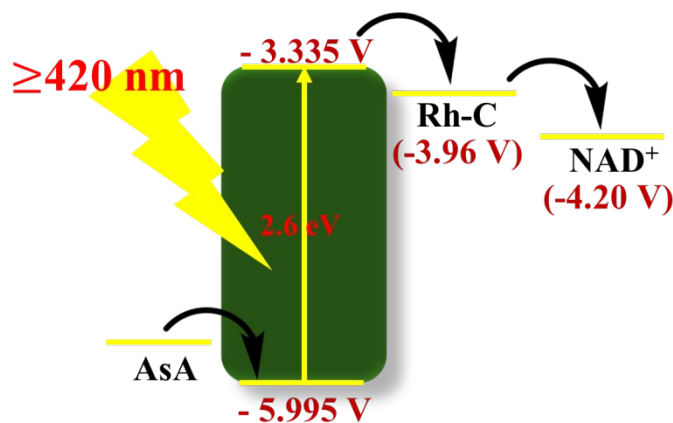


Figure 1. (a) UV-visible absorption spectra of the performed 1,4-NADH regeneration, (b) 1,4-NADH regeneration yield, and (c) Reusability result of 1,4-NADH from the EB-B-Se-g- C_3N_4 photocatalyst.



Scheme 4. Electron transfer mechanism for 1,4-NADH regeneration.

4. Discussion

4.1. UV-visible spectra & FTIR: Unlocking the secrets of molecular signatures

The electronic transitions, along with optical ability, have been studied using UV-visible spectra, which were consistent with previous literature [15,36]. The Figure 2(a) shows the absorption spectra of EB, B-Se-g-C₃N₄, and EB-B-Se-g-C₃N₄ photocatalyst. EB shows a narrower absorption band at 500-560 nm, while the B-Se-g-C₃N₄ shows no absorption in the interest area. However, the EB-B-Se-g-C₃N₄ photocatalyst shows a wider range of absorption spectra at 500-600 nm, implying the bathochromic shift (red shift). The optical band gap of EB-B-Se-g-C₃N₄ photocatalyst came to be ~2.29 eV by applying the Scherrer equation. In addition, the diffuse reflectance spectroscopy (DRS) also supports the wider absorption spectra of the EB-B-Se-g-C₃N₄ photocatalyst.

The Tauc plot was also calculated through DRS calculations, providing the band gap of EB-B-Se-g-C₃N₄ photocatalyst, which comes to be 2.5 eV, lying in the semiconductor region, indicating the semiconductor nature of EB-B-Se-g-C₃N₄ photocatalyst, as shown in Figure 2(b) [37]. Fourier transform infrared spectroscopy (FTIR) was used to demonstrate the group functionality and vibrational frequency through stretching vibrations. Figure 2(c) shows the EB FTIR spectra with characteristic peaks at 1561 cm⁻¹, 1750 cm⁻¹, and 3000 cm⁻¹, which correspond to the stretching vibrations of phenylic group, C-O stretching of carbonyl group, and C-H stretching, respectively [38]. The FTIR spectra of g-C₃N₄ and B-Se-g-C₃N₄ depict the characteristic peaks at 1408 cm⁻¹, 1519 cm⁻¹, and 2990 cm⁻¹, corresponding to C-N, C=C, and N-H stretching vibrations, respectively [34,39]. After coupling with EB, the composite EB-B-Se-g-C₃N₄ photocatalyst shows the shifting of N-H and C=O at 3300 cm⁻¹ and 1735 cm⁻¹ respectively, which implies the amide bond formation between EB and B-Se-g-C₃N₄ [40].

4.2. Powdered X-ray diffraction spectroscopy: X-ray vision into crystal structures

The powder X-ray diffraction (PXRD) pattern was used to analyse crystallinity and physical changes in the moieties. The PXRD pattern of B-Se-g-C₃N₄ shows characteristic peaks at 27.13° and 13.41° corresponding to (002) and (100) planes, which are consistent with the reported literature and g-C₃N₄ core.^{31,41} After coupling with EB, the resulting composite EB-B-Se-g-C₃N₄ photocatalyst shows the shifting in (002) and (100) planes, which implies the preservation of the B-Se-g-C₃N₄ moiety in the composite, Figure 2(d and e).

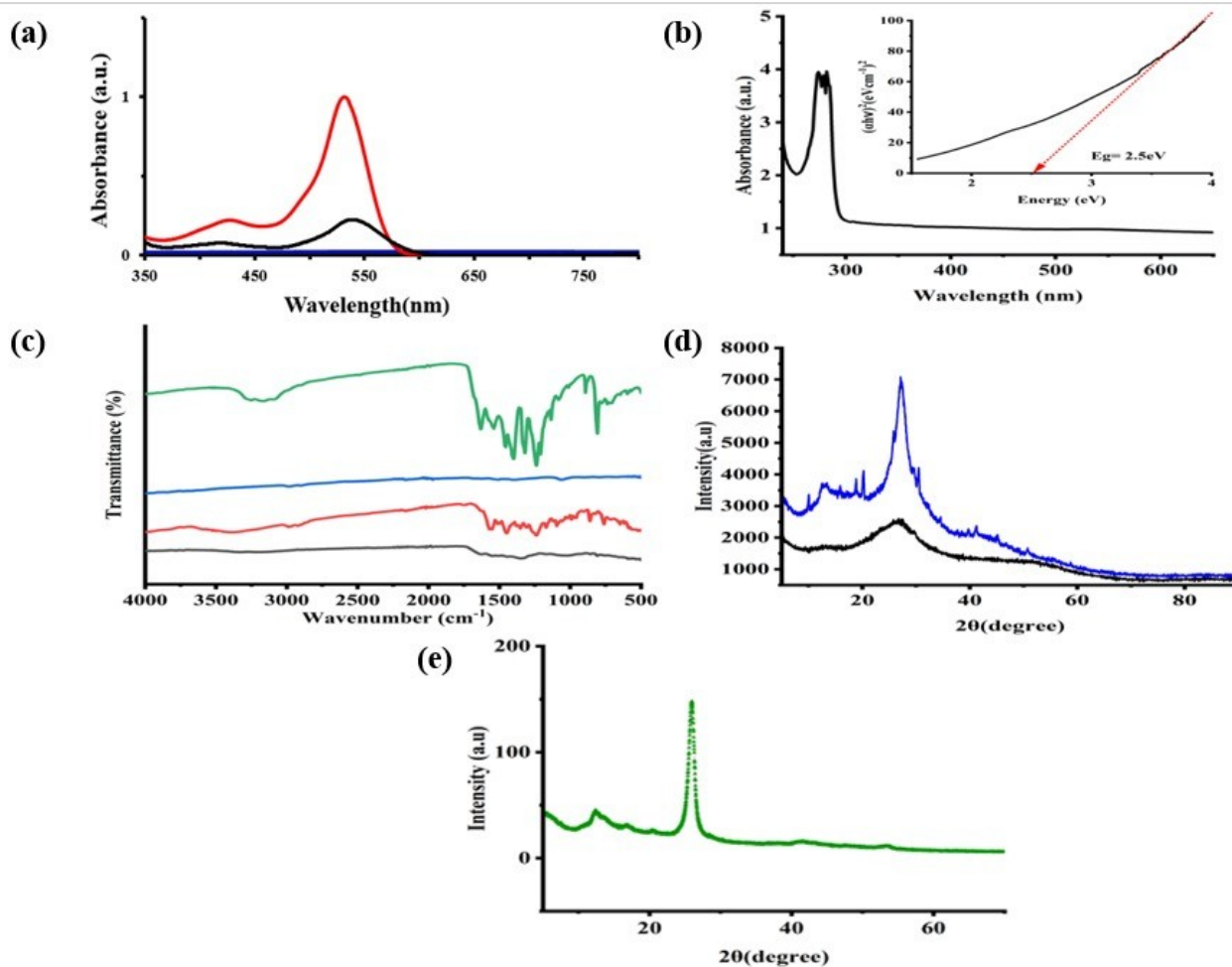


Figure 2. (a) UV-visible absorption spectra of EB (red line), EB-B-Se-g-C₃N₄ photocatalyst (black line) and B-Se-g-C₃N₄ (blue line), (b) Tauc plot of EB-B-Se-g-C₃N₄ photocatalyst to determine the optical band gap, (c) FTIR spectra of g-C₃N₄ (green), EB (red line), EB-B-Se-g-C₃N₄ photocatalyst (black line) and B-Se-g-C₃N₄ (blue line), (d) X-ray diffraction pattern of B-Se-g-C₃N₄ (blue line), EB-B-Se-g-C₃N₄ photocatalyst (black line), and (e) X-ray diffraction pattern of g-C₃N₄ (green).

4.3. Electrochemical analysis: Unveiling redox secrets

The electrochemical analysis was demonstrated through a three-electrode system dipped in a 0.01 M H₂SO₄ solution using calomel as a reference electrode, glassy carbon as a working electrode, and platinum wire as a counter electrode. The cyclic voltammetry was used to analyse the redox potential of moieties as shown in Figure 3(a). The reduction and oxidation potentials of EB-B-Se-g-C₃N₄ photocatalyst are -1.165 and 1.495 V, respectively. The energy levels, HOMO/LUMO, were calculated via these redox potentials, i.e. the HOMO and LUMO levels of EB-B-Se-g-C₃N₄ photocatalyst are -5.995 V and -3.335 V, respectively. The energy band gap observed through HOMO/LUMO energy levels is 2.6 for the EB-B-Se-g-C₃N₄ photocatalyst; this energy band was also supported by the Tauc plot calculated by DRS calculations [37]. Whereas for EB and B-Se-g-C₃N₄, it is 2.8 eV and 2.7 eV, respectively [35]. Electrochemical impedance spectroscopy (EIS) was used to analyse the separation and migration of charge carriers. The Nyquist plot was used to determine the arc radius of the moieties, Figure 3(b). The lower semicircular arc radius, i.e. EB-B-Se-g-C₃N₄ photocatalyst, implies the lower resistance of the moiety, which leads to better conductivity as reported literatures [42]. However, the Tafel plot also supports the charge transfer kinetics, Figure 3(c). A lower value of slope indicates lower potential required for charge separation, implying better charge migration, which results in better photocatalytic response [43]. Chronopotentiometry

is an electrochemical technique used for analysing the current-carrying capacity of the moieties. The subsequent results are demonstrated in Figure 3(d). The results imply the photoresponse across multiple on/off cycles with respect to visible light. The better photo-response of the EB-B-Se-g-C₃N₄ photocatalyst implies the better charge transfer and separation capability of the moiety, which was harmonious with previous studies [44].

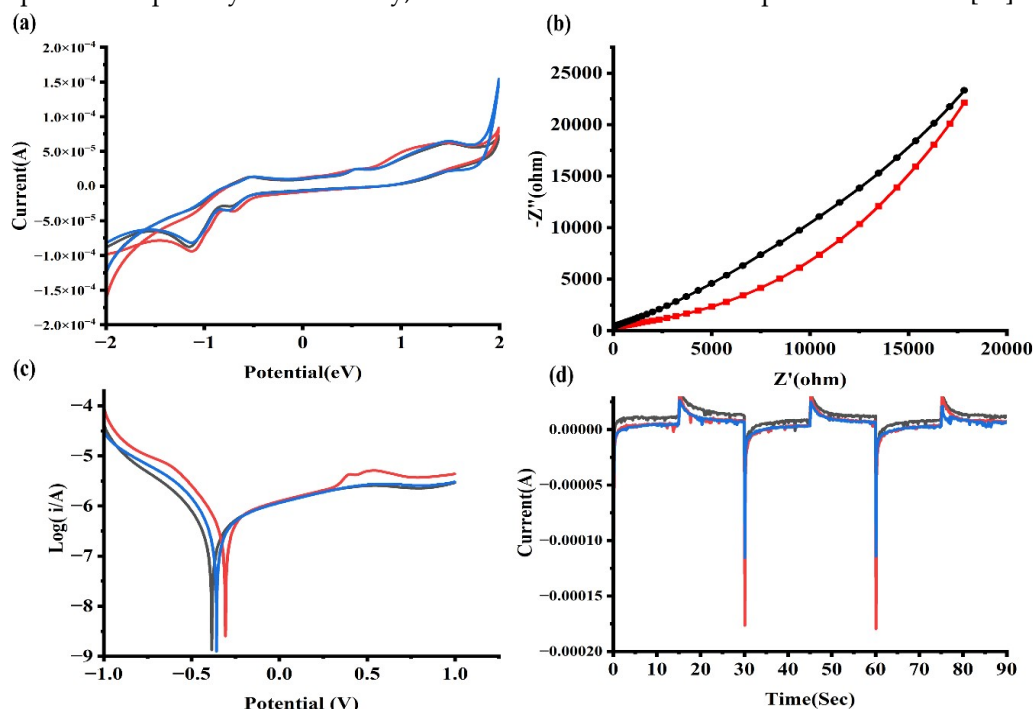


Figure 3. (a) Cyclic voltammetry of EB (red line), B-Se-g-C₃N₄ (blue line) and EB-B-Se-g-C₃N₄ photocatalyst (black line), (b) EIS of EB (red line), EB-B-Se-g-C₃N₄ (black line), (c) Tafel plot of EB (red line), B-Se-g-C₃N₄ (blue line) and EB-B-Se-g-C₃N₄ photocatalyst (black line), and (d) CP of EB (red line), B-Se-g-C₃N₄ (blue line) and EB-B-Se-g-C₃N₄ photocatalyst (black line).

4.4. Scanning electron microscopy (SEM) & Energy dispersive spectroscopy (EDS)

To explore the morphological behaviour of the moieties, scanning electron microscopy (SEM) was used. The morphology of the B-Se-g-C₃N₄ was observed to be cloth-like, while the morphology of the EB-B-Se-g-C₃N₄ photocatalyst was observed as a white, rock-like material. This change in morphology implies the EB-B-Se-g-C₃N₄ photocatalyst formation. Additionally, the occurrence of B, C, N, O, Na, Se and Br also signifies the coupling of EB and B-Se-g-C₃N₄, which was confirmed by energy dispersive elemental mapping and x-ray spectroscopy, Figure 4 [36,45].

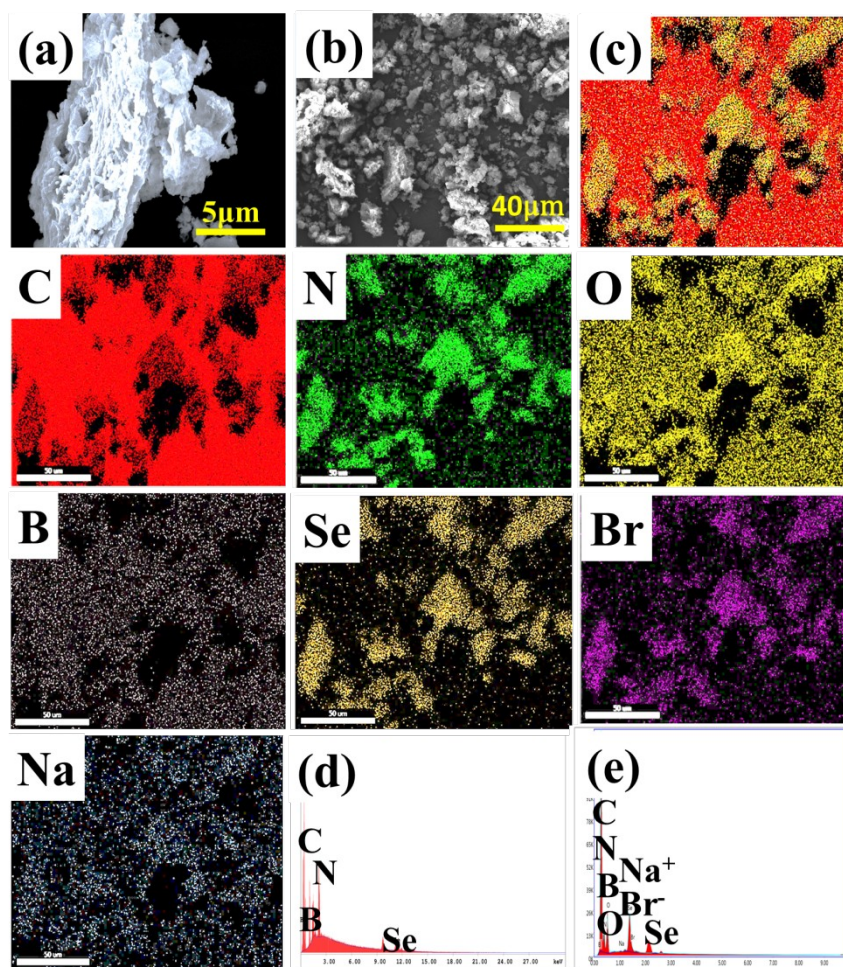


Figure 4. SEM micrographs of (a) EB-B-Se-g-C₃N₄ photocatalyst and (b) B-Se-g-C₃N₄, along with overlapped image of EB-B-Se-g-C₃N₄ photocatalyst showing carbon (red), nitrogen (green), oxygen (yellow), boron (light pink), selenium (dark yellow), bromine (pink), sodium (blue), and EDS spectra of (d) B-Se-g-C₃N₄ and (e) EB-B-Se-g-C₃N₄ photocatalyst.

5. Conclusions

In summary, we successfully synthesized an EB-B-Se-g-C₃N₄ photocatalyst via polycondensation, leading to the formation of amide bonds. The resulting EB-B-Se-g-C₃N₄ photocatalyst exhibits remarkable visible-light absorption ($\lambda > 420$ nm), enabling superior photocatalytic performance. Notably, it demonstrates an exceptional efficiency in the photocatalytic response up to 95.83%. Additionally, the EB-B-Se-g-C₃N₄ photocatalyst possesses key structural and electronic properties, contributing to its outstanding activity and stability. These findings highlight the potential of the EB-B-Se-g-C₃N₄ photocatalyst as a notably efficient photocatalyst for advanced photocatalytic regeneration under irradiation.

Availability of Data and Materials: The data supporting the findings of this study can be obtained upon request from the corresponding author. They are not publicly accessible due to privacy or ethical constraints.

Author Contributions: Surya Pratap Singh drafted the original manuscript and developed the methodology. Rajesh K. Yadav supervised the project and validated the findings. Shaifali Mishra, Rehana Shahin, Kanchan Sharma, Anurag Rai, and Anushka Pandey performed formal analysis. Surendra K. Jaiswal, Anupma Yadav, Rahul Maddeshiya, and Pragya Singh carried out data curation. Geeta Srivastava conducted data analysis. Vinay K. Mishra edited the manuscript. D. K. Dwivedi reviewed the manuscript and contributed to validation. All authors

contributed to editorial changes in the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agree to be accountable for all aspects of the work.

Acknowledgment: We sincerely thank Madan Mohan Malaviya University of Technology, Gorakhpur, for the research work.

Funding: This research received no external funding.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of AI and AI-assisted Technologies in the Writing Process: No AI or AI-assisted technologies were used in the preparation of this manuscript. The work has been verified through plagiarism screening, and the AI similarity index is 0% as confirmed by Turnitin.

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