Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 6 Issue 9 September 2022

Research Article

Isolation and Characterization and Cell-line Toxicity Study of Novel Biopolymer from Naturally Occurring Seeds

Sushant Kumar[1]*, Swarnima Pandey[2] and Anita Singh[3]

[1]*Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India*

[2]*Department of Pharmacy, Hygia Group of Institutions, UP, India*

[3]*Research Associate, Dr. SK Research and Development laboratory, Dewa, UP, India*

***Corresponding Author:** Sushant Kumar, Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India.

DOI: 10.31080/ASPS.2022.06.0896

Received: July 28, 2022

Published: August 22, 2022

Abstract

The aim of this research was to isolate and characterize the novel biopolymer from *Phaseolus vulgaris* seeds. The biopolymer was isolated by simplified and economical process and analysed for different physico-chemical and spectral properties. The isolated biopolymer appears as free flowing powder. The isolation procedures were optimized by repeating it for six times and thus were found to be economical and reproducible in nature. The different functional groups like presence of hydroxyl, alkanes and alkenes, carboxylic acid which confirms its polymeric nature. The other groups like aromatic ring at, ketone were found to be present in the FTIR spectra, NMR and mass spectra. The study finds that the isolated biopolymer from Phaseolus vulgaris seeds contains unusual inherent stabilizer and cum retardant capabilities that can be exploited to develop a lamotrigine-loaded bionanosuspension for long-term administration of drug candidate.

Keywords: Biopolymer; Biomaterial; SEM; Mass Spectra; FTIR Spectra; Economical

Introduction

The term "biopolymer" has piqued researchers' interest as a potential new biomaterial for the creation and design of tailored medication delivery systems [1]. Nowadays, the disadvantages of synthetic and semi-synthetic polymers are a difficult problem to solve when creating drug delivery systems. In the development of tailored medication delivery systems, synthetic and semi synthetic polymers are the preferred choice. However, these polymers have a number of adverse effects that can lead to patient incompatibility. So the biopolymers isolated from different natural sources like flower, seeds, barks and rhizomes have drawn the attention of researchers in designing of novel drug delivery systems [2].

It is possible to utilize the isolated biopolymer, which has novelistic polymeric features, as an intelligent biomaterial for the creation of drug delivery systems. These biopolymers are biodegradable and biocompatible in nature because they are derived from natural sources [3]. Other unique features such as excellent biodegradability, mucoadhesivity, filmability, retardibility, and release rate control properties have been demonstrated in studies [4]. These isolated biopolymers may be used to construct sustained, controlled, and prolonged release drug delivery systems because they have superior release rate regulating properties [5-8].

The isolated biopolymers was characterized for different physic-chemical properties, spectrophotometric characteristics [9-12].

Methods

Materials

The hybrid Phaseolus vulgaris seeds were purchased from a market in Lucknow, Uttar Pradesh. The rest of the solvents and compounds were analytical reagent-grade.

Isolation of biopolymer

Weighing 200 grams of seed and soaking it overnight in double distilled water The swelling seeds were removed from their outer coating. The exposed seeds were mashed into a paste in a grinder. During the grinding process, a small amount of distilled water can be added if necessary. The muslin cloth was used to filter the paste. The collected filtrate was centrifuged for 10 minutes at 5000rpm. The supernatant was obtained after centrifugation. To eliminate any residue, centrifugation was used. Then, in 1:1, 1:2, and 1:3 ratios, half of the supernatant was treated with acetone. Half of the supernatant was treated with methanol in the same proportions as the acetone. After that, they were placed in the refrigerator for the night. The mixtures were centrifuged for thirty minutes at 5000 rpm. After discarding the supernatant liquid, the biomaterial was recovered as sediment and air dried. For 48 hours, the product was kept in desiccators. The resultant biomaterial was sieved at number 200 before being stored for future use. This technique was optimized by performing it six times and calculating the percentage yield [13].

Isolated biopolymer characterization

The different biomaterials isolated from different natural sources which are edible in nature were characterized for their physico-chemical properties. The obtained isolated biopolymers were characterized for different parameters. The isolated biopolymers appear as free flowing powder. The isolation procedures were optimized by repeating it for six times and thus were found to be economical and reproducible in nature.

The isolated biopolymer's physicochemical properties were determined for following organoleptic properties tests like color, odor, taste. The solubility and chemical tests were also performed for the biomaterial.

Solubility

The solubility study of the isolated biopolymers were performed in different solvents like water, acetone, methanol, ethyl acetate, 10%w/v hydrochloric acid solution and diethyl ether and reported. The excess of the isolated biomaterial was added in 10ml of the specific solvent system in beaker gradually. The solution was dispersed well and kept for 24 hours on orbital shaker for achieving equilibrium state. Then the solution was centrifuged at 400rpm in centrifuge for 10 minutes and then filtered to get the clear solution. Then the filtrate was allowed for measurement in UV spectrophotometer machine (Mapada). The procedure was performed in triplicate for each isolated biopolymer [14].

Chemical testing for glucose, starch, and protein content were also carried out

Tests for carbohydrate

1ml of freshly prepared biomaterial solution (5% w/v prepared biomaterial solution in double distilled water) was taken in test tube. Add two drops of Molisch reagent. Add 1-2 ml of conc. Sulfuric acid in the test tube and purple color was observed at the at the interface of two layers formed. Test was performed and reported (Kokate, 1991).

Test for protein

For testing the presence of protein in the isolated biomaterial was treated with 0.1% solution of ninhydrin reagent and 10% tannic acid solution. The presence of blue color and yellow color precipitate indicates the presence of protein. Test was performed and reported [15].

Biuret test was performed for the confirmation of proteins. 2 ml of biomaterial was taken in test tube (5% biopolymer solution in distilled water), add 1 ml of sodium hydroxide solution with addition of copper sulphate solution drops. The mixture was kept aside for five minutes and observe any color changes. The appearance of violet color confirms the presence of proteins. Test was performed and reported [16].

Characterization via spectrophotometry

SEM examination, DSC testing, IR spectroscopy, mass spectroscopy, and NMR spectroscopy were all used to characterize the isolated biopolymer.

SEM examination

A scanning electron microscope was used to examine the isolated biopolymer. The external surface and internal structure were investigated via SEM examination. A modest amount of biopolymer was applied to aluminium studs before being coated with gold using a coater sputter under vacuum. Scanning electron micrographs of the biopolymer under observation were taken.

FTIR

The KBr discs were prepared for FTIR spectroscopy. 100mg of dried and desiccated solid KBr was combined with 1mg of isolated biopolymer. To remove any moisture, the material was crushed in a mortar and pestle and placed under an IR lamp. Under 10 tonnes of pressure, the mixture was transformed into a disc. In the line of IR radiation, the prepared disc was placed in a disc holder. The spectrum was captured in the 4000-200 cm^{-1} range [17,18].

DSC analysis (Differential scanning calorimetry)

DSC testing is a thermal analysis technique that determines how much heat flows into or out of a sample as a function of temperature. The sample was taken here and exposed to a temperature-controlled programme. The temperature of the glass transition has been determined. The temperature range for the heat flow was 50-300°C. The thermogram was taken using the DSC method [19,20].

Nuclear magnetic resonance spectraoscopy

The isolated biopolymer was subjected to NMR spectroscopy for spectrum analysis. A specific solvent, such as CDCl3, was used to dissolve the material. At a high rate of flow, the mixture was injected into the instrument. The flow was stopped using the valve switch. The measurement has been completed. The spectrum was processed and examined on an automated computer after the measurement was completed [19].

Results and Discussion

Biopolymer isolation

The process of isolation was optimized by repeating the determinations six times and means standard deviation (±) SD was calculated. With a yield of 11 ± 0.2%, the Phaseolus vulgaris biopolymer was found to be brownish-cream in color.

Isolated biopolymer characterization

The isolated biopolymer seemed to be creamy white. The biopolymer was discovered to be odorless and have a distinct flavor. It was discovered that it was just slightly soluble in water. Carbohydrate and protein tests were also positive. It shows the characteristics of an isolated biopolymer of Phaseolus vulgaris with observation.

Color

The biopolymer powder was found to be creamy white colour in appearance.

Odor

The biopolymer powder was found to have Characteristic odor.

Taste

The biopolymer powder was found to have Characteristic taste.

Solubility

The solubility study was performed in triplicate (n=3). The biopolymer was found to be Slightly soluble in water and soluble in acetone.

Chemical tests

- **Test for carbohydrate:** Molisch test of isolated biopolymers showed the presence of violet color ring at the interface thus confirmed the presence of carbohydrate.
- **Test for proteins:** The appearance of blue and yellow color precipitate after treatment with Ninhydrin reagent confirmed the presence of proteins. The Biuret test also confirmed the presence of proteins as all biopolymers showed the observation of violet color.

SEM analysis

Figure 1 shows a SEM image of an isolated biopolymer at 30000X and 70X magnification of biopolymer. Flaky structured amorphous particles were visible in SEM images. The biopolymer's surface was smooth and amorphous as the surface of standard polymer.

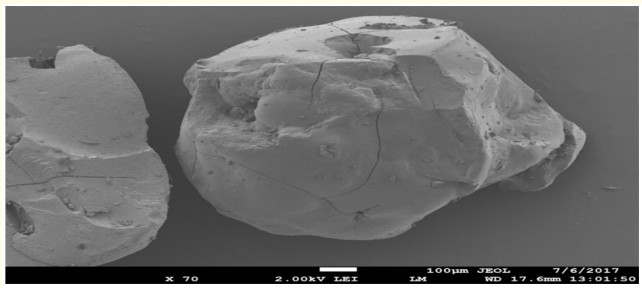

Figure 1: SEM of isolated biopolymer of biopolymer at 30000X.

Infrared spectroscopy (IR spectroscopy)

The presence of diverse functional groups such as hydroxyl (3402 cm^{-1}), alkenes (670 cm^{-1}), and carboxylic acid (1384.99 cm^{-1}) in the I.R. spectra of biopolymer verifies its polymeric features (Figure 2). The IR spectra revealed the presence of additional

groups such as aromatic ring at 1403.7 cm^{-1} due to c-c stretching vibrations-h bending at 771.84 cm^{-1}, amide at 1610.42 cm^{-1}, ester at 1384 cm^{-1}, and tertiary alcohol at 1082 cm^{-1}. The presence of these functional groups, like other conventional polymers, is responsible for drug release in retardant manner [18].

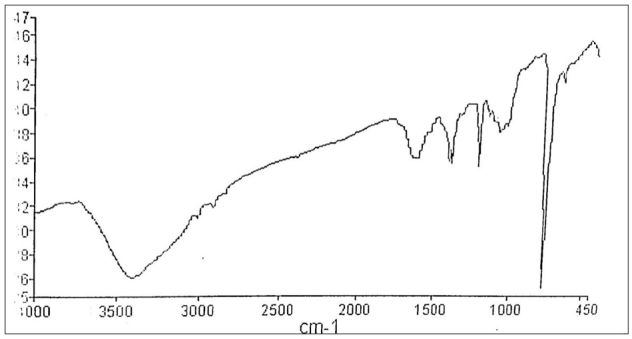

Figure 2: FTIR Spectra of biomaterial.

DSC

The biopolymer thermogram revealed an endothermic peak at 73.006 Celsius on the DSC thermogram (Figure 3). 94.3136 mj/mg was discovered in the area. The amorphous nature of the biopolymer is indicated by its broad peak.

Figure 3: DSC of biopolymer from *Phaseolus vulgaris.*

NMR spectroscopy

The appearance of distinct peaks at 0.011, 1.559, and 7.262 ppm (Figure 4) confirms the presence of methyl group, hydroxyl group, and aryl proton, respectively, in the NMR spectra. The presence of carbohydrate residue within the biopolymer is confirmed by the IHNMR spectra, which shows a shift of carbohydrate ring proton from 2-7 ppm [19]. Its polymeric nature is confirmed by the presence of these groups.

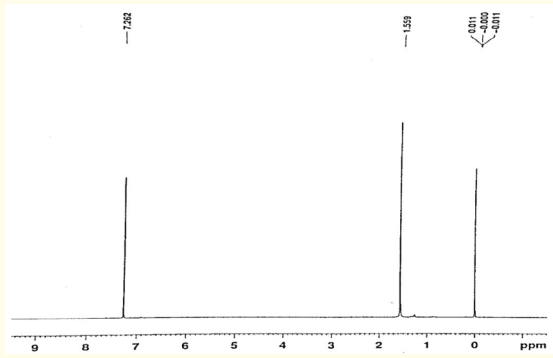

Figure 4: NMR Spectra of biopolymer from *Phaseolus vulgaris*.

Cell line toxicity of *biomaterial*

The cell line toxicity study of biopolymer from *Phaseolus vulgaris* in the concentration of 31.25,62.25, 125,250 and 500 (µg/ml) shows the mean % cell viability ranging from 129.93 ± 2.04% to 104.081 ± 12.92% with IC_{50} values (µg/ml) of >500. Thus the cell viability assay data demonstrate that there is no cell death observed in assay. Along with this the IC_{50} (Inhibitory concentration) value of the biopolymer was observed above 100 µg/ml. So the obtained data revealed that isolated biopolymer was safe and non-toxic in nature. So it can be safely used for the preparation of drug loaded bionanosuspension. Cell-line toxicity graph between concentration of biopolymer *Phaseolus vulgaris* v/s Mean % of cell viability is shown in figure 5.

Figure 5: Cell-line toxicity graph between concentration of biopolymer *Phaseolus vulgaris* v/s Mean % of cell viability. The results has been expressed as Mean ± SEM (n = 3).

The biomaterial isolated from different natural sources like seeds which are edible in nature was characterized for their physico-chemical properties. The obtained isolated biopolymer was characterized for different parameters. The isolated biopolymer appears as free flowing powder. The isolation procedures were optimized by repeating it for six times and thus were found to be economical and reproducible in nature [1-4].

The optimization and scaling up ability of the process confirmed the values of % yield. The yield was found to be reproducible in nature without any significant variation (Madhav and Varshney 2018).

The biopolymer was found to be flaky surface with rough with irregular shape. The pH study defines its similarity with the physiological pH that means the biopolymers are non-irritant.

Thus presence of these constituents like carbohydrate and proteins in chemical testing, confirmed its polymeric nature [5,6].

The SEM analysis of isolated biopolymer showed the rough and flaky structure. Granular and amorphous structure was also observed in SEM image which suggested polymeric nature of the biopolymer.

FTIR spectroscopy of biomaterial showed the different functional group like presence of hydroxyl, alkanes and alkenes, carboxylic acid which confirms its polymeric characteristics. The other groups like aromatic ring at, ketone were found to be present in the FTIR spectra. Thus the presence of these functional groups are responsible for the retardant property in drug release like standard polymer. This revealed its polymeric nature [7].

The mass spectrum of isolated biomaterial showed larger molecular weight structure similar to protenious nature, carbohydrate which confirmed the polymeric nature of biomaterial. It showed the parent peak with 338.3402 m/z.

The proton NMR spectra of biomaterial showed the presence of different peaks at 0.011, 1.559 and 7.262 ppm which confirms the presence of methyl group, hydroxyl group, aryl proton respectively. The HNMR spectra reveals the presence of carbohydrate residue in the biopolymer extracted as the shift of the carbohydrate ring proton form 2-7 ppm. The presence these groups confirmed its polymeric nature [18].

Isolated biomaterial showed the endothermic peak at 73.006°C The area was found to be 94.3136 mj/mg. The broad peak indicated amorphous nature of the biopolymer.

The obtained cell viability assay data demonstrates that there is no cell death observed in the isolated biopolymer. So it confirms that the isolated biopolymer can be safely used for the development of phenytoin loaded bionanosuspension without any toxicity. So it can be safely used for the preparation of drug loaded formulations in drug targeting [19,20].

Conclusion

The study finds that the isolated biopolymer from Phaseolus vulgaris seeds contains unusual inherent stabilizer and cum retardant capabilities that can be exploited to develop a lamotrigine-loaded bionanosuspension for long-term administration of drug candidate.

The isolated biopolymers still have not explored for their novel inbuilt characteristics in drug delivery, can be used as an alternative to standard polymers as these are biodegradable, biocompatible, bioretardant cum biostabilizer in nature. The biopolymers may be isolated in economical ways from the different edible natural sources.

Bibliography

1. Tangri P and Satheesh Madhav NV. "Formulation and evaluation of zidovudine bio-micro dwarfs using a novel bio muco resident from Artocarpus heterophyllus". *International Journal of PharmTech Research* 3 (2011): 169-174.

2. Shankar MSU and Madhav NVS. "A novel smart mucoadhesive biomaterial from *Lallimantia royalena* seed coat". *Journal of Science Asia* 37 (2011): 69-71.

3. Madhav NVS and Pangatey P. "Development of flexy bio-adhesive film loaded with nanosized zidovudine for brain specificity via novelistic soft palatal route". Indian Patent (2013).

4. Satheesh Madhav NV and Tangri P. "Design and evaluation of insulin loaded Bio- Flexy films for trans-soft palatal to brain delivery". 4 (2012): 10-18.

5. Satheesh Madhav NV and Yadav AP. "Development and evaluation of novel repaglinide biostrips for translabial delivery". *International Research Journal of Pharmacy* 4 (2013): 198-202.

6. Ojha A and Satheesh Madhav NV. "A novel potent muco-bioadhesant polymer from seeds of Ricinus communis". *World Journal of Pharmaceutical Sciences* 3 (2014): 2154-2165.

7. Satheesh Madhav N V and Yadav AP. "A novel translabial platform utilizing bioexcipients from Litchi chinensis for the delivery of rosiglitazone maleate". *Acta Pharmaceutica Sinica B* 3 (2013): 408-415.

8. Muller. "Nanosuspensions as particulate drug formulation in therapy rationale for development and what we can expect for the future". *Advanced Drug Delivery Reviews* 47 (2001): 3-19.

9. Rogawski MA and Loscher W. "The neurobiology of antiepileptic drugs". *Nature Reviews Neuroscience* 553-564.

10. Zhang J. "Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants". *International Journal of Pharmaceutics* 323 (2006): 153-163.

11. Patsalos PN., *et al.* "The importance of drug interactions in epilepsy therapy". *Epilepsia* 43 (2002): 365-385.

12. Dawood NM. "Formulation and characterization of lafutidine nanosuspension for oral drug delivery system". *International Journal of Applied Pharmaceutics* 10 (2018): 20-30.

13. Madhav S and Singh K. "Smart ungual biopenetrant from the roots of Beta vulgaris". *Journal of Applied Pharmaceutical Research* 5.2 (2017): 21-26.

14. Martin A. "Physical, Chemical Principles in the Pharmaceutical Sciences". 3rd ed. Varghese Publishing House, Bombay (2001).

15. Subrahmanyam CVS and Thimma Setty J. "Laboratory Manual of Physical Pharmaceutics". 2nd ed. Vallabh Prakashan, New Delhi (2002).

16. Kamila SN., *et al*. "Evaluation of effective Medhya formulation on trancranial treatment on rat". *International Journal of Biomedical Research* 5.6 (2014): 427-431.

17. Tyagi Y and Madhav NVS. "Design selegiline loaded bio-nanosuspension for the management of depression using novel bio-retardent from Manilkara zapota". *Drug Development and in Industrial Pharmacy* 45 (2019): 1351-1360.

18. Madhav NVS and Singh B. "A smart approach for delivering of nanosized olanzapine using piper betel biopolymer rate controlling flexi films for transvermillion delivery". *Asian Journal of Nanoscience and Materials* 2 (2019): 314-326.

19. Madhav NVS and Raina D. "Formulation and evaluation of duloxetine loaded bio-nanosuspension for brain specificity via acoustic meatus". *SOJ Pharmacy and Pharmaceutical Science* 4.1 (2017): 1-5.

20. Varshney S and Madhav NVS. "Smart approach for preparing nanosized topiramate loaded bio-flexy films using former from piper nigrum and screening its *In-vitro* performance". *Nanomedicine and Nanotechnology* 3.3 (2018): 000147.