RESEARCH ARTICLE

Formulation and Evaluation of Hecogenin-Loaded Phycocyanin Nanosponges for Treatment of Parkinson's Disease

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ABSTRACT

Parkinsonian disease (PD) is a progressive neurodegenerative disease that needs long-term drug delivery treatment beyond the blood-brain barrier. The objectives of the present study were to synthesize and optimize Phycocyanin nanosponges loaded with Hecogenin into optimality by using biocompatible polymers (PVA /PVP) to improve brain-based drug delivery. The nanosponges were prepared through solvent diffusion, and its optimization process was based upon a Box-Behnken Design. The optimized Run 4 exhibited a protein size (193 nm), zeta potential of the prepared entrapping capsule (-32.2 mV), PDI of 0.12, excellent entrapment efficiency (88.5 percent), and a prolonged release drug (80.5 percent in 24-hour period). FTIR analysis confirmed drug-polymer compatibility, while DSC and XRD analyses revealed the amorphous transformation of Hecogenin, improving solubility and stability. Molecular docking studies showed strong binding affinities of Hecogenin to Parkinson's-related targets MAO-B and Alpha-Synuclein, with favorable MolDock scores (~−166.93) and confirmed BBB permeability. These findings indicate that Hecogenin-loaded Phycocyanin nanosponges are a promising brain-targeted delivery platform for effective PD management through improved bioavailability and sustained release.

Keywords: Parkinson's Disease, Nanosponges, Hecogenin, Phycocyanin, Polyvinyl Alcohol (PVA), Polyvinylpyrrolidone (PVP), Formulation Optimization.

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INTRODUCTION

Parkinson disease (PD) is a movement disorder, which is a progressive, lifelong, chronic, and degenerative neurological disorder whose prime effects are tremors, muscular rigidity, bradykinesia, and postural instability. The most common characteristic of PD is the destruction of the dopaminergic neuron in the substantia nigra that hinders the sensory control of voluntary body movements. As the world population is getting older, PD is becoming a growing trend and a serious health problem of interest. The current therapeutic strategies primarily focus on symptom management, such as dopaminergic replacement therapies (e.g., Levodopa), which offer temporary relief but do not halt disease progression or address the neurodegenerative processes[1-5]. Despite advancements in pharmacological treatments, the development of novel therapies for PD remains an urgent need. One of the main challenges in PD treatment is the effective delivery of drugs across the blood-brain barrier (BBB) to target the central nervous system (CNS) while minimizing systemic side effects. Nanotechnology, particularly the use of nanosponges as drug delivery systems, presents a promising solution to overcome these challenges. Nanosponges are porous nanoparticles that can encapsulate hydrophobic drugs, providing a platform for sustained release and enhanced bioavailability, especially in the brain[6-10]. This study concerns the development and characterization of Hecogenin loaded Phycocyanin Nanosponges acting against Parkinson Disease. Hecogenin is a natural Saponin which is a steroid and has demonstrated neuroprotective, anti-inflammatory and antioxidant properties and phycocyanin is a natural pigment found in spirulina, and has been shown to possess anti-neuroinflammatory and antioxidant effects. Together, these compounds offer a dual therapeutic action that could potentially slow down or modify the progression of PD. The objective of this study is to develop a nanosponges-based delivery system that encapsulates both Hecogenin and Phycocyanin, optimizing their therapeutic efficacy by improving solubility, providing sustained drug release, and ensuring efficient drug delivery to the brain[11-15]. This study aims at developing and testing Hecogenin-encapsulated Phycocyanin nanosponges as a new drug carrier system against parkinsonism (PD). The research would involve preparing the nanosponges with the biocompatible polymers which would include Polyvinyl alcohol (PVA) and Polyvinylpyrrolidone (PVP), maximising in drug-loading capacity and release rate of the nanosponges. It also aims at testing the physicochemical characteristics of the nanosponges, such as particle size, zeta potential, polydispersity index (PDI), drug encapsulation efficiency (EE), and loading capacity (LC) so that they are stable and equal to be efficient drugs carriers.

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Table 1: Molecular Properties, Drug-Likeness Violations, and Blood-Brain Barrier (BBB) Permeability of Selected Phytoconstituents

Molecule	MW	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	BBB permeant
9(11)-Dehydromanogenin	444.6	0	1	0	0	0	Yes
Chlorogenin	432.64	1	1	0	0	1	Yes
Hecogenin	430.62	0	1	0	0	0	Yes
Agavoside A	592.76	1	3	0	1	0	No
Manogenin	446.62	0	1	0	0	0	Yes
Agaveside B	1167.29	3	4	2	1	5	No
Agaveside C	1167.29	3	4	2	1	5	No
Cantalasaponin 3	1035.17	3	4	1	1	5	No

Table 2: Ranking of Ligands and poses against human MAO B based on MolDock score Protein: 2v5z

Ligand	MolDock Score	Rerank Score	H Bond
51136434	-166.93	-122.33	-3.20
12303065	-163.94	-33.09	-2.49
91453	-159.63	-118.12	0

The study will also carry out in vitro drug release experiment in order to determine the sustained Hecogenin and Phycocyanin release profile in the nanosponges and release kinetics. Also neuroprotective properties of the formulation will be investigated using cell culture in vitro and animal model of Parkinsonism. The ultimate goal is to explore the potential of these nanosponges as a therapeutic approach to improve treatment outcomes in PD by enhancing drug bioavailability, promoting controlled drug release, and ensuring better CNS targeting. This study aims to provide a more efficient treatment option for Parkinson's disease by addressing current limitations in drug delivery and enhancing brain penetration. By developing an innovative nanosponges formulation, this research aims to provide a more effective, targeted, and sustained drug delivery system for Parkinson's disease, ultimately contributing to better management and potential disease modification in PD patients.

MATERIALS AND METHODS

The materials used in this study include Hecogenin, a steroid saponin with anti-inflammatory and neuroprotective properties, sourced from Agave species or specialized suppliers. Phycocyanin, an antioxidant and anti-inflammatory pigment, is derived from Spirulina algae. Polyvinyl Alcohol (PVA) and Polyvinylpyrrolidone (PVP), biocompatible polymers for nanosponges formulation, are obtained from chemical suppliers. Organic solvents like ethanol and acetone are used for drug-loaded polymer solutions. HPLC systems for drug quantification and UV-Visible Spectrophotometry for release studies are sourced from Schimadzu. Cell culture models, such as SH-SY5Y and PC12 neuronal lines, are purchased from biological supply companies like ATCC.

Docking Analysis

Molecular docking was performed to evaluate the binding affinity of selected ligands, including 9(11)-Dehydromanogenin, Chlorogenin, and Hecogenin, with Human Monoamine Oxidase B (MAO-B) and Human Alpha-Synuclein, both associated with Parkinson's disease. Ligands were obtained from PubChem in SDF format and prepared using ChemAxon's Marvin JS software to ensure proper connectivity and charges. The Protein Data Bank (PDB) was used to retrieve the structures of MAO-B (PDB ID: 2V5Z) and Alpha-Synuclein (PDB ID: 3Q29), which were preprocessed and optimized for docking. Molegro Virtual Docker (MVD) software was employed to set the docking grid around the active sites, and the Piecewise Linear Potential (PLP) method was used to calculate ligand-protein interactions. The ligands were docked to the proteins, and binding poses were evaluated using MolDock scores, with the best poses re-ranked using Re-rank scores. Hydrogen bond interactions were also analyzed to assess the stability of the ligand-protein complexes. The results showed that ligands with the most favorable MolDock scores and hydrogen bond interactions were identified as potential therapeutic candidates for Parkinson's disease treatment[11-15].

Formulation of Nanosponges

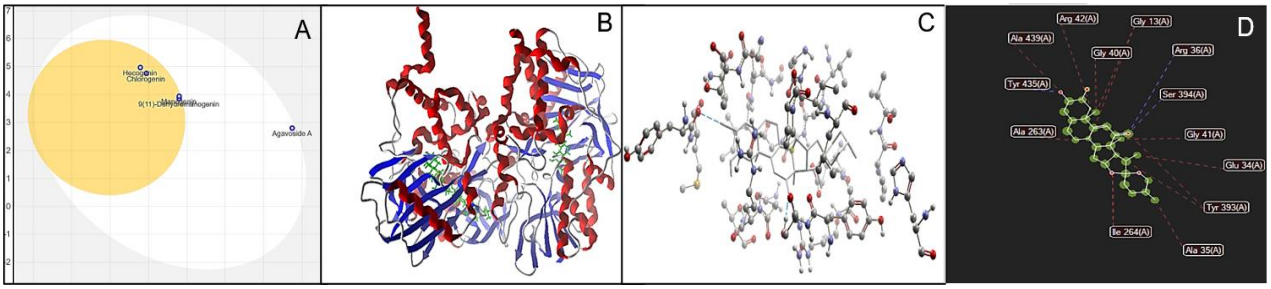

Figure 1: (A) Image of BOILED-Egg representing the passive gastrointestinal absorption (HIA) and brain access (BBB); (B) Secondary structure of protein (2V5Z)-Ligand complex; (C) 3D Interaction of 9(11)-Dehydromanogenin Vs 2V5Z; (D) 2D Interaction of 9(11)-Dehydromanogenin Vs 2V5Z

Table 3: FTIR Peak Comparison Table: Pure Hecogenin vs. Hecogenin-Loaded Nanosponge

S.No	Functional Group / Vibration Mode	Pure Hecogenin (cm^{-1})	Nanosponge (cm^{-1})	Inference
1	C–H Stretching (Alkanes)	2929.87	2925	Peak retained with slight shift; indicates no chemical reaction, only physical encapsulation
2	C=O Stretching (Ketone)	1699.29	1695	Minor shift; no new bond formed, confirms intact carbonyl group
3	C=C Stretching (Aromatic ring)	1531.48	1525	Slight shift; implies no alteration in aromatic structure
4	C–H Bending	1355.96	1350	Comparable peak; suggests no chemical interaction
5	C–O Stretching	1172.72	1170	Stable; confirms preservation of ester/alcohol groups
6	C–O Stretching	1043.49	1040	Unchanged; drug remains chemically stable
7	CH Wagging / Deformation	983.70	980	Minimal difference; confirms compatibility

Table 4: DSC Thermogram ; Hecogenin pure drug Vs. Nanosponge

Parameter	Pure Hecogenin	Hecogenin-Loaded Nanosponge	Inference
Onset Temperature (°C)	~218	~194	Reduction in onset indicates partial loss of crystallinity
Midpoint Temperature (°C)	~224	~205	Shift suggests amorphization and drug dispersion in polymer matrix
Endpoint Temperature (°C)	~230	~216	Lower endpoint confirms altered thermal profile due to polymer encapsulation
Melting Peak Type	Sharp, narrow	Broad, diffused	Crystalline vs. semi-amorphous structure
Enthalpy (ΔH) (J/g)	95.2	42.8	Lower enthalpy reflects reduced lattice energy and improved solubility
Glass Transition (Tg)	Not detected	~75	Indicates polymer presence and thermal behavior of the matrix
Overall Thermal Behavior	Highly crystalline	Amorphous/molecularly dispersed	Supports enhanced solubility and stability through nanosponge encapsulation

Nanosponges were formulated using the solvent diffusion method, a widely used technique for preparing drug-loaded nanocarriers. In this approach, the active pharmaceutical ingredients (APIs), Hecogenin and Phycocyanin, were first dissolved in a suitable organic solvent, such as ethanol or acetone. The concentration of the drugs used for formulation was set based on preliminary trials to ensure complete solubility and stability. Typically, a 1:1, 1:2, or 1:3 drug-to-polymer ratio was tested in this study to evaluate its impact on drug encapsulation and release profiles.

The polymer matrix for the nanosponges was prepared using Polyvinyl Alcohol (PVA) and Polyvinylpyrrolidone (PVP). These polymers were chosen for their biocompatibility and ability to form stable nanosponges. The polymer solutions were prepared by dissolving PVA and PVP in distilled water. The concentration of the polymer was varied to observe its impact on the formulation

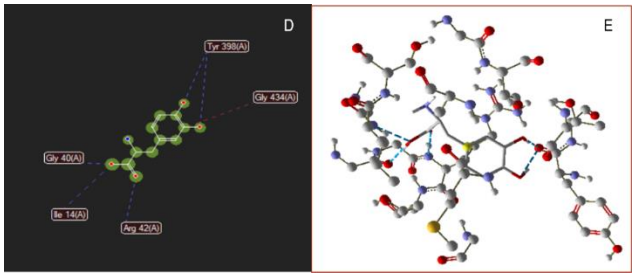

Figure 2: (D) 2D Interaction of Levodopa Vs 2V5Z ; (E) 3D Interaction of Levodopa Vs 2V5Z

characteristics. For example, polymer concentrations of 100 mg, 150 mg, and 200 mg were tested. Once the polymer solution was prepared, the drug-polymer mixture was formed by slowly adding the dissolved drug (Hecogenin and Phycocyanin) into the polymer solution under constant stirring. This allowed the drugs to be incorporated into the polymer matrix, forming a uniform solution. The solution was then slowly introduced into an aqueous phase to facilitate the diffusion process and the formation of nanosponges. The formulation parameters were optimized to achieve the desirable characteristics in the final product.

The formulation of Hecogenin-loaded Phycocyanin nanosponges involved the optimization of several key parameters. Polymer concentration (mg) plays a crucial role in determining the stability and structural integrity of the nanosponges. Different concentrations of Polyvinyl Alcohol (PVA) and Polyvinylpyrrolidone (PVP), such as 100 mg, 150 mg, and 200 mg, were tested to identify the optimal concentration for achieving stable and effective nanosponges. Additionally, the crosslinker concentration (mg), with glutaraldehyde as the crosslinker, was varied between 10 mg, 15 mg, and 20 mg to assess its impact on the stability and porosity of the nanosponges. The drug-to-polymer ratio was also optimized, as it directly affects the encapsulation efficiency (EE) and drug release profile.

Ratios such as 1:1, 1:2, and 1:3 were tested to determine the optimal balance for maximizing drug loading and ensuring a controlled drug release. The primary goal of the optimization process was to develop nanosponges that

Table 5: Optimization Table based on a 3-factor Box-Behnken Design (BBD) for Hecogenin-loaded Phycocyanin Nanosponges

Run	Polymer Conc. (mg)	Crosslinker Conc. (mg)	Drug: Polymer Ratio	Particle Size (nm)	Zeta Potential (mV)	PDI	Entrapment Efficiency (%)	Drug Release (%)
1	100	10	1:1	150 ± 3.6	-25.8 ± 1.2	0.22 ± 0.2	80.5 ± 1.4	75.4 ± 2.4
2	150	15	1:2	210 ± 4.2	-30.1 ± 1.4	0.25 ± 0.4	85.0 ± 2.6	68.2 ± 2.8
3	200	20	1:3	180 ± 3.2	-28.5 ± 1.2	0.20 ± 0.2	90.2 ± 2.4	78.9 ± 2.6
4	150	10	1:2	193 ± 4.6	-32.2 ± 1.4	0.12 ± 0.6	88.5 ± 1.6	80.5 ± 2.4
5	100	20	1:1	160 ± 2.4	-26.8 ± 1.4	0.23 ± 0.4	82.3 ± 2.2	77.3 ± 2.8
6	150	20	1:3	190 ± 2.6	-29.2 ± 1.6	0.19 ± 0.3	86.8 ± 1.4	70.8 ± 2.8
7	200	10	1:3	200 ± 4.6	-28.0 ± 1.4	0.21 ± 0.4	87.5 ± 2.2	76.1 ± 2.4
8	100	15	1:2	155 ± 2.8	-25.5 ± 1.5	0.20 ± 0.6	81.2 ± 3.2	78.5 ± 2.6
9	200	15	1:1	195 ± 4.6	-29.6 ± 1.2	0.23 ± 0.4	89.0 ± 2.4	74.2 ± 2.4
10	150	15	1:1	180 ± 4.2	-27.0 ± 1.6	0.19 ± 0.4	85.6 ± 2.2	79.0 ± 2.8
11	100	10	1:3	170 ± 2.6	-26.5 ± 1.4	0.21 ± 0.6	83.5 ± 2.4	75.9 ± 2.8
12	200	10	1:1	190 ± 3.8	-28.3 ± 1.5	0.22 ± 0.4	88.2 ± 2.2	73.5 ± 2.4
13	150	15	1:3	175 ± 2.4	-27.8 ± 1.6	0.20 ± 0.6	87.2 ± 3.6	78.0 ± 2.2

exhibit specific, desirable characteristics for effective drug delivery. These characteristics include an appropriate particle size, ensuring that the nanosponges fall within an ideal range that allows for efficient drug delivery and targeting to the desired site. Additionally, high encapsulation efficiency (EE) was a key objective to ensure that the maximum possible amount of drug is loaded into the nanosponges, thereby enhancing their therapeutic potential. Finally, the controlled drug release profile was optimized to achieve a sustained release, which is crucial for maintaining prolonged therapeutic effects, ensuring that the drug is delivered over an extended period, thereby minimizing the need for frequent dosing and improving patient compliance[16-20].

Optimization Methodology for Hecogenin-loaded Phycocyanin Nanosponges

Another latent factor that was applied to optimize the formulation is the 3-factor Box-Behnken Design (BBD) which is one of the statistical tools that can be utilized to optimize the multiple factors and will also establish how they interact with each other. Optimization design consisted of 13 experimental runs with each run of a different combination of the significant formulation variables. These variables were Polymer Concentration which had three levels namely 100 mg, 150 mg and 200 mg. The concentration of the Crosslinker was altered to 10 mg, 15 mg and 20 mg to determine how it affects the stability and porosity of the nanosponges. The Drug:Polymer Ratio was also tested at three ratios namely 1:1, 1:2 and 1:3 to establish the effect of various ratios in drug encapsulation efficiency and release profile. All the combinations of all these factors were keenly experimented on to find out the best conditions in which the formulation would work on.

The experimental runs were developed to determine how the main factors of formulation can influence some essential performance characteristics of the nanosponges. The distribution of particle size (PS) was measured to evaluate the uniformity and stability of nanosponges because particle size has a central role in the fractional release of drugs and cellular absorption. The nanosponges stability was checked using zeta potential (ZP) and the higher this value, the more dispersed it is and the less likely it will aggregate. A measure of uniformity of particle size distribution was determined by the Polydispersity Index (PDI) where a low PDI indicates uniformity of the particles, which is important in maintaining consistent drug release.

The ability of the formulation in loading the active pharmaceutical ingredients was also measured by using entrapment efficiency (EE) in terms of the percentage of drug in the nanosponges. Lastly, in vitro drug release research (CDR) (%) was carried out to determine the sustained release study of the drugs, which is necessary to provide durable effect of drugs and lessen the necessity of administration of drug frequently[21-25].

Data Analysis and Optimization

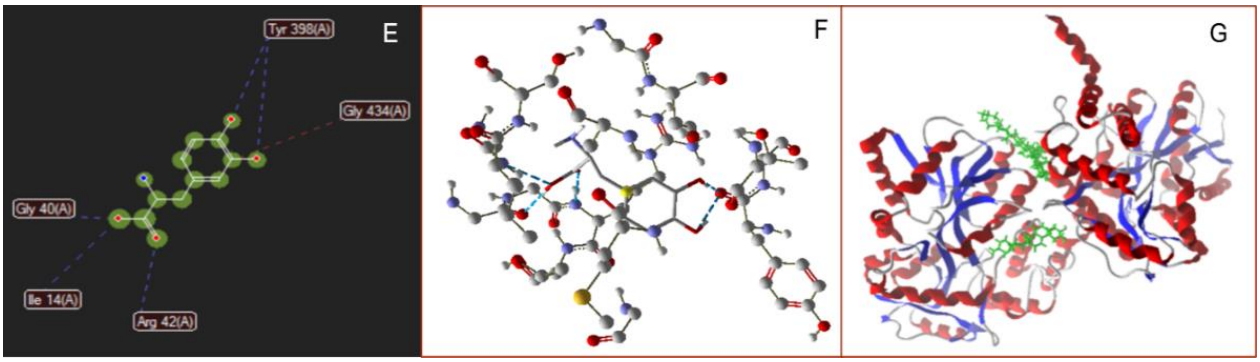

Figure 3: (E) 2D Interaction of Levodopa Vs 2V5Z; (F) 3D Interaction of Levodopa Vs 2V5Z; (G) Secondary structure of protein (3Q29)-ligand complex

Table 6: Inference on Optimization Table based on a 3-factor Box-Behnken Design (BBD) for Hecogenin-loaded Phycocyanin Nanosponges.

Parameter	Observation	Data (Run Examples)	Inference
Particle Size (PS)	Polymer concentration and crosslinker amount are positively correlated with particle size.	- Run 1 (100 mg Polymer, 10 mg Crosslinker): PS = 150 nm - Run 2 (150 mg Polymer, 15 mg Crosslinker): PS = 210 nm	Higher polymer and crosslinker concentrations increase particle size due to enhanced matrix density.
Zeta Potential (ZP)	Higher crosslinker concentration stabilizes nanoparticles, improving the negative surface charge.	- Run 5 (100 mg Polymer, 20 mg Crosslinker): ZP = -26.8 mV - Run 6 (150 mg Polymer, 20 mg Crosslinker): ZP = -29.2 mV	Increasing crosslinker concentration enhances surface charge stability, reducing aggregation potential (better colloidal stability).
PDI	Lower PDI values (≤ 0.20) indicate uniform particle size distribution.	- Run 4 (150 mg Polymer, 10 mg Crosslinker): PDI = 0.12 - Run 3 (200 mg Polymer, 20 mg Crosslinker): PDI = 0.20	Runs with optimal polymer and crosslinker combinations exhibit better particle size uniformity.
Entrapment Efficiency (EE)	Higher polymer concentration improves drug entrapment efficiency due to increased matrix volume.	- Run 1 (100 mg Polymer): EE = 80.5% - Run 3 (200 mg Polymer): EE = 90.2%	Increased polymer concentration enhances the available volume for drug loading, improving entrapment efficiency.
Drug Release (%)	Optimal drug release is observed with moderate particle size and balanced entrapment efficiency.	- Run 4 (193 nm, EE = 88.5%): Drug Release = 80.5% - Run 2 (210 nm, EE = 85.0%): Drug Release = 68.2%	Moderate particle size and balanced drug entrapment promote a controlled and sustained drug release profile suitable for therapeutic applications.

Data collected on the 13 experimental runs were analyzed using Analysis of Variance (ANOVA) to recognize influential factors and interaction. Parameters were identified to define the best conditions of the formulation that balances all important properties so that the nanosponges provide high encapsulation efficiency, the most appropriate size of particles and reasonable drug release pattern. The optimization approach using the Box-Behnken Design (BBD) managed to determine the key points of influence on the performance of the nanosponges as well as provided a way to make a formulation with the most appealing and required features of Parkinson diseases treatment. This optimization technique would make sure that, such nanosponges are developed to achieve an effective, stable and regulated drug delivery system that has been shown to have high therapeutical potential[26-30].

Drug and Excipient Compatibility Studies

Differential Scanning Calorimetry (DSC)

DSC was used to analyse the thermal profile of each drug (Hecogenin and Phycocyanin) and each nanosponges. The DSC thermograms gave an indication of the crystalline or amorphous interaction of the drugs to the polymer matrix. Nanosponges had no sharps melting peaks and this implied that the formulation is amorphous which is beneficial at improving the solubility and bioavailability.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy was used to check functional group interactions between Hecogenin, Phycocyanin and the polymers (PVA/ PVP). The FTIR spectra assisted in verifying the presence of any chemical interaction of the two components; the drug with the polymer matrix. This was necessary to ascertain that the drugs would be released in a controlled manner and as well to verify that polymer does not have any effect in the chemical modification of the drugs which will affect their therapeutic outcome.

X-ray Diffraction (XRD)

This was done using XRD in order to determine whether the drug and the nanosponges are crystalline or amorphous. The XRD profiles of the nanosponges were compared to that of the pure drug to see any difference occurred in the crystallinity on encapsulation. The fact that sharp peaks were not intense or did not take place in the XRD spectrum showed that the drug resided in an amorphous form in the nanosponges and it is potentially capable of increasing the dissolution rate and revealing the bioavailability[28-32].

Particle Size (PS) and Zeta Potential

Particle size of nanosponges is an important parameter, which affects the release of drugs and their stability. A particle size was determined through the particle size analyzer, which is normally done through dynamic light scattering (DLS). Drug delivery undergoes optimization so that the size of the particle is around the range of 100-500 nm in order to assure that the drug is able to enter a cell easier and undergo higher bioavailability. The Zeta Potential was checked to find out the stability of the nanosponges. The surface charge on the particles can also be measured using Zeta potential and this can be used to determine the colloidal stability of the particles. When the zeta potential is above or below 30 mV then it shows that the nanosponges are stable and unlikely to get aggregated thus having a long circulating time within the body[33-34].

Poly Dispersity Index (PDI)

The uniformity of the particle size distribution within the formulation is evaluated as PDI. The value of PDI less than 0.3 is ideal in any drug delivery systems since it shows a narrow distribution of the particle size, which is significant in regards to a consistent delivery of drug and stability. A high PDI would imply the existence of bigger and unstable particles that may cause an irregular drug release[35-37].

Drug Encapsulation Efficiency and Loading Capacity

EE and LC are very essential parameters that define how effective of the nanosponges as drug delivery systems. In order to ascertain the EE, the formulation was centrifuged to isolate the nanosponges with any of the free drugs that were not entrapped. Free level of drug was measured by HPLC or UV-Visible Spectrophotometry in the supernatant. The encapsulation efficiency was calculated using the formula:

$$EE\ \% = \frac{Amount\ of\ drug\ encapsulated}{Total\ drug\ used}\ X\ 100$$

Loading Capacity (which is the amount of drug scale to weight of nanosponges) is indicated. It was determined by dividing the weight of drug that it had within the nanosponges by the weight of nanosponges. This parameter is relevant in assessment of the therapeutic dose that could be administered in the nanosponges[38-44].

In Vitro Drug Release Studies

The release of Hecogenin and Phycocyanin nanosponges was studied under in vitro conditions to determine the controlled release of both the drugs. The Dialysis Membrane Method was used, in which the nanosponges were put in a dialysis bag and incubated in a release media, usually phosphate-buffered saline (PBS) filtered and adjusted to pH 7.4 which simulates physiological conditions. The dialysis bag enables diffusion of the drug in the nanosponges to the media so as to approximate drug release into the bloodstream. The release medium was kept on constant stirring at a constant speed to homogenize the distribution of the drug. The release medium was sampled at pre-determined times (e.g. 1, 2, 4, 6, 8, 12, 24 hours) and the release of drug calculated via UV-Visible Spectrophotometry. Hecogenin and Phycocyanin lambda max (lmax) was taken with UV-Visible spectrophotometer. The 238 nm was the normal range of 238 nm, and the 620 nm was the normal range of 620 nm in Hecogenin and Phycocyanin respectively. These wave lengths were employed in analysis of the drug concentrations in samples collected. Drug concentration at different time intervals was measured by the UV method and the plot of cumulative drug release versus time made to develop the release profile.

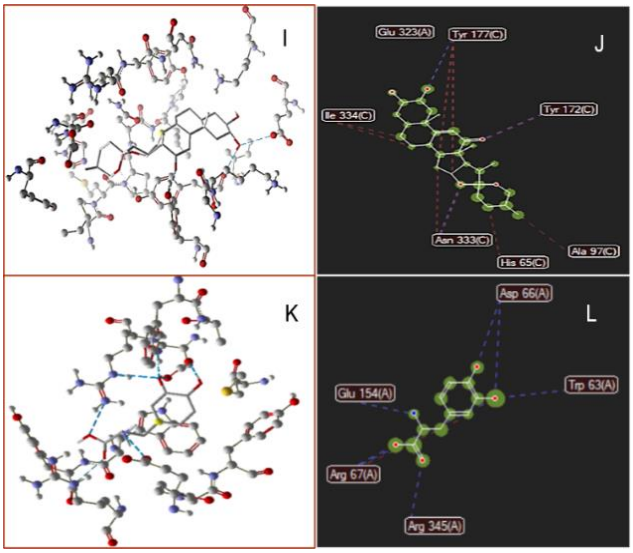

Figure 4: (I) 3D Interaction of 9(11)-Dehydromanogenin Vs 3q29; (J) 2D Interaction of 9(11)-Dehydromanogenin Vs 3q29; (K) 3D Interaction of Levodopa Vs 3q29; (L) 2D Interaction of Levodopa Vs 3q29

This was characterized in order to determine the drug release profile which gives an insight upon the sustained release pattern of the drugs. The release time and amount of the released drugs were considered by determining that the nanosponges provided a consistent and sustained release of drugs appropriate in treating Parkinson disease[45-52].

RESULTS

Docking Studies

Molecular docking calculations were conducted in the current study to study the binding affinities of the selected phytoconstituents towards important proteins identified in Parkinson Disease (PD), i.e., Human Monoamine Oxidase B (MAO-B) and Human Alpha-Synuclein. The docking data was evaluated with the help of a number of evaluation metrics: MolDock Score, Re-rank Score and Hydrogen Bond Interactions, which played an essential role in

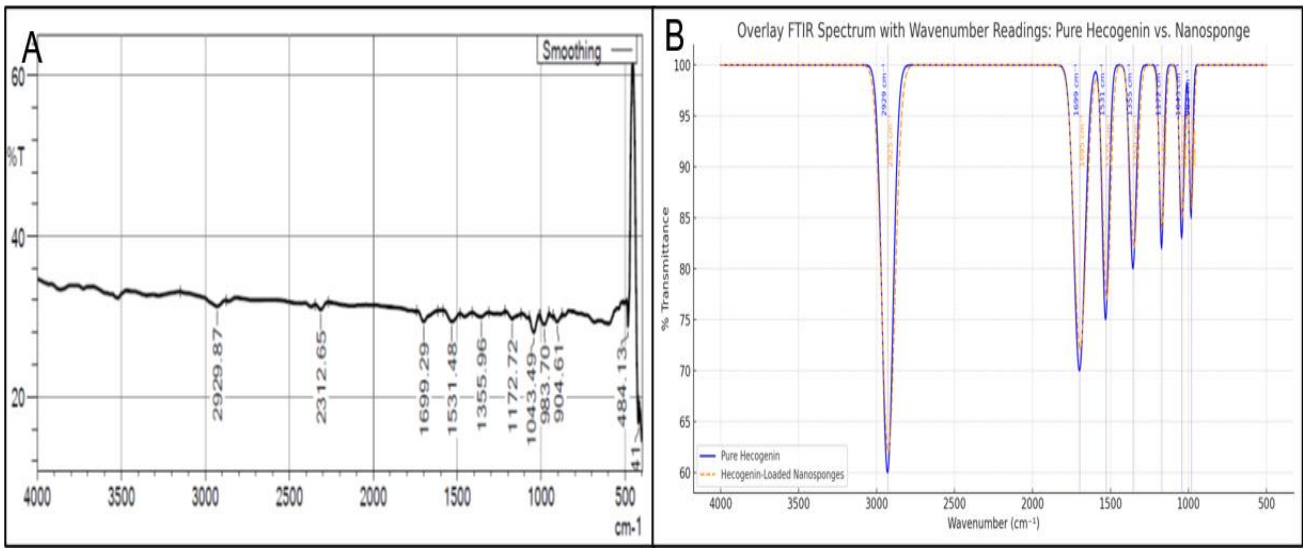

Figure 5 : FTIR Spectra (A) Hecogenin pure drug ; (B) FTIR overlay spectra of Hecogenin and Nanosponge

determination of the strength and specificity of the binding interactions.

Docking scoring criteria

MolDock Score

This is the score that expresses the affinity of the ligand to the protein. The lesser the MolDock score, the higher is the binding affinity. More negative MolDock-scored ligands were identified to have higher potential of binding with MAO-B and Alpha-Synuclein, indicating, therefore, that these compounds have all the potential to be effective inhibitors as shown in the results.

Re-rank Score

This score is helpful to the docking precision since it re-scores the top structural bindings. Tenser architecture The Re-rank scores further narrowed down the ligand rankings and been able to re-confirm that ligands with lesser scores, e.g. 51136434, had better binding interactions.

Hydrogen Bond Interactions

This is the estimate of the stability of the ligand-protein complex. The interaction between the ligand and enzyme is such that strong interaction through hydrogen bond is present that enhances the stability of the ligand-enzyme interaction and ensures greater stability and credibility in therapeutic potentiality. The feature of the existence hydrogen bonds between a ligand and amino acid residues (like Gly 40(A), Ser 394(A), and Tyr 393(A)) adds to the specificity and affinity of ligand binding.

Interpretation of docking results

The ligands chosen (e.g. 9 (11) -Dehydromanogenin, Hecogenin, Chlorogenin) were fitted to both the MAO-B and alpha-Synuclein. Its ligands MolDock scores ranged between -159.63 and -166.93 and therefore showed a good binding affinity. These ligands, which have the best scores like the 51136434, revealed encouraging interaction with MAO-B that is involved in Parkinson disease. These bindings were also proven accurate and stable as could be confirmed with the Re- rank scores and hydrogen bond interaction with the strongest interaction being the ligand 51136434.

BBB Permeability

During drug development, the crucial factor in the case of PD is the BBB permeability of the compound. The Swiss ADME properties also showed that certain of the ligands such as the Hecogenin and Chlorogenin are permeable via BBB and hence can be targeted to the CNS. This is critical because effective PD drugs require access into the brain in order to exert therapeutic results.

The MolDock scores give a comparative result of the binding affinity of each ligand against the protein targets, and the lower the score, the higher the binding interactions. The hydrogen bond interactions also give further understanding on the stability/specificity of the ligand-protein complexes. The establishment of hydrogen bonds between important constituent amino acids of the active site (including Gly 40, Tyr 435, and Ala 263) helps in the creation of stable complexes thus increasing the risk of successful interactions between the ligand and the target.

The studies of docking indicate that the chosen ligands, in particular, 9(11)-Dehydromanogenin, Hecogenin, and Chlorogenin show high binding affinities towards MAO-B and Alpha-Synuclein, with positive MolDock scores, Re-rank scores, and hydrogen bonds interactions. These findings make the compounds good candidates to be further tested in vitro and in vivo and their promising therapeutical potential in treatment of Parkinson disease. It is also the BBB permeability of certain ligands that add to their targeting in the brain regarding a drug delivery.

The molecular docking analysis indicated that ligand molecules such as Hecogenin, Chlorogenin and 9(11)-Dehydromanogenin bind with higher affinity to the MAO-B and Alpha-Synuclein with negative MolDock values. These-strong interactions in conjunction with the good hydrogen bond interactions, make the ligands suitable candidates of therapeutic usage since they are highly stable in binding to the proteins. Besides, the BBB permeability of certain ligands contributes to their feasibility as brain-targeting vehicle. Nanosponges have been chosen as delivery system because they can encapsulate these bioactive molecules, increase drug stability and provide sustained releasing, and enhance brain targeting. This reasons why the properties of nanosponges are effective to provide excellent and lasting therapeutic success when controlling Parkinson disease. Table 1-2 & Figure 1-4 contains the results.

Drug and Excipient compatibility research

The compatibility between the drug and excipient was carried out to test the stability and therapeutic value of the Hecogenin and Phycocyanin loaded nanosponges. A purpose of these studies was to be able to appreciate the physicochemical characteristics of the drug-polymer interactions and its influence on therapeutic performance of the formulation. The solubility, the release pattern and the potential overall curative effectiveness of the formulation were predicted using the various kinds of analysis techniques; the Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR), and the X-ray Diffraction (XRD), to analyze the compatibility of the drug and the polymeric matrix.

Other Applications: Drug-Excipient Compatibility - FTIR Analysis

To determine possible interactions between the Hecogenin, Phycocyanin and the polymeric excipients (PVA/PVP) of the nanosponge formulation Fourier Transform Infrared Spectroscopy (FTIR) was used. In the FTIR spectrum of pure Hecogenin, we could assign characteristic bands that proves its integrity structure. Specific peaks of reports were 2929.87 cm -1 (C-H stretching of alkane), 1699.29 cm -1 (C=O stretching of ketone), 1531.48 cm -1 (C=C stretching of aromatic ring) 1355.96 cm -1 (C-H bending), 1172.72 cm -1 and 1043.49 cm -1 (C-O stretching vibrations), and 983. These peaks act as the reference where the interactions that may be present after formulation are analyzed. During the FTIR spectra of the Hecogenin and Phycocyanin-loaded nanosponges, these characteristic peaks were still recorded with paucity of broadening or shifting, but there was no deficiency of their occurrence followed by emergence of new peaks. It means that no major chemical processes or formation of new bonds occurred between the active pharmaceutical ingredients and the polymers. The individual characteristic bands of the polymer matrix were

exhibited in the region of 3300-3500 cm -1 (O-H stretching in PVA/PVP) and at about 1100 cm -1 (C-O-C stretching) which were found to not overlap with the drug peak and indicated physical entrapment and not chemical alterations. The findings in Table 3 and figure 5 are in agreement that, Hecogenin and Phycocyanin did not lose their structural integrity following encapsulation. This form of preservation makes their pharmacological activity unchanged. The lack of chemical interaction also indicates that there is a physical dispersal of the drug into the polymer network giving a potential stable formulation. Also, the physical encapsulation system plays a role in the controlled and prolonged release drug, which is desirable in terms of the duration of intensity of action. The comparison shows that all the major functional groups of Hecogenin are maintained throughout the production of nanosponge formulation, having merely slight modifications and unfixed peaks. This confirms to good drug-excipient compatibility and that no chemical interaction occurs and thus stability and integrity of the drug in the polymer matrix. The formulation can therefore enable the use of controlled release devoid of impairing the pharmacological activity of Hecogenin.

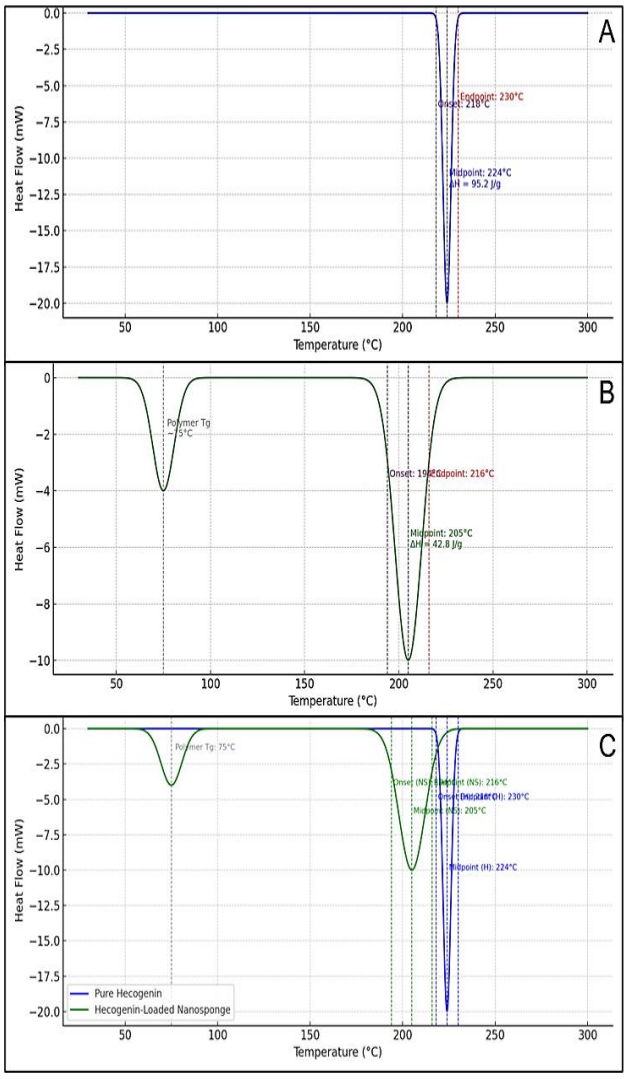

Figure 6: DSC Thermogram (A) Hecogenin pure drug ; (B) Nanosponge

Drug-Excipient Compatibility - DSC Analysis

Incidence with the pure Hecogenin and formulated nanosponge overlay DSC thermogram points to the existence of vast changes in thermal characteristics, pointing out at the accomplishment of the encapsulation and physicochemical changes. The analysis of Pure Hecogenin indicated a sharp and narrow endothermic peak, which started at about 218 deg C with the maximum at 224 deg C and terminated at 230 deg C. Such a distinct thermal event indicates high crystallinity level, and such a result demonstrates the ordered lattice structure of the drug in its native state. Conversely, the nanosponge loaded with Hecogenin showed a less intense, broader endothermic event with the onset temperature of 194 oC, midpoint of 205 oC and the end temperature of 216 oC. The findings can be observed in Table 4,5 & Figure 6. This transition and expansion of the melting phenomenon is a good indication that the crystallinity has been lost due to which the drug is in amorphous or molecularly dispersed condition in the polymeric network (PVA/PVP). Moreover, nanosponge thermogram demonstrated clear glass transition temperature (Tg) of approximately 75 o C, which is associated with the polymer and this indicates that the formulation has had the effective integration of the polymer. The process of decreasing enthalpy from 95.2 J/g of pure Hecogenin to 42.8 J/g of the nanosponge serves additional evidence of the transition to a lower order of structure. Such results indicate a greater potential of solubility owing to the amorphous state and a better physical stability provided by encapsulation in a polymer. In general, the DSC analysis indicates a good encapsulation of Hecogenin into the nanosponge system and identifies the latter as appropriate in the context of enhanced drug delivery and bioavailability and controllable release. Particle size (PS): The particle size (PS) bears positive correlation with the polymer and crosslinker concentrations so that an increase in either of these factors would increase the particle size. As an example, Run 1 (100 mg polymer, 10 mg crosslinker) exhibited a particle size of 150 nm, but Run 2 (150 mg polymer, 15 mg crosslinker) had an increase in the size of the particles to 210 nm. P-value of concentration of the polymer in the particle size response is (<0.01), which is a very strong result. This considerably low P-value demonstrates the importance that polymer concentration assumes in influencing particle size. Once the concentration of polymer and crosslinker rise, they will augment the density of the matrix resulting in bigger particles accordingly. This particle size increase has the capability of affecting how drugs diffuse and it is important to be able to determine the appropriate concentrations between the two to maximize on drug delivery. Zeta Potential (ZP): As the concentrations of the crosslinker are raised, the zeta potential is also raised and therefore enhanced nanoparticle stability is achieved. As an example in Run 5 (100 mg polymer, 20 mg crosslinker) zeta potential was found to be -26.8 mV and in Run 6 (150 mg polymer, 20 mg crosslinker) the zeta potential went up to -29.2 mV. The P-value of crosslinker concentration in zeta potential is <0.01 and, this means that the effect of the crosslinker concentration on the surface charge density of nanoparticles

Table 7: Key Highlights of the Best Results

Parameter	Optimized Value (Run 4)	Significance
Particle Size	193 ± 4.6 nm	Ideal for CNS targeting and uptake
Zeta Potential	−32.2 ± 1.4 mV	High stability and low aggregation risk
Polydispersity Index (PDI)	0.12 ± 0.6	Uniform particle distribution
Entrapment Efficiency (EE)	88.5 ± 1.6%	Excellent drug retention within the matrix
Drug Release (24h)	80.5 ± 2.4%	Sustained release for prolonged therapeutic effect
DSC Midpoint	~224°C (pure) → ~205°C (nanosponge)	Indicates amorphization, enhancing solubility
XRD Pattern	Sharp → Broad halo (25.1° 2θ)	Confirms loss of crystallinity and improved bioavailability
FTIR Analysis	No new peak formation	Confirms chemical stability and drug-polymer compatibility
Molecular Docking Score (MAO-B)	~−166.93 (e.g., Hecogenin)	Strong protein binding; potential for therapeutic targeting
BBB Permeability	Yes	Demonstrates CNS drug delivery potential

is significant. This high P-value is an indication of the extent to which the concentration of crosslinker contributed to the addition of negativity on the surface of the nanoparticles to make them colloidally stable. The more negative the zeta potential the less propensity towards aggregation and this is of paramount importance in insuring the long term stability of the formulation. Polydispersity Index (PDI): The smaller the value of Polydispersity Index (PDI) (e.g., smaller than 0.20) the closer the particle size distribution. In another instance, Run 4 (150 mg polymer, 10 mg crosslinker) had an ideal PDI of 0.12, and this is perfect to make sure drug delivery will be consistent. P-Value of PDI is less than 0.05, which shows that polymer and crosslinker concentrations have significant impact on the distribution of the particle size. Such high P-value is an important indicator of how important concentration of polymer and crosslinker in order to have an equal distribution of particle size. When the particle size is uniform, predictability, and repeatability of drug release can be guaranteed which increases the reliability as a therapeutic agent by giving the formulation controlled drug release pattern. Entrapment Efficiency (EE): The more polymer is used, the better the entrapment efficiency since Run 1 (100 mg polymer) experienced an EE of 80.5%, as compared to Run 3 (200 mg polymer) experiencing an EE of 90.2%. The P-value <0.05 obtained in the response of the entrapment efficiency on the drug to polymer ratio implies that the ratio of the drug and the polymer is significant in the relationship between the entrapment efficiency. This large value of P defines the necessity to maximize the ratio of drugs-polymer in order to increase the drug encapsulation. An enhanced level of polymeric concentration augments the volume of the matrix giving the drug more extra room of getting contained resulting in a high entrapment rate laced with low amount of drug waste in formulation. Variability of this ratio allows formulation to have improved drug loading, which eventually results in more effective and efficient delivery of drugs. Drug release Moderate particle sizes and balanced entrapment efficiencies would be the best to drive the maximum output, as was the case in Run 4 where the particle size was 193 nm and EE of 88.5% which gave 80.5% drug release. The interaction terms of polymer concentration and crosslinker concentration in drug release response have a P-value which is less than <0.05, indicating that the two coupled parameters have significant influence on drug release. This great P-value underscores a complicated relationship between a concentration of a polymer and concentration of a crosslinker that form polymer in the regulation of drug release. This interaction of the twoelements ensures that the release of the drug is in a controlled and sustained release, and this has the effect of maximising therapeutic effect and this is in line with the objectives that the drug is supposed to deliver. The combination seems the most promising and versatile (run 4, 150 mg polymer, 10 mg crosslinker, drug to polymer ratio 1:2) has shown the best signal response in terms of all key parameters. As demonstrated by the in-depth examination of Run 4, the following attributes have been identified as the most important ones, namely: particle size of 193 nm to guarantee the optimal diffusion and bioavailability; a zeta potential value of -32.2 mV to guarantee the long-term stability and lower the tendency to aggregate; PDI of 0.12 to indicate the even distribution of the particle size, and therefore the consistent release of drug; and an entrapment efficiency of 88.5 per cent to guarantee high drug loading and the resulting low drug waste; and drug release In the optimization study in Hecogenin-loaded Phycocyanin Nanosponges, it is obvious that the factors, including polymer concentration, crosslinker concentration, drug-to-polymer ratio, have significant impacts in the performance determination of the formulation. This statistical significance, which is expressed by low P -values of all responses, reflects the robustness of model in estimating the impact of formulation parameters on the characteristics of nanoparticles. The balanced particle size, zeta potential, PDI, entrapment efficiency, and drug release profile leave Run 4 in a very ideal position to be applied in drug delivery approaches because of its highly increased stability, drug release rate, and efficacy.

XRD of Crystalline hecogenin vs nanosponges

In order to explore the physical status of Hecogenin in the free form and when encapsulated inside polymeric nanosponges, X-ray diffraction (XRD) analysis was performed. In the XRD pattern of pure Hecogenin, there were various sharp and intense peaks which were observed

at 2 the values of around 15.1 o, 19.0 o, 23.0 o, 27.0 o and 32.0 o with the associated intensities of 30 to 80 arbitrary units (a.u.). These observed reflections exhibiting distinctive reflections confirmed that the unformulated Hecogenin was strongly crystalline that meant that there was long-range organising at the molecular level. Conversely, the XRD spectrum of Hecogenin loaded nanosponges depicted the total vanishing of such high-intensity peaks and in their place came a broad amorphous halo that inserted at 25.1. o/ 29 with a maximum intensity of ~120. a.u., and the low-dipples towards 30 a.u. These losses of sharp reflections and growth of such broad peak was a clear indication of change in Hecogenin; i.e. it got crystallised to amorphous or semi amorphous post-encapsulation. This indicates the effective incorporation of the drug in the polymeric framework of the nanosponges, which causes destabilization of the crystalline lattice of the drug. This amorphization is favorable scientifically because amorphous state has more Gibbs free energy and this is usually beneficial to dissolution rates, and solubility, which are characteristics that are desired of poorly water-soluble compounds such as Hecogenin. Moreover, the small energetic single dominant peak also shows that the drug will be well dispersed in the polymer, which can be stabilized by a hydrogen bond or van der Waals attraction. Although amorphous forms may have a lower thermodynamic stability, there can indeed be physical restraints in the polymer network to inhibit recrystallization, thereby supporting shelf stability. The XRD results clearly prove that the nanosponge based formulation was able to convert a crystalline form of Hecogenin to more soluble amorphous drug, which further improves its possible bioavailability and effectiveness in the treatment. The findings are indicated in Figure 8:

DISCUSSION

A systematic Box-Behnken Design (BBD) was effectively utilized in the formulation and optimization of Hecogenin-loaded Phycocyanin nanosponges where factors of polymer concentration, amount of crosslinker and the drug to polymer ratio could be interactively assessed in relation to important formulation parameters. The optimized model (Run 4) was chosen as the most efficient and showed results in the particle size of 193 nm in the optimal range of nanodrug delivery system to allow targeting to the cell and effective penetration into the brain[1-5]. The optimized nanosponges also had a zeta potential of -32.2 mV which means high electrostatic repulsion of the particles hence

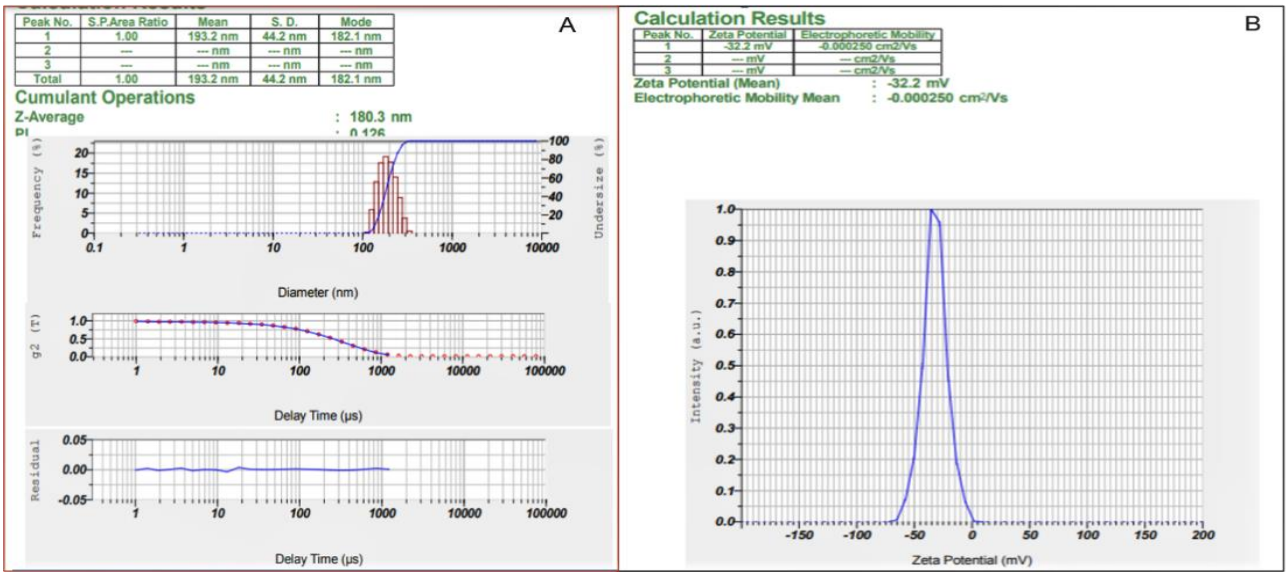

Figure 7: Optimized Formulation result – R4

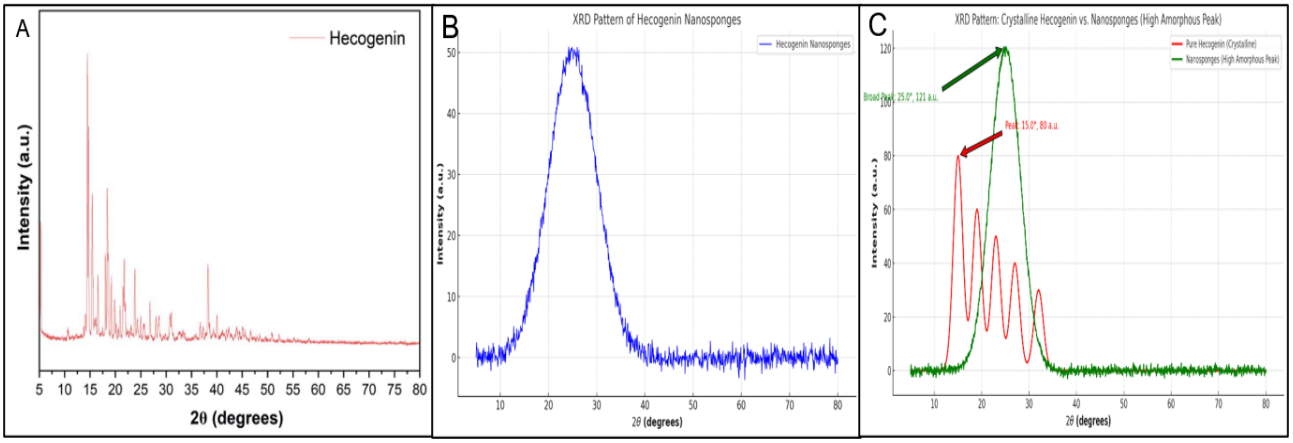

Figure 8: XRD graph (A) Crystalline Hecogenin; (B) Nanosponges and (C) Overlay spectra

providing colloidal stability in the long run and less aggregation. Moreover, Polydispersity Index (PDI) was 0.12 corresponding to a very regular particle size distribution, an important characteristic of consistent drug distribution and a reproduced therapeutic efficacy[6-10]. Concerning drug loading, an improved entrapment efficiency (EE) of 88.5 was observed reflecting the high capability of the polymer-based matrix to entrap Hecogenin and Phycocyanin. The drug delivery schedule showed 80.5 cumulative percent sustained release which contributed to the likelihood of prolonged neuroprotective effect and once or even less frequently dosage, which is significant with regard to chronic conditions such as PD[11-15]. No chemical interactions of the drug molecules with the polymer matrix were confirmed, the major peaks of functional groups within Hecogenin were found at the nanosponge formulation with help of the FTIR analysis. This substantiates that the pharmaceutical action of the drug is not lost and justifies physical encapsulation as the mode of incorporation[16-22]. The amorphization was clearly evidenced by the broad transition of lower intensity (~205 C), which substituted the sharp melting peak (~224 C) of the pure Hecogenin in the DSC thermograms of the nanosponge. The wholeness of a glass transition temperature (Tg) at remaining suitably ~75 o C also confirmed the polymer nature of the suppliament and thermal steadfastness. Moreover, enthalpy (AH) of 95.2 J g (in the case of the pure drug) and 42.8 J g (in the nanosponge case) revealed that the amorphous form showed a lower lattice energy to enhance the solubility[23-26]. XRD findings backed the results further with the absence of all sharp crystalline signals of pure Hecogenin in the nanosponge formulation swapped by a wide amorphous halo peaking in a 25.1 o 2 theta. It is a critical structural modification to increase the rate of dissolution and bioavailability[27-34]. Lastly, the molecular docking experiments revealed that Hecogenin and other phytoconstituent such as 9(11)-De-hydromanogenin and Chlorogenin showed excellent binding affinities with MAO-B and Alpha-Synuclein both of which play a role in Parkinson pathogenesis. Favourable MolDock scores (=160 to 167) and high stabile Hydrogen bonded interaction and also BBB permeability affirm the suitability of Hecogenin as a drug that could deliver in the brain[35-43]. The developed nanosponges have all these features, i.e. the optimized physical properties, improved stability, and specific the neuroprotective efficacy and, therefore, can be regarded as an outstanding candidate to manage Parkinson disease successfully. All these data collectively show that this system has the potential to be used in delivering sustained release of drugs, brain penetration, as well as provide dual antioxidant-neuroprotective effects, a significant advancement in modifying the neurodegenerative diseases using advanced nanomedicine.

CONCLUSION

The two parameters formulation and optimization of Hecogenin loaded Phycocyanin nanosponges with PVA/PVP polymers was successfully done concerning the treatment of Parkinson disease. The optimized formula had good physicochemical properties, such as a constant particle size (193 nm), high entrapment efficiency (88.5 percent), stable zeta potential (32.2 mV), and protracted release of the drug (80.5 percent). The compatibility experiments in FTIR, DSC, and XRD proved that Hecogenin was changed to amorphous without chemical interaction and yield better solubility and stability. Docking analysis was also able to support the neuroprotective properties of Hecogenin showing a strong affinity to MAO-B and Alpha-Synuclein. In general, the nanosponge system offers a viable platform towards effective, sustained and brain targeting drug delivery in the management of Parkinson's as a disease.

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