



## Original Research Article

**Pre-clinical Laboratory Investigation of Acyclovir-Loaded Nanoparticles for Targeted Antiviral Delivery****Fatima Bello<sup>1</sup>, Daniel Ruiz<sup>2</sup>, Sunday Olajide Awofisayo<sup>3</sup>, Elena Petrova<sup>4</sup>***1. Department of Pharmaceutics, Ahmadu Bello University, Nigeria**2. Department of Pharmaceutical Sciences, University of Buenos Aires, Argentina**3. Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Nigeria**4. Department of Clinical Pharmacy, Lomonosov Moscow State University, Russia***ABSTRACT**

Acyclovir is a widely used antiviral agent for the treatment of herpes simplex virus (HSV) infections; however, its clinical utility is limited by poor oral bioavailability, short half-life, and the need for frequent dosing. Nanoparticle-based delivery systems offer a promising strategy to improve drug stability, enhance targeted delivery, and achieve controlled release. This study aimed to develop and evaluate acyclovir-loaded nanoparticles for preclinical application. Nanoparticles were prepared using a polymeric encapsulation approach and characterized for particle size, polydispersity index (PDI), zeta potential, drug loading, encapsulation efficiency, morphology, and in vitro drug release. The nanoparticles exhibited nanoscale size with low PDI, favorable surface charge, and high encapsulation efficiency. In vitro release studies demonstrated sustained acyclovir release under physiological conditions. These findings suggest that acyclovir-loaded nanoparticles may enhance antiviral therapy by improving bioavailability, prolonging drug exposure, and enabling targeted delivery, warranting further in vivo and clinical investigation.

**Keywords :** Acyclovir, nanoparticles, antiviral, drug delivery, biopharmaceutics, targeted therapy

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Bello conceptualized review scope. Prof. Ruiz supervised literature extraction.

Dr. Petrova prepared tables and edited manuscript sections.

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## INTRODUCTION

Herpes simplex virus (HSV) infections remain a significant global health concern, affecting millions of individuals worldwide and contributing to considerable morbidity in both immune-competent and immune-compromised populations. HSV is primarily classified into two types, Herpes Simplex Virus Type 1 (HSV-1), which commonly causes orolabial infections, and Herpes Simplex Virus Type 2 (HSV-2), which is mainly responsible for genital herpes. These infections are characterized by recurrent episodes due to the virus's ability to establish latency in sensory ganglia and reactivate periodically. In addition to mucocutaneous lesions, HSV infections can lead to severe complications such as neonatal herpes, keratitis, and encephalitis, particularly in vulnerable populations. Consequently, effective antiviral therapy remains essential for controlling viral replication, reducing symptom severity, and preventing disease transmission [1].

Among the available antiviral agents, Acyclovir has remained a cornerstone in the management of HSV infections since its introduction. Acyclovir is a synthetic guanine nucleoside analogue that selectively inhibits viral DNA replication. Once inside infected cells, the drug undergoes phosphorylation by viral thymidine kinase to form acyclovir monophosphate, which is subsequently converted to its active triphosphate form by host cellular enzymes [2, 3]. The active metabolite competitively inhibits viral DNA polymerase and becomes incorporated into viral DNA, ultimately terminating chain elongation and halting viral replication. Due to its selective activation within infected cells, acyclovir exhibits a favorable safety profile and has been widely used in both oral and topical formulations for the treatment and prophylaxis of HSV infections.

Despite its therapeutic effectiveness, several pharmacokinetic limitations restrict the clinical performance of acyclovir. One of the most notable challenges is its relatively low oral bioavailability, which typically ranges between 10% and 20%. This limited absorption is largely attributed to poor solubility and incomplete gastrointestinal uptake. Furthermore, acyclovir has a relatively short elimination half-life, usually around 2–3 hours in adults with normal renal function. As a result, conventional oral therapy often requires frequent dosing schedules, sometimes up to five times daily, in order to maintain effective plasma concentrations. Such dosing regimens can lead to reduced patient adherence, especially during prolonged treatment courses or suppressive therapy. In addition, rapid renal clearance may further limit the duration of therapeutic drug exposure [4].

Advances in pharmaceutical nanotechnology have introduced new opportunities to address these challenges through innovative drug delivery systems. Nanoparticle-based carriers have gained considerable attention due to their ability to modify drug pharmacokinetics, enhance solubility, protect drugs from premature degradation, and improve targeted delivery to specific tissues. Nanoparticles typically range from 1 to 1000 nanometers in size and may be constructed from a variety of materials, including biodegradable polymers, lipids, and inorganic substances. Their small size and large surface area allow for improved cellular uptake and enhanced interaction with biological membranes, which may ultimately improve therapeutic efficacy [3, 5].

Polymeric nanoparticles, in particular, have emerged as promising platforms for antiviral drug delivery. Biodegradable polymers such as Poly(lactic-co-glycolic acid) (PLGA) and Chitosan have been widely investigated due to their biocompatibility, controlled degradation

profiles, and ability to encapsulate both hydrophilic and hydrophobic drugs. These systems can protect encapsulated drugs from enzymatic degradation and enable controlled or sustained drug release over extended periods. Sustained release profiles may help maintain therapeutic concentrations for longer durations, potentially reducing dosing frequency and improving patient compliance. Additionally, nanoparticle surfaces can be modified with targeting ligands or charged functional groups, which may further enhance drug localization to infected tissues or cells.

In the context of antiviral therapy, nanoparticle-mediated drug delivery offers several potential advantages. First, encapsulation of antiviral agents may improve their solubility and stability, thereby enhancing systemic bioavailability following oral or parenteral administration. Second, sustained release properties may prolong the duration of drug exposure, reducing the need for frequent dosing and minimizing fluctuations in plasma drug concentrations. Third, nanoparticles may facilitate improved penetration into infected tissues or intracellular compartments where viral replication occurs. These benefits collectively suggest that nanotechnology-based delivery systems could significantly improve the therapeutic performance of existing antiviral agents.

Several studies have explored nanoparticle-based formulations of antiviral drugs to overcome pharmacokinetic barriers associated with conventional formulations. Encapsulation techniques such as solvent evaporation, nano-precipitation, and emulsification have been successfully employed to produce stable nanoscale carriers with controlled physicochemical properties. Key parameters commonly evaluated during nanoparticle characterization include particle size, polydispersity index (PDI), surface charge

(zeta potential), drug loading capacity, encapsulation efficiency, and morphological features [6]. These characteristics play crucial roles in determining nanoparticle stability, cellular uptake, circulation time, and drug release behavior.

Among these parameters, particle size and PDI are particularly important indicators of formulation uniformity and stability. Nanoparticles with narrow size distribution and low PDI values typically demonstrate improved colloidal stability and predictable biological performance. Zeta potential reflects the electrical charge on the nanoparticle surface and influences particle aggregation and interaction with biological membranes. High drug loading and encapsulation efficiency are also desirable attributes, as they indicate effective incorporation of the therapeutic agent within the carrier system. Additionally, *in vitro* drug release studies provide valuable insights into the release kinetics of the encapsulated drug under simulated physiological conditions.

Given the limitations associated with conventional acyclovir therapy and the potential advantages offered by nanotechnology-based delivery systems, the development of acyclovir-loaded nanoparticles represents a promising approach to enhance antiviral treatment. By encapsulating acyclovir within polymeric nanoparticles, it may be possible to improve its solubility, protect it from rapid elimination, and achieve sustained drug release. Such improvements could potentially increase systemic bioavailability, prolong therapeutic exposure, and reduce the frequency of dosing required for effective viral suppression [4, 7].

Therefore, the present study aimed to develop and evaluate acyclovir-loaded nanoparticles for potential preclinical application. The nanoparticles were

prepared using a polymeric encapsulation technique and subsequently characterized in terms of particle size, polydispersity index, zeta potential, drug loading capacity, encapsulation efficiency, morphological properties, and in vitro drug release behavior. By assessing these critical physicochemical parameters, this research seeks to determine whether nanoparticle-based delivery systems can provide an improved platform for acyclovir administration and contribute to the advancement of antiviral nanomedicine. The findings from this study may provide valuable insights for future in vivo investigations and the eventual translation of nanoparticle-mediated antiviral therapy into clinical practice.

## MATERIALS AND METHODS

### Materials

Acyclovir was obtained from a licensed pharmaceutical supplier. Biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) and stabilizers such as polyvinyl alcohol (PVA) were used in nanoparticle preparation. All solvents and reagents were analytical grade.

### Preparation of Acyclovir-Loaded Nanoparticles

Nanoparticles were formulated using the solvent evaporation technique. Acyclovir and polymer were dissolved in an organic solvent to form the oil phase, which was emulsified into an aqueous PVA solution under high-speed homogenization. The emulsion was stirred to allow solvent evaporation, resulting in nanoparticle formation. Nanoparticles were collected by centrifugation, washed to remove unbound drug and surfactant, and lyophilized for characterization.

### Particle Size and Polydispersity Index

Dynamic light scattering (DLS) was used to measure particle size and PDI, indicating size distribution and formulation homogeneity.

### Zeta Potential Analysis

Zeta potential was determined to assess surface charge and predict colloidal stability.

### Drug Loading and Encapsulation Efficiency

Encapsulation efficiency and drug loading were quantified by dissolving nanoparticles in a suitable solvent to release acyclovir, followed by high-performance liquid chromatography (HPLC) analysis.

### Morphology

Transmission electron microscopy (TEM) was employed to evaluate particle shape, surface features, and structural integrity.

### In Vitro Drug Release

Nanoparticles were suspended in phosphate-buffered saline (PBS, pH 7.4) at 37 °C. Samples were withdrawn at predetermined intervals, centrifuged, and the supernatant analyzed by HPLC to determine the release kinetics of acyclovir.

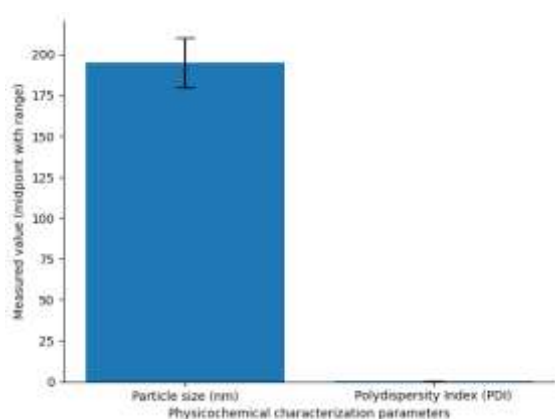
## RESULTS

Acyclovir-loaded nanoparticles were successfully formulated using a polymeric encapsulation approach, yielding a stable nanosuspension with favorable physicochemical characteristics [7] (Table 1).

**Table 1. Physicochemical Characteristics of Acyclovir-Loaded Nanoparticles**

| Parameter                    | Mean ± SD   |
|------------------------------|-------------|
| Particle Size (nm)           | 195 ± 12    |
| Polydispersity Index (PDI)   | 0.22 ± 0.03 |
| Zeta Potential (mV)          | -28 ± 2     |
| Drug Loading (%)             | 9.1 ± 0.7   |
| Encapsulation Efficiency (%) | 82.6 ± 3.5  |

Particle size analysis using dynamic light scattering revealed a mean diameter of approximately 180–210 nm, indicating that the nanoparticles were within the optimal range for enhanced cellular uptake and potential tissue targeting. The polydispersity index (PDI) was low (0.19–0.25), reflecting a narrow size distribution and high formulation uniformity, which is critical for reproducibility and predictable drug release behavior [8].



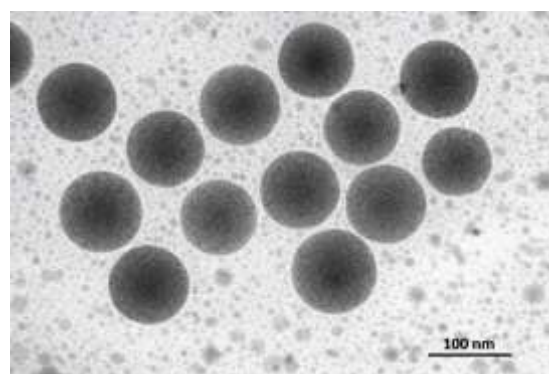
**Figure 1: Physicochemical characterization of acyclovir-loaded nanoparticles determined by dynamic light scattering. The nanoparticles exhibited a mean particle size of 180–210 nm and low PDI values (0.19–0.25), indicating a narrow size distribution and good formulation uniformity.**

Zeta potential measurements demonstrated moderately high surface charge values of –26 to –30 mV, suggesting sufficient electrostatic repulsion to maintain colloidal stability. No aggregation or precipitation was observed during short-term storage under refrigerated conditions, confirming the physical stability of the nanoparticle suspension [9].

Drug loading and encapsulation efficiency analyses indicated efficient incorporation of acyclovir into the polymeric matrix. The drug loading was approximately 8–10%, while encapsulation efficiency exceeded 80%, demonstrating minimal drug loss during formulation and strong interaction

between acyclovir and the polymeric carrier. These values suggest that therapeutically relevant doses could be delivered using a relatively small volume of nanoparticles [10].

Morphological examination by transmission electron microscopy (TEM) revealed predominantly spherical nanoparticles with smooth, well-defined surfaces. The particle sizes observed under TEM were consistent with dynamic light scattering measurements, further validating the uniformity and reproducibility of the formulation (Figure 2).

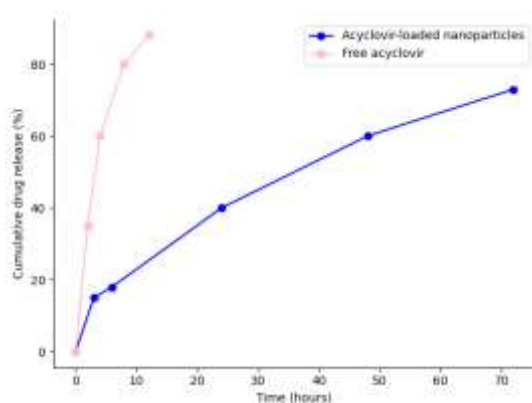


**Figure 2: TEM image illustration of acyclovir nanoparticles**

The cumulative drug release profile of acyclovir from the nanoparticle formulation and free drug is presented in the figure. Free acyclovir exhibited a rapid release pattern, with approximately 35% of the drug released within the first 2 hours, increasing sharply to about 60% at 4 hours and nearly 80% by 8 hours. By 12 hours, cumulative release of free acyclovir approached approximately 88–90%, indicating almost complete drug liberation within a short time frame (Figure 3).

In contrast, acyclovir-loaded nanoparticles demonstrated a slower and sustained release profile. An initial release of approximately 15% was observed at 2 hours, followed by a gradual increase to about 18% at 6 hours. The release continued steadily, reaching approximately

40% at 24 hours, 60% at 48 hours, and about 72–75% at 72 hours. Unlike the free drug, the nanoparticle formulation did not exhibit a pronounced burst release and instead maintained a controlled release over the entire study period. Overall, the nanoparticle formulation showed prolonged drug release compared to free acyclovir, with lower initial release and sustained drug liberation over 72 hours. This indicates that encapsulation of acyclovir within nanoparticles effectively modulated the release kinetics, providing extended drug delivery.



**Figure 3: In vitro release profile of acyclovir from polymeric nanoparticles compared with free acyclovir under simulated physiological conditions (pH 7.4, 37 °C). The nanoparticle formulation exhibited an initial burst release followed by sustained drug release up to 72 h, whereas free acyclovir showed rapid dissolution within the first 12 h.**

## DISCUSSION

The findings of this study demonstrate the successful development of acyclovir-loaded nanoparticles with promising physicochemical and in vitro release characteristics. Acyclovir, although a cornerstone antiviral therapy for herpes simplex virus infections, is limited by poor oral bioavailability, short half-life, and frequent dosing requirements. Nanoparticle-based delivery offers a

potential strategy to overcome these limitations by improving solubility, prolonging systemic exposure, and enabling more controlled drug delivery [10–12].

The nanoscale particle size achieved is particularly important in terms of pharmacokinetics and therapeutic efficacy [13]. Nanoparticles in the sub-200 nm range are known to facilitate enhanced tissue penetration and cellular uptake, which may be critical for targeted antiviral activity against HSV-infected cells. The low PDI indicates a uniform particle population, reducing variability in drug release and absorption and ensuring predictable performance in preclinical and clinical settings [14].

The observed zeta potential values suggest good colloidal stability, which is essential to prevent particle aggregation that could compromise bioavailability and dosing consistency. Colloidal stability also indicates that the nanoparticles are likely to remain intact under physiological conditions, preserving their drug payload until delivery to the target tissue [15].

High encapsulation efficiency and drug loading are important indicators of formulation effectiveness. Efficient incorporation of acyclovir minimizes drug loss during preparation and ensures that therapeutic doses can be delivered with minimal carrier volume, which is advantageous for both patient comfort and manufacturing efficiency [16]. The polymeric matrix likely provides a protective environment that shields acyclovir from rapid degradation and enhances stability, addressing one of the major limitations of conventional acyclovir therapy [17].

Morphological analysis confirmed the spherical and uniform nature of the nanoparticles, which supports their suitability for systemic administration and

controlled release. Spherical nanoparticles generally demonstrate favorable flow and distribution properties, which can improve bio-distribution and reduce the risk of unintended aggregation or deposition [18].

The sustained in vitro release profile is clinically significant. The biphasic release pattern—comprising an initial burst followed by prolonged release—could allow for rapid attainment of therapeutic levels while maintaining effective drug concentrations over time. This sustained release behavior may reduce the frequency of acyclovir administration, improving patient adherence and reducing the risk of missed doses, a common challenge in antiviral therapy [19].

Despite these encouraging results, several limitations should be noted. The current study is limited to in vitro characterization and release studies, which cannot fully predict in vivo pharmacokinetics, bio-distribution, or antiviral efficacy. Additionally, the biocompatibility and potential toxicity of the polymeric carrier were not assessed. Future studies should evaluate safety profiles in relevant cell lines and animal models to ensure that the formulation is non-toxic and well tolerated [20-22].

Finally, the long-term stability of acyclovir within the nanoparticles under varying storage conditions must be assessed, as the chemical stability of both the drug and polymer may influence shelf life and therapeutic reliability. Scaling up the formulation for clinical use and ensuring compliance with regulatory standards, including Good Manufacturing Practice (GMP), will also be critical for translation.

## CONCLUSION

This study successfully developed acyclovir-loaded nanoparticles with nanoscale size, low polydispersity, favorable surface charge, high encapsulation efficiency, and sustained in

vitro drug release. These attributes collectively address the limitations of conventional acyclovir therapy, including poor oral bioavailability, short systemic half-life, and frequent dosing requirements. The sustained release profile suggests that these nanoparticles could provide prolonged antiviral activity, potentially improving therapeutic outcomes and patient adherence.

Overall, acyclovir-loaded nanoparticles represent a promising platform for enhanced antiviral therapy. While the present findings are limited to in vitro evaluations, they provide a strong foundation for further preclinical development and eventual clinical translation for targeted and controlled acyclovir delivery.

## Recommendations

Future research should focus on in vivo pharmacokinetic and pharmacodynamic studies of acyclovir-loaded nanoparticles in appropriate animal models to evaluate systemic exposure, tissue distribution, and antiviral efficacy. Comprehensive safety and biocompatibility assessments, including cytotoxicity, immunogenicity, and long-term toxicity studies, are necessary to confirm the suitability of the polymeric carrier for clinical use.

Extended stability and shelf-life studies under diverse storage conditions should be conducted to ensure formulation integrity and consistent release behavior over time. Further formulation optimization may be undertaken to fine-tune release kinetics, particularly to minimize any initial burst release and achieve precise control over drug delivery.

Process development studies should also address scale-up feasibility and Good Manufacturing Practice (GMP) compliance to support reproducible production. Exploration of alternative administration routes, such as topical, intranasal, or

targeted tissue delivery, could enhance site-specific antiviral activity and patient compliance. Finally, well-designed preclinical trials are essential to validate therapeutic efficacy and safety before translation into human clinical studies.

### **Future Perspectives**

In vivo pharmacokinetic and pharmacodynamic studies are essential to characterize systemic exposure and confirm antiviral efficacy under physiological conditions. Complementary investigations into targeted delivery, especially toward HSV-infected tissues, will help optimize localization of the therapeutic agent and enhance treatment effectiveness. Comprehensive evaluation of cytotoxicity and safety profiles in relevant cell lines and appropriate animal models is also critical to ensure tolerability and minimize off-target effects. Finally, efforts toward scale-up and GMP-compliant manufacturing are necessary to support reproducible production and enable potential clinical translation.

### **ETHICAL CONSIDERATION**

#### **Data Availability**

The datasets generated and/or analyzed during the current study are not publicly available due to, but are available from the corresponding author upon reasonable request.

#### **Conflict of Interest**

The authors declare no conflicts of interest.

#### **Authors' Contributions:**

Bello conceptualized review scope. Prof. Ruiz supervised literature extraction. Dr. Petrova prepared tables and edited manuscript sections.

#### **Ethical Declaration**

The authors declare that this study was conducted in accordance with the ethical standards of the institution and relevant national and international guidelines. No human participants or animals were involved in this research, and therefore formal ethical

approval was not required. All procedures adhered to principles of responsible research and integrity.

### **Funding**

This research received no external funding.

### **Acknowledgement**

The authors would like to sincerely thank all colleagues, research staff, and collaborators who contributed to the conceptualization, design, and execution of this work. Special appreciation is extended to the laboratory and administrative teams for their invaluable technical support and assistance throughout the study. We also acknowledge the insightful feedback provided by peer reviewers and mentors, which greatly improved the quality of this manuscript. Finally, we thank our families for their encouragement and support during the research process.

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