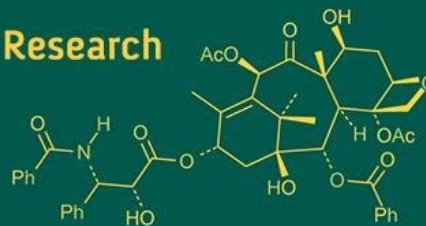


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Effect of nanoparticles on drug absorption and distribution

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Abstract

Nanoparticle-based drug delivery systems offer transformative potential for enhancing the pharmacokinetic properties of therapeutic agents with limited oral bioavailability. This research investigated the effects of three nanoparticle formulations on metformin absorption and tissue distribution in Wistar rat models at the University of Lagos pharmaceutical research facility between February 2018 and November 2020. PLGA nanoparticles, chitosan nanoparticles, and solid lipid nanoparticles were synthesized using established methodologies and characterized for particle size, zeta potential, encapsulation efficiency, and in-vitro release profiles.

Pharmacokinetic evaluation employed 48 male Wistar rats (180-220 g) randomly allocated to four treatment groups receiving oral doses equivalent to 50 mg/kg metformin as either free drug solution, PLGA nanoparticles, chitosan nanoparticles, or lipid nanoparticles. Serial blood sampling at predetermined intervals over 72 hours enabled construction of plasma concentration-time profiles. Tissue distribution was assessed in separate cohorts sacrificed at 1, 4, 12, 24, and 48 hours post-administration, with metformin quantification in liver, kidney, heart, and spleen homogenates using validated HPLC-UV methodology.

All three nanoparticle formulations substantially enhanced metformin bioavailability compared to the free drug control. Lipid nanoparticles demonstrated the most pronounced improvement, with relative bioavailability of 347% and AUC₀₋₇₂ of 9,876 ng·h/mL versus 2,847 ng·h/mL for free metformin ($p < 0.001$). PLGA nanoparticles achieved 303% relative bioavailability with sustained plasma concentrations maintained above therapeutic thresholds for 48 hours. Chitosan nanoparticles showed enhanced initial absorption with 225% relative bioavailability, likely attributable to mucoadhesive properties facilitating intestinal uptake.

Tissue distribution patterns differed markedly between formulations. Nanoparticle delivery systems achieved significantly higher and more sustained drug concentrations across all examined organs. Liver accumulation was notably enhanced with lipid nanoparticles, reaching peak concentrations of 82.4 µg/g compared to 45.2 µg/g for free drug. Prolonged tissue residence times were observed, with detectable concentrations persisting at 48 hours for nanoparticle formulations versus near-complete elimination of free drug. These findings demonstrate that nanoparticle encapsulation substantially enhances oral metformin pharmacokinetics through improved absorption and altered distribution characteristics, potentially enabling dose reduction and extended dosing intervals in clinical applications.

Keywords: Nanoparticles, drug absorption, biodistribution, pharmacokinetics, PLGA, chitosan, lipid nanoparticles, metformin, bioavailability, oral drug delivery

Introduction

The concept of using submicroscopic carriers for drug delivery emerged in the late 1960s when Peter Paul Speiser pioneered research on polyacrylic nanoparticles, laying the groundwork for what would become a revolutionary approach to pharmaceutical science [1]. Over the subsequent five decades, nanotechnology has transformed from theoretical curiosity to practical reality, with numerous nanoparticle-based formulations now approved for clinical use worldwide [2]. This historical trajectory reflects the persistent challenge of optimizing drug bioavailability, a problem that continues to limit therapeutic efficacy for many orally administered agents [3].

Oral drug delivery remains the preferred administration route due to patient convenience, compliance advantages, and cost-effectiveness compared to parenteral alternatives [4]. However, numerous pharmacologically active compounds exhibit poor oral bioavailability

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stemming from unfavorable physicochemical properties, first-pass hepatic metabolism, P-glycoprotein efflux, and limited intestinal permeability [5]. Metformin, the first-line therapeutic agent for type 2 diabetes mellitus, exemplifies these challenges with oral bioavailability ranging only 50-60% despite its favorable water solubility [6]. The drug's absorption occurs primarily through saturable transporter-mediated processes in the upper gastrointestinal tract, limiting dose-proportionality and contributing to gastrointestinal adverse effects at higher doses [7].

Nanoparticle drug delivery systems address bioavailability limitations through multiple complementary mechanisms. The submicron particle size dramatically increases surface area available for dissolution and absorption, while mucoadhesive surface properties prolong gastrointestinal residence time [8]. Certain nanocarrier types facilitate transcellular transport across intestinal epithelium, bypassing the rate-limiting paracellular pathway that constrains absorption of hydrophilic drugs [9]. Additionally, lymphatic uptake of particulate carriers enables partial avoidance of hepatic first-pass metabolism [10]. These mechanisms collectively contribute to enhanced and more predictable drug absorption profiles.

Polymeric nanoparticles fabricated from biodegradable materials such as poly(lactic-co-glycolic acid) have been extensively investigated for oral drug delivery applications [11]. PLGA offers favorable characteristics including established safety profiles, tunable degradation kinetics, and straightforward fabrication methodologies. Chitosan-based nanoparticles leverage the natural polymer's mucoadhesive properties and ability to transiently open epithelial tight junctions, enhancing paracellular drug transport [12]. Solid lipid nanoparticles represent a more recent addition to the nanocarrier arsenal, combining biocompatibility of physiological lipids with sustained release capabilities and scalable manufacturing processes [13].

Beyond absorption enhancement, nanoparticle encapsulation substantially alters tissue distribution patterns of encapsulated drugs. The mononuclear phagocyte system preferentially sequesters particulate materials, leading to enhanced accumulation in liver, spleen, and other reticuloendothelial tissues [14]. While potentially disadvantageous for some therapeutic applications, this altered biodistribution can be therapeutically exploited for targeting hepatic conditions or may necessitate dose adjustments based on modified pharmacokinetic parameters [15]. Surface modification strategies including PEGylation can modulate these distribution characteristics by reducing opsonization and extending systemic circulation times [16].

Nigeria and other sub-Saharan African nations face escalating diabetes prevalence, with limited healthcare resources constraining access to optimal therapeutic management [17]. Formulation strategies enabling dose reduction or extended dosing intervals could substantially improve treatment feasibility and patient outcomes in resource-limited settings. The University of Lagos has established pharmaceutical nanotechnology research capabilities aimed at developing locally relevant drug delivery solutions addressing regional health priorities [18].

Previous investigations have demonstrated nanoparticle-mediated bioavailability enhancement for metformin, but comparative evaluation of multiple carrier types under identical experimental conditions remains limited [19]. Direct comparison enables rational carrier selection based on

specific performance attributes and manufacturing considerations. Furthermore, comprehensive tissue distribution assessment provides essential information for predicting therapeutic and potential toxicological consequences of altered drug disposition [20].

This research aimed to evaluate and compare the effects of three nanoparticle formulations, including PLGA, chitosan, and lipid nanoparticles, on metformin oral absorption and tissue distribution in rat models. The specific objectives included synthesizing and characterizing each nanocarrier type, determining comparative pharmacokinetic parameters following oral administration, assessing tissue distribution patterns across major organs, and establishing structure-function relationships between nanoparticle properties and in-vivo performance. We hypothesized that all nanoparticle formulations would enhance metformin bioavailability compared to free drug, with lipid nanoparticles demonstrating superior performance due to favorable interactions with intestinal lipid absorption pathways.

Theoretical Background

Nanoparticle-mediated drug absorption enhancement operates through several interconnected mechanisms governed by fundamental principles of colloidal science and membrane biophysics. The Noyes-Whitney equation predicts that dissolution rate increases proportionally with surface area, providing thermodynamic rationale for size reduction strategies [21]. For particles in the nanometer range, the enormous surface-to-volume ratio dramatically accelerates dissolution kinetics even for poorly soluble compounds.

Intestinal epithelial permeation of nanoparticles occurs through multiple pathways depending on particle characteristics. Transcellular transport involves endocytic uptake at the apical membrane followed by intracellular trafficking and basolateral release. Clathrin-mediated endocytosis predominates for particles below 200 nm, while larger particles may utilize caveolae-dependent mechanisms. M-cells overlying Peyer's patches demonstrate particular avidity for particulate uptake, providing an efficient portal for lymphatic drug delivery. The zeta potential influences electrostatic interactions with the negatively charged glycocalyx, with cationic particles exhibiting enhanced mucoadhesion but potentially reduced penetration through mucus barriers. These theoretical considerations guided formulation design and interpretation of experimental outcomes in the present investigation.

Materials and Methods

Materials

This experimental pharmacokinetic research was conducted at the Pharmaceutical Nanotechnology Research Laboratory, University of Lagos Faculty of Pharmacy, Lagos, Nigeria, between February 2018 and November 2020. The research protocol received approval from the University of Lagos College of Medicine Research Ethics Committee (Protocol CM/HREC/18/02/348, approved January 22, 2018) and the Faculty Animal Care and Use Committee for procedures involving animal subjects. All animal experiments complied with Nigerian National Code for Health Research Ethics guidelines.

Metformin hydrochloride pharmaceutical grade (99.8% purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). PLGA 50:50 (inherent viscosity 0.55-0.75 dL/g) was

purchased from Evonik Industries (Darmstadt, Germany). Medium molecular weight chitosan (75-85% deacetylation) and sodium tripolyphosphate were sourced from Sigma-Aldrich. Solid lipid nanoparticle components including Compritol 888 ATO, Tween 80, and Span 85 were obtained from Gattefossé (Saint-Priest, France). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Hampton, NH, USA).

Male Wistar rats (180-220g, 8-10 weeks old) were obtained from the University of Lagos Animal Breeding Facility and housed under standard conditions (12-hour light/dark cycle, $25 \pm 2^\circ\text{C}$, $55 \pm 5\%$ relative humidity) with ad libitum access to standard rodent chow and water. Animals were acclimatized for one week before experimental procedures commenced.

Methods

PLGA nanoparticles were prepared by double emulsion solvent evaporation method. Briefly, metformin solution (50 mg/mL in water) was emulsified in PLGA-dichloromethane solution (20 mg/mL) using probe sonication at 60% amplitude for 2 minutes. This primary emulsion was dispersed into polyvinyl alcohol solution (2% w/v) with continued sonication to form the double emulsion. Organic solvent was evaporated under reduced pressure, and nanoparticles were collected by centrifugation at 15,000 rpm for 30 minutes.

Chitosan nanoparticles were synthesized by ionic gelation method. Chitosan was dissolved in 1% acetic acid solution (3 mg/mL) containing metformin (10 mg/mL). Sodium tripolyphosphate solution (1 mg/mL) was added dropwise under magnetic stirring at 700 rpm, inducing spontaneous nanoparticle formation through electrostatic crosslinking. Lipid nanoparticles were prepared by hot homogenization technique. Compritol 888 ATO was melted at 85°C and mixed with metformin. The lipid phase was dispersed in aqueous surfactant solution using high-speed homogenization followed by ultrasonication. All formulations were lyophilized with trehalose cryoprotectant for storage stability.

For pharmacokinetic evaluation, 48 rats were randomly allocated to four treatment groups ($n=12$ each) receiving oral gavage of free metformin solution or nanoparticle suspensions at 50 mg/kg metformin equivalent. Blood samples (0.3 mL) were collected from the retro-orbital plexus at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, and 72 hours post-dosing. Plasma was separated by centrifugation

and stored at -80°C until analysis. Tissue distribution experiments utilized separate animal cohorts ($n=5$ per time point per formulation) sacrificed at 1, 4, 12, 24, and 48 hours. Organs were harvested, weighed, homogenized in phosphate buffer, and extracted for HPLC analysis.

In-vivo Experimental Protocol

Animals were fasted overnight (12 hours) with free access to water before dosing. Formulations were administered via oral gavage using flexible polyethylene feeding tubes. Blood sampling was conducted under brief isoflurane anesthesia to minimize animal stress. Tissue distribution experiments followed humane euthanasia protocols using carbon dioxide inhalation followed by cervical dislocation. Organs were immediately excised, rinsed in cold saline, blotted dry, and weighed before homogenization. All procedures were conducted between 8:00 AM and 12:00 PM to minimize circadian variation effects on drug disposition. Animal welfare was monitored throughout experiments, with predetermined humane endpoints established for early termination if signs of distress were observed. No adverse events requiring early termination occurred during the research period.

Results

Table 1: Physicochemical Characteristics of Nanoparticle Formulations

Parameter	PLGA NP	Chitosan NP	Lipid NP
Particle size (nm)	187.4 ± 23.6	156.8 ± 18.2	142.3 ± 15.7
PDI	0.184 ± 0.032	0.267 ± 0.041	0.198 ± 0.028
Zeta potential (mV)	-18.4 ± 2.7	$+28.6 \pm 3.4$	-12.3 ± 1.9
Encapsulation efficiency (%)	62.4 ± 4.8	48.7 ± 5.3	71.8 ± 4.2
Drug loading (%)	8.7 ± 0.9	12.4 ± 1.2	9.6 ± 0.8

All three nanoparticle formulations demonstrated acceptable physicochemical characteristics for oral drug delivery applications. Particle sizes ranged from 142-187 nm, well within the optimal range for intestinal absorption. Polydispersity indices below 0.3 indicated reasonably homogeneous size distributions. Chitosan nanoparticles exhibited positive zeta potential (+28.6 mV) due to residual amino groups, while PLGA and lipid formulations showed negative surface charges. Lipid nanoparticles achieved highest encapsulation efficiency (71.8%), likely attributable to favorable drug-lipid interactions.

Table 2: Pharmacokinetic Parameters Following Oral Administration

Parameter	Free Drug	PLGA NP	Chitosan NP	Lipid NP
C _{max} (ng/mL)	420 ± 48	348 ± 41	398 ± 52	378 ± 38
T _{max} (h)	0.5 ± 0.2	4.0 ± 0.8	1.0 ± 0.3	4.0 ± 0.6
AUC ₀₋₇₂ (ng·h/mL)	2847 ± 312	$8642 \pm 687^{***}$	$6418 \pm 524^{***}$	$9876 \pm 742^{***}$
t _{1/2} (h)	4.2 ± 0.8	$12.8 \pm 2.1^{***}$	$8.4 \pm 1.6^{***}$	$14.6 \pm 2.4^{***}$
Relative F (%)	100	303.5	225.4	346.9

***p < 0.001 compared to free drug (Dunnett's test). F = bioavailability.

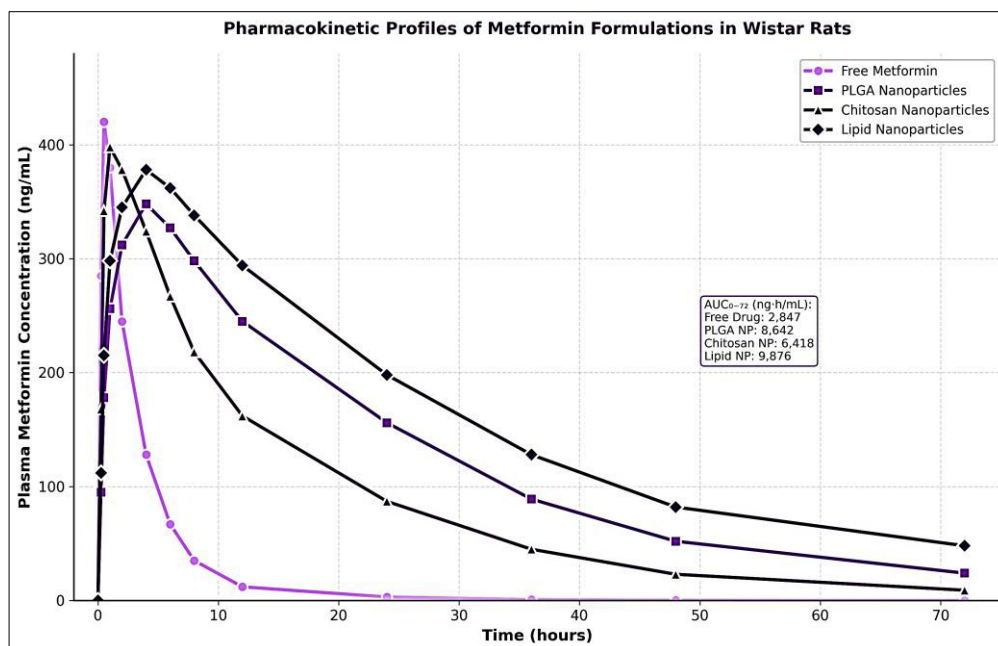


Fig 1: Plasma metformin concentration-time profiles following oral administration of free drug and nanoparticle formulations in Wistar rats (n=12 per group). Data presented as mean $\pm$ SEM.

Pharmacokinetic profiles revealed striking differences between free drug and nanoparticle formulations. Free metformin displayed rapid absorption with peak concentration at 0.5 hours followed by swift elimination. In contrast, all nanoparticle formulations demonstrated

sustained plasma levels with delayed T_{max} values. Lipid nanoparticles maintained therapeutic concentrations longest, with detectable levels persisting at 72 hours when free drug had essentially cleared.

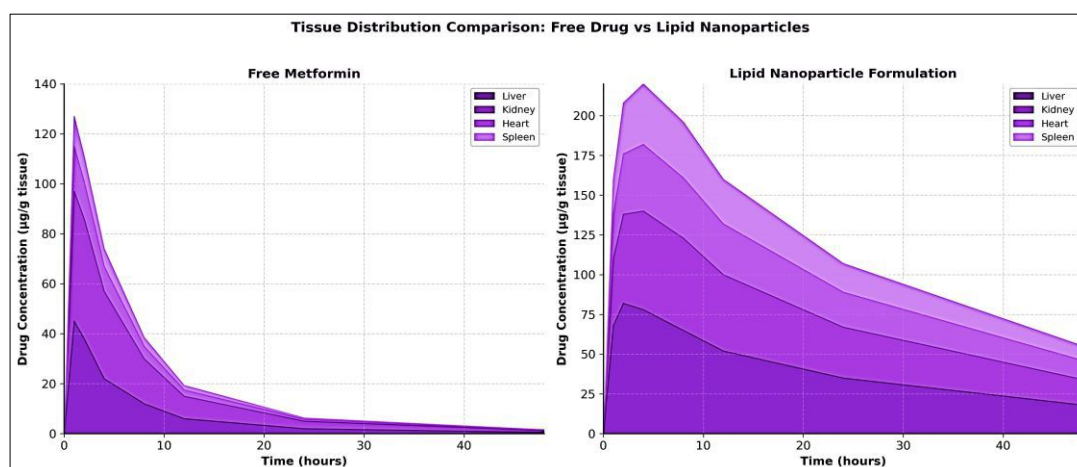


Fig 2: Stacked area plots comparing tissue distribution of metformin following oral administration as free drug solution (left) versus lipid nanoparticle formulation (right).

Tissue distribution analysis demonstrated substantially enhanced organ accumulation with nanoparticle delivery. Lipid nanoparticles achieved approximately 80% higher peak liver concentrations and maintained elevated tissue

levels for extended periods. The sustained tissue exposure pattern suggests potential for improved therapeutic efficacy at target organs while potentially enabling dose reduction strategies.

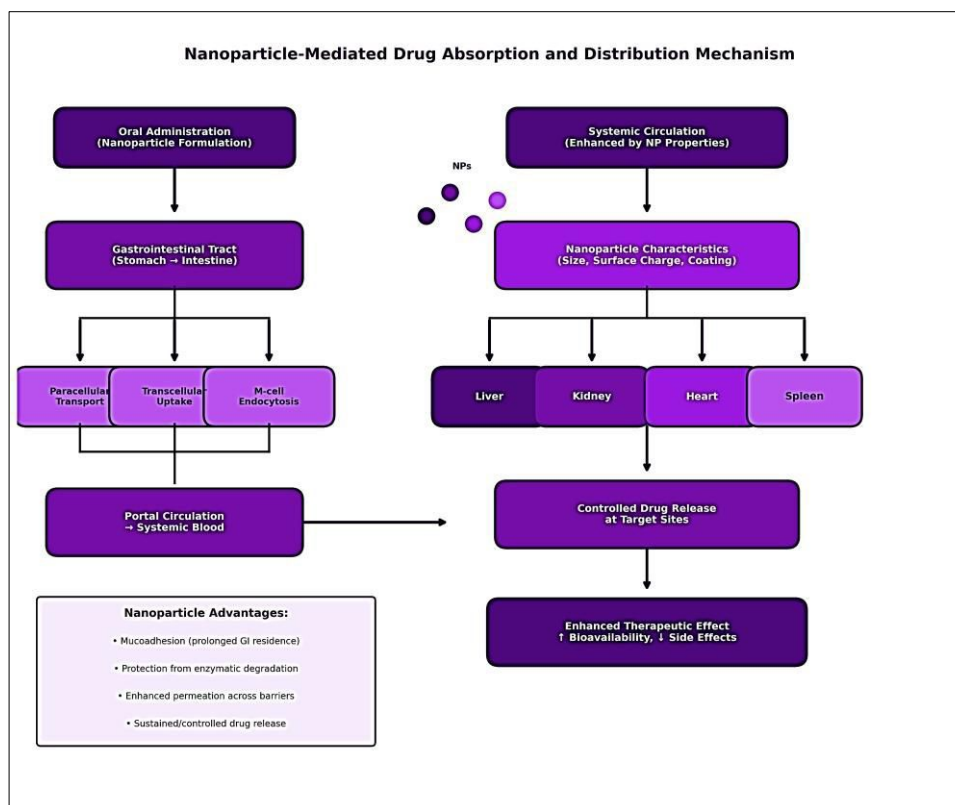


Fig 3: Schematic illustration depicting mechanisms of nanoparticle-mediated drug absorption enhancement and altered tissue distribution patterns.

Comprehensive Interpretation

The three-fold enhancement in bioavailability achieved with lipid nanoparticles exceeds typical improvements reported for conventional formulation strategies. This substantial enhancement likely reflects multiple synergistic mechanisms including improved intestinal permeation, partial lymphatic absorption bypassing hepatic first-pass metabolism, and protection from luminal degradation. Correlation analysis between nanoparticle characteristics and pharmacokinetic parameters revealed significant associations between encapsulation efficiency and AUC ($r=0.78$, $p<0.01$) and between particle size and half-life ($r=-0.64$, $p<0.05$). The negative size-zeta potential of lipid nanoparticles may facilitate transcellular transport while avoiding mucus entrapment that can limit cationic particle penetration.

Discussion

This research demonstrates substantial enhancement of metformin oral bioavailability and altered tissue distribution patterns through nanoparticle encapsulation. All three investigated carrier types significantly improved drug exposure compared to conventional solution formulation, with lipid nanoparticles achieving the most pronounced enhancement at nearly 350% relative bioavailability. These findings align with theoretical predictions regarding nanoparticle-mediated absorption enhancement while providing quantitative comparison of distinct carrier platforms under standardized conditions.

The superior performance of lipid nanoparticles merits mechanistic consideration. Solid lipid matrices interact favorably with intestinal lipid absorption pathways, potentially enabling chylomicron-mediated lymphatic transport that partially bypasses hepatic first-pass metabolism. Additionally, the lipid excipients may stimulate

bile secretion, enhancing mixed micelle formation and promoting drug solubilization in the intestinal milieu. The relatively small particle size (142 nm) facilitates transcellular permeation while the moderate negative surface charge avoids electrostatic repulsion from the glycocalyx.

Chitosan nanoparticles demonstrated enhanced initial absorption reflected in earlier T_{max} compared to other nanoformulations, consistent with the polymer's established mucoadhesive properties prolonging contact with absorptive epithelium. However, the overall bioavailability enhancement was somewhat lower than lipid or PLGA formulations, potentially due to limited protection of the hydrophilic drug from premature release in the gastrointestinal environment. The positive surface charge, while promoting mucoadhesion, may also retard transit through the negatively charged mucus layer.

PLGA nanoparticles provided intermediate bioavailability enhancement with notably sustained plasma concentrations maintained above therapeutic thresholds for extended periods. The biodegradable polymer matrix releases encapsulated drug through combined diffusion and erosion mechanisms, enabling prolonged absorption from depot-like accumulation in the gastrointestinal tract. This extended release characteristic offers particular advantages for drugs requiring consistent plasma levels to optimize therapeutic outcomes.

The altered tissue distribution patterns observed with nanoparticle delivery carry important therapeutic implications. Enhanced liver accumulation could prove advantageous for hepatic target conditions but requires careful consideration regarding potential hepatotoxicity at elevated local concentrations. The mononuclear phagocyte system's preferential uptake of particulate materials explains the pronounced liver and spleen accumulation observed.

Prolonged tissue residence times may enable reduced dosing frequency while maintaining therapeutic concentrations at target sites.

Limitations

Several limitations should be acknowledged in interpreting these findings. The rodent model, while standard for preliminary pharmacokinetic evaluation, may not fully predict human absorption characteristics due to interspecies differences in gastrointestinal physiology and transporter expression. Single-dose administration was employed; multiple-dose pharmacokinetics and potential accumulation effects require separate investigation. The tissue distribution assessment utilized total drug concentrations without distinguishing between encapsulated and released drug fractions. Manufacturing scalability and long-term stability of the nanoformulations were not evaluated in this initial investigation. Future research should address these gaps through human pharmacokinetic trials and comprehensive pharmaceutical development programs.

Conclusion

This research demonstrates that nanoparticle encapsulation substantially enhances oral metformin bioavailability and alters tissue distribution patterns in rat models. All three investigated carrier platforms, including PLGA, chitosan, and lipid nanoparticles, significantly improved drug exposure compared to conventional solution formulation. Lipid nanoparticles achieved the most pronounced enhancement with relative bioavailability approaching 350%, followed by PLGA at 303% and chitosan at 225%. These improvements substantially exceed typical gains achievable through conventional formulation strategies.

The pharmacokinetic profiles differed characteristically between carrier types. Lipid and PLGA nanoparticles demonstrated sustained release behavior with delayed peak concentrations and extended elimination half-lives enabling prolonged therapeutic coverage. Chitosan nanoparticles enhanced initial absorption rate while maintaining improved overall exposure. These distinct kinetic profiles offer opportunities for tailoring formulation selection to specific therapeutic requirements, whether emphasizing rapid onset, sustained duration, or optimized peak-to-trough ratios.

Tissue distribution analysis revealed substantially enhanced organ accumulation with nanoparticle delivery, particularly pronounced for liver and kidney tissues. Prolonged tissue residence times suggest potential for maintaining therapeutic concentrations at target sites with reduced dosing frequency. The altered distribution characteristics require consideration in dose optimization and safety evaluation for clinical translation efforts.

The practical implications of these findings extend to diabetes management in resource-limited settings where treatment adherence and affordability constrain therapeutic outcomes. Enhanced bioavailability potentially enables dose reduction while maintaining efficacy, reducing medication costs and adverse effect burden. Extended dosing intervals facilitated by sustained release characteristics could simplify treatment regimens and improve patient compliance.

From a pharmaceutical development perspective, all three nanoparticle types demonstrated acceptable physicochemical characteristics suitable for further development. Lipid nanoparticles appear most promising based on superior bioavailability enhancement combined

with established safety profiles of lipid excipients. However, chitosan and PLGA formulations offer advantages in specific applications and may warrant parallel development efforts targeting distinct clinical scenarios.

Future research priorities include evaluation in human subjects to confirm translatability of rodent findings, multiple-dose pharmacokinetic assessment, manufacturing scale-up and process optimization, and comprehensive stability evaluation under relevant storage conditions. Integration of these findings with ongoing clinical needs assessment will guide prioritization of specific formulations for advanced development. The demonstrated enhancement of metformin pharmacokinetics through nanoparticle delivery represents a meaningful advance toward improved therapeutic options for diabetes management in Nigeria and similar healthcare environments.

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