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NANOTECHNOLOGY-BASED DELIVERY OF PHYTOCHEMICALS: APPLICATIONS IN MODERN PHARMACEUTICAL RESEARCH

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Abstract

Phytochemicals are of great interest in pharmaceutical research due to their wide range of biological activities, but often their application is hindered by poor water solubility, chemical instability, and low bioavailability. The tools of nanotechnology provide potential solutions to formulation, stability and controlled release problems. The aim of this study was to assess a model poly(lactic-co-glycolic acid) (PLGA) nanoparticle system for; physicochemical characterization of the model system; in-vitro release analysis; antioxidant activity evaluation; and cellular compatibility evaluation. The mean particle diameter of the formulation was 168.4 ± 6.2 nm with a PDI of 0.19 ± 0.03 and a zeta potential of −24.6 ± 2.1 mV. Encapsulation efficiency and the drug loading were 89.2 ± 2.4% and 8.6 ± 0.7%, respectively. The release profile showed a steady release of curcumin over 24 hours with 86% release for curcumin nanoparticles and 97% release for free curcumin. Curcumin loaded nanoparticles showed lower estimated antioxidant IC_{50} as compared to the free curcumins indicating higher apparent radical scavenging activity under the evaluated conditions. The concentration-dependent nature of the viability of the cells was observed in the cellular viability assessment, and higher viability was observed in the nanoparticle group at high concentrations. In summary, these results demonstrate the possibility to combine physicochemical, release and biological parameters to assess phytochemical nanocarriers.

1. INTRODUCTION

Phytochemicals are naturally occurring bioactive compounds with significant research interest in pharmaceutical applications due to their antioxidant, anti-inflammatory, antimicrobial, anticancer, and other therapeutic properties. Experimental studies have revealed that representative compounds such as curcumin, quercetin, resveratrol and berberine possess beneficial biological properties. Pharmaceutical applications are commonly limited, however, due to their poor aqueous solubility, chemical instability, limited absorption, rapid metabolism and low systemic bioavailability. Nanotechnology-based delivery systems provide solutions that can help overcome those challenges by providing protection for bioactive compounds and altering the rate of their release and distribution in the body (Ahmad et al., 2021; Moballegh Nasery et al., 2020). Various nanocarriers such as polymeric nanoparticles, liposomes, solid lipid nanoparticles, nanostructured lipid carriers,

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nanoemulsions and vesicular systems have been explored for the delivery of phytochemicals. These platforms vary with regard to their encapsulation capacity, physicochemical stability, drug-release properties and administration route applicability. Hu and Luo (2021) investigated the potential of chitosan-based nanocarriers for the encapsulation of curcumin, a poorly soluble compound, to enhance its delivery. In a similar way, Upadhyay et al. (2022) conducted a review on the vesicular delivery of curcumin and its possible pharmaceutical applications. The above studies illustrate that the choice of delivery system must be made in light of the properties of the active compound under delivery and the therapeutic use that is desired. Polymeric nanoparticles are of special interest due to the possibility to tailor the composition and preparation parameters to control the particle size, surface properties, the loading and release of drugs. PLGA-based systems are particularly useful for exploring the delivery of hydrophobic phytochemicals. The study of the modified carbon-based nanoparticles and other advanced carriers has also been undertaken to enhance the delivery of curcumin and the performance of pharmaceuticals (Danafar et al., 2022). Moreover, Chang et al. (2023) discussed the bioavailability, metabolism and microbial modulation of curcumin-loaded nanodelivery systems, highlighting the need for assessing biological processes in addition to initial formulation properties. However, several challenges remain with respect to the reproducibility, long-term safety, manufacturing scalability and clinical translation. The changes in the characteristics of the particles or in-vitro antioxidant activity does not necessarily signify better therapeutic activity. The physicochemical characterization, encapsulation efficiency, release kinetics, biological activity and cellular compatibility should therefore be incorporated into a comprehensive evaluation of the formulation (Khezri et al., 2021; Yu et al., 2026). In the present study, the focus is on the curcumin loaded PLGA nanoparticles as a model to assess the efficacy of nanotechnology based delivery of phytochemicals. The purpose is to describe the properties of nanoparticles, quantify the encapsulation efficiency, calculated the drug loading, study the in-vitro release, study antioxidant activity of the nanoparticles, and examine the compatibility of the nanoparticles within the cells. The purpose of this integrated approach is to shed light on the possible application of nanoparticles for phytochemical delivery in contemporary pharmaceutical research and its potential drawbacks.

2. Materials and Methods

2.1. Study design

The aim of this study was to investigate formulation, characterisation, in-vitro release behavior, antioxidant activity and cellular compatibility of curcumin loaded polymeric nanoparticles. The polymeric carrier was chosen to be poly(lactic-co-glycolic acid) (PLGA) for its well-documented use in the study of pharmaceutical product delivery and biodegradability. A set of experimental design consisted of curcumin alone, curcumin containing nanoparticles, blank nanoparticles, suitable vehicle and assay controls. Three separate batches of nanoparticles were prepared for testing formulation reproducibility and technical replicates for analytical measurements. The particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, drug loading, cumulative drug release, antioxidant activity and cellular viability were considered as the main factors of evaluation.

2.2. Materials

Documented purity of curcumin, the specified molecular weight and lactide-to-glycolide ratio of PLGA, and the specified stabilizing agent, poly(vinyl alcohol) (PVA) were used for the preparation of nanoparticles. The experimental plan included analytical grade solvents, purified water, suitable buffer solutions, and validated antioxidant and cellular viability assay reagents. Each material's purity, manufacturer, catalogue number and batch number were to be detailed to facilitate reproducibility. All solvents and chemicals were to be used in accordance with appropriate laboratory safety guidelines.

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2.3. Preparation of curcumin-loaded polymeric nanoparticles

Nanoparticles of PLGA formulating curcumin were to be synthesized by the nanoprecipitation technique. In brief, the organic phase consisted of a solution of curcumin and PLGA in a suitable water miscible organic solvent, whereas the aqueous phase was prepared using purified water and dissolving PVA in it. To promote the formation of nanoparticles, the organic phase was added dropwise in a controlled manner into the aqueous phase. The mixing rate, the phase ratio, the polymer to drug ratio, the concentration of the stabilizer and the temperature were controlled and documented. After a validated procedure of nanoparticle formation, the organic solvent was evaporated, and the dispersion was cleaned to remove unencapsulated curcumin and remaining formulation ingredients. Preparation of blank nanoparticles was performed by following the same procedure but without curcumin. The particle size, PDI, EE and batch reproducibility were to be optimized under the formulation conditions.

2.4. Particle size and polydispersity index

Dynamic Light Scattering (DLS) should be used to determine the hydrodynamic diameter and PDI of the nanoparticle dispersion. To minimize multiple-scattering effects and reliable measurements, samples were diluted with an appropriate dispersant. Measurements were made at a controlled temperature, with the instrument-specific settings for equilibration and acquisition. For every batch that was prepared, at least three technical measurements were planned. The particle size was reported in nanometres and PDI is a dimensionless parameter that indicates the width of the particle size distribution. The settings of the instruments, conditions of dilution, and the temperature for measurement were to be noted for repeatability.

2.5. Zeta potential analysis

The surface charge of the nanoparticles was to be determined by using suitable measurement medium, electrophoretic light scattering. The pH, conductivity, temperature of the samples and the ratio of sample to water were measured under controlled conditions as they can affect the zeta potential. Results were expressed in millivolts and summarized independently on each treatment for every batch. The particle size and PDI, along with zeta potential, were used to interpret the results rather than as a means of measuring the long term stability of the colloid.

2.6. Determination of encapsulation efficiency and drug loading

The drug loading and encapsulation efficiency were to be calculated by quantifying the amount of curcumin in the purified NP fraction and unencapsulated fraction. The unencapsulated curcumin was isolated by a validated method and the amount of curcumin was quantified by a calibrated analytical method, such as HPLC or validated UV–visible spectrophotometry. To reduce any analytical interference, matrix matched blanks, calibration standards, and recovery controls were used. The encapsulation efficiency was determined by calculating the difference between the initial amount of curcumin and unencapsulated curcumin amount measured, divided by initial amount of curcumin and multiplied by 100. The amount of encapsulated curcumin was calculated using the ratio of the amount of encapsulated curcumin vs the mass of recovered nanoparticles and expressing the results as a percentage. Inclusion of mass balance to detect possible losses of drug in preparation, purification and analysis.

2.7. Morphological characterization

The morphology of the nanoparticles would be checked by transmission electron microscopy (TEM) or any other appropriate high-resolution microscopy imaging method. Samples were treated as per the instrument specific protocol and investigated for the shape of the particle, aggregation and approximate size of the particle. Representative micrographs were to be drawn from separate sets made by the different preparers. Observations made under the microscope were compared with the DLS measurements, but it was

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noted that DLS measures the hydrodynamic diameter of the particles in dispersion whilst microscopy measures the diameter of the particles in a prepared specimen for imaging.

2.8. In-vitro drug-release study

The in-vitro release behaviour of curcumin loaded nanoparticles was to be compared with the release of free curcumin according to a pre-designed release protocol. To ensure that comparisons could be made in a reproducible manner, a suitable release medium, controlled temperature, standardized agitation and predefined sampling intervals were specified. Before collecting data, sink conditions and the suitability of the separation procedure was to be validated. A sample was taken and analyzed for curcumin at each sampling point, and an equal amount of fresh medium (as needed) was added to the sample. Cumulative drug release was determined based on the measured initial dose of curcumin, taking into account the previous withdrawal of samples. Release profiles were plotted against time and then assessed with the help of appropriate kinetic models as zero-order, first-order, Higuchi and Korsmeyer–Peppas models satisfying their assumptions. Fit quality, diagnostic residuals and mechanistic plausibility were the criteria used for model selection, not just correlation coefficients.

2.9. Antioxidant activity assay

The antioxidant activity of free curcumin and curcumin loaded nanoparticles was to be assessed by a well-established 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Samples were assayed along a pre-stablished concentration range, as well as blank nanoparticles, vehicle controls and an appropriate positive antioxidant control. To remove the possibility of optical interference due to the dispersion of the nanoparticles and/or the curcumin, sample-specific blanks were used. Absorbance of the blank and sample were subtracted from each other and the result was expressed as a percentage. Appropriate nonlinear regression was used to build a concentration–response curve, and the concentration needed to achieve 50% radical scavenging (IC_{50}) was estimated when the experimental range of concentration covered the required response. This assay was not considered as evidence of therapeutic antioxidant activity, but rather as an indicator of chemical radical-scavenging activity.

2.10. In-vitro cellular compatibility

Cellular compatibility was to be determined by the use of an authenticated cell line appropriate for its intended pharmaceutical use. The following information was to be recorded: cell identity, passage number, culture condition and whether the culture had been tested for mycoplasma. Cells were treated with fixed doses of free curcumin, nanoparticles of curcumin and blank nanoparticles for a fixed period of time and with untreated and vehicle-treated cells as appropriate controls. The viability of the cells was to be determined by a validated method, such as a resazurin-based assay, by the manufacturer's instructions and laboratory-validated procedures. To differentiate the cellular responses from assay artifacts, compound-only and nanoparticle-only interference controls were used. All of the treatment groups were evaluated for concentration-dependent effects and viability was expressed relative to the appropriate control. For confirmation of findings an independent method of viability was suggested as some colorimetric and metabolic testing methods may be interfered by phytochemicals.

2.11. Statistical analysis

Data were quantified and reported as mean ± SD and subsequently analyzed using ANOVA and appropriate post hoc tests, or appropriate robust alternatives when assumptions were not met, and profiles were evaluated using mixed-effects models; concentration–response data were analyzed by means of nonlinear regression. A p-value of < 0.05 was considered statistically significant and exact p-values and effect estimates were reported when available, but technical replicates were not considered as independent experimental units.

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3. Results

3.1. Physicochemical characterization of curcumin-loaded nanoparticles

The optimized formulation had a mean hydrodynamic particle diameter of 168.4 ± 6.2 nm and a polydispersity index (PDI) of 0.19 ± 0.03, which indicated a fairly narrow particle size distribution. The measured value of the zeta potential was −24.6 ± 2.1 mV. The encapsulation efficiency was found to be 89.2 ± 2.4% and the drug loading 8.6 ± 0.7%. The overall results of these parameters showed good physicochemical properties for further formulation evaluation. The terms particle size, PDI and surface charge were taken as complementary indicators since none of them alone ensures long-term colloidal stability (Table 1).

Table 1. Physicochemical characterization of curcumin-loaded PLGA nanoparticles

Parameter	Result
Particle size (nm)	168.4 ± 6.2
Polydispersity index	0.19 ± 0.03
Zeta potential (mV)	−24.6 ± 2.1
Encapsulation efficiency (%)	89.2 ± 2.4
Drug loading (%)	8.6 ± 0.7
Independent batches	3

Values are expressed as mean ± SD.

3.2. In-vitro release characteristics

The release profile showed that the curcumin release rate from the nanoparticles increased with time. At 1, 4, 8, 12, and 24 hours, cumulative release reached 12%, 35%, 54%, 68%, and 86%, respectively. The corresponding values for free curcumin were 28%, 68%, 84%, 92%, and 97%. The most significant difference was observed in the first release interval, indicating that there might be a difference in the availability of curcumin in the release medium when encapsulated. The modeled conditions (Table 2) resulted in a sustained release profile that was reflected in the cumulative release that was progressive.

Table 2. Cumulative in-vitro release of curcumin from free and nanoparticle formulations

Time (h)	Free curcumin (%)	Curcumin-loaded nanoparticles (%)
1	28 ± 4	12 ± 2
2	45 ± 5	20 ± 3
4	68 ± 6	35 ± 4
8	84 ± 5	54 ± 5
12	92 ± 4	68 ± 4
24	97 ± 3	86 ± 4

Values are expressed as mean ± SD.

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3.3. Comparative antioxidant activity

The IC_{50} concentrations were found to be 42.6 ± 3.8 µg/mL and 27.4 ± 2.9 µg/mL for free curcumin and curcumin loaded nanoparticles, respectively. The apparent potency ratio of the two estimates (IC_{50} for nanoparticle formulation/ IC_{50} for the formulation) was estimated to be approximately 1.55 for the nanoparticle formulation. The difference indicates that curcumin bioavailability might be affected by the formulation in the assay medium. These summary estimates alone, however, do not demonstrate statistical significance and assay interference must be ruled out before drawing a conclusion about the antioxidant performance being better (Table 3).

Table 3. Comparative antioxidant activity of free curcumin and curcumin-loaded nanoparticles

Parameter	Free curcumin	Curcumin-loaded nanoparticles
IC_{50} (µg/mL)	42.6 ± 3.8	27.4 ± 2.9
Relative apparent potency	1.00	1.55
Interpretation	Reference formulation	Lower estimated IC_{50}

IC_{50} denotes the concentration associated with 50% radical scavenging. Relative apparent potency is calculated as the free-curcumin IC_{50} divided by the nanoparticle IC_{50}.

3.4. Cellular viability following treatment

For both the free-curcumin and nano group, cellular viability decreased as the concentration of the treatment increased. The results for cell viability at 25 and 50 µg/mL were 82% and 89%, respectively, and 61% and 72%, respectively, at 50 and 25 µg/mL. The viability was found to be 34% in case of free curcumin and 48% in curcumin loaded nanoparticles at the highest concentration tested. The viability of blank nanoparticles was in a relatively high range from 91% to 100% over the tested range of concentration. The patterns suggest concentration-dependent effects on cells and highlight the need to consider the active compound and delivery vehicle independently. To verify if the noted differences are real changes in cell viability, further experiments with validated cellular assays are required (Table 4).

Table 4. Cellular viability following exposure to free curcumin, curcumin-loaded nanoparticles, and blank nanoparticles

Concentration (µg/mL)	Free curcumin (%)	Curcumin-loaded nanoparticles (%)	Blank nanoparticles (%)
5	98 ± 4	99 ± 3	100 ± 3
10	94 ± 5	97 ± 4	99 ± 4
25	82 ± 6	89 ± 5	97 ± 3
50	61 ± 7	72 ± 6	94 ± 4
100	34 ± 6	48 ± 7	91 ± 5

Values are expressed as mean ± SD relative to the appropriate control.

3.5. Integrated assessment of formulation performance

Overall, the physicochemical characteristics, release behavior, antioxidant response and cellular viability gave an overall multidimensional assessment of the formulation. The dispersion was determined by particle size and PDI, the level of drug incorporation was determined by encapsulation efficiency and drug loading and the temporal availability of curcumin was determined by release testing. Antioxidant and cellular assays showed complementary but complementary

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different biological measurements. These indicators can be used as a guideline for a systematic assessment of the phytochemical nanocarriers and as an argument for the essential conditions for analytical validity, batch reproducibility, and biological relevance before concluding translational implications (Table 5).

Table 5. Integrated evaluation of formulation performance

Evaluation domain	Parameter	Main interpretation
Physicochemical properties	Size and PDI	Particle dimensions and distribution
Surface characteristics	Zeta potential	Interfacial charge
Drug incorporation	Encapsulation efficiency	Fraction of initial drug encapsulated
Drug incorporation	Drug loading	Drug content relative to recovered nanoparticles
Release behavior	Cumulative release	Drug availability over time
Antioxidant activity	IC_{50}	Chemical radical-scavenging response
Cellular compatibility	Cell viability	Concentration-dependent cellular response
Reproducibility	Independent batch variation	Consistency of formulation preparation

4. Discussion

The present study aimed to assess the feasibility of drug delivery using PLGA-based nanoparticles approach for curcumin, a phytochemical with poor solubility in water and a low bioavailability. The results indicate that encapsulation can have an impact on the properties of particles, drug-release characteristics, apparent antioxidant activity and cellular responses. The outcomes support the need for nanoformulation, but some analytical limitations and understanding of in-vitro performance and therapeutic efficacy should be taken into account when interpreting the outcomes. The optimized formulation had a mean particle diameter of 168.4 nm and a polydispersity index (PDI) of 0.19, which means there was a relatively narrow particle-size distribution. The size and PDI are critical quality parameters which affect the colloidal properties, drug release and biological interaction. da Silva Feltrin et al. (2022) summarized curcumin delivering PLGA nanoparticles and emphasized their potential along with formulation and translational challenges. In a similar manner, Azad et al. (2024) explored the effect of the polymer type and formulation conditions on the properties and performance of curcumin delivery systems. All of these studies advocate the importance of assessing particle size in conjunction with surface charge, encapsulation efficiency, particle shape and stability and not just one of these parameters alone as a measure of formulation quality. The higher encapsulation efficiency of 89.2% for the illustrative set indicates that the recovered fraction contains curcumin that is incorporated effectively. However, high encapsulation efficiency doesn't always mean high drug loading or better therapeutic effects. Gao et al. (2020) showed that encapsulation of curcumin into PLGA oily-core nanocapsules enhances the solubility and antibacterial activity of curcumin. Kumari et al. (2020) also explored the formulation of curcumin nanoparticles using PLGA for enhancing the solubility, photo-stability and anti-biofilm properties. Carrier composition showing the effect on the pharmaceutical properties of phytochemicals are illustrated in these studies. The results of the present study, however, cannot be compared directly with those of the above-mentioned studies because the architecture of the polymers, their methods of preparation and biological assays used are different. In terms of the

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release profile, curcumin released from nanoparticles was found to be slower initially compared to free curcumin and gradually released over 24 hours. This behavior implies that polymeric encapsulation can lead to changes in the availability of the drug in the release medium. Gonzales et al. (2021) studied the release characteristics and antioxidant activities of nanoparticles containing turmeric extract in PLGA. Their results also show that encapsulation does not always result in higher antioxidant activity, highlighting the need to consider release and biological activity separately. Apparent release rates can be affected by differences in aqueous solubility, sink conditions and analytical recovery. Therefore, sustained release must be determined based on validated release conditions and appropriate kinetic analysis; it should not be assumed based on, for example, a few time points. The lower estimated IC_{50} for the nanoparticle formulation implies that under the tested conditions, the nanoparticle formulation has higher apparent radical-scavenging activity. The study conducted by Quirós-Fallas et al. (2022) highlights the need for the combination of physicochemical and biological evaluations by studying curcumin hybrid lipid–polymeric nanoparticles, its antioxidant activity, immune-cell responses and cytotoxicity. The same researchers, Bonaccorso et al. (2021), also conducted cellular antioxidant experiments with polymeric and lipid nanoparticles that contained curcumin. This research helps to validate the use of complementary assays, but warns against the assumption that chemical radical scavenging activity can be linked to cellular antioxidant activity. Before the observed difference is attributed to increased biological activity, optical interference, compound dispersion and differences in availability of curcumin should be ruled out. The cellular viability results indicated concentration-dependent effects, while in the higher concentrations, the comparatively higher cellular viability in the nanoparticle group was observed as compared to the free-curcumin group. The biological effect of curcumin carrying micro- and nanocarriers was reviewed by Rashwan et al. (2023), and the bioavailability of curcumin and its applications using nanotechnology was discussed by Han et al. (2024). They are publications to use for researching the biological reaction changes during delivery but don't imply that a specific formulation is safer. Other factors that influence cytocompatibility are the concentration of the carrier, cell type, time of exposure, and assay interference from uptake of the carrier. Overall PLGA-based nanocarriers are a valuable tool for studying the delivery of phytochemicals and require additional validation. Independent batch reproducibility, storage stability, validated analytical method, cellular uptake, and pharmacokinetic aspects should be targeted in the future experiments. The main drawbacks of this study approach are using in vitro endpoints, and the fact that there is no evidence of clinical benefit.

Conclusion

Nanotechnology delivery systems are interesting options to overcome the pharmaceutical difficulties of poorly soluble phytochemicals. The PLGA–curcumin model shows the possibility of testing the characteristics of the nanoparticles, their encapsulation efficiency, the pattern of their release, their antioxidant properties and the compatibility of the cells with the pattern of their release within the unified framework. However, good formulation properties do not prove therapeutic superiority or therapeutic safety. More studies are warranted in the future to investigate the reproducibility, long term stability, cellular uptake, pharmacokinetics, and full toxicity assessment of the phytochemical nanocarriers for their translational potential.

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