International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 21s, 2025
https://theaspd.com/index.php

Preparation And Characterization Of Mangiferin Encapsulated Albumin Nanoparticles For The Treatment Of Inflammation

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Abstract
The objective of the current investigation was to develop albumin nanoparticles loaded with mangiferin for prolonged release and improving half-life and bioavailability of manfigerin. The nanoparticles were prepared from bovine serum albumin using ethanol as the desolvating solvent and glutaraldehyde as the crosslinking agent. The optimization of pH, crosslinking time and volume of desolvating solvent was done to obtain lowest particles size, high entrapment efficiency. All the formulations were obtained in yield ranging from 73.5 to 88.7%. The particle size of the nanoparticles ranged from 167.3 to 267.1 nm. The zeta potential of all the formulations was negative and ranged from -29.1 to -31.5 meV. The high zeta potential helps in preventing the aggregation of the particles. The percent entrapment of mangiferin in the formulations ranged from 60.7 to 88.6%. F7 was selected as the best formulation (88.6% entrapment and 195.6 nm size) prepared using 50 mL desolvating solvent (ethanol), pH 8.0 and crosslinking time of 24 hours. Mangiferin was released from the standard solution in 4 hours (100.6%). On the other hand 98.2% mangiferin was released from the albumin nanoparticles F7 in 24 hours. The mangiferin loaded albumin nanoparticles exhibited the inhibition of albumin denaturation comparable to pure mangiferin. The albumin nanoparticle formulation F7 had shown the inhibition capacity (84.63 ± 0.249%). The inhibition protein denaturation by 100 µg/mL solution of standard drug mangiferin was found to be 87.60 ± 0.509%.
Keywords: *Mangiferin, nanoparticles, albumin, bioavailability, desolvating*

INTRODUCTION

Nanotechnology has shown great potential in the area of drug delivery. In particular, nanomaterials allowed the development of platforms for the efficient administration, protection, transport, and specific delivery of challenging therapeutic or diagnostic cargos, such as poorly soluble drugs, proteins, and gene therapeutics, in biological fluids toward cellular and intracellular targets.[1,2]

Albumin is one of the most abundant and important proteins in the body and has high binding capacities for both hydrophobic and hydrophilic drugs, relatively long half-life, specific targeting of inflammation sites, as well as virtually minimal toxicity and immunogenicity.[3] Additionally, natural affinity of many drugs for binding to albumin has been known and this allows for the possibility for chemical conjugation of drugs to albumin nanoparticles. Also the surface of the albumin-based nanoparticle can also be functionalized with ligands due to the presence of functional groups to which different types of linkers or spacers can be attached.[4]

Mangiferin is a flavonoid obtained from Mangifera indica and has been found to have role in treating diabetes, as antioxidant and anti-inflammatory agent. It has a short half-life and exhibits poor bioavailability.[5] Hence it was thought upon to utilize bovine serum albumin for preparing nanoparticle loaded with mangiferin in order to help its localization in the inflammation site and to prolong the release of the encapsulated drug (improved half-life and bioavailability). The objective of the present work was to prepare and evaluate mangiferin loaded albumin nanoparticles and compare the anti-inflammatory activity of the nanoparticle with the pure drug.

MATERIAL AND METHODS

Mangiferin was purchased from Yucca enterprises, Mumbai; bovine serum abumin (BSA) was purchased from Himedia. All reagents/chemicals were used without purification.

Preformulation studies

The melting point was determined by open capillary method and solubility was qualitatively assessed.[6] Calibration curve of mangiferin was prepared in methanol by measuring absorbance of solution at 257 nm using UV-Visible spectrophotometer (Labtronics,LT-2201).[7]

Preparation of Albumin Nanoparticles

The albumin nanoparticles were prepared by desolvation method. An appropriate amount of bovine serum albumin was accurately weighed and dissolved in 10 mM sodium chloride solution in water (Table 1). The pH of this solution was adjusted to 4.9, 8 or 9 using 0.1 N sodium hydroxide solution. A magnetic stirrer at 500 rpm at room temperature was used to prepare mangiferin-loaded nanoparticles. First, mangiferin (50 mg) was dissolved in ethanol (the desolvating agent), and then was continuously added to the previously prepared solution at a rate of 1 mL/min. After the addition of ethanol, cross-linking of the nanoparticles was induced by adding 8% glutaraldehyde solution in distilled water. The cross-linking process was continued by stirring for the specified period of time. The nanoparticles were further purified by the complete removal of ethanol using vacuum evaporation, followed by re-dispersion of the pellets in the same volume of 10 mM solution of NaCl in water solution.[8,9]

Table 5.1 Design table for formulating albumin nanoparticles

Formulation	BSA (mg)	pH	Ethanol (mL)	Crosslinking Time
F1	50	8.0	5	12
F2	50	9.0	5	12
F3	50	8.0	50	12
F4	200	9.0	50	12
F5	200	8.0	5	24
F6	200	9.0	5	24
F7	200	8.0	50	24
F8	200	9.0	50	24

Characterization of nanoparticles

Yield, particle size and zeta potential

To determine the percent yield of synthesis, the NP powder sample obtained was weighed, and the percent yield was calculated using the formula:

$$\text{Percent Yield} = \frac{\text{Weight of nanoparticles}}{\text{Weight of drug} + \text{BSA}} \times 100$$

The size of prepared albumin nanoparticles was measured by laser light scattering technique using particle size analyser.

Entrapment Efficiency

The amount of drug adsorbed or encapsulated in the albumin NPs was determined from the amount of free mangiferin in the supernatant after the centrifugation process, determined by UV spectroscopy. The nanoparticles after purification were centrifuged at 5000 rpm and the supernatant was collected and analyzed for the amount of non-encapsulated mangiferin. The percent entrapment was calculated using the formula:

$$\text{Percent entrapment} = \frac{\text{Total mangiferin used} - \text{Mangiferin in supernatant}}{\text{Total Mangiferin}} \times 100$$

In vitro drug release

Dialysis bag method was used to assess the in vitro release of mangiferin from albumin nanoparticles, and the corresponding mangiferin standard dispersion in water. Briefly, mangiferin albumin nanoparticles corresponding to 3 mg mangiferin or 3 mg mangiferin in 10 mM solution of NaCl in water, were placed in cellulose dialysis bags (cutoff 12,000–14,000 Da), which were immersed in 100 mL phosphate buffer saline (PBS) with pH 7.4 at 37 °C, under constant shaking at 100 rpm. The dialysis bags, presoaked in PBS, were firmly clipped from both ends before being immersed in the release medium. Two-milliliter samples were withdrawn at 0, 0.5, 1, 2, 4, 8, 12, and 24 h, and were replaced with fresh buffer to maintain sink conditions. The samples were measured spectrophotometrically to determine the concentration of each sample.[10]

In vitro anti-inflammatory activity

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution. The mangiferin albumin nanoparticles was dissolved in DMSO and appropriately diluted to prepare sample solution of concentration 1 µg/mL. A solution of 1% BSA in deionized water was prepared for the test. Tramadol solution of concentration 1 µg/mL was used as the positive control. The test containers were filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solution. Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels in place of extract. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined.[11,12]

Results and Discussion

The procured sample of mangiferin was white in color with melting temperature range of 157-159°C. It was partially soluble in water and soluble in ethanol and methanol. The calibration curve was prepared for a range of 10-50 µg/mL (Figure 1).

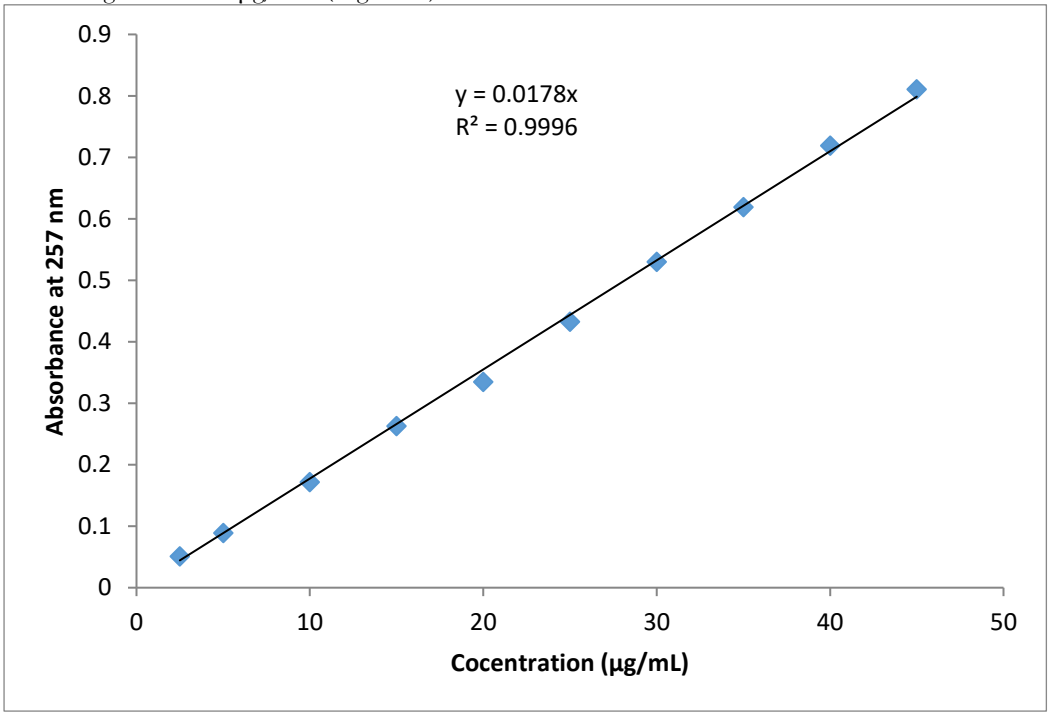

Figure 1 Standard curve of mangiferin

Preparation of albumin nanoparticles

The mangiferin loaded albumin nanoparticles were prepared by desolvation method. The effect of pH, desolvation volume and crosslinking time on entrapment efficiency and particle size of the nanoparticles was studied. Changing pH significantly alters the net charge on the protein surface, since it consists of hydrogens of amino acids that interact with different ions in solution. The isoelectric point of albumin is around 4.9, and at this pH the net charge on the protein surface is zero. At pH 4.9, there is a lack of electrostatic repulsion, and thus amorphous aggregates are readily formed through nonspecific interactions, mainly hydrophobic in nature.

At other pHs, positive charges may be generated by protonation of primary amino groups (particularly lysine), while negative charges are generated from deprotonation of carboxylic acid groups (glutamate and aspartate). Charges are generated on the whole surface of the protein, leading to greater electrostatic repulsion between molecules and decrease in hydrophobic interactions, thereby reducing aggregation and favoring a structural reorganization of the protein. Moreover, they can significantly alter the interaction and/or binding of compounds (drugs).

Another important component in the process of NP formation is the amount of cross-linking agent (glutaraldehyde) added. Glutaraldehyde does not change the size of the NPs but significantly alters the

charge on the surface of the NPs obtained. Glutaraldehyde is a low-cost, water-soluble bifunctional reagent with high reactivity.

The effect of formulation variables (pH, crosslinking time and volume of desolvating agent) on particle size and entrapment efficiency were studied. The particle size and in vitro release of the formulation were also studied.

Characterization of nanoparticles

Yield

The yield of albumin nanoparticles were determined by weighing the lyophilized nanoparticles. All the formulations were obtained in yield ranging from 73.5 to 88.7%. It was found that pH of formulation affected the yield with a higher yield at pH 9.0. Also an increase in the desolvating solvent increased the yield of the formulations (Table 2).

Particle size

The effect of formulation parameters on particle size was determined by measuring the particle size of the formulations using particle size analyzer (Table 6.6). The particle size was significantly affected by pH and crosslinking time. It was found that at pH 9.0, smaller particles were obtained in comparison to pH 8.0 (Table2). Crosslinking for 24 hours was able to produce smaller particles.

Zeta potential

The ability of the particles to overcome electrostatic attraction as well as vanderwaals interaction is measured by the zeta potential. The zeta potential of all the formulations was negative and ranged from -29.1 to -31.5 meV. The high zeta potential helps in preventing the aggregation of the particles (Table 2).

Entrapment Efficiency

The entrapment of manfigerin in the albumin nanoparticles was affected by the crosslinking time, pH and volume of desolvating solvent. At higher desolvating volume, lower pH and high crosslinking time, better entrapment was achieved (Table 2).

The best formulation was selected on the basis of particle size and entrapment efficiency. The formulation with lowest particle size and highest entrapment of mangiferin was selected for in vitro release and anti-inflammatory study. The highest entrapment was found in F7 (88.6%). Though F8 presented lower particle size (167.3 nm), due to low entrapment compared to F7, it was not selected as the best formulation. Hence F7 was selected as the best formulation (88.6% entrapment and 195.6 nm size).

Table 2 Yield, size and potential of formulations

Formulation	Yield (%)	Particle size (nm)	Zeta potential (nm)	Percent Entrapment
F1	73.5	267.1	-29.1	68.4
F2	77.2	235.3	-30.8	60.7
F3	78.1	242.7	-31.3	77.5
F4	82.3	211.3	-31.6	72.4
F5	76.2	222.9	-30.5	74.1
F6	80.4	201.4	-29.8	70.2
F7	84.6	195.6	-29.8	88.6
F8	88.7	167.3	-31.5	79.7

In vitro release

The release of mangiferin from albumin nanoparticle as well as standard solution was studied using dialysis bag method. Mangiferin was released from the standard solution in 4 hours (100.6%). On the other hand 98.2% mangiferin was released from the albumin nanoparticles F7 in 24 hours (Figure 2).

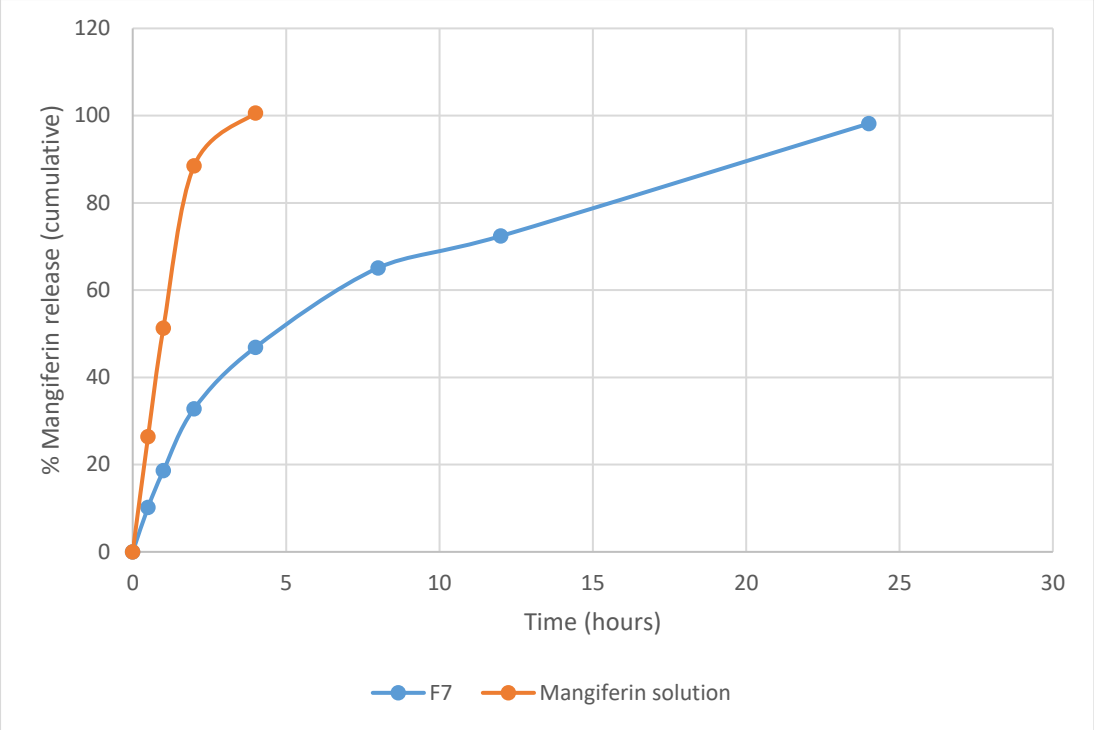

Figure 2 Release of mangiferin

Anti-inflammatory activity

The mangiferin loaded albumin nanoparticles exhibited the inhibition of albumin denaturation comparable to pure mangiferin (Table 3). The albumin nanoparticle formulation **F7** had shown the inhibition capacity (84.63 ± 0.249%). The inhibition protein denaturation by 100 µg/mL solution of standard drug mangiferin was found to be 87.60 ± 0.509%.

Table 3 Albumin denaturation inhibition activity

Treatment	% Inhibition of albumin denaturation
Mangiferin solution	87.60 ± 0.509
F7	84.63 ± 0.249

CONCLUSION

Over decades, nanoparticulate systems have been developed as drug delivery system to provide better oral bioavailability consideration, controlled & targeted release of the encapsulated drug and improve the stability. The present formulation study on mangiferin loaded albumin nanoparticles led us to conclude that albumin nanoparticles exhibited the potential to overcome the issues of short half-life and low bioavailability associated with mangiferin. The release of mangiferin from the nanoparticles for 24 hours duration will be helpful in preventing the quick metabolism of the drug and in turn improve its half-life and bioavailability. Further studies for establishing the pharmacokinetic parameters of manfigerin loaded albumin nanoparticles are warranted to reinforce and confirm our hypothesis.

Acknowledgement

The authors are thankful to SOPS, RGPV for providing particle size and zeta potential study results. The authors would like to acknowledge RB Science, Bhopal for carrying out the anti-inflammatory studies.

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