ejpmr, 2025, 12(1), 46-50

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND EVALUATION OF ASCORBIC ACID-LOADED POLYMERIC NANOPARTICLES FOR CONTROLLED RELEASE AND ENHANCED STABILITY

Supachai Chumchuen*, Thanpisit Yuenwong, Julalak Kantanalit and Yoskrai Sridhanasakulchai

Research and Development Department, Scigen Lab Co., Ltd 1401 21st Street, Sacramento, 95811, USA.

***Corresponding Author: Supachai Chumchuen**

Research and Development Department, Scigen Lab Co., Ltd 1401 21st Street, Sacramento, 95811, USA.

Article Received on 16/11/2024 **Article Revised on 06/12/2024** **Article Accepted on 26/12/2024**

ABSTRACT

Ascorbic acid has essential biological functions but faces stability challenges, limiting its therapeutic use. This study explores the use of polymeric nanoparticles, made from Eudragit L100 and chitosan, to improve the stability and controlled release of ascorbic acid. Nanoparticles were prepared using the coacervation method and characterized for particle size, morphology, entrapment efficiency, and drug loading. The nanoparticles exhibited a spherical shape, with an average size of 200.87 ± 12.22 nm and a low polydispersity index (PDI) of 0.321 ± 0.32, indicating uniformity. Entrapment efficiency was 98.76%, and drug loading was 78.98%. *In vitro* release studies showed a sustained release of ascorbic acid over 10 hours, compared to the rapid release of free ascorbic acid. Stability tests revealed that the nanoparticles effectively protected ascorbic acid from oxidation under heat stress. These findings suggest that Eudragit L100 and chitosan-based polymeric nanoparticles are a promising system for improving the stability, bioavailability, and controlled release of ascorbic acid in therapeutic applications.

KEYWORD:- Ascorbic acid, Polymeric nanoparticles, Controlled release, Eudragit L100, Chitosan.

INTRODUCTION

Ascorbic acid (vitamin C) is an essential water-soluble vitamin with numerous biological functions, including antioxidant activity, immune system support, collagen synthesis, and iron absorption. However, its clinical use is limited by its instability in aqueous solutions, susceptibility to oxidation, and short half-life in the body.[1-2] These challenges hinder the effective delivery of ascorbic acid in therapeutic applications, particularly for sustained or controlled release. Consequently, novel drug delivery systems, such as polymeric nanoparticles, have been investigated to enhance the stability, bioavailability, and controlled release of ascorbic acid. Polymeric nanoparticles are promising carriers for bioactive compounds, including vitamins, due to their ability to encapsulate, protect, and release drugs in a controlled manner. Nanoparticles offer several advantages over conventional delivery systems, including the ability to protect sensitive drugs from degradation, enhance solubility, increase tissue penetration, and provide sustained or targeted drug release. Furthermore, polymeric nanoparticles can be engineered to respond to specific environmental conditions such as pH, temperature, or ionic strength, allowing for the development of systems that release their payload in a controlled and predictable manner.

Polymeric nanoparticles are composed of biodegradable and biocompatible polymers, which make them suitable for a wide range of pharmaceutical and biomedical applications. These nanoparticles can be prepared by various methods, such as solvent evaporation, nanoprecipitation, and coacervation, which allow for the encapsulation of hydrophilic, lipophilic, or amphiphilic drugs.[3-5] The advantages of polymeric nanoparticles in drug delivery include their ability to encapsulate a wide variety of drugs, their small size (typically in the range of 10–1000 nm), and the ability to control the drug release profile. The controlled release of drugs from nanoparticles is primarily governed by the properties of the polymer matrix, the type of drug, and the release mechanism. Common mechanisms of drug release from polymeric nanoparticles include diffusion, polymer degradation, and erosion, which can be influenced by the physicochemical properties of both the drug and the carrier material.

To overcome these challenges, researchers have turned to nanotechnology, specifically polymeric nanoparticles, as a promising delivery system for ascorbic acid. Polymeric nanoparticles offer several advantages when used for loading and delivering ascorbic acid, making them a highly efficient and effective method of therapeutic delivery.

The aim of this study is to develop a process for evaluating the controlled release of ascorbic acid by encapsulating it in Eudragit L100 crosslinked with chitosan.

METHODS

Reagents and Equipment

Ascorbic acid, Eudragit L100 and chitosan were purchased from Sigma-Aldrich (USA)., Sodium hydroxy and Hydrochloric acid was purchased from MACHEREY-NAGEL Corporation, (Germany)., Ultrasonic centrifugation Made in (China)., and Microplate reader 10 digital V, IKA, (English), Black-Box Type UV Analyzer model BTU-6 Made in (China).

Preparation ascorbic acid-loaded polymeric nanoparticles

Polymeric nanoparticles were prepared using the coacervation method. Briefly, 1200 mg of ascorbic acid was dissolved in 1 mL of deionized water. 1 mg/mL solution of Eudragit L-100 was prepared in deionized water at pH 7.0. The ascorbic acid solution (1.2 g/mL) was then added to the Eudragit L-100 solution. Subsequently, 2 mg/mL solution of chitosan was added dropwise to the mixture while stirring at 20,000 rpm for 90 minutes. Finally, the mixture was centrifuged at 30,000 rpm for 60 minutes. The supernatant was discarded, and the nanoparticle pellet (undernatant) was freeze-dried for further studies.

Characterization of ascorbic acid-loaded polymeric nanoparticles

Morphology

Ascorbic acid-loaded polymeric nanoparticle was conducted by transmission electron microscopy (TEM). Briefly, 1 mL of nanoparticle suspension was dried by cover glass and then sample was staining with uranyl acetate for 30 min. Afterward, sample was screening by TEM.

Particle size

The size distribution and polydispersity index (PDI) of the nanoparticles were measured using Dynamic Light Scattering (DLS). 1 mg/mL of nanoparticle was dispersed into 100 mL of deionized water. Then, there solution was analyzed.

Percent of entrapment (PE)

10 mg/mL of nanoparticles were dispersed in deionized water. The solutions were then centrifuged at 20,000 rpm for 30 minutes. The amount of ascorbic acid in the supernatant was measured by UV-Visible spectrophotometry at 245 nm. The percent entrapment (PE) was calculated using the following formula:

PE=(The amount of initial ascorbic acid−The amount of ascorbic acid in supernatant)X
(The amount of initial ascorbic acid)$^{-1}$ X 100

Percent of drug loading (PD)

500 mg of nanoparticle was dissolved into 50 mL of deionized water at pH 9. 1 mL of solution was measured by UV-Visible spectrophotometry at 245 nm. The percent drug loading (PD) was calculated using the following formula:

PD = (The amount of ascorbic acid in solution) X (500)$^{-1}$ X 100

In Vitro Release Study

100 µg/mL of nanoparticles were placed in a dialysis bag (molecular weight cutoff 30 kDa) and immersed in 500 mL of 0.1 M phosphate buffer solution (pH 6.8). The release medium was stirred gently using a magnetic stirrer at 37°C ± 0.5°C. At predetermined time points (0.5, 1, 2, 4, 6, 8, and 12 hours), 200 µL of sample was withdrawn, and the sampled volume was replaced with an equal volume of fresh phosphate buffer solution (pH 6.8) to maintain sink condition.

Stability studies

The powder of ascorbic acid-loaded polymeric nanoparticles was stored at 45°C for 4 months. It was then characterized and compared with the powder that was stored at 25°C for the same period.

Statistical analysis

Statistical analysis was performed ANOVA method, confidence level 99 % of the comparison was compared by individual pair Tukey's test.

RESULTS AND DISCUSSION

Characterization of ascorbic acid-loaded polymeric nanoparticles

In the figure 1, ascorbic acid-loaded polymeric nanoparticle has sphere shape, which were fabricated using Eudragit L100, crosslinked with chitosan, to enhance the stability and controlled release of ascorbic acid. These nanoparticles were systematically characterized in terms of their size, entrapment efficiency, and drug loading capacity to assess their suitability for use as a drug delivery system.

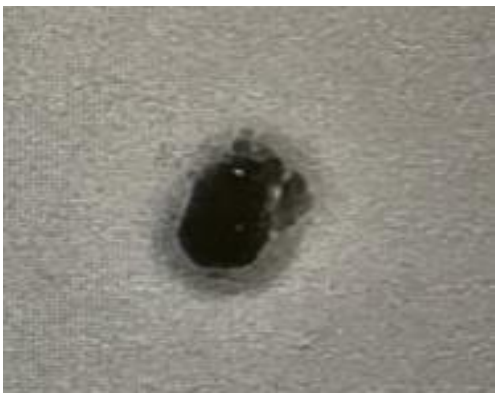

Figure 1: Nanoparticle suspension.

Particle Size and Poly distribution index (PDI)

One of the key parameters that influence the performance of drug delivery systems, especially for

controlled release, is the particle size. The particle size and distribution of the ascorbic acid-loaded nanoparticles were determined using Dynamic Light Scattering (DLS), which is a widely used method for analyzing the size distribution of nanoparticles in suspension. The nanoparticles were found to have an average particle size in the range of 200.87 ± 12.22 nm to 398.56 ± 45.12 nm, which is within the ideal size range for drug delivery purposes. In the case of PDI was 0.321 ± 0.32, indicated this system is homogeneous colloidal systems. This particle size range is particularly significant because it falls within the typical 100 nm to 1000 nm range that is most effective for nanoparticles intended for oral or parenteral drug delivery.[6-10] Nanoparticles in this size range are small enough to avoid rapid elimination by the immune system, yet large enough to provide a sustained release profile, enabling them to maintain prolonged drug activity in the body. Additionally, the relatively narrow size distribution (as indicated by the low polydispersity index) suggests a homogeneous batch of nanoparticles, which is essential for reproducible performance in drug delivery applications.

Entrapment Efficiency (PE)

The entrapment efficiency (PE) is a critical parameter that reflects the ability of the nanoparticle system to encapsulate the active pharmaceutical ingredient (API), in this case, ascorbic acid. A high entrapment efficiency ensures that a significant portion of the active compound is retained within the nanoparticles, thus maximizing its therapeutic potential and minimizing drug loss during storage and release. For the ascorbic acid-loaded polymeric nanoparticles, the entrapment efficiency was found to be 98.76%, which is exceptionally high. This result indicates that the majority of the ascorbic acid was successfully encapsulated within the nanoparticles, with minimal loss during the preparation process. The high entrapment efficiency suggests that the polymeric matrix (Eudragit L100 and chitosan) is highly effective in stabilizing and protecting the ascorbic acid from degradation, oxidation, and environmental factors that typically compromise its stability. Furthermore, this high entrapment efficiency ensures that the drug is protected from premature degradation, which is a significant concern for sensitive compounds like ascorbic acid. A high entrapment efficiency is particularly important in the context of controlled release formulations, as it minimizes the amount of drug that is lost in the early stages of release.[11-12] By encapsulating the drug efficiently, the formulation can deliver the drug over an extended period, reducing the frequency of dosing and improving patient compliance.

Drug Loading (PD)

Drug loading (PD) is another key parameter that describes the amount of drug incorporated into the nanoparticle formulation relative to the total weight of the nanoparticles. It is an important factor in determining the overall drug release capacity and therapeutic potential of the formulation. In this study, the drug loading capacity of the ascorbic acid-loaded polymeric nanoparticles was found to be 78.98%. This relatively high drug loading indicates that a substantial amount of ascorbic acid is incorporated into the nanoparticle matrix, which is advantageous for achieving the desired therapeutic effect. A high drug loading means that a smaller volume of nanoparticles is required to deliver the same amount of drug, which can be beneficial in reducing the overall size and dosage of the formulation, especially for parenteral or oral administration.[13-15] The high drug loading capacity observed in this study suggests that the combination of Eudragit L100 and chitosan is effective not only in encapsulating ascorbic acid but also in maintaining a high concentration of the drug within the nanoparticle system. This result is critical for achieving the desired therapeutic outcomes, as it ensures that the drug is delivered in an efficient and sustained manner over time.

Drug release studies

From Figure 2, the drug release study of ascorbic acid-loaded polymeric nanoparticles (Inno-pharmar Tech®), conducted using the dialysis bag method in phosphate buffer solution (pH 6.8), demonstrated controlled and sustained release characteristics over a 10-hour period. The release profile was significantly different ($p < 0.05$) when compared to pure ascorbic acid, which released the drug within 3 hours. The release pattern indicated that the nanoparticles were able to release ascorbic acid in a sustained manner, with an initial burst release followed by a gradual and consistent release over time.[14-16] This release profile suggests that the formulation is capable of providing controlled delivery of ascorbic acid, which is beneficial for therapeutic applications requiring prolonged drug action and minimal fluctuations in plasma drug concentrations.

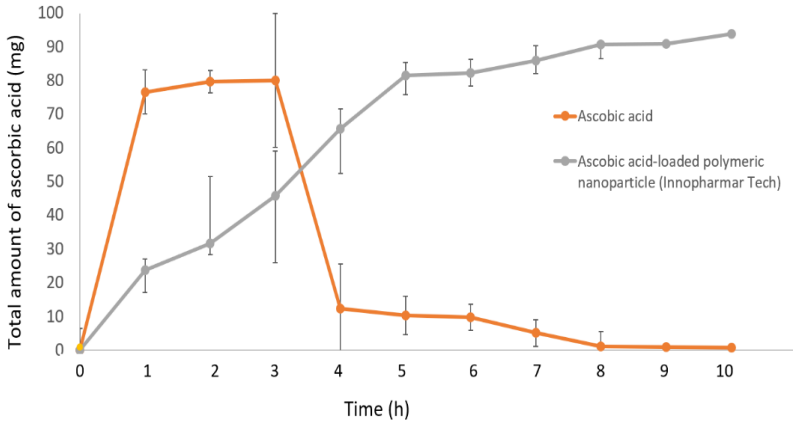

Figure 2: Drug release profile.

Stability studies

In Figure 3, the presence of a brownish color on the surface of the powder was observed when it was stored at 45°C for seven days. This phenomenon likely indicates that the ascorbic acid underwent oxidation, possibly due to the heat, resulting in the formation of brownish-dark clumps of powder. Ascorbic acid is known to be sensitive to heat and light, which can cause its degradation. In this case, the elevated temperature could have accelerated the oxidation process, leading to the appearance of the black dots. The transformation of ascorbic acid into a darker color under heat stress suggests that the compound is unstable in its pure, solid form when exposed to environmental stressors such as heat. Interestingly, this phenomenon was not observed in the ascorbic acid-loaded polymeric nanoparticles. In these cases, the ascorbic acid remained protected, and no black dots or signs of degradation were evident, even after the same storage conditions. This suggests that the polymer matrix in the nanoparticle formulation effectively shields the ascorbic acid from oxidative damage. The polymer likely prevents the ascorbic acid from interacting with external environmental factors, such as heat and oxygen, that would normally contribute to its degradation. This finding highlights the potential of using polymeric nanoparticles as a protective delivery system for sensitive compounds like ascorbic acid.[13]

Furthermore, the absence of the black dots in the nanoparticle formulation suggests that the polymer encapsulation may not only protect ascorbic acid from oxidation but may also inhibit any undesirable chemical interactions that could otherwise lead to degradation. In contrast, when ascorbic acid is stored in its naked, unencapsulated form, it is more vulnerable to environmental factors, which can lead to the formation of black spots as a result of chemical reactions with heat and oxygen.

The black dots observed on the naked ascorbic acid could also be the result of interactions between the ascorbic acid and the packaging material. It is possible that certain components of the packaging could catalyze or facilitate the degradation process of ascorbic acid. This interaction is worth investigating further, as it could provide valuable insights into how packaging materials can impact the stability of sensitive compounds during storage.[9]

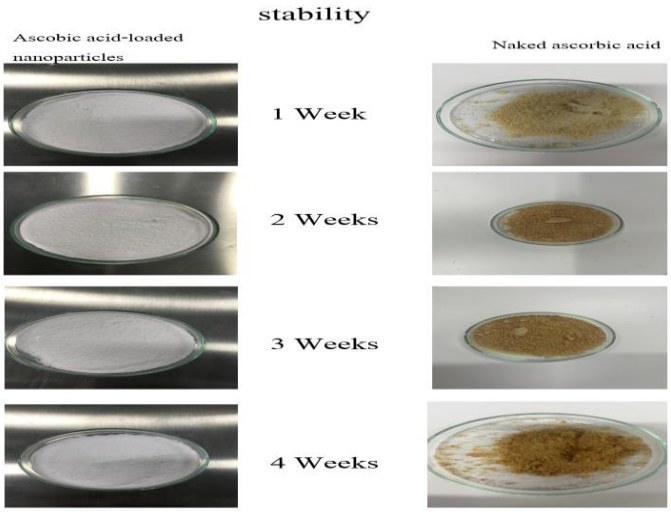

Figure 3: Ascorbic acid-loaded nanoparticle Innophamar Tech® (Left) Naked ascorbic acid (Right) at 45 °C for 4 weeks.

REFERENCES

1. Güney, G., Kutlu, H. M., & Genç, L. Preparation and characterization of ascorbic acid loaded solid lipid nanoparticles and investigation of their apoptotic effects. Colloids and Surfaces B: Biointerfaces, 2014; 121: 270-280.
2. Moribe, K., Limwikrant, W., Higashi, K., & Yamamoto, K. Drug nanoparticle formulation using ascorbic acid derivatives. Journal of drug delivery, 2011; 2011(1): 138929.
3. Jang, K. I., & Lee, H. G. Stability of chitosan nanoparticles for L-ascorbic acid during heat treatment in aqueous solution. Journal of agricultural and food chemistry, 2008; 56(6): 1936-1941.
4. Cano, A., Ettcheto, M., Chang, J. H., Barroso, E., Espina, M., Kühne, B. A., & García, M. L. Dual-drug loaded nanoparticles of Epigallocatechin-3-gallate (EGCG)/Ascorbic acid enhance therapeutic efficacy of EGCG in a APPswe/PS1dE9 Alzheimer's disease mice model. Journal of Controlled Release, 2019; 301: 62-75.
5. Abdel-Hakeem, M. A., Abdel Maksoud, A. I., Aladhadh, M. A., Almuryif, K. A., Elsanhoty, R. M., & Elebeedy, D. Gentamicin–Ascorbic Acid Encapsulated in Chitosan Nanoparticles Improved In Vitro Antimicrobial Activity and Minimized Cytotoxicity. Antibiotics, 2022; 11(11): 1530.
6. Jabali, M. K., Allafchian, A. R., Jalali, S. A. H., Shakeripour, H., Mohammadinezhad, R., & Rahmani, F. Design of a pDNA nanocarrier with ascorbic acid modified chitosan coated on superparamagnetic iron oxide nanoparticles for gene delivery. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2022; 632: 127743.
7. Othman, N., Md. Jamil, S. N. A., Masarudin, M. J., Abdullah, L. C., Daik, R., & Sarman, N. S. L-Ascorbic acid and thymoquinone dual-loaded palmitoyl-chitosan nanoparticles: Improved preparation method, encapsulation and release efficiency. Processes, 2020; 8(9): 1040.
8. Stevanović, M., Savić, J., Jordović, B., & Uskoković, D. Fabrication, in vitro degradation and the release behaviours of poly (DL-lactide-co-glycolide) nanospheres containing ascorbic acid. Colloids and Surfaces B: Biointerfaces, 2007; 59(2): 215-223.
9. Rossi, S., Vigani, B., Puccio, A., Bonferoni, M. C., Sandri, G., & Ferrari, F. Chitosan ascorbate nanoparticles for the vaginal delivery of antibiotic drugs in atrophic vaginitis. Marine drugs, 2017; 15(10): 319.
10. Moaddab, M., Nourmohammadi, J., & Rezayan, A. H. Bioactive composite scaffolds of carboxymethyl chitosan-silk fibroin containing chitosan nanoparticles for sustained release of ascorbic acid. European polymer journal, 2018; 103: 40-50.
11. An, Q., Sun, C., Li, D., Xu, K., Guo, J., & Wang, C. Peroxidase-like activity of Fe3O4@ carbon nanoparticles enhances ascorbic acid-induced oxidative stress and selective damage to PC-3 prostate cancer cells. ACS applied materials & interfaces, 2013; 5(24): 13248-13257.
12. Jang, K. I., Lee, J. S., & Lee, H. G. Physicochemical properties and cell permeation efficiency of l-ascorbic acid loaded nanoparticles prepared with N-trimethyl chitosan and N-triethyl chitosan. Food Science and Biotechnology, 2014; 23: 1867-1874.
13. Li, L., Luo, P., Wu, S., & Wang, Y. Deciphering the neuroprotective effect of ascorbic acid mediated synthesis of iron oxide nanoparticles against Parkinson's disease: an in vitro and in vivo approach. Macromolecular Research, 2023; 31(10): 949-960.
14. Rezvani, M., Manca, M. L., Caddeo, C., Escribano-Ferrer, E., Carbone, C., Peris, J. E., & Manconi, M. Co-loading of ascorbic acid and tocopherol in eudragit-nutriosomes to counteract intestinal oxidative stress. Pharmaceutics, 2019; 11(1): 13.
15. Shatilo, V., Antonyuk-Shcheglova, I., Naskalova, S., Bondarenko, O., Monastyrov, M., & Prikhna, T. Positive effects of Quercetin, Iron Oxide Nanoparticles and Ascorbic Acid composition in the treatment of women with metabolic syndrome. Ageing and longevity, 2023; 4(1): 8-15.
16. Mallakpour, S., & Naghdi, M. Design and preparation of poly (Vinyl alcohol) flexible nanocomposite films containing silica nanoparticles with citric acid and ascorbic acid linkages as a novel nanofiller through a green route. International Journal of Polymer Analysis and Characterization, 2016; 21(1): 29-43.