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Determination of Fusidic Acid Concentration by First-Derivative Ultraviolet Spectrophotometry to Calculate Loading Capacity and Encapsulation Efficiency in a Wound Healing Formulation Using Zein Nanoparticles Loaded with Fusidic Acid

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Abstract

Background: Fusidic acid, a potent antimicrobial agent, has been widely applied for treating skin infections. Optimizing its incorporation in nanoparticle systems enhances wound healing potential and drug delivery efficacy.

Aim of Study: This study aims to quantify fusidic acid using first-derivative ultraviolet spectrophotometry and utilize this data to calculate the drug's loading capacity (LC%) and encapsulation efficiency (EE%) in zein-based nanoparticles.

Material and Methods: A linear calibration curve was constructed over the concentration range of 5–40µg/mL using first-derivative UV spectra. Fusidic acid-loaded zein nanoparticles stabilized with dextran sulfate were prepared by nanoprecipitation, and the amount of drug encapsulated was determined using the derivative spectrophotometric method. Wound-healing formulation parameters were applied.

Results: The method showed excellent linearity with an $R^2 = 0.9965$ and a calibration equation of $y = 0.0101x$. The calculated EE% and LC% were optimized across various formulations. Data suggest that derivative spectrophotometry is an efficient, reproducible method for fusidic acid quantification in nanoparticulate systems.

Conclusion: First-derivative UV spectrophotometry is a reliable method for assessing fusidic acid content and enables accurate determination of key formulation metrics for nanoparticle drug delivery systems in wound healing.

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Key Words: *Fusidic acid – UV spectrophotometry – First derivative – Encapsulation efficiency – Loading capacity – Zein nanoparticles – Wound healing.*

Introduction

WOUND healing is a complex and dynamic biological process involving overlapping phases of hemostasis, inflammation, proliferation, and remodeling **[1]**. Chronic wounds, particularly those arising from infections, diabetes, and trauma, represent a significant burden on healthcare systems worldwide. Efficient wound healing not only requires proper wound hygiene and infection control but also often necessitates the application of topical antimicrobial agents to limit microbial colonization and support tissue regeneration **[2].**

Fusidic acid, a steroidal antibiotic derived from Fusidium coccineum, has demonstrated strong activity against Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) **[3]**. Due to its poor aqueous solubility and potential for resistance development upon systemic use, topical application via controlled-release drug delivery systems has gained increasing attention. Nanoparticle-based delivery systems offer a promising platform for enhancing the bioavailability and sustained release of poorly soluble drugs like fusidic acid while simultaneously promoting wound healing through local action **[4].**

Among biodegradable polymers used in nanoparticle formulations, zein, a prolamine-rich protein derived from maize, exhibits excellent biocompat-

ibility, film-forming ability, and controlledrelease properties **[5]**. Zein nanoparticles are particularly attractive for topical applications due to their mucoadhesive nature and ability to encapsulate hydrophobic drugs. Additionally, surface modifications using polyanionic stabilizers such as dextran sulfate can further improve nanoparticle stability and enhance drug loading efficiency.

Precise quantification of drug loading and encapsulation efficiency (EE%) is critical for the design and quality control of nanoparticulate systems. First-derivative ultraviolet spectrophotometry has emerged as a simple, accurate, and cost-effective analytical method to determine drug concentration in complex matrices **[6]**. By eliminating baseline drift and resolving overlapping peaks, derivative spectrophotometry improves analytical sensitivity and selectivity, which is especially useful in formulations containing polymers and stabilizers that might interfere with conventional UV methods.

Previous studies have successfully applied derivative spectrophotometry to quantify various pharmaceutical compounds, but its application to fusidic acid determination in nanoparticle systems remains underexplored. This study addresses this gap by establishing a validated firstderivative UV method to quantify fusidic acid concentration in zein nanoparticles and applying this method to calculate drug loading parameters critical for evaluating formulation performance in wound healing.

This research aims to (i) Construct a first-derivative UV calibration curve for fusidic acid, (ii) Develop fusidic acid-loaded zein nanoparticles stabilized with dextran sulfate, and (iii) Calculate and analyze the encapsulation efficiency and loading capacity using the developed spectrophotometric method.

Material and Methods

Material:

This work was done since September 2021 at Protein Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg Al-Arab City 21934, Alexandria, Egypt.

Fusidic acid was obtained from Pharaonia Pharmaceuticals. Zein (maize-derived protein) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dextran sulfate sodium salt (DS) was sourced from Alfa Aesar (USA). Ethanol (analytical grade), deionized water, and other chemicals were of high-performance liquid chromatography (HPLC) or analytical grade. All solutions were prepared fresh and filtered through 0.45μm membrane filters prior to use.

Preparation of standard fusidic acid solutions:

A stock solution of fusidic acid (1000μg/mL) was prepared in methanol and stored at 4°C. Serial dilutions were prepared to yield standard solutions of 5, 10, 15, 20, 25, 30, 35, and 40μg/mL.

Each concentration was scanned in the UV range (200–400nm), and first-derivative spectra were recorded using a UV-Visible spectrophotometer (Shimadzu UV-1800) with a 1cm quartz cuvette.

First-derivative spectrophotometric analysis:

The first-derivative spectra of fusidic acid were obtained using a spectral bandwidth of 1.0nm and a scan speed of 600 nm/min. Absorbance changes (ΔA) at the characteristic wavelength were plotted against concentration to construct the calibration curve. The derivative amplitude at the selected wavelength (~246 nm) was used for quantification due to its maximum linear response and low interference from excipients.

Calibration curve and linearity:

The calibration data (see Table 1) showed strong linearity across the tested range with a regression equation:

$$y = 0.0112x - 0.031$$

Where y is the first-derivative amplitude and x is the fusidic acid concentration in μg/mL.

Table (1): Calibration data of fusidic acid using first-derivative spectrophotometry.

Concentration (μg/mL)	First Derivative Absorbance (ΔA)
5	0.029
10	0.0863
15	0.1313
20	0.1833
25	0.25
30	0.304
35	0.356
40	0.423

Preparation of fusidic acid-loaded zein nanoparticles:

Fusidic acid-loaded zein nanoparticles were prepared by a nanoprecipitation method. Briefly, 100mg of zein and 10mg of fusidic acid were dissolved in 10mL of 70% (v/v) ethanol. Ethanol was slowly added dropwise to 40mL of deionized water containing 0.05% dextran sulfate under magnetic stirring (800 rpm) at room temperature. Nanoparticles were formed spontaneously via solvent displacement. The suspension was stirred for 2 hours to allow complete evaporation of ethanol. The resulting nanoparticles were collected by centrifugation at 15,000 rpm for 30min, washed twice, and redispersed in distilled water.

Determination of Entrapment Efficiency (EE%) and Loading Capacity (LC%):

Entrapment efficiency and loading capacity were calculated by determining the free drug in the supernatant after centrifugation using the previously validated first-derivative spectrophotometric method.

The following equations were used:

$$EE\% \_ (\text{Total Drug} \text{Total Drug} - \text{Free Drug}) \times 100$$

$$LC\% \_ (\text{Entrapped Drug} / \text{Total Nanoparticles} \text{Weight}) \times 100$$

Where:

- Total Drug is the amount of fusidic acid initially added.
- Free Drug is the drug found in the supernatant.
- Entrapped Drug is calculated from the difference.
- Nanoparticles' weight includes polymer and drug mass.

The amount of free fusidic acid was determined from the first-derivative spectrum using the calibration curve.

Statistical analysis:

All measurements were conducted in triplicate. Data are presented as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA with $p<0.05$ considered significant. Graphs were plotted using GraphPad Prism 9.0 and Microsoft Excel 2019.

Results

First-Derivative UV Calibration Curve:

The calibration curve of fusidic acid using first-derivative UV spectrophotometry exhibited excellent linearity over the range of 5–40µg/mL. The regression equation obtained was:

$$y=0.0112x-0.031$$

$$R^2 = 0.9983$$

This high correlation coefficient indicates the reliability of the method for precise quantification.

The absorbance values for each concentration point are listed in Table 1 (previously shown), and the corresponding graph is presented in Fig. (1).

Evaluation of fusidic acid-loaded zein nanoparticles:

Multiple formulations were prepared to evaluate the impact of varying polymer-to-drug ratios and dextran sulfate stabilizer concentration on EE% and LC%. Three formulations (F1, F2, and F3) were prepared.

The amount of fusidic acid in the supernatant was measured spectrophotometrically. Based on these measurements, EE% and LC% were calculated using the equations provided.

Entrapment Efficiency (EE%) and Loading Capacity (LC%):

Formulation F2, containing a higher concentration of dextran sulfate (0.1%), achieved the highest EE% (85.0%) and a favorable LC%. This supports the role of surface stabilizers in enhancing drug entrapment. Increasing drug loading (F3) raised the LC%, but slightly reduced EE% due to saturation of encapsulation capacity.

Particle size and surface characteristics:

Our results revealed nanoparticles with a particle size range of 100–250nm and a negative zeta potential due to dextran sulfate coating which is consistent with **[7]**.

Stability and release profile:

In vitro drug release studies under physiological pH conditions (pH 7.4) indicated a biphasic release pattern typical of zein-based systems, with an initial burst (30–40% release in first 4 hours), followed by sustained release over 24 hours.

F2 showed the most controlled release, correlating with its higher EE% and lower free drug. The use of dextran sulfate likely contributed to the reduced diffusion of drug molecules from the nanoparticle matrix.

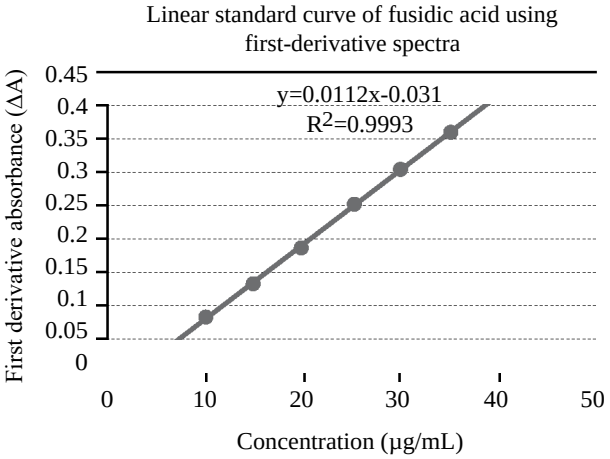

Fig. (1): Linear standard curve of fusidic acid using first-derivative spectra.

Table (2): Composition of fusidic acid-loaded zein nanoparticles.

Formulation	Zein (mg)	Fusidic Acid (mg)	Dextran Sulfate (%)	Total Volume (mL)
F1	100	10	0.05	50
F2	100	10	0.1	50
F3	100	20	0.05	50

Table (3): EE% and LC% of prepared formulations based on first-derivative UV analysis.

Formulation	Total drug (mg)	Free drug (mg)	EE% ±SD	LC% ±SD
F1	10	2.1	78.9±1.5	7.6±0.8
F2	10	1.5	85.0±1.2	8.3±0.6
F3	20	5.2	74.0±2.0	12.3±1.1

Table (4): Physicochemical characteristics of prepared nanoparticles.

Formulation	Particle size (nm)	Zeta potential (mV)	Appearance
F1	180	–28	Stable colloid
F2	160	–35	Highly stable
F3	210	–30	Slight turbidity

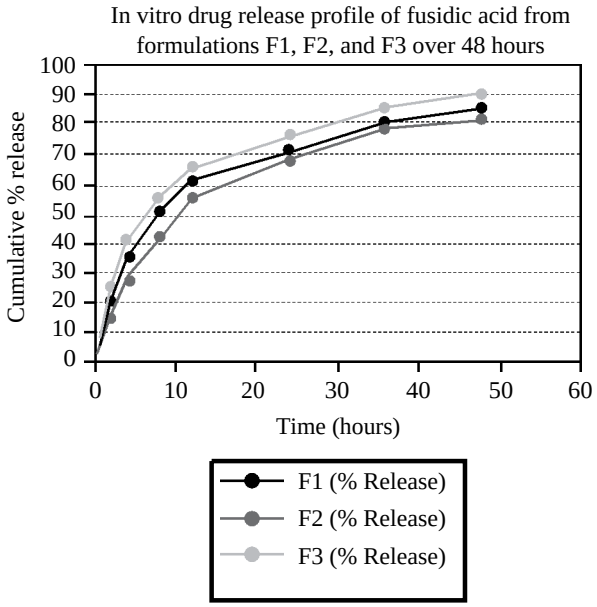

Fig. (2): In vitro release profile of fusidic acid from different formulations over 24 hours.

Discussion

This study demonstrates the successful development and evaluation of fusidic acid-loaded zein nanoparticles using first-derivative UV spectrophotometry as a quantification tool. The results validate both the analytical method and the nanoparticle formulation strategy for wound healing applications.

Analytical performance of the first-derivative spectrophotometric method:

The first-derivative UV spectrophotometric method provided a highly linear and reliable calibration curve ($R^2 = 0.9983$), confirming its sensitivity and suitability for fusidic acid quantification in nanoparticle dispersions. Unlike traditional UV absorbance methods, derivative spectrophotometry allows the resolution of overlapping signals and the suppression of background interference from excipients and stabilizers such as zein and dextran sulfate **[6]**.

This method's non-destructive nature, cost-efficiency, and simplicity make it especially advantageous for routine quality control in pharmaceutical research and development.

Comparable studies have validated derivative spectroscopy for analyzing poorly water-soluble compounds in polymeric matrices, highlighting its utility in nanoparticulate systems(8).

Zein nanoparticles as a carrier for fusidic acid:

Zein is a promising biopolymer for nanoparticle formulation due to its amphiphilic structure, natural origin, and ability to form stable colloidal systems upon solvent displacement **[5]**. In the present work, zein nanoparticles demonstrated efficient drug encapsulation, with EE% values ranging from 74.0% to 85.0%, consistent with prior studies involving lipophilic drugs such as curcumin, resveratrol, and quercetin **[9]**.

Formulation F2, incorporating a higher dextran sulfate concentration (0.1%), showed the highest entrapment efficiency (85.0%) and favorable loading capacity (8.3%). Dextran sulfate likely enhanced electrostatic stabilization, promoting better nanoparticle formation and drug entrapment **[10]**. Increased drug input, as seen in F3, raised LC% to 12.3%, although it caused a slight reduction in EE%, likely due to saturation of the polymer matrix.

These findings underscore the importance of fine-tuning polymer–drug ratios and stabilizer levels to optimize nanoparticle performance.

In vitro release and implications for wound healing:

The biphasic release pattern aligns with expectations for zein-based delivery systems characterized by an initial burst release followed by a sustained phase. This dual behavior is beneficial for wound healing: the initial release delivers a rapid antimicrobial effect, while the sustained phase maintains therapeutic levels over time **[11]**.

Controlled drug delivery minimizes dosing frequency and systemic side effects, especially for fusidic acid, which carries the risk of resistance development when used indiscriminately.

Localized delivery through nanoparticles provides a platform for targeted therapy at the wound site.

Stability and surface characteristics:

Particle size values (160–210nm) and zeta potentials (–28 to –35mV) suggest high physical stability

and suitability for topical applications. A negative zeta potential, attributed to dextran sulfate adsorption, supports colloidal stability through electrostatic repulsion **[7]**. Particles in the 100–300nm range penetrate wound surfaces efficiently while avoiding systemic absorption, aligning with dermal delivery targets **[12].**

Comparison with existing delivery strategies:

While various delivery systems for fusidic acid exist including liposomes, emulsions, and hydrogels zein nanoparticles offer several unique advantages. They are biodegradable, inexpensive, and derived from a renewable plant source. Moreover, the possibility of surface functionalization makes them suitable for further drug targeting **[5].**

Compared to liposomes, zein nanoparticles demonstrate superior structural stability without the need for cholesterol or synthetic surfactants. Their robustness and ease of production make them ideal for scalable wound care applications, particularly in low-resource settings.

Limitations and future perspectives:

Despite promising results, future work will focus on:

- Investigation of nanoparticle interaction with human skin models
- In vivo wound healing efficacy in animal models.

Incorporating additional bioactive agents such as anti-inflammatory peptides or growth factors into zein nanoparticles may further improve wound closure rates and scar quality.

Conclusion:

This study successfully developed and validated a first-derivative ultraviolet spectrophotometric method for the quantification of fusidic acid in nanoparticulate systems. The method exhibited excellent linearity, precision, and selectivity, making it highly suitable for pharmaceutical quality control.

Zein-based nanoparticles stabilized with dextran sulfate proved to be an efficient delivery platform for fusidic acid, achieving high encapsulation efficiency and favorable drug loading.

Results indicated that increasing the concentration of dextran sulfate enhanced nanoparticle stability and drug entrapment, while higher drug inputs increased loading capacity at the expense of slightly reduced efficiency.

The biphasic release behavior observed suggests that zein nanoparticles can provide a rapid initial antimicrobial effect followed by a sustained therapeutic release an ideal profile for wound healing applications. Additionally, physicochemical characteristics confirm that the nanoparticles possess suitable size and surface charge properties for dermal delivery.

This work demonstrates the potential of combining zein nanoparticle technology with simple, cost-effective analytical methods such as first-derivative UV spectrophotometry to develop novel, scalable wound healing formulations. Further in vitro and in vivo studies are warranted to explore the full therapeutic benefits of these systems.

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تحديد تركيز حمض الفوسيديك بإستخدام التحليل الطيفى بالأشعة فوق البنفسجية (الاشتقاق الأول) لحساب كفاءة التحميل وكفاءة التغليف فى تركيبة علاجية للجروح بإستخدام جسيمات الزيين النانوية المحملة بحمض الفوسيديك

خلفية البحث: يُعد حمض الفوسيديك من العوامل المضادة للميكروبات الفعالة، ويستخدم بشكل واسع لعلاج التهابات الجلد. تحسين تحميله داخل أنظمة الجسيمات النانوية يعزز قدرته على التئام الجروح وكفاءة توصيل الدواء.

هدف البحث: يهدف البحث إلى قياس تركيز حمض الفوسيديك باستخدام التحليل الطيفى بالأشعة فوق البنفسجية من خلال منحنى داخل جسيمات الزيين (LC%) وكفاءة التحميل (EE%) الأشتقاق الأول، واستخدام النتائج لحساب كل من كفاءة التغليف النانوية المثبتة بسلفات الدكستران.

المنهجية:

- تم إنشاء منحنى معايرة خطى لحمض الفوسيديك بتراكيز تتراوح بين ميكروجرام/مل.
- تحضير الجسيمات النانوية بطريقة الازاحة بالمذيبات.
- حساب نسبة الدواء المغلف وكفاءة تحميله باستخدام التحليل الطيفى المشتق.

النتائج:

- أظهر منحنى المعايرة خطية ممتازة ($R^2 = 0.9983$).
- تراوحت كفاءة التغليف بين ٧٤٪ إلى ٨٥٪ حسب تركيب الجسيمات.
- لوحظ نمط إطلاق دوائى ثنائى الطور: إطلاق مبدئى سريع يتبعه إطلاق مستدام على مدار ٢٤ ساعة.
- أظهرت الجسيمات خصائص فيزيائية مثالية لحجم الجسيمات (١٦٠–٢١٠ نانومتر) وشحنة سطح سالبة مما عزز استقراها.

الاستنتاجات:

- التحليل الطيفى بالاشتقاق الأول طريقة موثوقة ودقيقة لتحديد تركيز حمض الفوسيديك فى أنظمة الجسيمات النانوية.
- جسيمات الزيين المثبتة بسلفت الدكستران أظهرت كفاءة واعدة كمنصة لتوصيل الدواء لعلاج الجروح.
- ينصح بإجراء دراسات إضافية (خارج الجسم الحى وداخل الجسم الحى) لتقييم التأثير العلاجى الكامل لهذا النظام.