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Production and characterization of low molecular weight heparin obtained by modified double emulsion method with solvent evaporation

Abstract

The main anticoagulant agents currently used in the treatment of coagulation disorders are low molecular weight heparin (LMWH) and unfractionated heparin (UFH). LMWH is formed by smaller UFH chains obtained by multiple chemical or enzymatic depolymerization processes and has advantages over UFH in that it does not cause severe toxicity and has more predictable pharmacokinetic properties. The use of LMWH, even with its advantages, is still limited due to the need for daily subcutaneous injections because it is of animal origin and does not have a definite chemical composition. These factors motivated the researchers to explore new delivery systems of this medication for better therapeutic results. The objective of this study was to produce and characterize LMWH nanoparticles through the solvent-evaporated double emulsion method using poly lactic-co-glycolicacid (PLGA) and polyvinyl alcohol (PVA), calculate encapsulation efficiency (EE) and evaluate stability for future in vitro and in vivo testing. The methodology used was effective and produced LMWH nanoparticles of medium diameter 224.8±0.85, zeta potential -17.27±1.10 and polydispersity index 0.07±0.05. Despite the hydrophilic characteristic of LMWH, the obtained EE was 66.5%, a very promising result that suggests the effectiveness of the nanoencapsulation method used resulting in an alternative for the development of new anticoagulant therapies.

Keywords: low molecular weight heparin, anticoagulants, nanoparticles

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Abbreviations: LMWH, low molecular weight heparin; UFH, unfractionated heparin; PLGA, lactic-co-glycolic acid; PVA, polyvinyl alcohol; EE, encapsulation efficiency

Introduction

Currently, thromboembolic diseases are responsible for a significant portion of morbidity and mortality.[1] The drugs of choice for coagulation disorders are unfractionated heparin (UFH) and low molecular weight heparin (LMWH). UFH has had problems with its use as an unpredictable effect requiring monitoring, risk of bleeding and thrombocytopenia because it acts directly on fibrin formation. Besides that, this drug can cause osteoporosis by binding to osteoblasts, and due to its animal origin may present a risk of contamination by pathogens.[2] Studies state that LMWH has advantages over UFH, as a more predictable response, lower risk of bleeding and thrombocytopenia, and is the most suitable anticoagulant for pulmonary embolism and deep vein thrombosis.[2] However, it is known that the use of this heparin still has complications due to the need for daily subcutaneous injections, parenteral administration route, high molecular weight and anionic character.[3]

As the pharmacokinetic properties of LMWH are more predictable, there was a need to work with new drug delivery systems to achieve better therapeutic results, better bioavailability and fewer side effects.[4] Nanocapsulation of a drug may be a good alternative for improving its pharmacokinetic profile and efficacy, as well as reducing side effects and increasing its stability.[5] Studies have reported promising results in the development of micro and nanoparticles using LMWH1-co-loaded polylactic glycolic acid (PLGA). PLGA is widely used because it is biodegradable, biocompatible and versatile and has already been approved by the European Food and Drug Administration as an excipient for parenteral products.[6] Thus, the purpose of this study was the LMWH nanoencapsulation and characterization of nanoparticles obtained from the solvent-evaporated double emulsion method, using PLGA and polyvinyl alcohol (PVA) for future in vitro and in vivo efficacy tests.

Methodology

Nanoparticle Development: Commercially purchased aqueous LMWH solution (Clexane 100 mg/ml) was dissolved in 5ml diclomethane: PLGA (50:50) solution and sonicated (Sonifier® Model W-450D Branson) at 50% amplitude for 15 seconds in an ice bath forming the first emulsion. Immediately thereafter, a 1.0% PVA solution was added and sonication again forming the double emulsion. The suspension was allowed to stir for 6 hours to fully evaporate the solvent (dichloromethane). Subsequently, the suspension was added to a separating filter (Amicon Ultra-15ml) and centrifuged for 15 min at 3000rpm (Figure 1).[6]

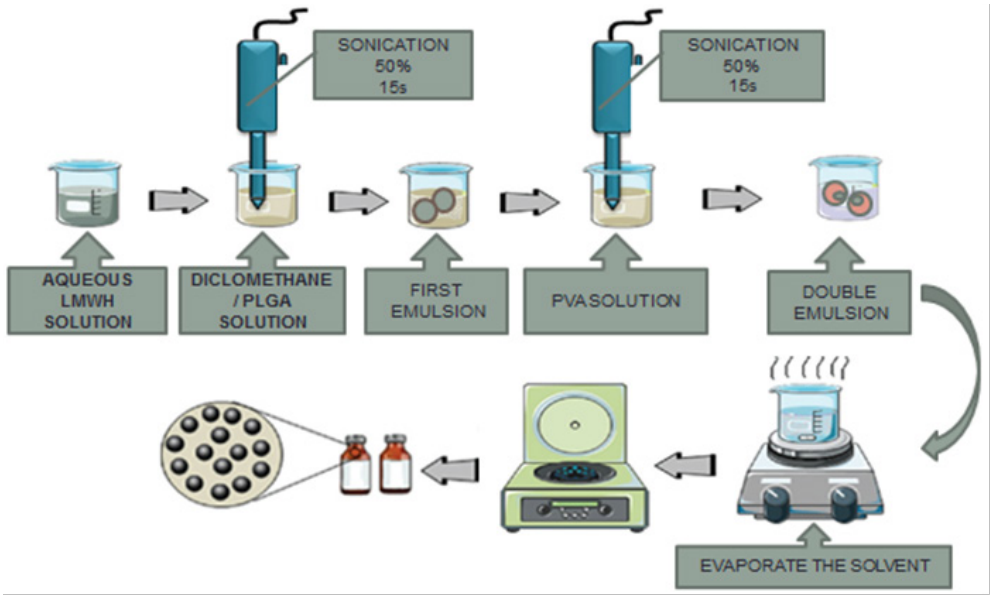

Figure 1 Nanoparticle development.

Characterization: Nanoparticles were characterized by surface morphology, surface coating, particle size, polydispersity index, zeta potential, drug loading and encapsulation efficiency by photon correlation spectroscopy (PCS) by Zeta Sizer Malvern (Malvern Instruments Corp). Measurements were made in KCL solution (1mM). Stability was measured at times 0 and 30 days; For this, the dispersions were kept at room temperature and protected from light and heat for 30 days.[3]

Nanoecapsulation efficiency tests: Nanoparticulate encapsulated LMWH was quantified by turbidimetric method. To perform the calibration curve a stock solution of 10mg/ml LMWH was prepared. From this solution successive dilutions were made to obtain the concentrations: 4mg/ml, 3mg/ml, 2mg/ml, and 1mg/ml. The absorbances of these solutions were read on a UV-Vis spectrophotometer (Thermo Scientific Genesis 10S UV-Vis Spectrophotometer) at a wavelength of 290nm in triplicate.[8] Then the supernatant was measured at a wavelength of 290 nm. LMWH encapsulation efficiency (EE) was calculated by the following equation:

$$EE\left(\%\right) = \frac{\left[Intial\ drug\ total\right] - \left[Drug\ in\ the \sup erna\ \tan t\right]}{\left[Intial\ drug\ total\right]} \times 100$$

Results and discussion

Nanoencapsulations

Homogeneous and stable LMWH nanoparticles were obtained with 217.9±2.5nm of particle diameter (Dm), zeta potential (PZ) -17.06±0.51mV and polydispersion index (IPD) of 0.067±0.05. Surface chemistry critically affects the way nanoparticles interact with each other and with the environment, and even more with cells.[5] Nanoparticles prepared with PLGA have invariably negative charge.[9] The prepared samples presented satisfactory Dm of particles for nanostructured systems with low standard deviation, indicating robustness in the preparation. In addition, the low polydispersion value (IPD) obtained revealed the homogeneity of the samples.

Similar results were obtained by Chereddy KK and colleagues, Dm 163.0±2.2, PZ −19.2±2.5mV and IPD 0.15±0.05, when nanoencapsulating a healing peptide using a similar methodology.[6] In the article by Prado LB et al., LMWH was nanoencapsulated using poly (E- caprolactone) (PCL) and chitosan and the results obtained were Dm 512.5nm, IPD 0.409 and PZ + 30.9mV of chitosan in the process. In this study the solvent-evaporated double emulsion method produced nanoparticles larger than 400nm and IPD greater than 0.2 characterizing system polydispersion, in other words, nanoparticles of varying sizes,[7] suggesting that the efficiency of developing a nanocarrier system is related to the methodology employed and the selected inputs.

Stability

After preparation, the nanoparticles resulted in formulations with homogeneous macroscopic appearance, milky, white and opalescent appearance. The suspensions showed bluish reflection, resulting from the Brownian motion of the suspended nanoparticles. The visual appearance of LMWH nanoparticle suspensions was similar to that described in the literature and remained homogeneous over the stability study time. The results are described in Table 1.

Table 1 Nanoparticle stability

Time	Particle diameter (nm)	Zeta potential (mV)	Polydispersion index (IPD)
0 day	217,9±2,5	-17,06±0,51	0,067±0,03
30 days	228,4±3,2	-17,3±0,91	0,07±0,05

Encapsulation efficiency

As LMWH is hydrophilic, the literature reports that the encapsulation of this type of substance is complex, since there is a greater diffusion of the active to the external aqueous phase before the precipitation of the polymer, thus reducing the encapsulation efficiency.[7] In this study, the LMWH Encapsulation Efficiency was 66.5%, showing that despite the hydrophilic characteristics of the

drug, the methodology employed in the development of the carrier system was satisfactory, a result similar to that obtained by Jogala et al, 2015 which obtained 46-70% EE, when nanoencapsular LMWH using PVA and PLGA9 double emulsion methodology. Thus, it can be stated that the EE obtained with the described methodology agrees with the literature and, therefore, satisfactory.

Conclusion

The present study confirmed that even though LMWH is hydrophilic, and it can be nanoencapsulated by solvent-evaporated double emulsion method using Poly (lactic-co- glycolic acid) (PLGA) and Polyvinyl Alcohol (PVA). The encapsulation efficiency obtained was satisfactory when compared to others described in the literature. The nanoparticles obtained may represent an interesting and viable alternative for the treatment of thromboembolic disorders.

Acknowledgments

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Conflicts of interest

Authors declare that there is no conflict of interest.

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