Gelucire-Based Nanoparticles for Curcumin Targeting to Oral Mucosa: Preparation, Characterization, and Antimicrobial Activity Assessment

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ABSTRACT: The purpose of the study was to prepare and characterize curcumin (Cur) solid lipid nanoparticles (CurSLN) with a high-loading capacity and chemical stability for the treatment of oral mucosal infection. CurSLN were formulated using different lipids, namely, Gelucire 39/01, Gelucire 50/13, Precirol, Compritol, and poloxamer 407 as a surfactant. Formulae were evaluated for their entrapment efficiency, particle size, and *ex vivo* mucoadhesion test. Microbiological evaluation was carried out on six microorganisms, five of which are the most commonly affecting oral cavity in terms of determination of minimum inhibitory concentration (MIC), and minimum bactericidal concentration. Transmission electron microscopy was conducted for ultrathin section for *Candida albicans*-treated with formulated Cur. The results showed high entrapment efficiency and stability enhancement for Cur powder. Significant amount of Cur was retained onto the mucosal tissue indicating preferential mucosal uptake. CurSLN showed higher antimicrobial activity as compared with Cur raw material and chemically stabilized Cur where it showed MIC (0.185, 0.09375, 0.75, 3, 1.5, and 0.1875 mg/mL) against *Staphylococcus aureus*, *Streptococcus mutans*, *Viridansstrept*, *Escherichia coli*, *Lactobacillus acidophilus*, and *Candida albicans*, respectively. The prepared lipid nanoparticles maintained Cur chemical stability and microbiological activity. The lack of local antimicrobial therapeutics with minimum side effects augments the importance of studying natural products for this purpose. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3913–3924, 2015

Keywords: curcumin; nanoparticles; drug delivery system; buccal; mucosal delivery; lipid; antimicrobial activity; cell disintegration

INTRODUCTION

The oral mucosa is targeted by infectious agents such as viral, fungal, and bacterial species. Being exposed to infectious agents, or changes in the oral environment, interactions with the oral microbiome and reduced defenses all potentially contribute to the development of opportunistic and nonopportunistic infections of the oral mucosa.[1] The most commonly used formulations include therapeutic agents; suffer serious side effects, in addition to resistance shown by microorganisms. This is the most common problem encountered in managing oral infection and is related primarily to systemic drug therapy. Targeted oral delivery as an alternative can be a promising approach to overcome such resistances.

Combination of herbal medicine with nanotechnology has been widely proposed, as nanostructured systems might be able to potentiate the action of plant extracts, reducing the required dose, side effects, and improving activity. Unlike conventional treatments, nanosystems can deliver the active constituent at a sufficient concentration during the entire treatment period, directing it to the desired site of action.[2]

Curcumin (Cur), 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, the natural precious gift, is a natural polyphenolic phytochemical extracted from the powdered rhizomes of turmeric (*Curcuma longa*).[3] Cur, the golden spice is commonly known as turmeric, displaying miraculous and numerous pharmacological activities such as antioxidant, anti-inflammatory, antitumoral, and antimicrobial ones.[4] Over the past 30 years, extensive research has shown that it plays an important role in the prevention and treatment of various proinflammatory chronic diseases including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune, and malignant diseases.[5] However, its clinical application has been limited because of poor aqueous solubility, rapid hydrolysis at neutral and basic pH, fast metabolism, and systemic elimination, which together are responsible for its low bioavailability.[6,7]

Different approaches are being taken to overcome problems of unstable drug candidates, of the most promising is the solid lipid nanoparticles (SLN) providing an alternative to polymeric nanoparticles and liposomes. SLN could be developed for both lipophilic and hydrophilic drugs. They are biocompatible, stable during storage and manufacturing, suitable for scaling up, and organic solvent-free preparation. Moreover, SLN rigid morphology is a fundamental advantage over other lipid-based colloidal drug delivery systems such as liposomes and nanoemulsions. With these features, SLN formulations have the potential to improve the bioavailability and stability of drugs for topical therapy.[8] Therefore, SLN have been intensively explored for drug delivery in the last decade and have been studied for different routes of administration; oral,[9] ocular,[10] nasal,[11] topical,[12] for both local,[13,14] and systemic[15] effect; however, only few works have been introducing their use for treatment of buccal mucosal

Abbreviations used: Cur, curcumin; SLN, solid lipid nanoparticles; PX407, poloxamer; G, Gelucire; Cp, Compritol; Pr, Precirol.

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diseases, in which Karavana et al.[8] introduced a new approach for the treatment of recurrent aphthous stomatitis using SLN-containing cyclosporine, dispersed in a gel matrix.

Few studies have introduced Cur solid lipid nanoparticles in an attempt to increase oral bioavailability,[16–18] but none introduced them for buccal mucosal treatment, nor used Gelucire50/13 for Cur delivery.

Commonly used solid lipids to produce SLN include glyceryl-behenate (Compritol), glyceryldistearate (Precirol), and cetyl-palmitate. Gelucires® are multifunctional lipid excipients composed of mono-, di-, and triglycerides and mono- and difatty acid esters of polyethylene glycol (PEG). Because it exhibits unique compositions with surfactants, cosurfactants, and lipid phases, it has interesting properties such as emulsification, drug solubility enhancement, and granule formation acting as lipid matrix in drug delivery systems.[19] Gelucires® are generally recognized as safe.[20] Its incorporation in lipid nanocarriers can prove to be helpful in increasing drug loading of lipophilic compounds as well as it can help stabilization of the lipid nanosystem. It is worth mentioning that Gelucires® are usually described with two numbers, the former is representing the lipid melting point, whereas the latter denotes the HLB value.

Therefore, this study aimed at formulating Cur as SLN to maintain its stability, improving its solubility and release as well as studying the feasibility of preparing Cur as Gelucire 50/13-based nanoparticles. Investigating the use of different lipids and stabilizers will also be studied for comparison. The potential of the delivery system adopted for enhancement of the antimicrobial activity of Cur for treatment of oral mucosal infection will be investigated.

MATERIAL AND METHODS

Curcumin (Hebeifood Additive Company, Ltd., ShiJiaZhuang, China) (purity >95%), lipids (Gelucire 39/01, Gelucire 50/13, Compritol 888 ATO, Precirol ATO5) (sample gift from Gattefosse, Lyon, France), poloxamer 407 (Kolliphore 407) (kind gift from BASF, Ludwigshafen, Germany), PEG 7-glyceryl-cocoate (Galaxy, Navi Mumbai, India), PEG 400, sodium lauryl sulfate (SLS), and potassium dihydrogen phosphate (El-Nasr Pharmaceutical Company, Alexandria, Egypt). Mucin was obtained from porcine stomach, type II, (Sigma, St. Louis, Missouri). *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Streptococcus mutans* ATCC25175 (*S. mutans*), *Viridans Streptococci* (clinical isolate), *Lactobacillus acidophilus* DSM20079 (*L. acidophilus*), *Escherichia coli* (ATCC 25922) (*E. coli)*, *Candida albicans* ATCC36802 (*C. albicans*).

METHODS

Solubility Study of Cur in Lipids (Semiquantitative Method)

An accurately weighed amount of each lipid, 500 mg, was melted in a beaker placed in a water bath adjusted to be 5°C above the lipid melting point. Cur was then added in increments of 5 mg and stirred on a magnetic stirrer until no more Cur was dissolved.[21] Total Cur increments showing no precipitation in the melted lipid is considered as solubility of Cur.

Formulation and Optimization of Cur SLN

Preliminary investigation was conducted for screening of the most suitable stabilizer concentration. Different concentrations of Gelucire50/13 (2%, 4%, 6%, 8%) were examined. Gelucire39/01 was used in this preliminary investigation. Eight different SLN formulations, each containing 60 mg Cur, were prepared using Gelucire 39/01, and 50/13, Compritol and Precirol. Gelucire50/13 and poloxamer 407 were used separately as stabilizer in all formulations.

Curcumin solid lipid nanoparticles (CurSLN) were prepared by hot homogenization method.[22] The lipid was melted at a temperature (5°C) above its melting point followed by the addition of Cur. Calculated amount of aqueous phase (water to 100%) with and /or without 8% poloxamer 407 maintained at same temperature squirted gently into the lipid (oil) phase under magnetic stirring at 600 rpm. Next, the mixture underwent high-shear dispersion at 12,000 rpm for 5 min using T18 ULTRA-TURRAX® homogenizer (IKA, Staufen, Germany). The emulsion obtained was cooled gradually to room temperature forming SLN. Blank SLN containing no drug were prepared in similar manner. The formulations were optimized with respect to lipid concentration, type, and concentration of surfactant. Composition of CurSLN formulations is shown in Table 1.

Characterizations of Cur SLN

Measurement of Particle Size and Polydispersity Index

The average of three for particle size and polydispersity index (PDI) of the SLN formulae was determined using Nano-ZS

Table 1. Composition of CurSLN Dispersion Each Containing 60 mg Cur/10 mL Dispersion

	Ingredients (mg)/10 mL Dispersion					
	Lipids				Stabilizers	
Formula Code	Gelucire 39/01	Gelucire 50/13	Compritol	Precirol	Gelucire 50/13	Poloxamer 407
G39/01-G50/13	500	–	–	–	800	–
G50/13-G50/13	–	500	–	–	800	–
Cp-G50/13	–	–	500	–	800	–
Pr-G50/13	–	–	–	500	800	–
G39/01-PX407	500	–	–	–	–	800
G50/13-PX407	–	500	–	–	–	800
Cp-PX407	–	–	500	–	–	800
Pr-PX407	–	–	–	500	–	800

G, Gelucire; Cp, Compritol; Pr, Precirol; PX407, poloxamer 407.