Design and Optimization of Self-Nanoemulsifying Delivery System to Enhance Quercetin Hepatoprotective Activity in Paracetamol-Induced Hepatotoxicity

OSAMA A. A. AHMED,[1] SHAIMAA M. BADR-ELDIN,[2] MONA K. TAWFIK,[3] TAREK A. AHMED,[4] KHALID M. EL-SAY,[4] JIHAN M. BADR[5]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Minia University, Minia, Egypt
[2]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[3]Department of Pharmacology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
[4]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
[5]Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt

Received 1 November 2013; revised 4 December 2013; accepted 6 December 2013

Published online 6 January 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23834

ABSTRACT: The present study aimed to develop optimized quercetin (QT)-loaded self-nanoemulsifying drug delivery system (SNEDDS) that offers protective effect against liver damage. Solubility study of QT in different oils, surfactants, and cosurfactants was performed. Ternary phase mixtures of the selected components were constructed to select a suitable range for each component. Experimental mixture design was utilized to optimize SNEDDSs that possess smaller globule size with enhanced emulsification and dissolution rates. QT SNEDDS was compared with QT suspension control and silymarin. *In vivo* evaluation and histopatholgical study of the selected QT SNEDDSs were achieved after administration of paracetamol over dosage to albino rats. Two optimized formulations were selected; one based on Sefsol and the other based on linoleic acid as an oily phase, Tween® 80 and polyethylene glycol 400 as surfactant and cosurfactant, respectively. Both Sefsol and linoleic-acid-optimized SNEDDS formulation showed no symptoms associated with toxicity and offered protective effect against paracetamol-induced hepatotoxicity by scavenging free radicals, attenuating lipid peroxidation, and enhancing the activity of antioxidants. The histopatholgical observations revealed that the inflammatory infiltrations induced by paracetamol were significantly ameliorated. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:602–612, 2014

Keywords: quercetin; SNEDDS; mixture design; hepatoprotective activity; nanotechnology; optical activity; dynamic light scattering; emulsion; nanoparticles

INTRODUCTION

The advances in combinatorial chemistry and screening strategy resulted in new chemical compounds with higher molecular weight and enhanced lipid solubility.[1,2] Thus, the successful formulation of most of the newly discovered drugs has become a challenge because of their inherent low aqueous solubility that generally leads to low oral availability, increased variation between and within subjects, and lack of dose-response proportional relationship.[3,4] Recently, lipid-based formulations have been evolving as an effective approach for enhancing oral bioavailability of lipophilic drugs. This approach comprises loading of drugs into lipid carriers such as surfactant or oils dispersions,[5] liposomes,[6] solid lipid nanoparticles, lipid nanocarriers,[7] micro- and nano-emulsions,[8,9] and self-nanoemulsifying drug delivery systems (SNEDDSs).[10]

SNEDDS are composed of natural or synthetic oils along with surfactants and cosurfactants that undergo spontaneous emulsification in the aqueous gastrointestinal tract (GIT) medium to form oil in water emulsion with globules having nanosize range.[11,12] SNEDDS offers several advantages including high solvent power, improved permeability across the GIT membrane, decreased or diminished food effect, and improved drug bioavailability.[13,14] The bioavailability enhancing property of the self-emulsifying formulations is not only because of improvement of drugs dissolution, but also associated with several mechanisms including reduction of first-pass drug metabolism in the liver. The reduced first-pass effect could be attributed to the association of lipid components with selective drug engulfing into the lymphatic transport system, and to the ability of lipid excipients and their metabolites to cause changes in the GI fluid-promoting drug absorption.[15] Moreover, the drug loading in the internal phase of SNEDDS can guard the drug against hydrolysis by the GIT enzymes.[16]

Parameters related to formulation, such as the concentration of surfactant, oil to surfactant ratio, and globule size, determine the self-emulsification ability of the formulation, and potentially the effectiveness of the drug oral absorption. Thus, only specific combinations of oils, surfactants, and cosurfactants will lead to optimized self-emulsifying systems.[17] The development of such efficient systems can be time-consuming and labor intensive because of the complex composition and the multiple components involved. Mixture experimental design has been proven to be an efficient strategy for the development and optimization of SNEDDS and to obtain the essential information to outline the relationship between the variables and performance.[18,19]

Quercetin (QT) [3, 3′, 4′, 5, 7-pentahydroxyflavone] is a flavonoid of plant origin. It is a common constituent of most edible fruits and vegetables. Previous preliminary studies

Correspondence to: Tarek A. Ahmed (Telephone: +20-1220610496; Fax: +20 2 2611404; E-mail: dr_tarek_nour@yahoo.com, drtareknour@gmail.com)

Journal of Pharmaceutical Sciences, Vol. 103, 602–612 (2014)

Table 1. Solubility of QT in Various Oils, Surfactants, and Cosurfactants at 25°C

Vehicle	Solubility (mg/mL) ± SD, $n = 3$
Sefsol	9.35 ± 0.32
Triacetin	3.53 ± 0.42
Isopropyl myristate	0.57 ± 0.05
Oleic acid	3.22 ± 0.33
Linoleic acid	9.43 ± 0.42
Tween® 80	32.63 ± 1.04
Tween® 60	4.89 ± 0.75
Span 80	4.76 ± 0.55
PEG 4000 (10%)	0.09 ± 0.02
PEG 6000 (10%)	0.13 ± 0.05
PEG 200	36.43 ± 2.32
PEG 400	47.47 ± 2.43
Propylene glycol	18.55 ± 1.11
Ethanol	21.56 ± 1.04
Propanol	21.66 ± 1.65

Table 2. Composition and Assessment of SNEDDS Constructing Ternary Phase Diagram A

Formula	Sefsol (%)	PEG (%)	Tween® 80 (%)	Visual Grading[a]	Globule Size (nm)	UV Absorbance at 638.2 nm
A1	85	10	5	C	5140	1.9239
A2	75	15	10	C	4450	1.5637
A3	65	20	15	C	3160	1.1246
A4	55	25	20	C	3920	1.3858
A5	45	30	25	C	2610	1.199
A6	40	30	30	C	2500	1.0016
A7	30	40	30	B	437	0.4924
A8	25	55	20	B	409	0.3288
A9	15	65	20	A	173	0.0075
A10	10	80	10	A	101	0.0039
A11	30	30	40	A	202	0.0126
A12	25	20	55	A	88	0.0037
A13	15	20	65	A	76	0.0016
A14	10	10	80	A	114	0.0043

[a] Visual grading system: A, a rapidly forming (within 30 s) microemulsion that was clear in appearance; B, a rapidly forming, slightly less clear emulsion that had a yellowish white appearance; and C, a milky white emulsion that formed within 2 min.

reported the hepatoprotective effect of QT.[20–22] It shows a wide range of pharmacological effects, such as antiproliferative and anti-inflammatory effects on many human cancer cell lines, antiviral properties, and also against aging.[23] However, the extremely low water solubility of QT combined with its extensive metabolism by the gut microorganisms result in reduced oral bioavailability.[24] This study aimed to formulate an optimized SNEDDS of QT containing the least surfactant and maximum lipid amounts that are characterized by having small globule size and high emulsification and dissolution rates. The study also aimed at investigating the optimized formulation hepatoprotective and antioxidant activity compared with silymarin, a market widely used hepatoprotective drug, in paracetamol-induced liver damaged rats.

MATERIALS AND METHODS

MATERIALS

Sefsol 218 was a kind gift from Nikko chemicals Company, Ltd. (Chuoku, Tokyo, Japan). Linoleic acid and isopropyl myristate were purchased from Acros organics (Fair Lawn, New Jersey). Triacetin was purchased from Spectrum Chemical Mfg. Corporation (New Brunswick, New Jersey). Propylene glycol was obtained from TEDIA company, Inc. (Fairfield, Ohio). Paracetamol and silymarin were from Sigma Chemical Company (Cairo, Egypt). QT, oleic acid, Tween® 60, Tween® 80, Span 80, polyethylene glycol (PEG) 4000, PEG 6000, PEG 200, PEG 400, propranolol, ethanol, and acetonitrile were procured from Sigma–Aldrich (St. Louis, Missouri). All other reagents and chemicals were of analytical grade.

METHODS

Solubility Studies

The solubility of QT in different oils, surfactants, and cosurfactants was assessed (Table 1). Excess amount of the drug was added to screw capped glass vials containing 3 mL of each of the studied vehicles. The mixtures were placed in a thermostatically controlled shaking water bath (Model 1031; GFL Corporation, Burgwedel, Germany) at 25 ± 0.5°C for 72 h. Samples were analyzed every day until equilibrium is reached (the solubility values on 2 consecutive days vary by not more than 5%). The mixtures were then subjected to centrifugation at 905 g for 15 min (labofuge 400; Heraeus GmbH, Hanau, Germany). Filtration of the supernatant was then performed through Acrodisc® syringe filter 0.45 μm Supro® membrane. The drug concentration in the filtrate quantified spectrophotometrically (Lambda 25 UV/Vis spectrophotometer; PerkinElmer, Shelton, Connecticut) for dissolved QT at 372 nm using methanol as a blank. All experiments were carried out in triplicate. Results were presented as mean value (mg/mL) ± SD.

Phase Equilibrium in Three Component System

Construction of Ternary Phase Mixtures

Aiming to recognize the zone of nanoemulsion formation, ternary mixtures of the selected surfactant, cosurfactant, and oil were constructed. For each ternary phase, 14 ternary mixtures were prepared with varying concentrations of Sefsol or linoleic acid (oil phase), Tween® 80 (surfactant phase), and PEG 400 (cosurfactant phase) as shown in Tables 2 and 3. For any mixture, the total of the three components always added to 100%.

Visual Assessment, Globule Size Analysis, and Emulsification Ability of the Ternary Mixtures

The efficiency of the prepared SNEDDS was assessed by diluting 100 mg of each mixture to 20 mL double distilled water. The diluted mixture was then gently agitated by means of a magnetic stirrer. The resulting dispersions were visually evaluated for the tendency to emulsify spontaneously and the final emulsion appearance. The number of volumetric flask inversions necessary to provide uniform emulsion was used as an indication for the ease of emulsion formation. The formed emulsions were visually observed for their relative turbidity, and their ultra violet transmittance was determined spectrophotometrically at 638.2 nm against blank of double distilled water after standing for 2 h.[16] Dynamic light scattering (DLS) using a