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# Quillaja saponin: A prospective emulsifier for the preparation of solid lipid nanoparticles

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ABSTRACT

Quillaja saponin (QS) is a non-ionic amphiphilic surfactant of natural origin. In the present study, we evaluated its potential to form solid lipid nanoparticles (SLNs) in the presence of stearic acid (SA) as a lipid carrier and imatinib mesylate (IM) as a model drug. IM loaded solid lipid nanoparticles (IMSLNs) were prepared using the hot homogenisation method. Characterisation of IMSLNs revealed that they were quasi-spherical in shape, neutrally charged and 143.5–641.9 nm in size. Haemolysis, a toxicity issue of QS was not observed in SLNs. Comparative in vitro cytotoxicity analyses performed in human breast cancer cell line MCF7 revealed that IMSLNs were more toxic than IM. On the other hand, in vitro viability studies in the RAW264.7 cell line did not show any sign of toxicity by IMSLNs. Our results indicate that QS hold great potential in nano drug delivery as an emulsifier.



## 1. Introduction

Applications of solid lipid nanoparticles (SLNs) have increased tremendously in recent times due to their major advantages such as biocompatibility, biodegradability, ease of preparation, potential for scale up and cost effectiveness. SLNs consist of a solid lipid core dispersed in aqueous solutions of stabilisers or surfactants at room temperature [1]. Stearic acid, palmitic acid, glyceryl behenate, etc., are the commonly used lipids; whereas, poloxamers, polysorbates, phospholipids, bile salts, sodium oleate, cremophor EL, etc. are commonly used as stabilisers in the preparation of SLNs. In this communication, we report on the efficient preparation of SLNs loaded with an anticancer drug using Quillaja saponin (QS) for the first time.

QS is a non-ionic amphiphilic surfactant extracted from the bark of *Quillaja saponaria* (Soap bark tree). QS is a glycoside (Fig. 1) with a lipophilic backbone of quillaic acid and gypsogenic acid (a triterpene aglycone), to which hydrophilic polysaccharide moieties like rhamnose, xylose, arabinose, galactose, fucose, and glucuronic acid are attached [2]. Thus, QS can perform the role of a surfactant by adsorbing at the oil and water interface to form an emulsion [3]. Despite the characterisation of the micellar properties of QS as early as 1800 AD and approval by the US Food and Drug Administration (FDA) [4], its application has not gained prominence in the pharmaceutical sector so far. In addition to its emulsifying property, QS also possesses many bioactivities (larvicidal, antitumor, antimicrobial etc.) and is used as a natural foaming agent in cosmetic products, and as an adjuvant in vaccine delivery [5–7]. Furthermore, a product based on the *Q. saponaria* bark extract is marketed under the trade name Q-Naturale and approved by FDA for use as emulsifier in beverages.

The aim of the present study was to investigate the possibility of using QS as a surfactant in the preparation of SLNs containing an anticancer drug. Briefly, we have used stearic acid (SA), an 18-carbon fatty acid as the lipid core to encapsulate imatinib mesylate (IM), a hydrophilic anticancer drug. The prepared SLNs, loaded with IM (IMSLN), were physicochemically characterised for particle size distribution, morphology, zeta potential, crystallinity, entrapment efficiency and drug release. In addition, they were analysed for in vitro haemolytic activity, cytotoxicity and viability using cell lines. Our results demonstrate that QS is an excellent stabiliser for the preparation of SLNs without any haematotoxicity.

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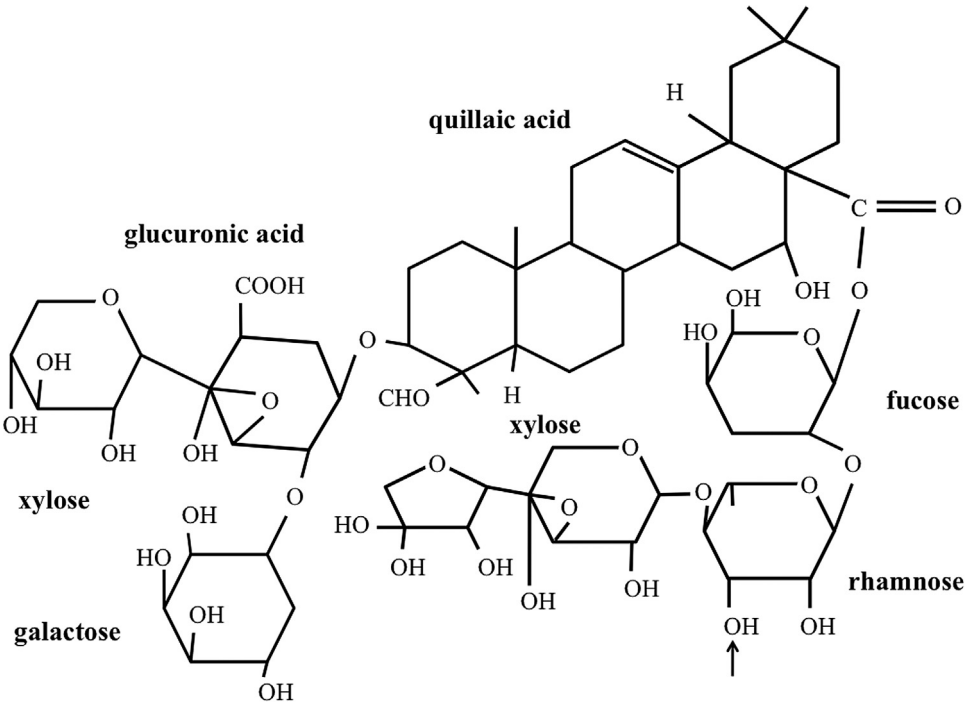


**Fig. 1.** Structure of Quillaja saponin.

## 2. Experimental

### 2.1. Materials

IM was obtained as a gift sample from NATCO Labs (Hyderabad, India). SA and QS were purchased from Sigma Aldrich, Germany.

### 2.2. Preparation of imatinib mesylate SLNs

IMSLNs were prepared via a hot homogenisation method followed by ultrasonication [8]. Briefly, SA was melted at 75 °C in a water bath. IM was added to the melted lipid to form a clear, homogeneous lipid phase. Simultaneously, an aqueous solution of QS preheated to 75 °C was dispersed in the lipid phase using a high shear homogeniser (POLYTRON PT 3100 D) at 13,000 rpm for 4 min at 75 °C. This pre-emulsion was sonicated in an ultrasonic homogeniser (SONICS) for 2 min at 40% amplitude and cooled down in an ice bath to form IMSLNs. Blank SLNs (BSLNs) were prepared in a similar manner but, in the absence of IM. The composition of various formulations is provided in Table 1.

### 2.3. Characterisation

Prepared IMSLN formulations were characterised for their particle size, polydispersity index and zeta potential by Malvern Zetasizer (Nano ZS90, Malvern Instruments). Atomic force microscopy (AFM, NTMDT, NTEGRA prima, Russia) and high-resolution transmission electron microscopy (TEM, JEOL JEM 2100) techniques were used to characterise the morphology of IMSLNs. To measure the endothermic melting temperatures of the SLNs, part of the formulations were dried in a LYODEL freeze drier at −30 °C under −1 mbar pressure and analysed by differential scanning calorimetry (DSC, Universal V4.7A TA Instruments). Briefly, 10 mg sample (IM or SA or QS or IMSLNs or BSLNs) was placed on an aluminium pan and scanned between 25 °C and 300 °C at a rate of 5 °C/ min under nitrogen gas.

To calculate encapsulation efficiency, 2 ml of the formulation was centrifuged at 20,000 rpm for 45 min at 10 °C and the supernatant was analysed for free drug concentration using UV spectroscopy at 256 nm [9]. BSLNs were used as a control. Encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{amount of drug added during preparation} - \text{amount of free drug in the supernatant} \times 100}{\text{amount of drug added during preparation}}$$

### 2.4. In vitro drug release

In vitro drug release profiles of IMSLN formulations and IM solubilised in phosphate buffered saline (PBS, pH 7.4) was studied by a dialysis bag method. Briefly, 1 ml sample was loaded into a dialysis bag with a molecular weight cut off of 12,000–14,000 Da and both its ends were sealed with clips. The dialysis bag was then placed in a beaker containing 50 ml of PBS maintained at 37 ± 1 °C under stirring at 50 rpm using a magnetic stirrer. After various time points (0.5, 1, 2, 3, 4, 6, 8 and 10 h), 2 ml of PBS was withdrawn from the beaker. Each withdrawal was compensated by addition of 2 ml of fresh PBS to the beaker. The drug content of the collected samples was determined by UV spectroscopy at 256 nm.

### 2.5. Analysis of toxicity

Because of its smaller particle size, decent entrapment efficiency and high concentration of QS, the formulation 3 (IMSLN3) was selected for toxicity evaluation.

#### 2.5.1. In vitro haemolysis test

The in vitro haemolytic potential [10] of QS (50 μg/ml solution in saline), IM (10 μg/ ml and 20 μg/ ml in saline), IMSLN3 (equivalent