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*Original Citation:*

Enhanced curcumin permeability by SLN formulation: The PAMPA approach / Righeschi, Chiara; Bergonzi, Maria Camilla; Isacchi, Benedetta; Bazzicalupi, Carla; Gratteri, Paola; Bilia, Anna Rita. - In: LEBENSMITTELWISSENSCHAFT + TECHNOLOGIE. - ISSN 0023-6438. - STAMPA. - 66:(2016), pp. 475-483. [10.1016/j.lwt.2015.11.008]

*Availability:*

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*Published version:*

DOI: 10.1016/j.lwt.2015.11.008

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# Accepted Manuscript

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Chiara Righeschi, Maria Camilla Bergonzi, Benedetta Isacchi, Carla Bazzicalupi,  
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PII: S0023-6438(15)30299-1

DOI: [10.1016/j.lwt.2015.11.008](https://doi.org/10.1016/j.lwt.2015.11.008)

Reference: YFSTL 5076

To appear in: *LWT - Food Science and Technology*

Received Date: 28 May 2015

Revised Date: 2 November 2015

Accepted Date: 4 November 2015

Please cite this article as: Righeschi, C., Bergonzi, M.C., Isacchi, B., Bazzicalupi, C., Gratteri, P., Bilia, A.R., Enhanced curcumin permeability by SLN formulation: the PAMPA approach, *LWT - Food Science and Technology* (2015), doi: 10.1016/j.lwt.2015.11.008.

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**Enhanced curcumin permeability by SLN formulation: the PAMPA approach.**

Chiara Righeschi<sup>a‡</sup>, Maria Camilla Bergonzi<sup>a‡\*</sup>, Benedetta Isacchi<sup>a</sup>, Carla Bazzicalupi<sup>b</sup>,  
Paola Gratteri<sup>c</sup>, Anna Rita Bilia<sup>a</sup>

<sup>‡</sup> These authors contributed equally to this work.

<sup>a</sup> *Department of Chemistry “Ugo Schiff”, University of Florence, via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy*

<sup>b</sup> *Department of Chemistry “Ugo Schiff”, University of Florence, via della Lastruccia 3-13, 50019 Sesto Fiorentino, Florence, Italy*

<sup>c</sup> *Department of NEUROFARBA-Pharmaceutical and Nutraceutical Section-Laboratory of Molecular Modeling Cheminformatics & QSAR, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy*

\*Corresponding author: Maria Camilla Bergonzi: *E-mail address: mc.bergonzi@unifi.it*

Phone: +39 055 4573678.

29 **Abstract**

30 Curcumin health benefits are strongly limited by its poor aqueous solubility and low oral  
31 bioavailability. This work was focused on the development and characterization of solid lipid  
32 nanoparticles (SLNs) for the encapsulation of curcumin for oral administration. High shear  
33 homogenization and ultrasonication techniques were employed to prepare Compritol SLNs.  
34 The physicochemical characterization of round shaped curcumin-loaded SLNs was carried out  
35 by monitoring particle size (lower than 300 nm), zeta potential (-33 mV), drug loading  
36 capacity (1.60%), drug entrapment efficiency (80%), TEM analysis and *in vitro* drug release.  
37 Stability (4°C) was investigated over one month. Parallel Artificial Membrane Permeability  
38 Assay (PAMPA) showed a considerable increase of curcumin permeated when formulated as  
39 SLNs. A modified release profile suggested that curcumin molecules are solubilized into the  
40 solid lipid matrix. The developed SLNs were produced without the use of solvents and all  
41 excipients were GRAS ingredients; both technology and composition were suitable for food  
42 application.

43

44 **Keywords:** curcumin, solid lipid nanoparticles, Parallel Artificial Permeability Assay, *in vitro*  
45 release, stability studies.

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## 58 1. Introduction

59 Curcumin is a yellow-colored phenolic natural constituent derived from the rhizome of the  
60 spice herb *Curcuma longa* L., widely known as turmeric. It has a broad spectrum of biological  
61 activities, principally antioxidant (Sharma, Manoharlal, Puri, & Prasad, 2010; Selvam,  
62 Subramanian, Gayathri, & Angayarkanni, 1995; Ruby, Kuttan, Babu, Rajasekharan, & Kuttan  
63 1995) anti-inflammatory (Menon & Sudheer, 2007; Rao, Basu, & Siddiqui, 1982),  
64 antibacterial (Negi, Jayaprakasha, Jagan Mohan Rao, & Sakariah, 1999), antifungal (Sharma,  
65 1976). Its chemopreventive (Devasena, Rajasekaran, Gunasekaran, Viswanathan, &  
66 Venugopal, 2003; Park, 2010) activity has received considerable attention because curcumin  
67 influences multiple signaling pathways, modulating more than 30 different proteins, including  
68 thioredoxin reductase, cyclooxygenase-2 (COX-2), protein kinase C (PKC), 5-lipoxygenase,  
69 activated AMP-activated protein kinase (AMPK) and tubulin. Other molecular targets  
70 modulated by this substance included transcription factors, growth factors and their receptors,  
71 cytokines, enzymes, and genes regulating cell proliferation and apoptosis (Aggarwal, Kumar,  
72 & Bharti, 2003; Shishodia, Singh, & Chaturvedi, 2007; Aggarwal, Kuiken, Michelle, Laxmi,  
73 Kuzhuvilil, & Bokyoung, 2009).

74 Curcumin shows a good safety profile, no studies in either animals or humans have  
75 demonstrated any toxicity associated with its use, even at high doses (Shankar, Shantha,  
76 Ramesh, Murthy, & Murthy, 1980; Lao, Ruffin, Normolle, Heath, & Bailey, 2006). This safe  
77 profile has been reflected by the continuous increase of preparations based on curcumin  
78 marketed as food ingredient or constituent of dietary supplements. Despite multiple health  
79 benefits, its utility is strongly limited by its poor aqueous solubility and low oral  
80 bioavailability. The latter is attributed to poor absorption, extensive intestinal and hepatic  
81 metabolism, rapid elimination and clearance from the body (Pan, Huang, & Lin, 1999; Anand,  
82 Kunnumakkara, Newman, & Aggarwal, 2007). Formulation of curcumin in effective dosage  
83 forms represented a challenge to overcome its poor physicochemical properties. Several  
84 strategies such as nanoparticles, liposomes, solid dispersions, microemulsions and  
85 complexation with phospholipids and cyclodextrins have been developed to improve the  
86 bioavailability of curcumin (Bisht, Feldmann, Soni, Ravi, Karikar, Maitra, & Maitra, 2007;  
87 Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007; Tiyaboonchai, Tungpradit, &  
88 Plianbangchang, 2007; Sanoj Rejinold, Sreerexha, Chennazhi, Nair, & Jayakumar, 2011;  
89 Bergonzi, Hamdouch, Mazzacuva, Isacchi, & Bilia, 2014; Chaurasia, Patel, Chaubey, Kumar,  
90 Khan, & Mishra, 2015).

91 This study was focused on the preparation and characterization of solid lipid nanoparticles  
92 (SLNs) for oral administration of curcumin (Dhillon, Aggarwal, Newman, Wolff,  
93 Kunnumakkara, & Abbruzzese, 2008). Lipid-based drug delivery systems are promising  
94 formulations, since lipids are known oral drug absorption enhancers (Porter, Wasan, &  
95 Constantinides, 2008; Chakraborty, Shukla, Mishra, & Singh, 2009) and they can be  
96 developed in small particle sizes ranging from micro to nanometers (Jia, 2005; Pouton, 2006),  
97 that can increase the absorption in the hydrophilic environment of the gastrointestinal tract.  
98 SLNs combine these features: they are colloidal carriers of submicron size constituted of solid  
99 lipids at body and room temperatures. Substances used to prepare these nanoparticles are  
100 physiologic and generally recognized as safe compounds (GRAS ingredients); this aspect  
101 makes SLNs carriers without toxicity in humans (Müller, Mehnert, Lucks, Schwarz, Zur  
102 mühlen, & Meyhers, 1995). Moreover, they retain the advantages of traditional colloidal  
103 systems (Müller, Mader, & Gohla, 2000) such as enhanced physical stability, protection of  
104 drug molecules from degradation in the body, controlled drug release, organ or tissue specific  
105 targeting, biocompatibility, laboratory to industrial-scalability. SLNs, consist of a lipid core  
106 and an outer shell of amphiphilic surfactant. If drug is loaded in the outer shell and on the  
107 particle surface, it is quickly released, displaying a burst effect. On the other hand, if it is  
108 incorporated into the particle core, it is released in a prolonged way. This make possible a  
109 controlled drug release from these carriers, representing an important tool to obtain a  
110 prolonged release of the drug (Müller et al., 2000; Reddy, 2005).

111 A few studies concerning the development of curcumin SLNs have been reported in the  
112 literature, but only one paper is dealing SLN for oral administration (Kakkar, Singh, Singla, &  
113 Kaur, 2011). This study described the preparation of SLNs using Tween 80, soy lecithin and  
114 Compritol with microemulsion technique. *In vivo* pharmacokinetics performed in rats after  
115 oral administration of SLNs revealed significant improvement of curcumin bioavailability,  
116 but the percentage of Tween 80 in the SLNs is quite high (more than six times higher than the  
117 lipid phase). In other studies, the percentage of surfactant is the same or much higher than that  
118 of the lipid (Kakkar, Mishra, Chuttani, & Kaur, 2013; Aditya, Macedo, Doktorovova, Souto,  
119 Kim, Chang, & Ko, 2014; Hazzah, Farid, Nasra, EL-Massik, & Abdallah, 2015; Sun, Bi,  
120 Chan, Sun, Zhang & Zheng, 2013).

121 In the present study, the preparation of curcumin SLNs for a suitable oral dosage form was  
122 investigated, by using 50 g/kg fat phase and a low content of surfactant (25 g/kg), very  
123 suitable for oral administration.

124 In addition, the development of SLNs was carried out by homogenization-ultrasonication  
125 technique without the use of the solvents, technology that can be applied also to an industrial  
126 process.

127 Another key aspect was the analyses by DSC and X-ray diffractometry to investigate the  
128 drug-lipid interactions and the crystallinity of both curcumin and lipids, because this aspect  
129 could influence the release. Particle size analysis is a necessary, but not a sufficient step to  
130 characterize SLN quality. Special attention must be paid to the characterization of the degree  
131 of lipid crystallinity and the modification of the lipid, because these parameters are strongly  
132 correlated with drug incorporation and release profiles. DSC uses the fact that different lipid  
133 modifications possess different melting points and melting enthalpies. By means of X-ray  
134 scattering it is possible to assess the length of the long and short spacings of the lipid lattice.

135 Finally, stability test, release profile and permeability assay were performed to confirm the  
136 optimized biopharmaceutical properties of the developed nanovectors. The results confirmed  
137 that the developed SLNs offer a promising delivery system for enhancing the oral absorption  
138 of curcumin, suitable either for food fortification or as dietary supplements.

139

## 140 **2. Materials and methods**

### 141 *2.1 Materials*

142 Compritol 888 ATO, a mixture of mono-, di- and triglycerides of behenic acid (C22), was a  
143 gift of Gattefossè (Milan, Italy). Curcumin, Pluronic F68 and 1,7-octadiene were purchased  
144 from Sigma Aldrich Corporation (St. Louis, MO, USA). Lecithin was kindly provided by  
145 Galeno (Comeana, Prato, Italy). Lipase from porcine pancreas, pepsin from porcine gastric  
146 mucose, bile salts and cholesterol were analytical grade from Sigma Aldrich (Milan, Italy).  
147 96-well MultiScreen PAMPA filter plate were purchased from Millipore Corporation,  
148 (Tullagreen, Carrigtwohill, County Cork, Ireland). All the solvents used were HPLC grade  
149 from Merck (Darmstadt, Germany); 85% formic acid was provided by Carlo Erba (Milan,  
150 Italy). Ethanol analytical reagent grade was from Riedel-de Haen Laborchemikalien GmbH &  
151 Co. KG, (Seelze, Germany). Water was purified by a Milli-Q<sub>plus</sub> system from Millipore  
152 (Milford, MA). Phosphotungstic acid (PTA) was from Electron Microscopy Sciences  
153 (Hatfield, USA). Hydriion Buffer chemvelope pH 7.40±0.02 was purchased from Micro  
154 Essential Laboratory (Brooklin, New York, USA).

155

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## 158 2.2 High shear homogenization-ultrasonication method

159 Blank and drug loaded SLNs were prepared using hot homogenization process followed by  
160 ultrasonication (Castelli et al., 2005). Two formulations were investigated (SLN-1 and SLN-  
161 2) loaded with two different concentrations of curcumin: 0.01 g/g and 0.02 g/g respect to the  
162 lipid phase, respectively.

163 Briefly, different amounts of curcumin, (respectively 75 and 150 mg) were accurately  
164 weighed and added to melted Compritol (7.5 g, 80°C). Pluronic F68 (3.75 g) was dissolved in  
165 distilled water (138 g) and heated at 85°C in a beaker. When a clear homogenous lipid phase  
166 was obtained, hot aqueous surfactant solution was added to hot lipid phase and homogenized  
167 at 9660 x g, by using a high-speed stirrer (Ultra Turrax T25, IKA-Werke GmbH&Co. KG,  
168 Staufen, Germany) for five minutes. The temperature was maintained at 80°C during this step.  
169 Then, the coarse emulsion was subjected to probe sonication (Sonopuls HD 2200, 200 W  
170 power, probe MS 72, Bandelin Electronic GmbH, Berlin, Germany) for different times. Probe  
171 sonication process was suspended for 2' intervals during each cycle, to prevent increase of  
172 temperature. Temperature was monitored during the process. Sonication was applied  
173 maximum for 15 minutes to avoid metal contamination, by considering also previously  
174 published experimental conditions (Silva, González-Mira, García, Egea, Fonseca, Silva,  
175 Santos, Souto, & Ferreira, 2011; Müller, Rühl, Runge, Schulze-Forster, & Mehnert, 1997;  
176 Nassimi, Schleh, Lauenstein, Hussein, Hoymann, & Koch, 2010; Nayak, Tiyaboonchai,  
177 Patankar, Madhusudhan, & Souto, 2010; Vitorino, Carvalho, Almeida, Sousa, & Pais, 2011).  
178 Working temperature was kept at least 5°C above the lipid melting point (85°C) to prevent  
179 recrystallization during homogenization and ultrasonication. After ultrasonication, the  
180 obtained emulsion (O/W) was cooled in an ice bath in order to solidify the lipid matrix and to  
181 form SLNs. Blank and curcumin loaded SLNs were prepared and characterized as well. SLNs  
182 dispersions were stored at 4°C for further analyses.

183

## 184 2.3 Particle size analysis and zeta potential measurements

185 Mean diameter of the population and polydispersity index (P.I.) as a measure of the width of  
186 particle size distribution together with the measure of zeta potential values were assessed by  
187 photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments,  
188 UK). Samples were prepared diluting 10 µl of SLN suspension with 2 ml of deionized water.  
189 All measurements were done in triplicate. pH was measured prior to and after dilution,

190 because it value could alter the zeta-potential. pH resulted 6.1 prior to dilution and 6.3 after  
 191 dilution.

#### 192 *2.4 Determination of encapsulation efficiency*

193 The percentage of curcumin entrapped in the lipid matrix was determined as follows: a fixed  
 194 amount of SLNs dispersion was purified by dialysis method, using a membrane  
 195 (MW=12.400, Sigma Aldrich, Milan, Italy). Then, an amount of material retained in the bag  
 196 was freeze-dried at -40°C for 24 h, for following stability studies. The freeze drying process  
 197 was optimized to obtain a homogenous porous solid and according to TEM and light  
 198 scattering analyses.

199 Sample was dissolved in MeOH under stirring at 80°C for 10 min and then cooled to room  
 200 temperature to preferentially precipitate the lipid (Nayak, et al., 2010; Tiyaboonchai,  
 201 Tungpradit, & Plianbangchang, 2007; Sanna, Gavini, Cossu, Rassu, & Giunchedi, 2007). The  
 202 suspension was centrifuged for 30 minutes at 13148 x g and the supernatant was analyzed by  
 203 HPLC/DAD analysis using curcumin as external standard. Calibration curves were performed  
 204 on six solutions in the concentration range 1.38–138 µg/ml. The squared correlation  
 205 coefficient was >0.99. Curcumin encapsulation efficiency was expressed as drug recovery and  
 206 calculated from the following equation:

$$207 \text{ Drug encapsulation efficiency (\%)} = \frac{\text{mass of active in nanoparticles}}{\text{mass of active fed to the system}} \times 100$$

210 The drug loading content was the ratio of incorporated drug to lipid (w/w):

$$211 \text{ Drug loading (\%)} = \frac{\text{mass of active in nanoparticles}}{\text{weight of lipid}} \times 100$$

212 No lipid interference occurred during UV determination of curcumin (data not shown).  
 213 Curcumin loaded concentration was assayed by HPLC/DAD analysis performed using a HP  
 214 1100 Liquid Chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a  
 215 HP 1040 Diode Array Detector (DAD), an automatic injector, an auto sampler and a column  
 216 oven and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA).  
 217 The UV-Vis spectra were recorded between 220–500 nm and the chromatographic profiles  
 218 was registered at 420 nm. Separations were performed on a reversed phase column Luna C18  
 219 (150 x 4.6 mm, 5 µm, Phenomenex) maintained at 27°C. The eluents were H<sub>2</sub>O at pH 3.2 by  
 220 formic acid (Solvent A) and acetonitrile (Solvent B), using a multi-step linear gradient of 32  
 221

222 min at a flow rate of 0.4 ml/min: 0.10 min 72% A and 18% of B; 10.0 min 56% A to 44% B;  
223 27.0 min 52% A to 48% B; 27.0 min 52% A to 48% B; 32.0 min 20% A to 80% B .

#### 224 2.5 Transmission electron microscopy (TEM)

225 The morphological characterization of the systems was obtained by using transmission  
226 electron microscopy technique (TEM, CM12 Philips, Netherlands). Samples were deposited  
227 on a *formuvar* film-coated copper grid and then stained with one drop of 20 g/L aqueous  
228 solution of phosphotungstic acid (PTA), allowing to dry before TEM observation.

229

#### 230 2.6 Differential scanning calorimetry (DSC)

231 DSC was carried out using a Mettler TA4000 apparatus equipped with a DSC 25 cell.  
232 Samples (about 10 mg) were accurately weighed (Mettler M3 Microbalance) directly in  
233 pierced aluminum pans and scanned between 30 and 250°C at a heating rate of 10 K min<sup>-1</sup>  
234 under static air. DSC thermograms of pure curcumin, Compritol, and curcumin-loaded SLNs  
235 were compared.

#### 236 2.7 X-ray powder diffractometry

237 X-ray powder diffractograms were obtained with a Bruker D8 advance powder  
238 diffractometer, equipped with Cu K $\alpha$  radiation and operating in  $\vartheta$ -2 $\vartheta$  Bragg Brentano  
239 geometry at 40 kV and 30 mA. The “SolX” solid-state detector was used. C/Ni Goebel  
240 mirrors for the incident beam were used. The samples were analyzed at ambient temperature  
241 over the 10–35° 2 $\vartheta$  range at a scan rate of 0.02 deg s<sup>-1</sup>.

#### 242 2.8 In vitro release studies

243 Dialysis bag method was applied to study the drug release using a mixture of PBS (pH 6.8)  
244 and EtOH (150 mL/L ethanol to maintain the sink conditions) or simulated intestinal fluid as  
245 dissolution media. Simulated intestinal conditions contained intestinal enzymes (lipase 0.4  
246 mg/ml, bile salts 0.7 mg/ml and pancreatin 0.5 mg/ml) and calcium chloride solution 750 mM  
247 at pH 7.0, (Aditya, et al., 2014). Release was monitored for 12 h. The dialysis bags were  
248 hydrated in PBS before use. Two milliliter of SLNs dispersion was introduced into the  
249 dialysis bag. The bag was placed in a beaker containing 200 mL of dissolution medium  
250 maintained at 37°C under magnetic stirring (50 rpm). Aliquots of the dissolution medium

251 were withdrawn at different time intervals and replaced with the same volume of fresh  
252 medium to maintain the sink conditions. The samples were suitably diluted and analyzed for  
253 curcumin determination. All the operations were carried out in triplicate.

## 254 2.9 Stability studies

255 Stability of curcumin SLNs was studied over 1 month. SLNs were kept at  $4\pm 1^\circ\text{C}$  and at fixed  
256 time intervals; they were assayed for their physical stability. Physical stability was checked by  
257 monitoring size, zeta potential and polydispersity of formulation.

## 258 2.10 Parallel artificial membrane permeability assay (PAMPA)

259 The PAMPA assay is a method for predicting passive intestinal absorption. The assay is  
260 carried out in a 96-well, MultiScreen-IP PAMPA (Millipore corporation) filter plate. The  
261 ability of compounds to diffuse from a donor compartment, through a PVDF membrane filter  
262 pretreated with a lipid-containing organic solvent, into an acceptor compartment is evaluated.  
263 5  $\mu\text{L}$  of lecithin (10g/L) and cholesterol (8g/L) in 1,7-octadiene solution were added to the  
264 filter of each well. Immediately after the application of the artificial membrane, 150  $\mu\text{L}$  of  
265 drug containing donor solutions (free drug or curcumin-SLNs diluted in 0.05 mL/mL  
266 DMSO/PBS) were added to each well of the donor plate. In details, SLNs suspension was  
267 diluted to obtain in each donor compartment a final concentration of curcumin of 160  $\mu\text{g}$ . A  
268 saturated solution of curcumin (0.3  $\mu\text{g}$  of curcumin solubilized in the donor compartment)  
269 was used as control. 300  $\mu\text{L}$  of buffer (0.05 mL/mL DMSO/PBS, pH 7.4) were added to each  
270 well of the acceptor plate. The acceptor plate was then placed into the donor plate, ensuring  
271 that the underside of the membrane was in contact with buffer. The plate was covered and  
272 incubated at room temperature under shaking for 24 hours and permeation was evaluated at 1,  
273 2, 4, 6, 19, 24 hours.

## 274 3. Results and Discussion

### 275 3.1 Preparation of SLNs

276 The use of solid lipids instead of liquid oils is a very attractive idea to achieve controlled drug  
277 release, because drug mobility in a solid lipid should be considerably lower, compared with a  
278 liquid oil. SLNs are non-toxic, high biocompatible and easy to produce in large scale. SLN  
279 are drug carriers composed of a solid core suitable to target drugs to specific intestine  
280 associated systems. The nanoparticles are in the submicron size range. At room temperature

281 the particles are in the solid state. Therefore, the mobility of incorporated drugs is reduced,  
282 which is a prerequisite for controlled drug release.

283 Selected SLNs consisting of a core of Compritol were stabilized with Pluronic F68. Compritol  
284 was selected because it is a mixture of mono, di and triglycerides. Moreover, Compritol in  
285 enhancing the oral bioavailability in comparison to other lipid matrices was previously  
286 highlighted (Paliwal, Rai, Vaidya, Khatri, Goyal, Mishra, Mehta, & Vyas, 2009).

287 Compritol-based nanoparticles were heterogeneous with better drug-loading and release  
288 characteristics as compared with the other formulations. Accordingly, SLNs containing lipids  
289 with highly crystalline structure can give drug expulsion. On the other hand, the imperfections  
290 (lattice defects) of the lipid structure could offer more loading space to accommodate drugs  
291 (Westesen, Bunjes, & Koch, 1997; Muller et al., 2000; Silva, González-Mira, García, Egea,  
292 Fonseca, & Silva, 2011; Freitas & Müller, 1999; Mehnert & Mäder, 2012) while the use of a  
293 mixture of different oils as lipophilic can benefit the encapsulation efficiency. Compritol was  
294 also chosen as the lipid component because it gives stable dispersions with smaller particles.  
295 Two formulations with different amount of curcumin (0.01 g/g and 0.02 g/g respect to the  
296 lipid phase, SLN-1 and SLN-2 respectively) were prepared and fully characterized.

297 Homogenization-ultrasonication method was selected because economical, efficient and  
298 reproducible process to produce SLNs. Effect of different process variables on size, P.I., zeta  
299 potential, encapsulation efficiency was analyzed, and the results were reported on Table 1.  
300 Homogenization time was maintained constant at 10 minutes, time necessary to obtain a good  
301 emulsification of lipid (Das, Ng, Kanaujia, Kim, & Tan, 2011).

302 Sonication time (5, 10 and 15 minutes) showed huge influence on particle size. Sizes  
303 significantly decreased with increasing sonication time and the samples resulted always  
304 homogeneous. The best results in terms of P.I. values were found in the formulations SLN-1  
305 and SLN-2, when 15 minutes of sonication were applied.

306 All formulations were negatively charged, the zeta potential varied from -10 to -45 mV  
307 indicating a relatively good stability and dispersion of the system. The negative value of zeta  
308 potential of SLNs was attributed to the presence of behenic acid into the lipid matrix surface  
309 and also to Pluronic. This is a non-ionic surfactant used in the production of relatively stable  
310 dispersions. Although non-ionic surfactant could not interact with charging group like ionic  
311 ones, but it can influence the particle/water interface and electric double layer. Pluronic can  
312 also provide additional steric stabilization of particles (Schwarz & Mehnert, 1999; Lim &  
313 Kim, 2002).

314 The quantity of curcumin loaded in the system increased the dimensions of SLNs: with 2%  
315 (0.02 g/g) the sizes ranged from 415 to 270, by increasing the sonication time. Particle size  
316 slightly increased with higher drug concentration; this is probably due to the presence of  
317 curcumin inside of lipidic core.

318 The data in Table 1 shows that all formulations possessed high entrapment efficiency (E.E.  
319 %) ranged from  $70.7\pm 2\%$  to  $80.2\pm 2\%$ . Encapsulation efficiency was not significantly  
320 different among SLN-1 and SLN-2, resulting around of 70%; such values might be related to  
321 the structure of the lipid which had a great influence on drug incorporation. Lipids which  
322 form highly crystalline particles with a perfect structure lead to drug expulsion (Westesen et  
323 al., 1997), while, more complex lipids such as Compritol, being mixtures of mono-, di- and  
324 triglycerides, produce less perfect structures with many imperfections offering space to  
325 accommodate curcumin (Müller et al., 2000). Another feature of Compritol that favors the  
326 encapsulation of lipophilic curcumin was the high hydrophobicity due to the long chain fatty  
327 acids attached to the triglycerides.

328 The formulation selected on the basis of the best properties in terms of size, P.I. and zeta  
329 potential was SLN-2-15, loaded with 0.02g/g of curcumin and sonicated for 15 minutes: it  
330 showed also an encapsulation efficiency of 80% and was selected for further investigations.

331

### 332 3.2 TEM analysis

333 TEM was conducted to investigate the morphology of solid lipid nanoparticles SLN-2-15. It  
334 was evident from TEM images that nanoparticles were almost spherical with smooth  
335 morphology, appeared as black dots, well dispersed and separated (Figure 1). This description  
336 agrees with a previous observation that the use of chemically heterogeneous lipids in  
337 combination with heterogeneous surfactants favors the formation of ideally spherical lipid  
338 nanoparticles (Mehnert & Mader, 2001). The mean diameter was in the range of 250-300 nm.

### 339 3.3 DSC and X-ray diffractometry assay

340 DSC and X-ray diffraction were performed for the assessment of the drug–lipid interactions  
341 and the crystallinity of curcumin and lipid matrices, because this aspect could influence the *in*  
342 *vitro* and *in vivo* release of the compound from the systems (Müller et al., 2000). Lipid  
343 crystallization is an important point for the performance of the SLN carriers. In fact, less  
344 perfect crystals with many imperfections can offer space to accommodate the drug (Müller et  
345 al., 2000) and also it can modulate the mobility of the drug during the release process

346 (Mehnert & Mäder, 2001; Silva et al., 2011; Mehnert & Mäder, 2012). The lack of  
347 crystallinity is also highly required to avoid extrusion of drug during storage.

348 The following samples were analysed: pure curcumin, Compritol, unloaded and loaded SLNs  
349 SLN-2-15. Figure 2 depicts the DSC thermograms obtained. As Compritol is not composed of  
350 pure triacylglycerols, the observed melting peak at 72.1°C might be due to a mixture of  
351 metastable polymorphic  $\beta$  and  $\beta'$  forms. The thermogram showed also a relatively small  
352 endothermic shoulder at around 55°C; this small shoulder corresponds to the melting of a very  
353 unstable modification of Compritol which is the  $\alpha$  modification (Souto et al., 2006), that  
354 clearly disappears after the nanoparticles preparation. DSC analysis of curcumin showed the  
355 melting point at 173.17°C. In the DSC thermograms of blank SLNs (unloaded) and loaded  
356 SLNs, a small endothermic peak was observed at 50°C. This peak indicates the presence of  
357 Pluronic either in the form of coating surrounding the nanoparticles or as residue after dialysis  
358 and lyophilization.

359 Curcumin melting peak was not recorded in the SLN formulation attributed to the solubility  
360 of the drug within the solid lipid matrix.

361 X-ray diffraction studies evidenced that diffraction pattern of bulk Compritol (Figure 3)  
362 presents two main typical signals at 21.5° (2 $\theta$ ) and 23.5° (2 $\theta$ ), significantly modified when  
363 formulated into nanoparticles. Besides, another signal arises at 19.4° (2 $\theta$ ) after SLN  
364 preparation, corresponding to the most stable polymorphic form of triacylglycerols  $\beta$  (Souto,  
365 Mehnert, & Müller, 2006). These results might indicate that the final formulation is composed  
366 of the most stable polymorphic state of Compritol: SLNs seem to lose part of their  
367 crystallinity by transforming from a mixture of  $\beta$  and  $\beta'$  polymorphs in the most stable  $\beta$   
368 polymorph and allowing curcumin to penetrate in the molecular gaps.

### 369 3.4 *In vitro* release studies

370 The release of curcumin from SLN-2-15 formulation was tested *in vitro* in of PBS (pH 6.8)  
371 and simulated intestinal medium. Due to the poor aqueous solubility of curcumin, ethanol  
372 (150 mL/L) was used in the receptor medium to mimic sink condition, as already described in  
373 literature (Kakkar et al., 2011; Mulik, Mönkkönenc, Juvonend, Mahadika, & Paradkarb,  
374 2010). Cumulative drug release percentage versus time was plotted to demonstrate the release  
375 patterns (Figure 4). In both dissolution media a sustained/prolonged release. Curcumin  
376 reached about 40% of the amount loaded in the formulation during 12 hours in PBS. This may  
377 be attributed to the curcumin released slowly from the lipidic solid matrices, through diffusion  
378 and dissolution mechanisms (zur Mühlen et al., 1998; Mehnert & Mäder, 2012). Although the

379 release rate of SLNs could be influenced by complex factors, it was reported that among the  
380 factors, the large surface area and high diffusion coefficient due to small molecular size or  
381 low viscosity in the matrix are preponderant (zur Mühlen, Schwarz, & Mehnert, 1998).

382 The release profile was similar, but more intense during the incubation of SLNs in simulated  
383 intestinal medium. After 12 h the percentage reached about 60%. The presence of enzymes  
384 and bile salts caused a pronounced release, probably due to degradation of the lipid carrier  
385 and the subsequent solubilization of curcumin in colloidal species, like mixed micelles and  
386 swollen micelles (Noack, Oidtmann, Kutza, & Mäder, 2012).

387

### 388 *3.5 Stability studies*

389 Stability of curcumin solid lipid nanoparticles was studied over 1 month. Physical stability  
390 was checked by monitoring size, zeta potential and P.I. during time, by DLS measurements.  
391 There was no modification neither of the particle size nor of the zeta potential (Figure 5).  
392 Furthermore, polydispersity was stable over time, no vesicle size alterations occurred over the  
393 tested period.

394

### 395 *3.6 Parallel artificial membrane permeability assay (PAMPA)*

396 An approach for rapid assessment of absorption potential include the Parallel Artificial  
397 Membrane Permeability Assay (PAMPA) (Kansy, Avdeef, & Fischer, 2004). Pampa is based  
398 on a 96-well microplate technology and allows reasonable throughput, although it lacks  
399 similarity to natural membranes because it does not possess pores or active transport  
400 mechanism. It enables fast determination of the trends in the ability of the compounds to  
401 permeate membrane by passive diffusion and it is thus suited for the screening of large  
402 libraries. Due to their small particle size, SLNs may exhibit bioadhesion properties to the  
403 gastrointestinal tract wall or enter in the intervillar spaces thus increasing their residence time  
404 in the gastrointestinal tract and releasing the active drug encapsulated. This increase in  
405 adhesion results in enhanced bioavailability.

406 The experiment was carried out measuring the ability of curcumin to diffuse from our SLNs  
407 suspension to a donor compartment through a PVDF membrane.

408 A showed a strong increase of permeated curcumin was found in the case of SLNs suspension  
409 when compared with a saturated solution of curcumin used as control (Figure 6). In the case  
410 of SLNs, 4.2  $\mu\text{g}$ , 7.9  $\mu\text{g}$  and 9.3  $\mu\text{g}$  of curcumin permeated to the acceptor compartment after  
411 6, 19 and 24h, respectively; within the same timeframe, 0.03  $\mu\text{g}$ , 0.03  $\mu\text{g}$  and 0.04  $\mu\text{g}$

412 respectively of curcumin permeated from saturated solution. We can observe also that in the  
413 case of aqueous saturated solution, the amount of permeated curcumin remains constant  
414 during the test while, in the case of SLNs, permeated curcumin increases with time during 24  
415 h of test.

416

#### 417 **4. Conclusions**

418 In this study the poorly-soluble curcumin was incorporated into SLNs by homogenization and  
419 ultrasound technique without the use of solvents.

420 Compritol demonstrated good solubilization of curcumin and formulation was obtained in  
421 terms of drug loading, surfactant percentage and sonication time. Pluronic F68 was selected  
422 as surfactant in the amount of 28 g/L of the formulation. SLN-2-15 formulation showed good  
423 values of encapsulation efficiency, size, P.I., zeta potential and stability.

424 These SLNs resulted round shape with homogeneous size distribution, as confirmed by TEM  
425 analysis. Size of the particles is a key factor for improve oral performance of poorly soluble  
426 drugs and the average particle size of the developed nanoparticles was maintained below 300  
427 nm, like other effective formulations, such as microemulsions or submicron emulsions. The  
428 increased permeability of curcumin loaded in SLNs was confirmed by PAMPA, a simple  
429 technique to evaluate *in vitro* passive gastrointestinal performance of innovative carriers. A  
430 prolonged release profile was observed suggesting that curcumin is solubilized into the solid  
431 lipid matrix. An increase in saturation solubility and, consequently, an increase in the release  
432 rate of the drug allows it to reach high concentrations in the gastrointestinal tract. It is  
433 expected a better *in vivo* performance of SLN because Pluronic may increase the permeability  
434 through the intestinal membrane and they may promote the bioadhesion to the GI wall. Also,  
435 the incorporation of curcumin into SLNs solid lipid matrix reduces its enzymatic degradation  
436 during the process of absorption.

437 SLNs are very versatile carriers, particularly for the oral administration, they can be easily  
438 transformed into powder (by spray-drying or lyophilization) and converted into solid dosage  
439 forms, such as tablets, hard gelatin capsules, pellets or powders. The obtained powders can be  
440 also dispersed in water or juice prior to administration.

441

442

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446 **References**

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- 662

**Figure captions**

**Figure 1:** TEM micrographs of curcumin of SLN-2-15 sample (loaded with 0.02g/g of curcumin and obtained with 15 min of sonication) (A) with details of single SLN (B; C).

**Figure 2:** DSC thermographs of Compritol; pure curcumin; blank and loaded solid lipid nanoparticles (SLN-2-15 sample, loaded with 0.02g/g of curcumin and obtained with 15 min of sonication).

**Figure 3:** X-ray diffraction pattern of Compritol; pure curcumin; blank and loaded solid lipid nanoparticles SLN-2-15 sample, loaded with 0.02g/g of curcumin and obtained with 15 min of sonication).

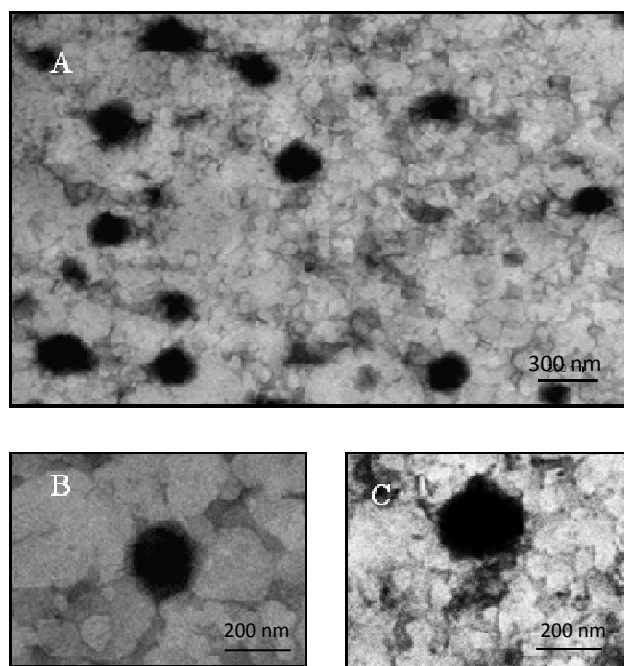
**Figure 4:** *In vitro* release profile of curcumin from SLN-2-15 sample (loaded with 0.02g/g of curcumin and obtained with 15 min of sonication). Each data represent the mean  $\pm$  standard deviation of three experiments.

**Figure 5:** Particle size (A), polydispersity index (P.I., B) and zeta potential (C) evolution of curcumin solid lipid nanoparticles (SLN-2-15 sample, loaded with 0.02g/g of curcumin and obtained with 15 min of sonication) at storage conditions (30 days, 4°C). (Mean $\pm$ S.D.; n=3).

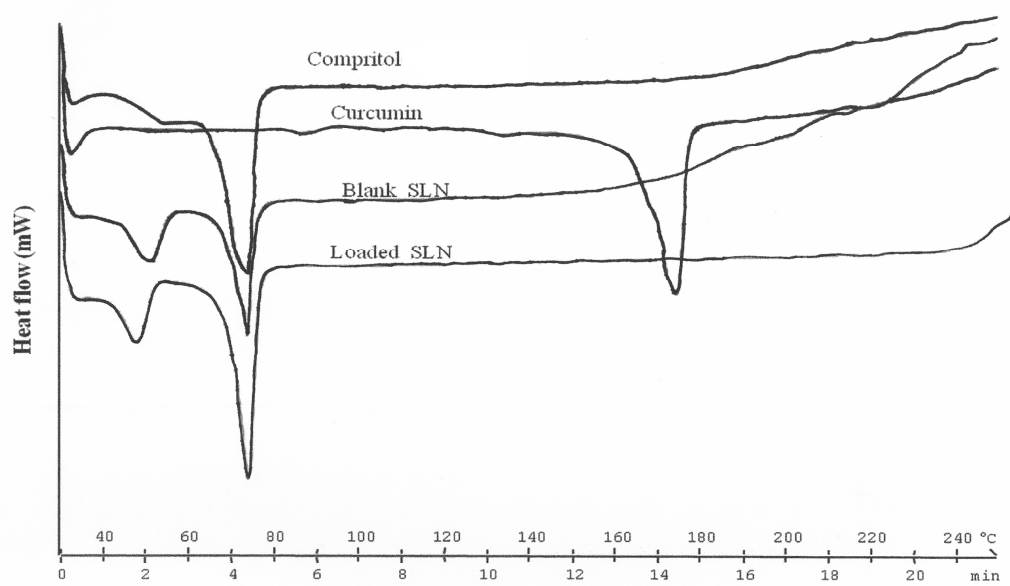
**Figure 6:** Permeation profile of free curcumin and curcumin loaded SLNs by Parallel artificial membrane permeability assay (PAMPA). Amount of curcumin is referred to each well. Black: control; grey: SLN-2-15 sample, loaded with 0.02g/g of curcumin and obtained with 15 min of sonication. (Mean $\pm$ S.D.; n=3).

**Table 1.** Characterization of developed blank and curcumin loaded SLNs; effect of sonication time and amount of curcumin on size, polydispersity index (P.I.), encapsulation efficiency (EE%) and drug loading (D.L.%). SLN-1, SLN-2 are solid lipid nanoparticles loaded respectively with 0.01 and 0.02g/g of curcumin. 5, 10 and 15 indicate sonication time. Each data represent the mean  $\pm$  standard deviation of three experiments.

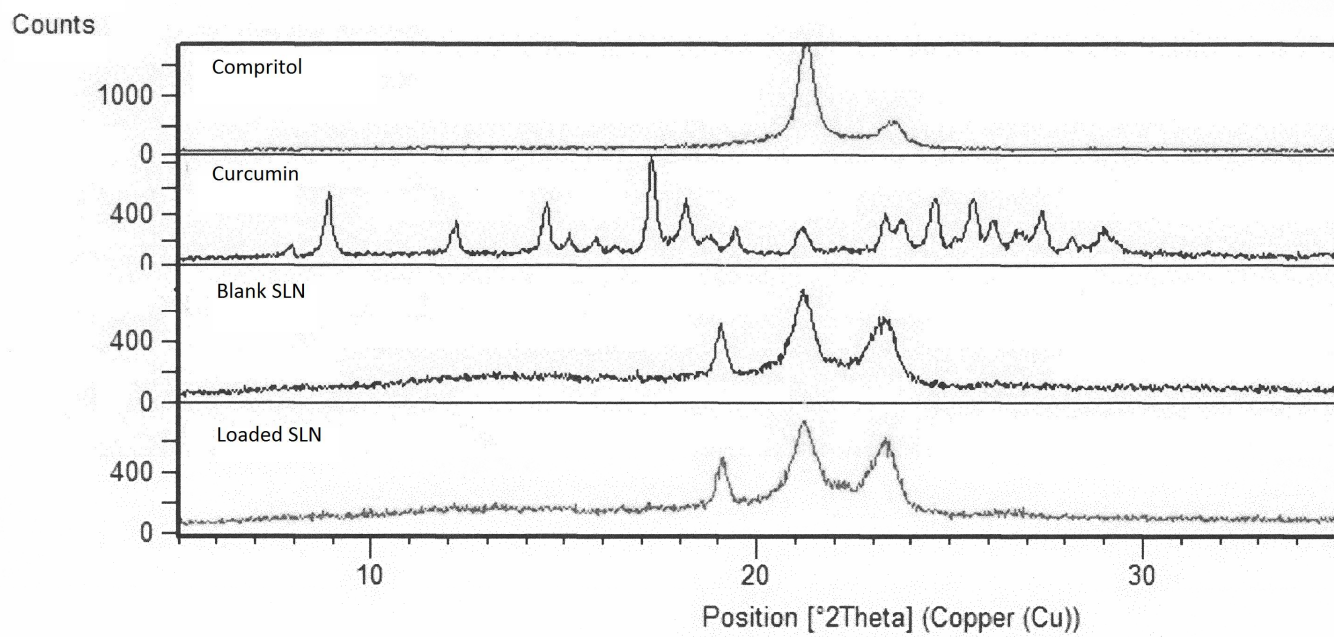
Formulation	Time of sonication (min)	Curcumin (g/g)	Size (nm)	P.I.	$\zeta$ Potential (mV)	EE (%)	DL (%)
Blank SLN	5	-	333 $\pm$ 1	0.28 $\pm$ 0.02	-36 $\pm$ 3	-	-
	10	-	254 $\pm$ 6	0.25 $\pm$ 0.01	-24 $\pm$ 2	-	-
	15	-	241 $\pm$ 9	0.33 $\pm$ 0.03	-10 $\pm$ 1	-	-
SLN-1-5	5	0.01	320 $\pm$ 18	0.31 $\pm$ 0.03	-45 $\pm$ 2	76.4 $\pm$ 4.9	0.76
SLN-1-10	10	0.01	238 $\pm$ 4	0.31 $\pm$ 0.04	-34 $\pm$ 4	74.2 $\pm$ 16	0.74
SLN-1-15	15	0.01	199 $\pm$ 23	0.26 $\pm$ 0.02	-39 $\pm$ 4	76.4 $\pm$ 4.6	0.76
SLN-2-5	5	0.02	415 $\pm$ 6	0.31 $\pm$ 0.08	-39 $\pm$ 3	70.7 $\pm$ 2	1.41
SLN-2-10	10	0.02	313 $\pm$ 13	0.33 $\pm$ 0.01	-29 $\pm$ 4	77.8 $\pm$ 8	1.56
SLN-2-15	15	0.02	270 $\pm$ 15	0.29 $\pm$ 0.01	-33 $\pm$ 3	80.2 $\pm$ 2	1.60



**Figure 1**



**Figure 2**

**Figure 3**

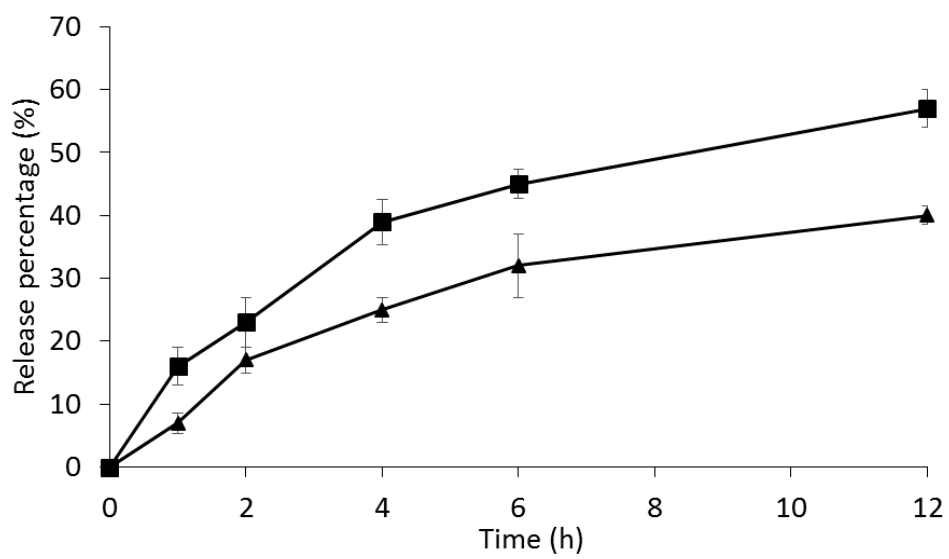


Figure 4

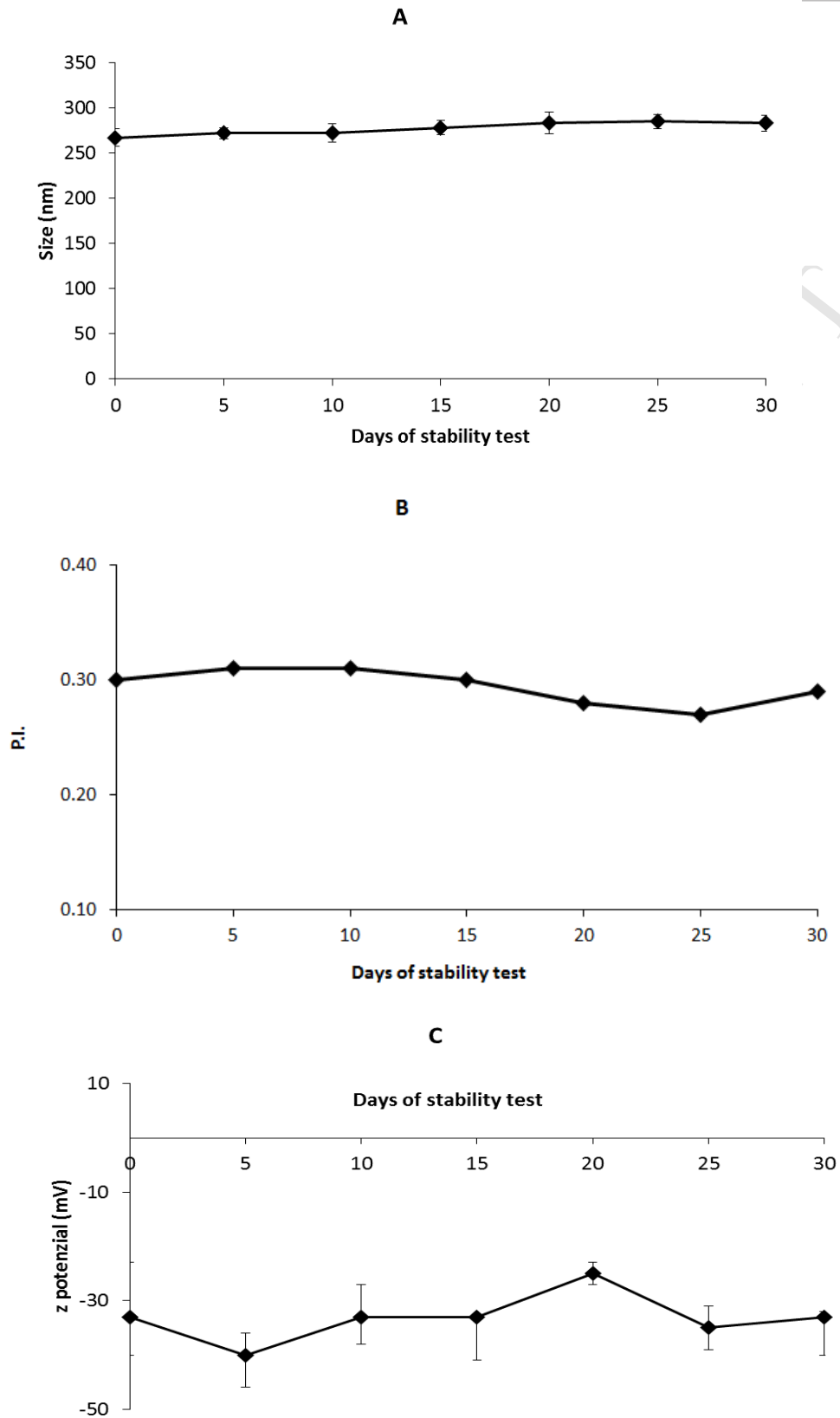


Figure 5

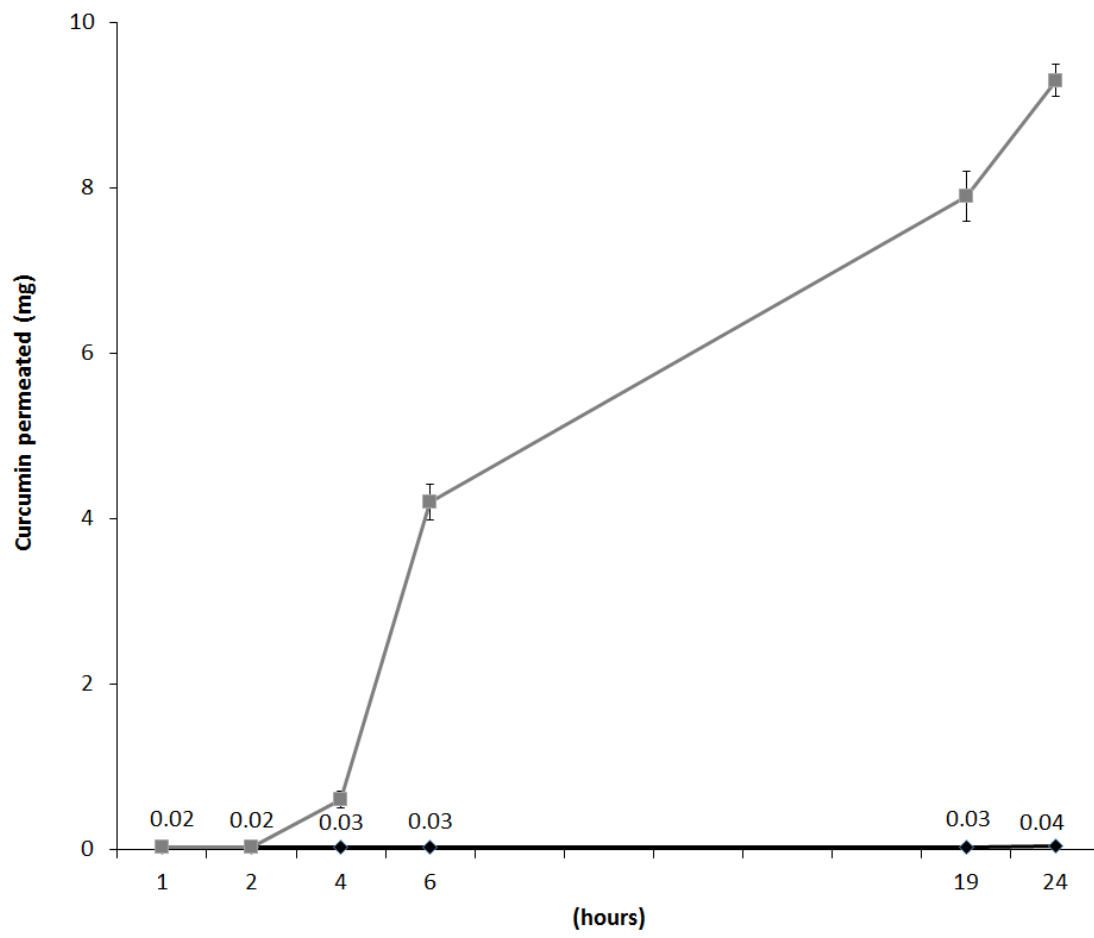


Figure 6

**Highlights:**

1. Compritol and Pluronic were used to prepare curcumin oral solid lipid nanoparticles.
2. Solid lipid nanoparticles resulted stable over 1 month at  $4\pm 1^{\circ}\text{C}$ .
3. *In vitro* studies showed a good release of curcumin from lipid nanoparticles.
4. Formulation increases the amount of curcumin permeated by 2 orders of magnitude.