The pancreatin solution is prepared by adding 1 g of pancreatin powder in 5 mL of lipolysis buffer. After 10 minutes of magnetic stirring, the solution is centrifuged at 2800g for the complete solubilization of lecithin.

Surfactants which are able to self-emulsify in contact with water and leading to clear and transparent dispersions. These surfactants dissolve more than 30 mg/mL of curcumin, the target dose for this study. We decided then to select as prototype formulations all mixtures of curcumin with water-soluble surfactants.

Excipients exhibiting the highest solvent capacity were water-soluble surfactants and Transcutol®, the hydrophilic co-solvent (Table 1). All these excipients were able to dissolve more than 30 mg/mL of curcumin, the target dose for this study. We decided then to select as prototype formulations all mixtures of curcumin with water-soluble surfactants.

Solubility in liquid excipients

Solubility of curcumin in liquid excipients is assayed by HPLC. In brief, the drug is added in excess to 60 mL amber borosilicate glass bottle containing 10 g of liquid-based formulation. Bottles are allowed to equilibrate at 37°C under magnetic stirring with periodic vortex mixing to ensure that undissolved drug particles are homogeneously suspended in the lipolysis fluid. At intervals, bottles are sampled and these aliquots are centrifuged (Universal centrifuge 335R) at 2800g and 37°C for 30 min (2). Samples are separated into a solid pellet phase and a particle-free supernatant. The supernatant is sampled and diluted with methanol for further HPLC analysis. The solubility of the drug is considered as reached when the difference between two consecutive values is less than 5%. For most of the excipients, the equilibration is reached in less than 3 days.

Solubility in solid excipients

For solid lipid-based excipients, the solubility of curcumin is assayed by differential scanning calorimetry (DSC). According to the Raoult’s law, the variation of melting point depends on the lipid excipient’s enthalpy related to the concentration of drug dissolved within the excipient. Samples are prepared by mixing the excipient and dissolving a specified amount of drug under stirring. These samples are left at 50°C to equilibrate overnight, vortexed, then aliquoted in aluminum pans (2.5-3 mg) and left at 20°C to solidify as crystals for 24 hours before DSC analysis (Perkin Elmer Pyris Diamond). Thermal analysis is carried out between 20 and 120°C with a heating rate of 3°C min⁻¹. The drug solubility is observed when there is a change of slope in the curve fitting the evolution of melting enthalpy of the excipient as a function of drug concentration in the sample (3). Solubility is then confirmed by polarized light hot stage microscopy (PL-HSM, Zeiss AquisScope A1).

Dispersion of selected formulation in water

A dispersion test is performed on selected mixtures in a 250-ml beaker. A gram of mixture is introduced in 200 mL of purified water at 37°C under agitation with a paddle at 100 rpm. Performance criteria are ease of emulsification, homogeneity, fineness of the dispersion, and absence of curcumin precipitation. The fineness of the dispersion is assayed by dynamic light scattering (DLS, Particle Sizing Systems Nicomp). The precipitation of curcumin is checked by polarized light microscopy (PLM).

Preparation of media and enzyme suspension

The lipolysis buffer is prepared by adding Tris (0.474 g/L), CaCl₂ (0.016 g/L) and NaCl (0.811 g/L) in Mill-Q water. The pH is adjusted at 4.5 with either HCL 1 M or NaOH 0.6 M. The lipolysis medium is prepared by adding Lipophospholipid/cholesterol (0.576 g/L) and NaCl/DC (0.515 g/L) in the lipolysis buffer. The medium is airdried overnight to allow the complete solubilization of lecithin.

The pancreatic solution is prepared by adding 1 g of pancreatin powder in 5 mL of buffer. After 10 minutes of magnetic stirring, the solution is centrifuged at 2800g and 37°C for 10 min. The supernatant is sampled and added to the lipolysis test (2).

In vitro lipolysis test

The experimental setup consists in a pH stat apparatus (Metrohm®), comprising a Titrando 802 propeller stirrer/804 Ti Stand combination, a glass pH electrode (iUnitrode) and a BOD Dosino dosing units coupled to 5 mL syringe. The apparatus is connected to a PC and operated using Tomo 2.0 software. The experiment is thermostated at 37°C and filled with lipolysis medium, to mimic fasted state in the small intestine. During the digestion of lipolysis-based formulations by pancreatic lipases, pH is maintained constant by the addition of NaOH 0.1 N.

Before the lipolysis test, the specific activity of pancreatic lipases is checked on tributyrine (model substrate). The substrate must be in excess to have a reaction rate directly proportional to the enzyme concentration. Five hundred microlitre of tributyrine and 1.5 μL of pancreatin suspension are introduced in 28 mL of lipolysis medium containing the digested lipid formulation. In this case only one sampling is performed at 45 min. The micellar phase is obtained at the end of lipolysis experiment after centrifugation at 21 000g and 37°C for 30 minutes. The supernatant is sampled and diluted with methanol for further HPLC analysis.

Pharmacokinetic calculations are also performed in order to determine the solubility of the drug in the lipolysis medium containing the digested lipid formulation. In this case only one sampling is performed at 45 min. The micellar phase is obtained at the end of lipolysis experiment after centrifugation at 21 000g and 37°C for 30 minutes. The pellets phase and the aqueous micellar phase. Each lipolysis sample is then immediately filtered on 0.45µm syringe-filter before dilution in methanol. In the in vitro digestion test was able to discriminate prototype formulations (Figure 1). Semi-solid and solid formulations take more time to hydrate and deliver curcumin in the micellar phase in comparison to the liquid one. However, after the 10-minute digestion phase all formulations are able to release 73 to 80% of the dose in the aqueous phase. The effect of lipolysis can be observed for formulations containing Tween® 80, Kolliphor® RH40 and Gelucire® 44/14. A slight decrease of the concentration of curcumin in the micellar phase is observed after 5 minutes and continues for 1 h. Gelucire® 48/16 is the only excipient able to maintain more than 80% of the drug in the solution during 1 h. The calculation of the SRM of these prototype formulations was not possible for the previous experiment, even if Kolliphor® 48/16 led to one of the lowest SRM (0.8), in comparison to other surfactants (Kolliphor® RH40 – SRM=1.6, and Gelucire® 44/14 – SRM=3.4).

RESULTS AND DISCUSSION

Solubility determination and formulation selection

Exipients exhibiting the higher solvent capacity were water-soluble surfactants and Transcutol®, the hydrophilic co-solvent (Table 1). All these excipients are able to dissolve more than 30 mg/mL of curcumin, the target dose for this study. We decided then to select as prototype formulations all mixtures of curcumin with water-soluble surfactants which are able to self-emulsify in contact with water and lead to clear and transparent dispersions.

Dispersion test

All prototypes were able to form transparent and homogeneous dispersions in contact with water and to keep curcumin in solution (Table 2). A swelling of the colloidal phase is observed for all prototype formulations addition of the compound to the pigments. No precipitation was observed by PLM. Classic dispersion test was not able to discriminate the four prototype formulations.

REFERENCES


CONCLUSION

LFC type IV formulation with Gelucire® 48/16 can efficiently address the low water-solubility of curcumin, pH-stat testing is a valuable tool to discriminate formulations in addition to classic dispersion test.