

# Formulation and investigation of fenugreek extract SEDDS systems

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## Introduction

Fenugreek (*Trigonella foenum-graecum* L) is a herb with several pharmacological effects researched, such as carminative, gastric stimulant, antioxidant, anti-inflammatory, anticarcinogenic and hepatoprotective effects (Yadav & Baquer, 2014). The antidiabetic effects of fenugreek have also been studied (Zia et al., 2001).

Self-emulsifying drug delivery systems (SEDDS) are mixtures of oils, surfactants and occasionally co-solvents. After oral administration, they form micro- or nanoemulsions containing the active pharmaceutical ingredient (APIs). SEDDS are easily manufactured and physically stable formulations that can improve the dissolution and absorption of lipophilic drugs and drug compounds, enhancing bioavailability.

SEDDS formulations of herbal medicines not only increases the solubility, and therefore the bioavailability, of the active ingredients, but also improves the stability, permeability and the dissolution profile, making controlled or sustained release achievable, and helping avoid the occasional irritation of the gastrointestinal tract (Zhang et al., 2015).

A wide variety of ground fenugreek seed products are available commercially, although studies have shown that the use of dried fenugreek extract instead of direct plant materials is more effective and has less side effects. The Caco-2 permeability of *Trigonella* compounds ranges from poor to good, and can be improved by penetration enhancers such as  $\beta$ -cyclodextrins (Okawara et al., 2014).

The study's objective was to formulate SEDDS containing *Trigonella foenum-graecum* extract to improve the stability of the herbal extract and to increase its permeability through a Caco-2 monolayer.

## Materials and methods

Fenugreek seeds were ground and extracted with boiling methanol, filtered, and evaporated to dryness in a rotary evaporator. This dry extract was re-dissolved in PBS and sterile filtered. The concentrated liquid extract in PBS contained 7.833 mg mL<sup>-1</sup> trigonelline and 8.258 mg mL<sup>-1</sup> 4-hydroxyisoleucine.

Table 1. Composition of the examined products.

	TFG	S1	TFG + S1	S2	TFG + S2
Fenugreek extract	6 mL	-	6 mL	-	6 mL
PBS	3 mL	6 mL	-	6 mL	-
isopropyl myristate	-	1 mL	1 mL	0.25 mL	0.25 mL
Transcutol HP	-	1 mL	1 mL	1.5 mL	1.5 mL
Labrasol	-	1 mL	1 mL	0.5 mL	0.5 mL
Kolliphor RH40	-	-	-	0.5 mL	0.5 mL
Capryol 90	-	-	-	0.25 mL	0.25 mL

The components listed in Table 1. were mixed well in a plastic tube. All the experiments were performed immediately after preparation.

Particle size analysis and zeta potential analysis measurements were carried out using a Malvern Zetasizer Nano ZSP. 1 mL of the sample SEDDS composition was diluted with 900 mL distilled water. The samples were allowed to equilibrate for 5 min at 25 °C before performing 5 measurements.

The antioxidant capacities of the compositions were determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Here, 1 mg/mL ascorbic acid solution was used as the positive control. 1 mL of the samples was added to 2 mL 0.06 mM DPPH and the absorbance was measured at 517 nm.

For the permeability assay, Caco-2 cells were seeded on 24-well polycarbonate filter inserts at 80,000 cells/insert. The culture medium was replaced with fresh every 3–4 days. Trans-epithelial electronic resistance was measured using a Millicell ERS-2 voltohmmeter with a chopstick electrode pair. All cell monolayers presented TEER values between 800 and 1000  $\Omega$  cm<sup>2</sup>.

The inserts were placed into the wells of a fresh plate. The permeability assay was commenced with the addition of 400  $\mu$ L of the sample solution to the apical chambers of the inserts. A 50  $\mu$ L aliquot was taken from the basal chamber containing PBS immediately, and at 15, 30, 60, 120 and 240 min. The samples taken from the basal chamber were replaced with PBS. LC-MS method was used to determine the permeated quantity of herbal compounds in the samples. During the permeability assay, the plate was placed in an incubator (37 °C, 35 rpm).

The MTT method was used for the cytotoxicity assay to determine the viability of Caco-2 cells after the treatment with compositions TFG, TFG + S1 and TFG + S2. A blank PBS treatment was used as a negative control, and the Triton X-100 treatment as the positive control. The cells were seeded 96-well tissue culture plates, and were allowed to grow for 7 days.

## Results and discussion

Based on the results of the particle size analysis, we can determine that our product TFG + S1 is a self-microemulsifying drug delivery system (SMEDDS), while TFG + S2 is a self-nanoemulsifying drug delivery system (SNEDDS).

The average zeta potential of TFG + S1 was -71.3 mV, with a standard deviation of 11.8 mV. The average zeta potential of TFG + S2 was -38.5 mV, with a standard deviation of 7.47 mV. The stability of the composition TFG + S1 was considered excellent, and that of the composition TFG + S2 was considered moderate.

Significantly more compound permeated from the SEDDS formulations TFG + S1 and TFG + S2 than from the native fenugreek extract TFG.

According to the MTT test results, the Trigonella extract and the SEDDS compositions were also well tolerated by the Caco-2 cells, as all cell viability values were over 74%. A cell survival rate over 70% is required to consider a material non-toxic. The Trigonella extract alone showed the highest cell survival rate, while there was no significant difference between the cytotoxicity values of the different SEDDS compositions.

## Conclusion

Based on our results, a modern, non-toxic, cytocompatible fenugreek SEDDS formulation with high antioxidant capacity was developed in order to improve the permeability and bioavailability of all components.

## References

- Okawara, M.; Tokudome, Y.; Todo, H.; Sugibayashi, K.; Hashimoto, F. Effect of  $\beta$ -Cyclodextrin derivatives on the diosgenin absorption in caco-2 cell monolayer and rats. *Biol. Pharm. Bull.* 2014, 37, 54–59.
- Yadav, U.C.S.; Baquer, N.Z. Pharmacological effects of *Trigonella foenum-graecum* L. in health and disease. *Pharm. Biol.* 2014, 52, 243–254.
- Zhang, L.; Zhang, L.; Zhang, M.; Pang, Y.; Li, Z.; Zhao, A.; Feng, J. Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug Deliv.* 2015, 22, 475–486.
- Zia, T.; Hasnain, S.N.; Hasan, S.K. Evaluation of the oral hypoglycaemic effect of *Trigonella foenum-graecum* L. (methi) in normal mice. *J. Ethnopharmacol.* 2001, 75, 191–195.