Methylselenocysteine loaded chitosan:zein nanoparticles: Formulation, characterisation, and in-vitro evaluation

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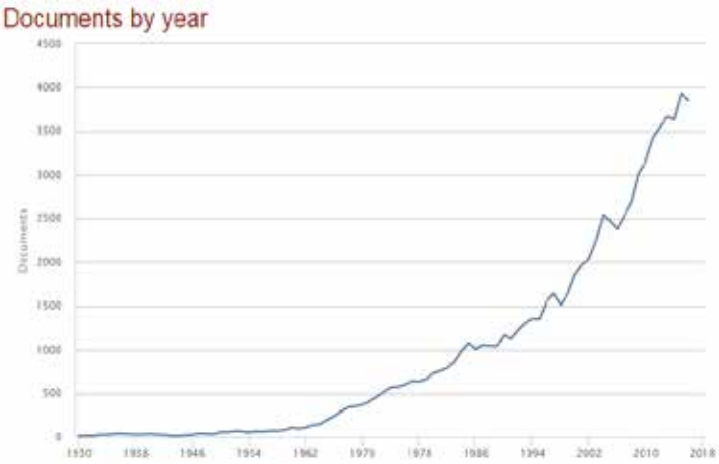
Introduction

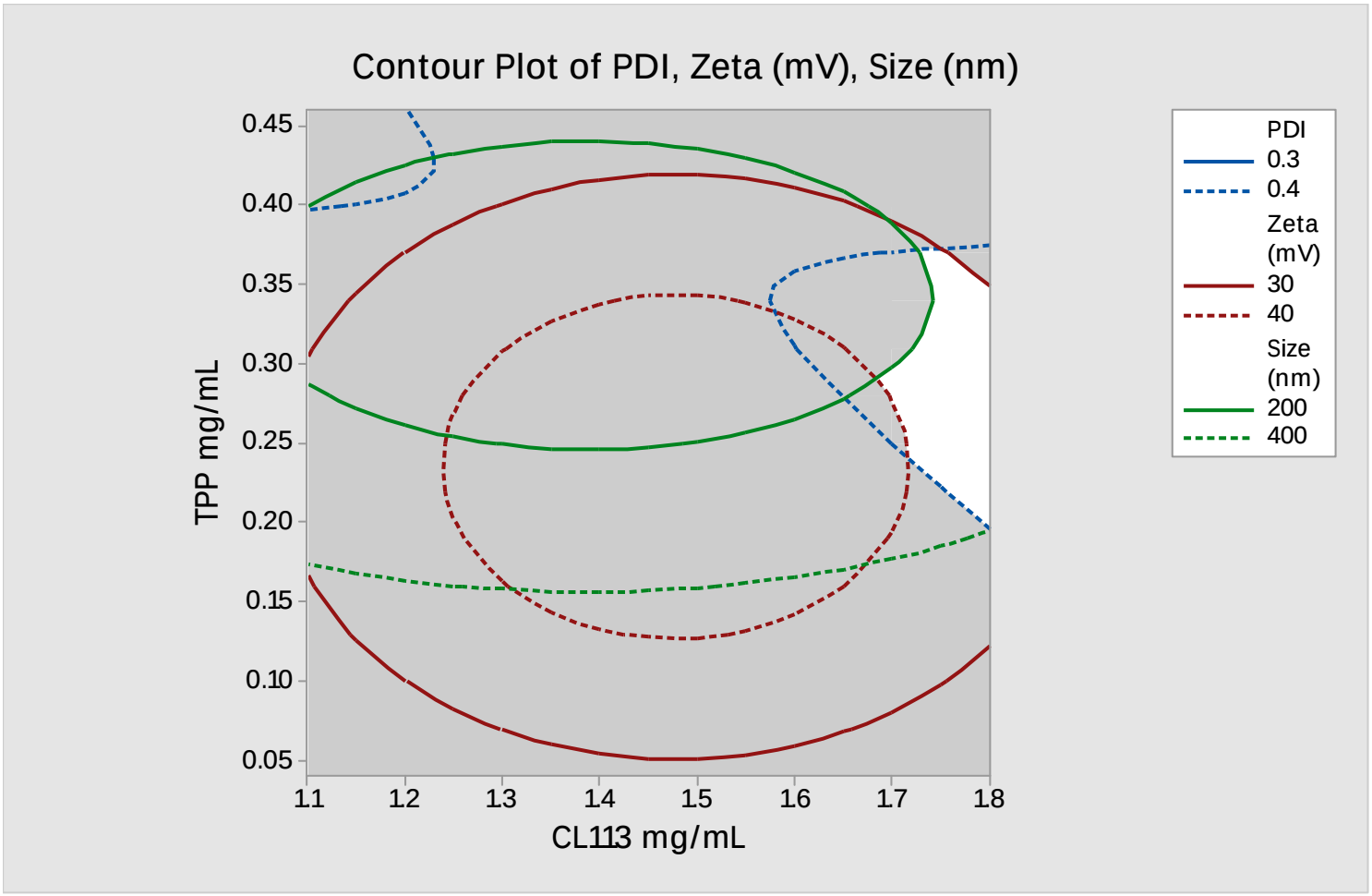


Problems with oral delivery of Se

- Narrow range between therapeutic and toxic concentrations
- Organic species known to be more bio-available than inorganic (Se amino acids)
- Metal containing organics – susceptible to oxidation
- Low intestinal epithelial absorption

TECHNOLOGY
NANOPARTICLES

Optimisation prediction via response surface design

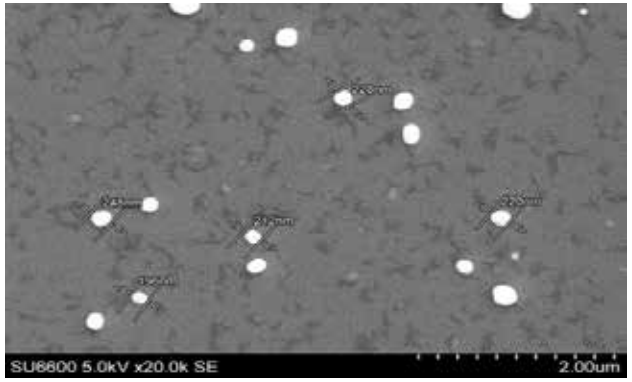


Target: PDI ≤0.4, Zeta ≥30 mV and Size ≈ 300 nm

Encapsulation Efficiency maximisation

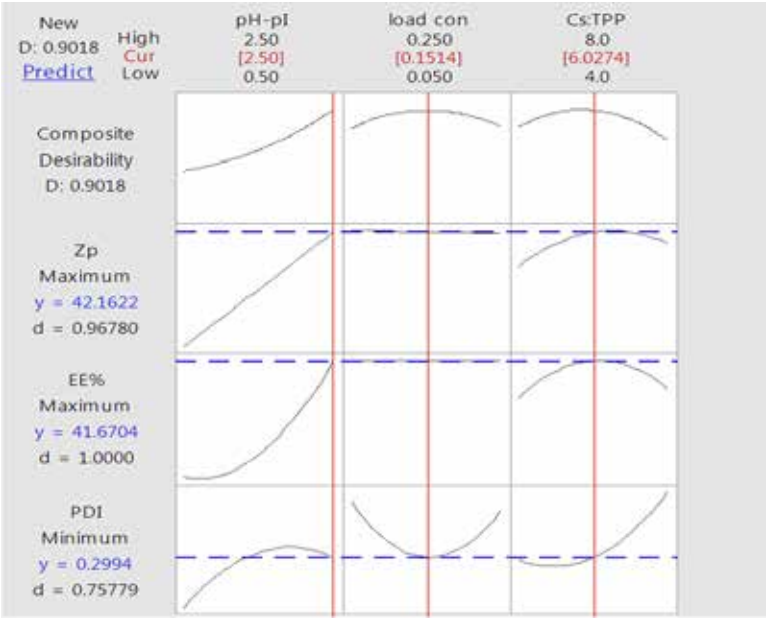
Step I.

- Box-Behnken design
  - Distance from Se-Met Isoelectric point
  - Load concentration
  - Ratio TPP:Cs



- Encapsulation efficiency and physicochemical properties analysed

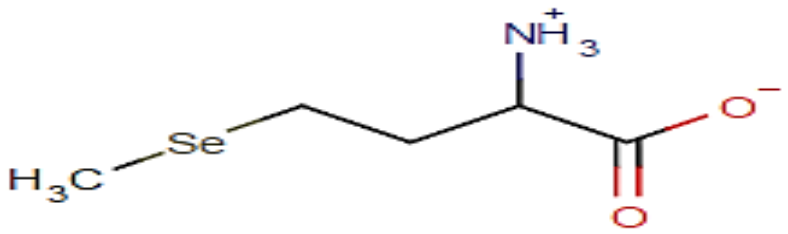
Property	Min	Max
Size (nm)	155	315
PDI	0.22	0.37
Zp (mV)	32	42
EE %	20	43



EE% maximisation Step II.

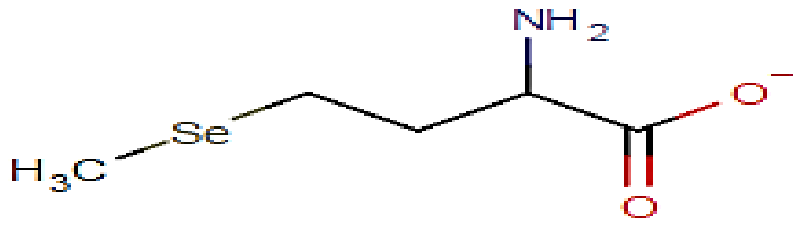
- Original Se-Met in Cs (pH=5)

EE>40%



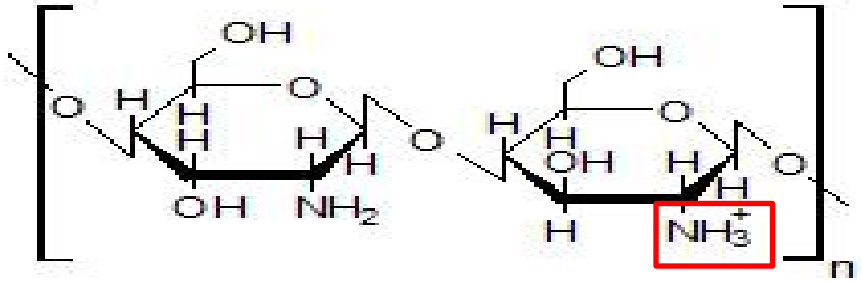
- Se-Met in TPP with water + NaOH (pH=12)

EE>55%



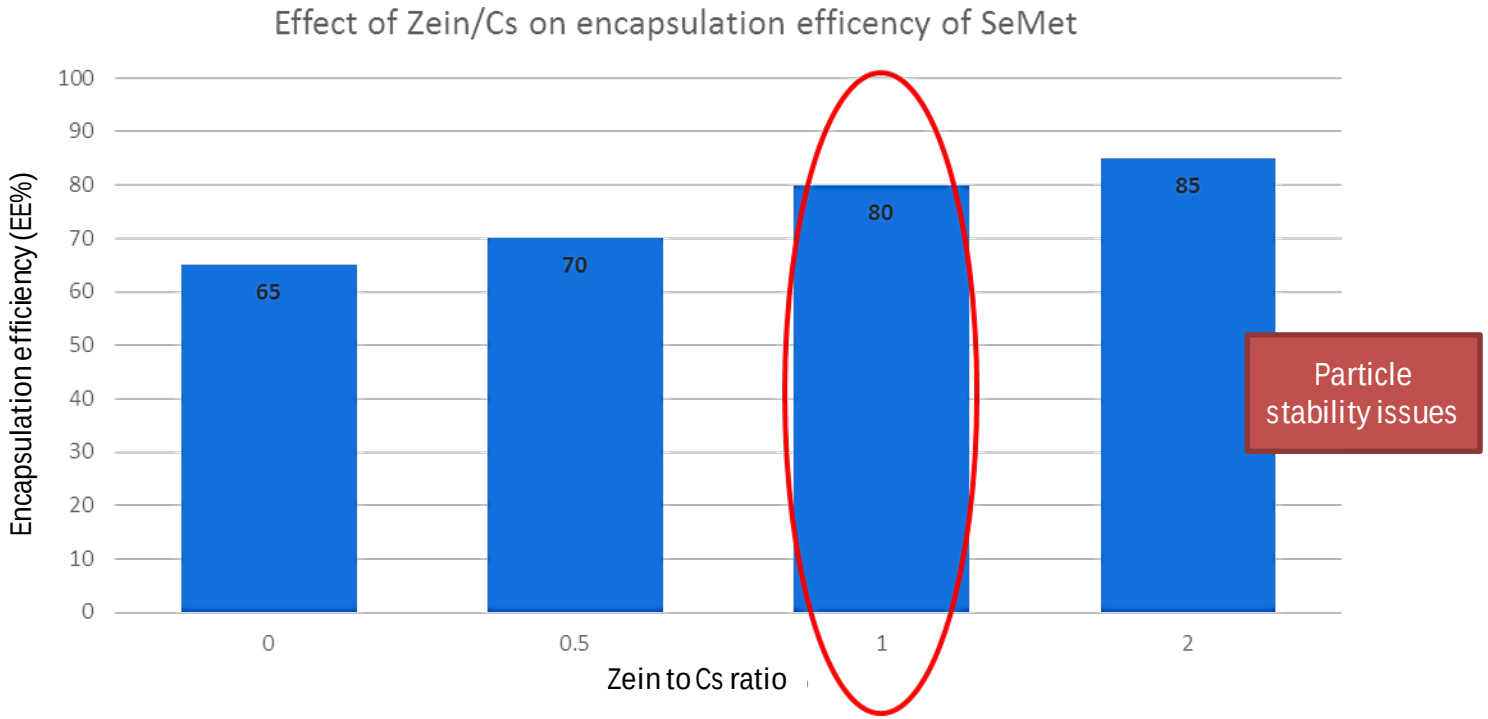
- Chitosan buffered in pH=3 medium

EE>65%



EE% maximisation Step III.

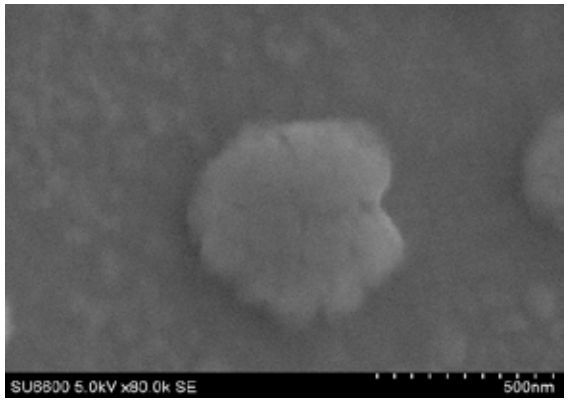
- **EE% >80% 1:1 Zein:Chitosan**
- **Physico-chemical properties not changed (size increased slightly)**

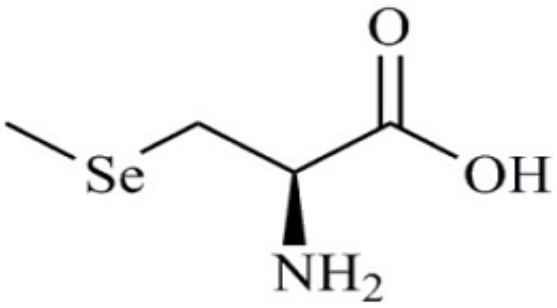


Property	Avg	±SD
Size (nm)	377	47
PDI	0.325	0.136
Zp (mV)	35	6
EE %	81	1.2

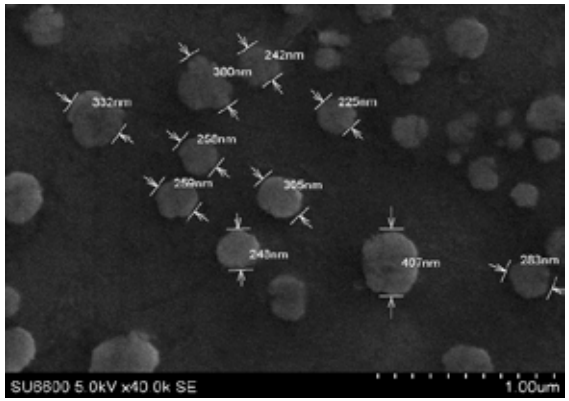
Formulation of Methylselenocysteine (MSC) loaded NPs

MSC 1:1 Zein:Cs				
Property	**Size (nm)**	**PDI**	**ZP (mV)**	**EE%**
Average	333.4	0.297	**21.9**	82.3
St Dev	20.3	0.049	1.7	4.5
MSC 0.75:1 Zein:Cs				
Property	**Size (nm)**	**PDI**	**ZP (mV)**	**EE%**
Average	252.6	0.163	**34.1**	80.7
St Dev	56.2	0.049	4.4	4.4

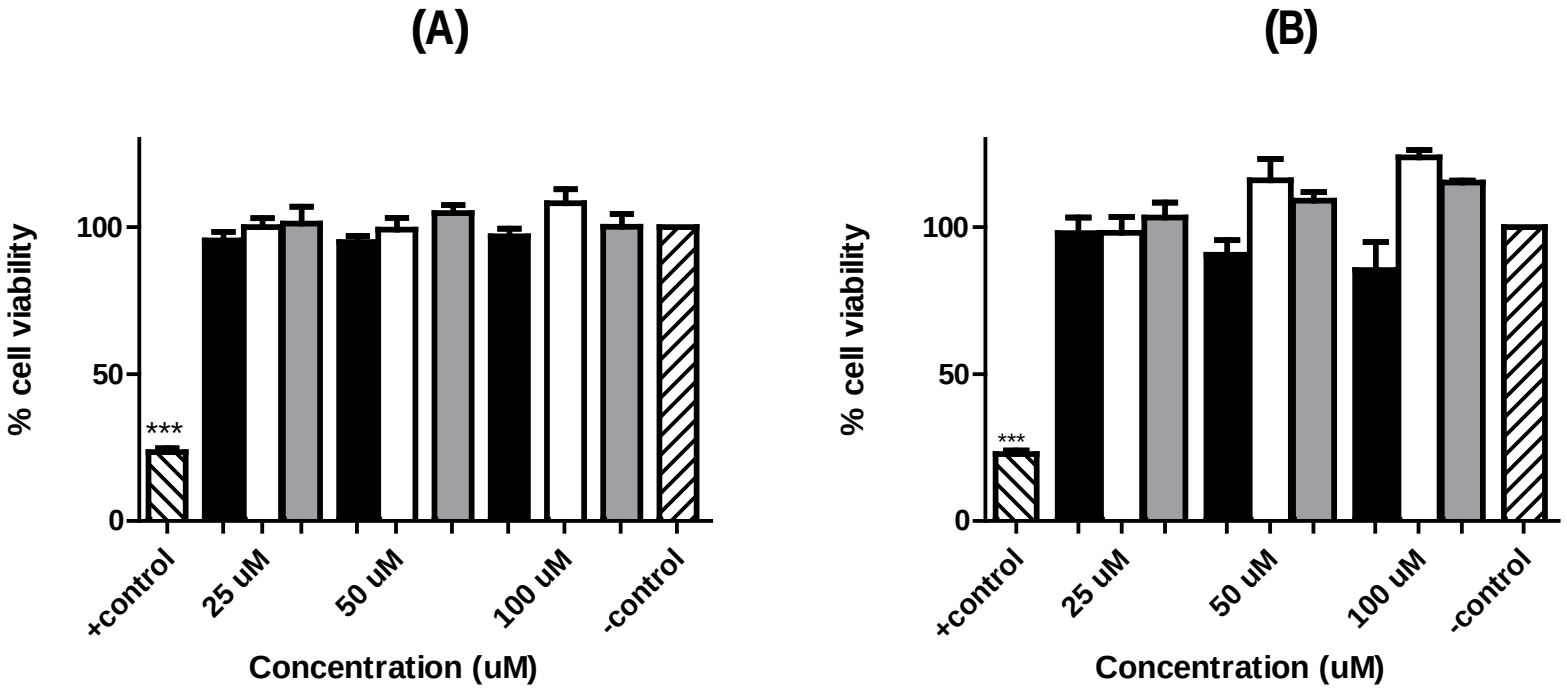




Methylselenocysteine



MSC loaded NP cytotoxicity assessment

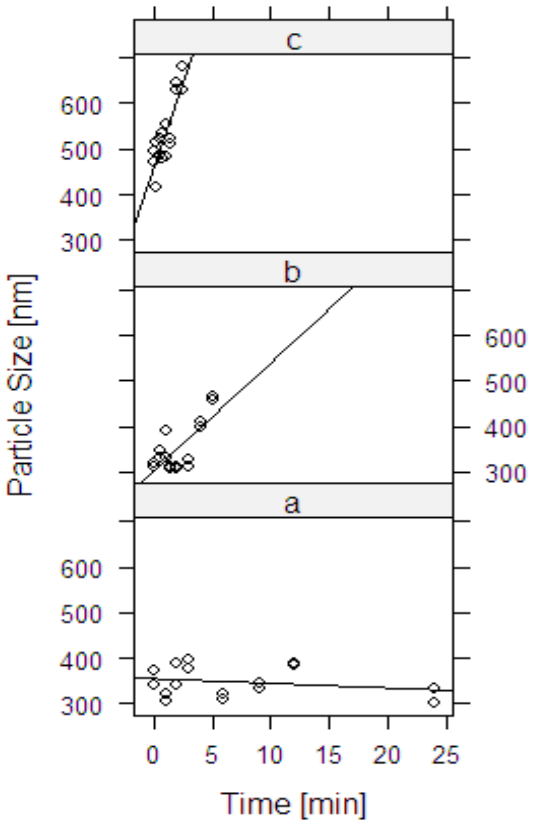


Cytotoxicity assessment of ■ MSC, □ unloaded NPs and ▨ MSC loaded NPs, exposed for **(A)** 4h in Caco2 cell lines and **(B)** 72h in HepG2 cell line at 25 uM, 50 uM and 100 uM concentration. Triton X (0.1 %) was used as positive control. Percentage (%) of MTS converted was compared to untreated control. 1-Way ANOVA with Dunnetts's post-test *** $P < 0.001$. N=3

Accelerated stability analysis of MSC loaded NPs coated with zein

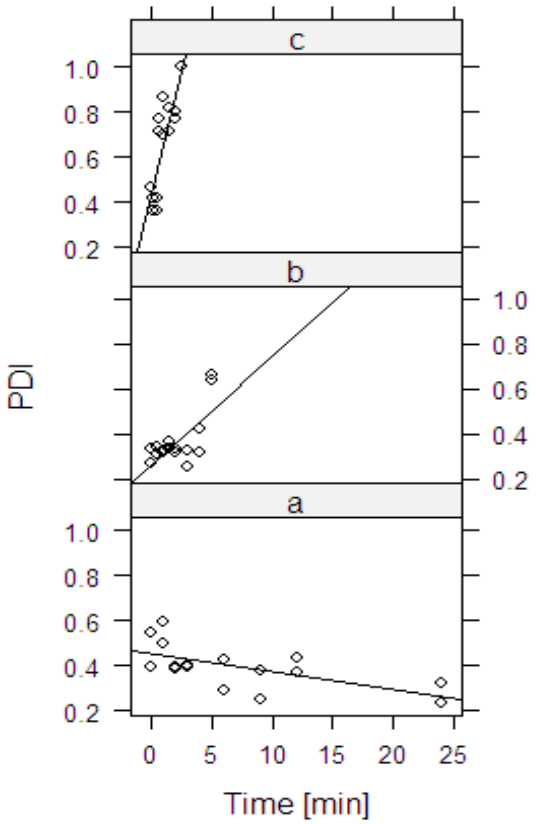
(1) Particle size, (2) PDI and (3) ZP analysis of MSC loaded NPs exposed to (a) 60 °C, (b) 70 °C and (c) 80 °C, over time periods of 120, 300 and 720 min, respectively. N = 3.

(1)



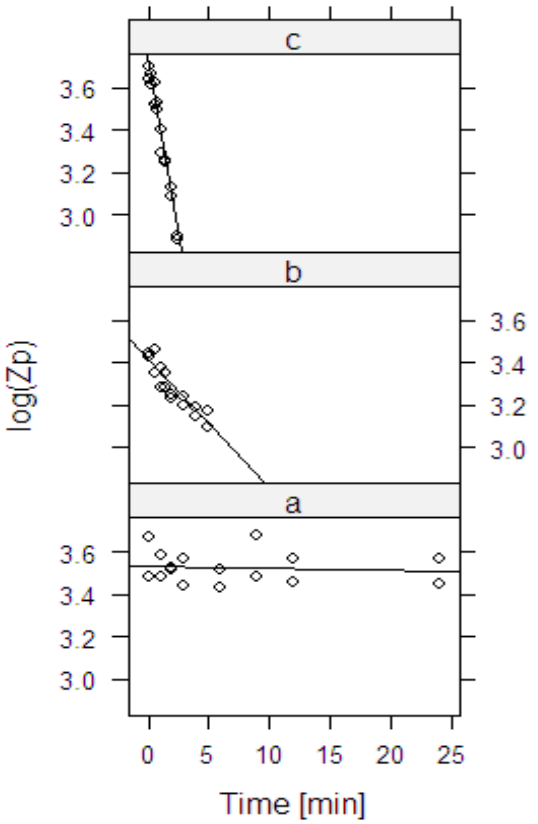
ln (kref@70 °C) = 1.66 ± 1 min^{-1}
Ea = 570.59 ± 103 kJ/mol

(2)



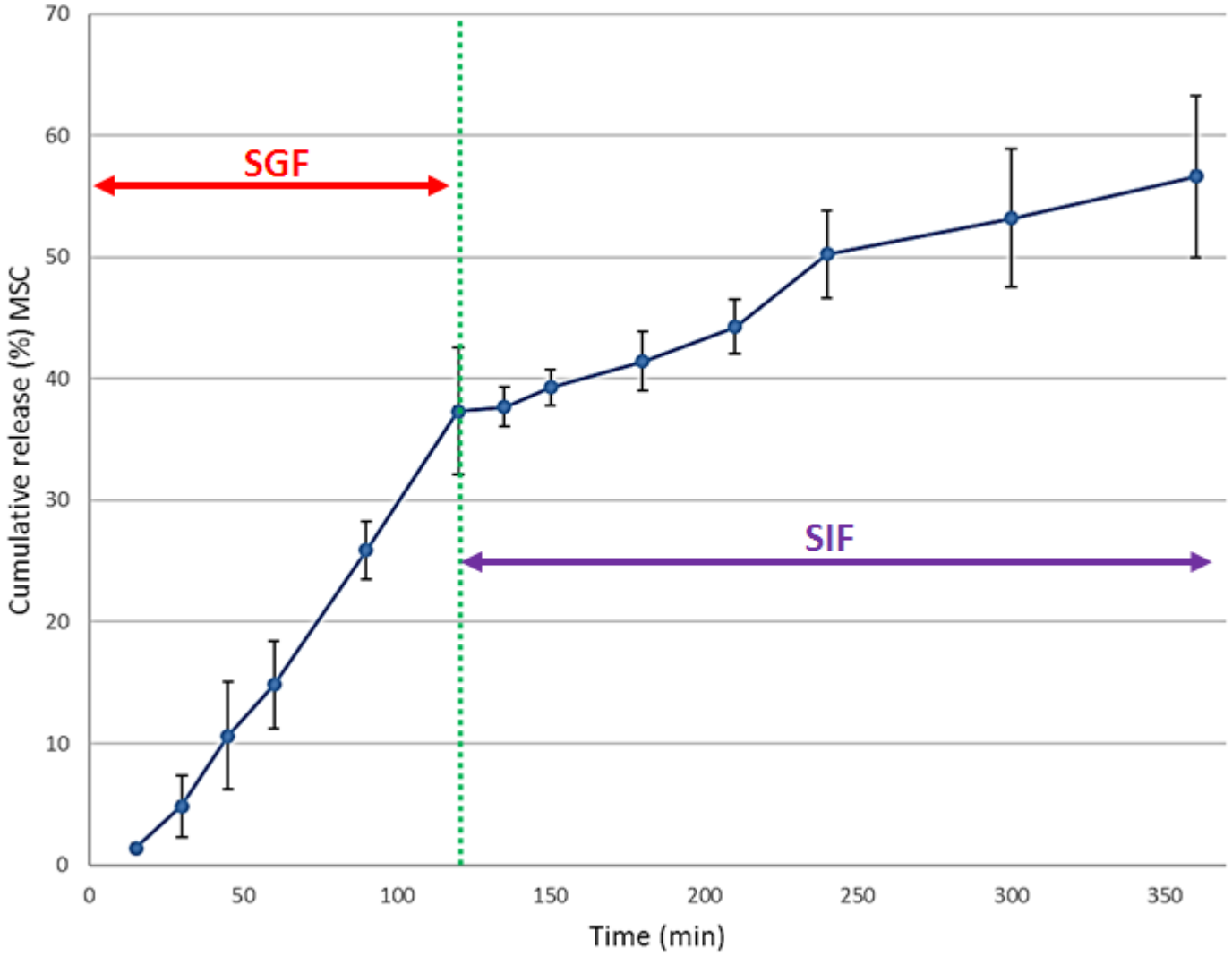
ln (kref@70 °C) = 0.029 ± 0.014 min^{-1}
Ea = 182.310 ± 42.646 kJ/mol

(3)



ln (kref@70 °C) = 0.038 ± 0.01 min^{-1}
Ea = 205.706 ± 25.65 kJ/mol

Cumulative release of MSC after 2 hr in SGF (pH 1.2) and 4 hr SIF (pH 6.8)



Conclusions

- DoE is a useful method for the optimisation and formulation of NPs
- MSC loaded NPs nanoparticles coated with zein, can be successfully prepared under mild conditions
- Chitosan nanoparticles may constitute an optimal delivery system for MSC with the appropriate physical properties for oral delivery
- Exploitation of ionisable groups on target encapsulates can significantly increase EE%
- Zein coating can help increase the encapsulation efficiency

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