**Supplementary Materials**

# Sodium Carboxymethyl Cellulose-Stabilised Multiple Emulsions with pH-Sensitive Behaviour, Enhanced Stability and Mucoadhesion for Oral Delivery of Chemopreventive Agents

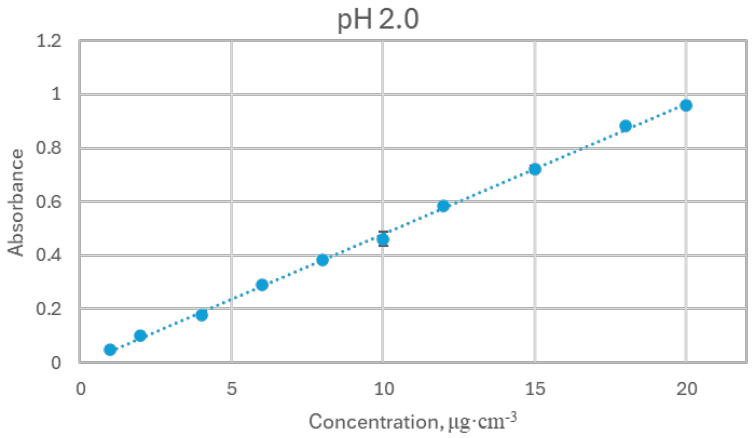
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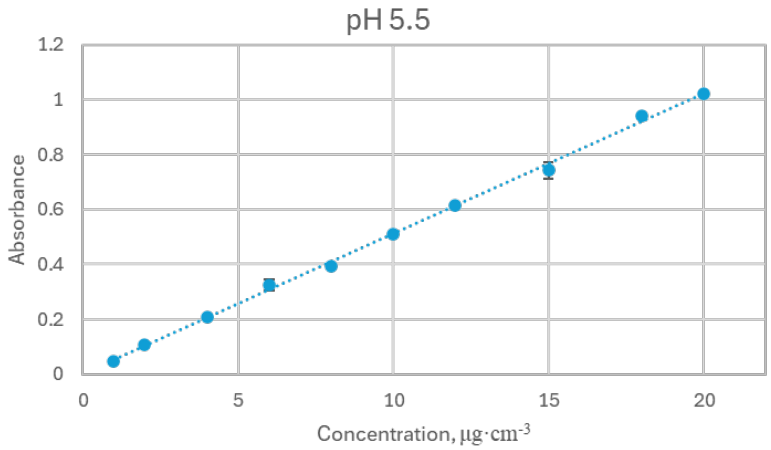
**S1. Calibration and validation of the spectrophotometric assay for trans-resveratrol**

Trans-resveratrol calibration curves were established in phosphate-buffered saline (PBS) and adjusted to pH 2.0, 5.5, and 7.0. Fresh standard solutions (1–20 μg·cm$^{-3}$) were prepared in each buffer immediately before measurement and protected from light. Absorbance was measured at 305 nm against a pH-matched blank using 1 cm quartz cuvettes using a Jasco FP-6500 spectrophotometer (Japan). Calibration curves ($A_{305}$ vs concentration) were constructed by least-squares linear regression (Figure S1). Limits of detection (LOD) and quantification (LOQ) were estimated as 3.3σ/S and 10σ/S, respectively (σ — the standard deviation, S — the slope of the calibration curve (from linear regression)), Table S1.

(A)




(B)




(C)

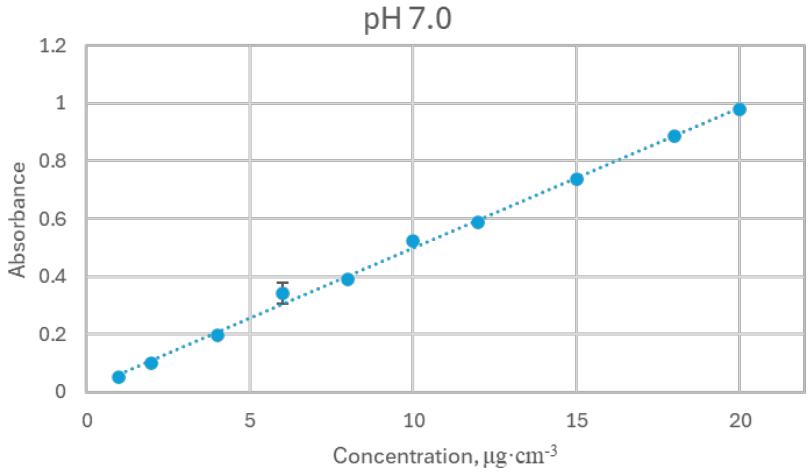



**Figure S1.** Calibration curves for trans-resveratrol at pH (A) 2.0, (B) 5.5 and (C) 7.0 (PBS, 305 nm). Linear regression equations and $R^2$ values are given in Table S1. Data represent mean ± SD (n = 3), ($p > 0.05$). Points represent error bars; not visible, mean errors are the same size or smaller than the symbol for a given measurement.

**Table S1.** Calibration parameters for trans-resveratrol at different pH values

| pH | Linear range μg·cm$^{-3}$ | Regression equation A = aC + b | $R^2$ | LOD μg·cm$^{-3}$ | LOQ μg·cm$^{-3}$ |
|---|---|---|---|---|---|
| 2.0 | 1-20 | A = 0.048C + 0.002 | 0.996 | 0.20 | 0.60 |
| 5.5 | 1-20 | A = 0.051C + 0.001 | 0.998 | 0.18 | 0.55 |
| 7.0 | 1-20 | A = 0.049C + 0.002 | 0.997 | 0.19 | 0.57 |

A — absorbance of the solution, C — concentration of trans-resveratrol (μg·cm$^{-3}$).

Regression equations obtained from calibration curves were used to determine trans-resveratrol concentrations in the external phase of multiple emulsions under different pH conditions.

Statistical evaluation: One-way ANOVA (factor: trans-resveratrol concentration, 1–20 μg·cm$^{-3}$) confirmed a significant effect of concentration on absorbance at 305 nm for all tested pH values ($p < 0.0001$). No significant lack-of-fit was observed ($p > 0.05$), and residuals were randomly distributed around zero, confirming the linearity of the calibration models. Residuals for calibration curves (pH 2.0, 5.5, 7.0) showed no systematic deviation; therefore, the models were considered valid within the working range (1–20 μg·cm$^{-3}$).

**S2. Calibration curve for selenium**

Selenium, originating from sodium selenite, was determined using a modified spectrophotometric procedure of Bera and Chakrabartty (1968, Analyst, 93, 50–53). The method is based on forming a yellow Se(IV)–2-mercaptobenzothiazole (MBT) complex in a strongly acidic medium, measurable at 370 nm. The pH of the samples was first adjusted to 7.5 ± 0.2 with 0.1 M HCl/NaOH to promote complex formation. For the assay, 1 cm$^3$ of the sample or standard was mixed with 15 cm$^3$ of 12 M HCl and 4 cm$^3$ of 0.1 % (w/v) MBT solution in 50 % ethanol, then diluted with 2 M HCl to a final

volume of 20 $cm^3$. The reaction mixture was vortexed, incubated for 10 min at room temperature, and the absorbance ($A_{370}$) was measured against a reagent blank using a 1 cm quartz cuvette.

Calibration standards were prepared from sodium selenite in the range 0–8 $\mu g \cdot cm^{-3}$ Se(IV) in a matrix-matched blank (PBS mixed with the emulsion external phase at a 10:1 (v/v) ratio, adjusted to pH 7.5, and treated identically to the samples). The calibration curve followed the equation:

$$A = (0.003 \pm 0.004) + (0.1574 \pm 0.0008) \cdot c$$

with $R^2 = 0.9989$ for eight points (0.5–8 $\mu g \cdot cm^{-3}$), ($p > 0.05$; no deviation from linearity) (Figure S2).

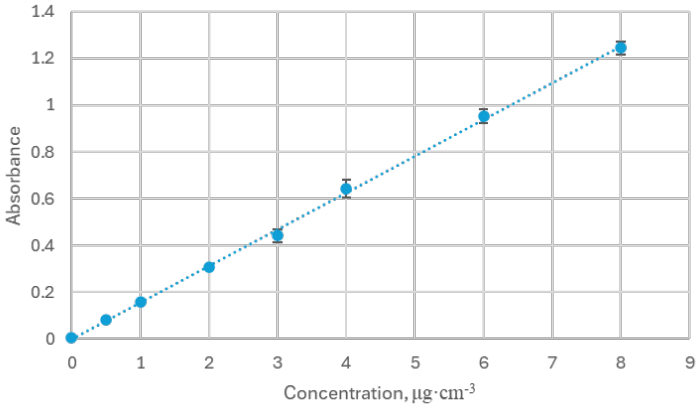



**Figure S2** Calibration curve for Se(IV)–MBT complex at 370 nm (mean ± SD, n = 3). Points represent error bars; not visible, mean errors are the same size or smaller than the symbol for a given measurement. Linear regression: A = 0.003 + 0.1574 c ($\mu g \cdot cm^{-3}$); $R^2 = 0.999$, ($p > 0.05$).

The calibration range covered the expected selenium concentrations released from emulsions under all tested conditions."

One-way ANOVA (factor: Se(IV) concentration) was applied to verify the significant effect of concentration on absorbance as an additional confirmation, Table S2.

**Table S2.** One-way ANOVA results confirming the linearity of the Se(IV)–MBT spectrophotometric calibration model.

| Source | df | SS | MS | F | p-value |
|---|---|---|---|---|---|
| Between concentrations | 7 | 2.252 | 0.322 | 974.3 | < 0.0001 |
| Within (error) | 16 | 0.0053 | 0.00033 | | |
| Total | 23 | 2.257 | | | |

df – degrees of freedom; SS – sum of squares; MS – mean square; F – F-statistic

The analysis confirmed a statistically significant effect of Se(IV) concentration on absorbance ($F(7,16) = 974.3$, $p < 0.0001$). No lack-of-fit was detected ($p > 0.05$), confirming a linear response within the working range.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated as "LOD"=3.3·σ/b, "LOQ"=10·σ/b, where σ = standard deviation of 10 blank readings (0.0043 AU) and b = slope of the calibration curve, yielding LOD = 0.076 μg·cm$^{-3}$ and LOQ = 0.23 μg·cm$^{-3}$.

Precision and accuracy results are summarised in Table S3.

**Table S3.** Precision and accuracy of the MBT spectrophotometric method for Se(IV) determination.

| Target spike μg·cm$^{-3}$ | Found (mean ± SD) μg·cm$^{-3}$ | Recovery (%) | RSD (%) |
|---|---|---|---|
| 0.5 | 0.49 ± 0.02 | 98 | 4.1 |
| 2.0 | 2.02 ± 0.05 | 101 | 2.5 |
| 6.0 | 5.88 ± 0.18 | 98 | 3.1 |

Repeatability (3 μg·cm$^{-3}$, n = 6): mean = 3.02 μg·cm$^{-3}$, RSD = 0.9 %. All acceptance criteria were met ($R^2 \geq 0.995$; RSD ≤ 5 %; recovery 95–105 %; LOD ≤ 0.1 μg·cm$^{-3}$).

Robustness and interferences: Small variations in reaction time (8–12 min) or temperature (20–25°C) caused a ≤ 3 % change in absorbance. Matrix blanks were used for background correction. No drift in $A_{370}$ was observed within 60 min. Sample pH (7.5 ± 0.2) was adjusted prior to acidification; no carry-over was observed.

Residual analysis confirmed the linearity of the calibration model (Figure S3).

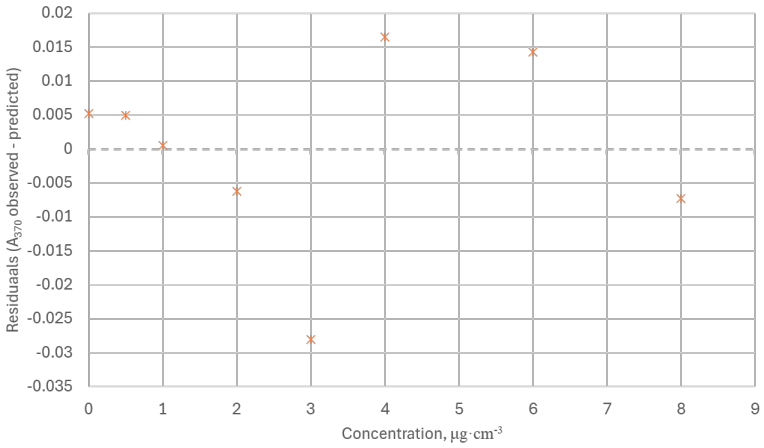



**Figure S3** Residuals of the calibration fit for Se(IV)–MBT complex showing random distribution around zero, confirming linearity.

**S3. Relative change in droplet size (|%ΔD$_{32}$|) during simulated gastrointestinal pH transition**

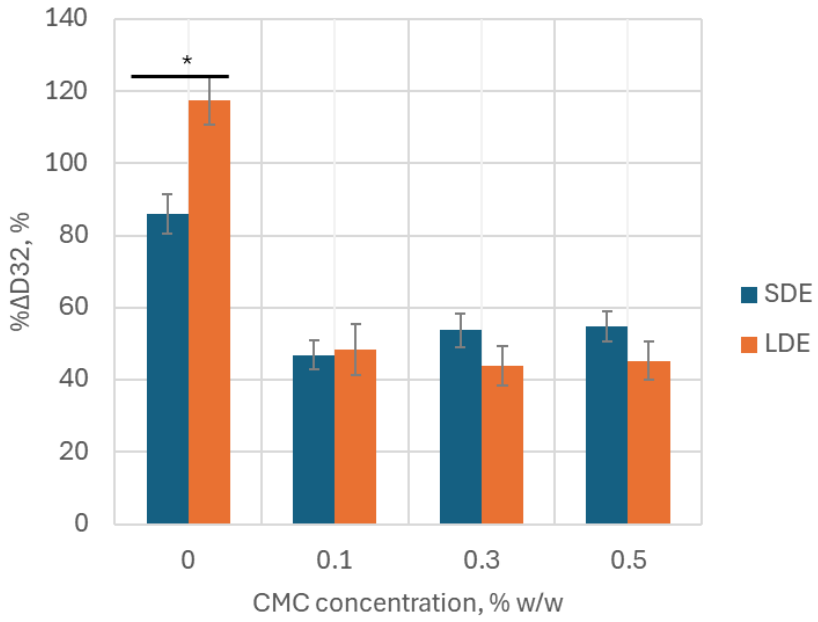



**Figure S4** Percentage change in droplet diameter ($|\%\Delta D_{32}|$ = ($D_{32}$_intestinal − $D_{32}$_initial)/$D_{32}$_initial × 100) for small-droplet (SDE) and large-droplet (LDE) emulsions containing various concentrations of CMC. Bars represent mean ± SD (n = 3). Asterisks indicate significant differences between SDE and LDE at the same CMC level ($p < 0.05$; two-way ANOVA with Tukey's test).

**S4. Relative change in ζ-potential during simulated gastrointestinal pH transition**

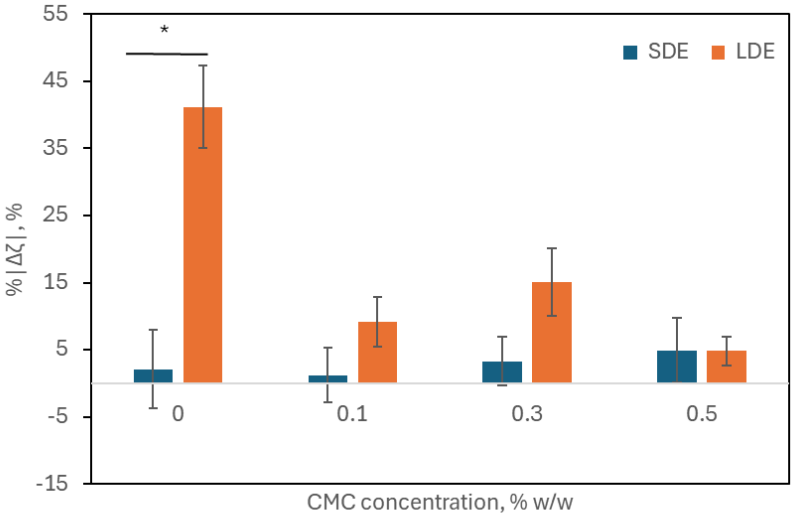



**Figure S5.** Relative change in ζ-potential ($|\%\Delta\zeta|$= (ζ_intestinal − ζ_initial)/ ζ_initial × 100) for small-droplet (SDE) and large-droplet (LDE) emulsions containing different concentrations

of CMC after transition from initial (pH 7.4) to intestinal conditions (pH 7.0). Bars represent mean ± SD (n = 3). Asterisks indicate significant differences between SDE and LDE at 0.0 % w/w CMC (* $p < 0.05$; two-way ANOVA with Tukey's test).

**S5. Relative change in interfacial CMC (|%ΔΓ|) during simulated gastrointestinal pH transition**

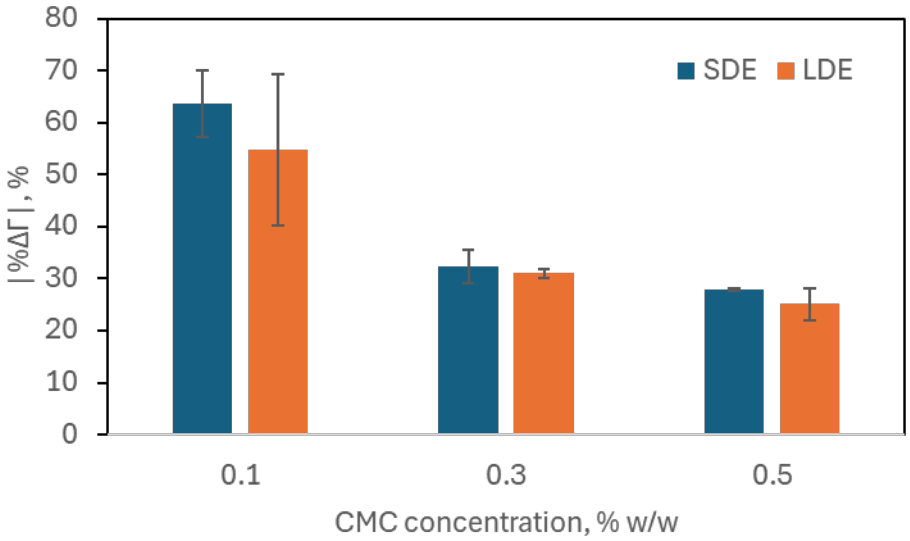



**Figure S6.** Relative change in interfacial CMC (|%ΔΓ||= (Γ_intestinal − Γ_initial)/ Γ_initial × 100) for SDE and LDE emulsions after exposure to simulated gastrointestinal pH transition (intestinal – initial). Values are presented as mean ± SD (n = 3). Statistical analysis (two-way ANOVA with Tukey's test) showed no significant differences between emulsion types ($p > 0.05$).