Supporting Information

Unveiling the flavone-solubilizing effects of α-glucosyl rutin and hesperidin: Probing structural differences through NMR and SAXS analyses

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1.1 SEM images

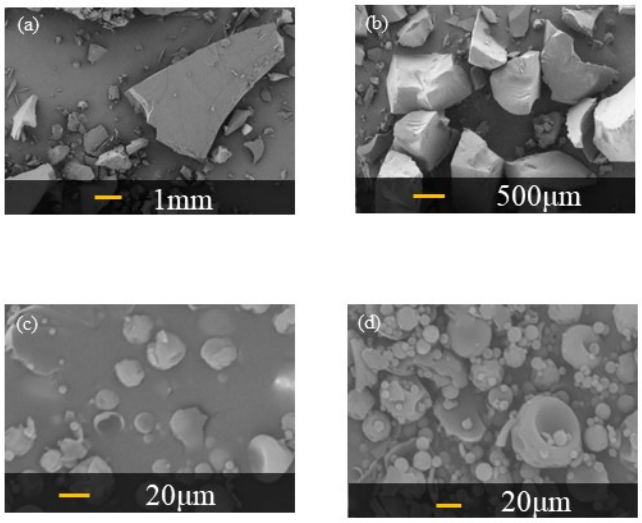

Figure S1. Scanning electron microscopy images of evaporated samples of (a) flavone/Hsp-G = 1:10 and (b) flavone/Rutin-G = 1:10, and of spray-dried particles of (c) flavone/Hsp-G = 1:10 and (d) flavone/Rutin-G = 1:10.

1.2 Powder X-ray diffraction patterns

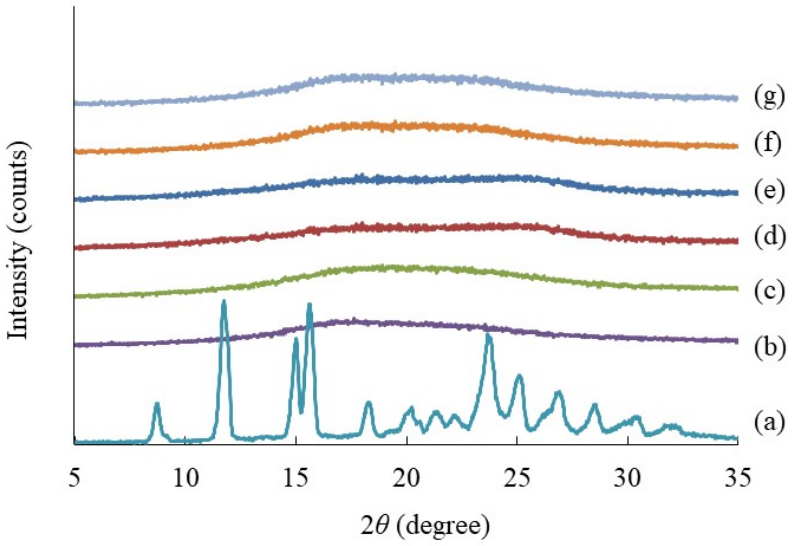

Figure S2. Powder X-ray diffraction patterns of (a) untreated flavone, (b) untreated Hsp-G, (c) untreated Rutin-G, (d) evaporated powder of flavone/Hsp-G (1:10), (e) evaporated powder of flavone/Rutin-G (1:10), (f) spray-dried particles of flavone/Hsp-G (1:10), and (g) spray-dried particles of flavone/Rutin-G (1:10).

2. Rutin-G SAXS Analysis

2.1 Polydisperse Sphere Model

The scattering intensity used for Rutin-G aggregates was modeled using the polydisperse sphere model, which gives the scattering for sphere with radius R as

$$I(q) = N\int_0^\infty p(R,\mu,\sigma)F^2(q,R)dR + BG, \#(S1)$$

where N is the number density of spheres, BG accounts for background scattering and

$$F(q,R) = 3V\Delta\rho\frac{\sin(qR) - qR\cos(qR)}{(qR)^3}, \#(S2)$$

$\Delta\rho$ is the scattering length density difference between the solvent and the spheres and $V = (4/3)\pi R^3$ is the volume. The log-normal distribution was used to model the Rutin-G aggregate radius polydispersity:

$$p(R,\mu,\sigma) = \frac{1}{R\sigma\sqrt{2\pi}}\exp\left(-\frac{\ln(R/\mu)^2}{2\sigma^2}\right), \#(S3)$$

where σ^2 and μ are the variance and median of $\ln(R)$, respectively.

3.2 Best-Fit Model Parameters

Table S1: Best-fit parameters of the polydisperse sphere model for Rutin-G aggregates and reduced χ^2 statistic as Rutin-G concentration (c) is varied: σ is the number distribution width parameter, μ is the number distribution median, $\Delta\rho$ is the scattering length density difference, and BG is the constant background term.

c (mg/mL)	χ^2	σ	μ (Å)	$\Delta\rho$ ($\times10^{-7}$)	BG ($\times10^{-7}$)
1	1.17	0.34 ± 0.05	7.1 ± 1.4	5.6 ± 1.9	0 ± 100
2	1.38	0.34 ± 0.01	7.9 ± 0.4	7.0 ± 0.6	0 ± 3000
3	1.08	0.336 ± 0.007	8.5 ± 0.2	7.5 ± 0.3	11.6 ± 0.6
4	1.22	0.343 ± 0.004	8.6 ± 0.2	8.6 ± 0.2	15.3 ± 0.6
5	1.11	0.347 ± 0.003	8.7 ± 0.1	9.5 ± 0.2	21.6 ± 0.5
6	1.20	0.350 ± 0.002	8.84 ± 0.09	10.1 ± 0.2	20.9 ± 0.5
8	1.27	0.355 ± 0.002	8.95 ± 0.06	11.5 ± 0.1	24.1 ± 0.5
10	1.31	0.364 ± 0.001	8.90 ± 0.05	12.6 ± 0.1	3.4 ± 0.4
15	1.99	0.3758 ± 0.0009	8.75 ± 0.03	14.60 ± 0.08	5.2 ± 0.4

2.3 Supporting Figures

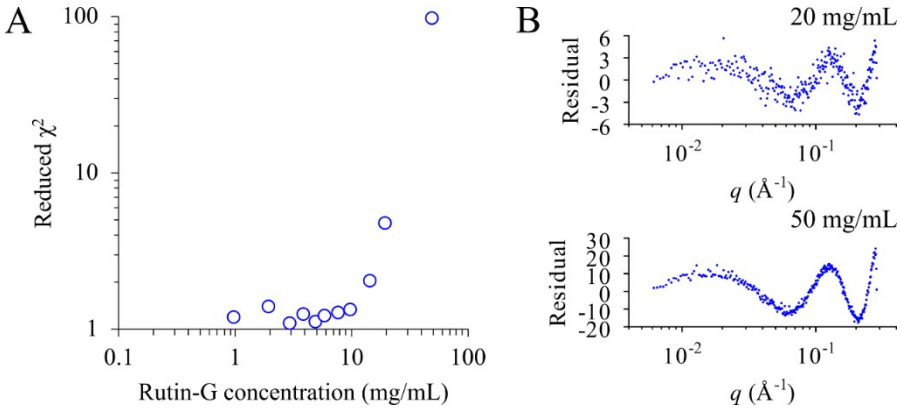

Figure S3: (A) Reduced χ^2 values of best-fit polydisperse sphere models for Rutin-G aggregates. (B) Normalized residuals for 20 and 50 mg/mL Rutin-G concentrations. Residuals for other concentrations are shown in the main text.

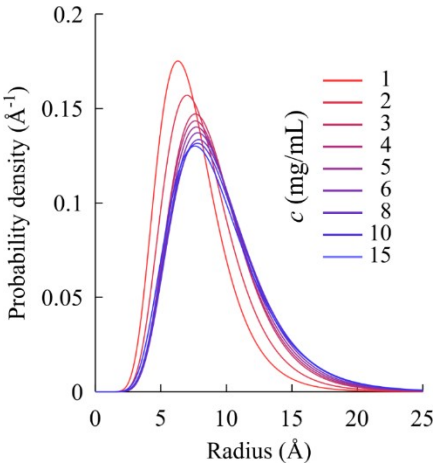

Figure S4: Number size distributions of Rutin-G aggregates determined from SAXS data analysis using the polydisperse sphere model.

3. Hsp-G SAXS Analysis

Hsp-G at concentrations below the CMC was modeled using the monodisperse sphere model, while above the CMC the core-shell sphere model was used.

3.1 Core-Shell Sphere Model

The scattering intensity used for Hsp-G micelles is given by[1]

$$I = P(q) + Aq^{B} + BG, \#(S4)$$

where Aq^{B} was used to include the contribution from the low-q power law scattering, BG was used to account for the background scattering and $P(q)$ is the core-shell sphere form factor given by

$$P(q) = \frac{scale}{V} F^{2}(q), \#(S5)$$

where V is the micelle volume and

$$F(q) = \frac{3}{V_{tot}}\left[V_{core}(\rho_{core} - \rho_s)\frac{\sin(qR) - qR\cos(qR)}{(qR)^3} + V_{shell}(\rho_{shell} - \rho_s)\frac{\sin(qR_{tot}) - qR_{tot}\cos(qR_{tot})}{(qR_{tot})^3}\right. \quad (S6)$$

where $V_{tot} = (4/3)\pi(R+t)^3$, $V_{core} = (4/3)\pi R^3$ and $V_{shell} = (4/3)\pi[(R+t)^3 - R^3]$ are the total, core and shell volumes, respectively, ρ_s, ρ_{core} and ρ_{shell} are the SLDs of the solvent, core and shell, respectively, R is the core radius, t is the shell thickness. The solvent and core SLDs were fixed to $\rho_s = 9.429 \times 10^{-6}$ and $\rho_{core} = 11.258 \times 10^{-6}$, respectively.

Consistency between best-fit ρ_{shell} and ρ_{shell} calculated from the aggregation number of Hsp-G,

$$\rho_{shell} = \phi_{head}\rho_{head} + \phi_s\rho_s = \frac{N_{agg}v_{head}}{V_{shell}}\rho_{head} + \frac{N_s v_s}{V_{shell}}\rho_s, \#(S7)$$

was enforced by utilizing the fit constriction:

$$\rho_{shell} = \frac{V_{core}v_{head}}{V_{shell}v_{tail}}(\rho_{head} - \rho_s) + \rho_s, \#(S8)$$

where v_{head} and v_{tail} are Hsp-G head and tail group volumes estimated using CRYSOL[2] software package, and ρ_{head} is the head SLD of Hsp-G.

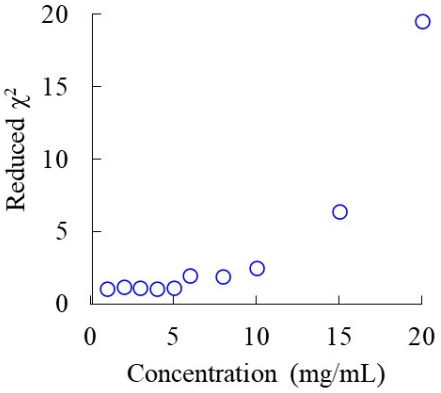

Figure S5: Goodness-of-fit value of monodisperse sphere model for Hsp-G SAXS data.

3.2 Best-Fit Model Parameters

Table S2: Best-fit parameters of the SAXS models used for Hsp-G aggregates and the reduced χ^2 statistic as Hsp-G concentration (c < CMC) is varied.

c (mg/mL)	χ^2	R (Å)	$\Delta\rho$ ($\times10^{-7}$ Å^{-2})	A	B	BG ($\times10^{-7}$)
1	1.04	9.1 ± 0.1	2.6 ± 1.4	$2.6\times10^{-18} \pm 5.4\times10^{-7}$	−5.99 ± 0.04	−0.01 ± 1.42
2	1.22	11.9 ± 0.4	1.5 ± 0.2	$1.7\times10^{-16} \pm 1.7\times10^{-6}$	−5.29 ± 0.02	3.72 ± 47
3	1.16	12.3 ± 0.2	1.8 ± 0.1	$8.4\times10^{-16} \pm 2.4\times10^{-6}$	−5.05 ± 0.01	6.49 ± 40

Table S3: Best-fit parameters of the SAXS models used for Hsp-G micelles and the reduced χ^2 statistic as Hsp-G concentration (c > CMC) is varied.

c (mg/mL)	χ^2	R (Å)	t (Å)	ρ_{shell} ($\times10^{-6}$ Å^{-2})	scale ($\times10^{-6}$)	A ($\times10^{-15}$)	B	BG ($\times10^{-6}$)
4	1.08	8.1 ± 0.1	4.7 ± 0.2	11.0 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	−5.03 ± 0.02	0.91 ± 0.03
5	1.28	8.0 ± 0.5	6.1 ± 1.3	10.5 ± 0.3	2.8 ± 0.7	1.3 ± 1100	−5.1 ± 0.5	1.3 ± 0.4
6	2.01	8.0 ± 0.3	6.2 ± 0.8	10.4 ± 0.19	3.8 ± 0.4	6.2 ± 640	−4.8 ± 0.6	1.4 ± 0.4
8	1.89	8.0 ± 0.3	7.0 ± 0.9	10.3 ± 0.1	7.1 ± 0.8	6.0 ± 540	−4.9 ± 0.4	1.9 ± 0.5
10	2.32	8.1 ± 0.2	7.6 ± 0.8	10.2 ± 0.1	11.5 ± 1.9	7.9 ± 870	−4.9 ± 0.5	2.6 ± 0.6
15	5.43	8.10 ± 0.08	8.4 ± 0.2	10.05 ± 0.02	22.7 ± 1.2	6.6 ± 120	−4.6 ± 0.2	3.7 ± 0.2

4. ^{1}H NMR spectra

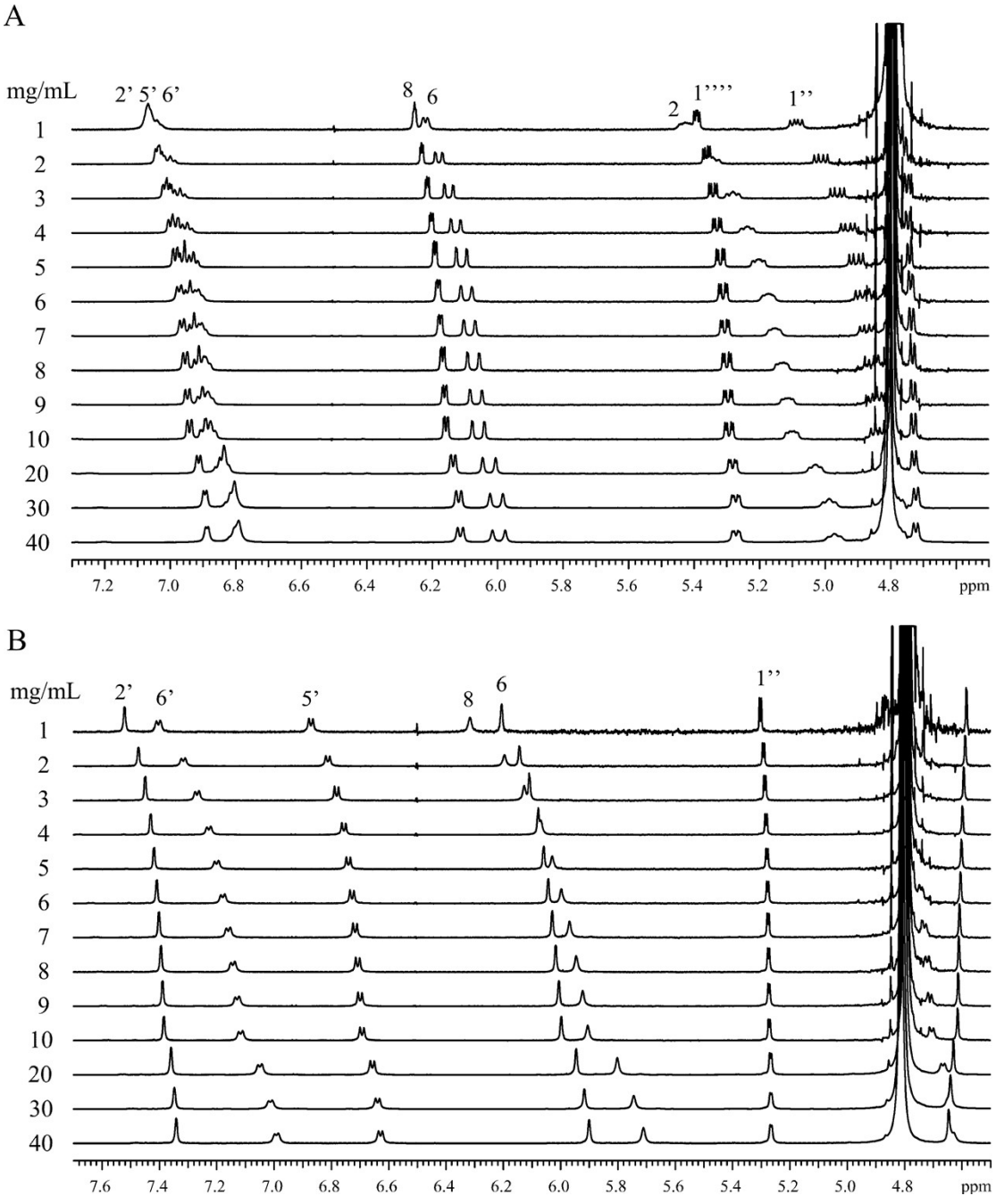

Figure S6. ^{1}H NMR spectra of Hsp-G (A) and Rutin-G (B) solutions at concentrations 1–40 mg/mL in D_2O at 25 °C.

References

1. Guinier, A.; Fournet, G. *Small-Angle Scattering of X-Rays*; John Wiley & Sons, Ltd, 1955.

2. Svergun, D.; Barberato, C.; Koch, M. H. CRYSOL - A Program to Evaluate X-Ray Solution Scattering of Biological Macromolecules from Atomic Coordinates. *J. Appl. Crystallogr.* **1995**, *28*, 768–773.