Electronic Supplementary Information

Tailoring Alginate Nanoparticles: Influence of Reverse Micelle Templates on Structure, Size, and Encapsulation Properties

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Experimental Section

Preparation of the different RMs solutions:

Stock solutions were prepared by dissolving AOT (0.1 M) in isopropyl myristate (IPM), BHDC (0.1 M) in toluene, and Triton X100 (0.7 M) in cyclohexane, followed by volumetric dilution. Precise amounts of these stock solutions were used to prepare RM solutions with varying water contents, defined by $W_0 = [water]/[surfactant]$. Water and reactants were added to each micellar solution using calibrated microsyringes to ensure accuracy. The resulting solutions were clear and homogeneous, ready for use in subsequent synthesis experiments.

For this study, the following RM systems were employed: AOT/IPM at W_0 values of 5, 12, 15, and 20; BHDC/toluene at W_0 values of 5, 7, 12, and 15; and TX-100/cyclohexane at W_0 values of 3, 5, and 8. The diameters of the ALG-NPs synthesized in these systems were determined using the DLS technique, as previously described. It was not possible to obtain higher values of W_0 due to turbidity problems.

Preparation of ALG-NPs:

A previously developed methodology for synthesizing ALG-NPs in AOT RMs was adapted for this study.¹ The preparation procedure for ALG-NPs is schematically illustrated in Figure S1. ALG-NPs were synthesized using AOT/IPM, BHDC/toluene, and TX-100/cyclohexane RMs as nanoreactors at varying W_0 values. Please note that unlike AOT and TX-100 RMs, using BHDC as a nano-template required careful control over the order of reactant addition, given the cationic nature of the RMs interface. Since alginate carries a negative charge, adding it before the calcium salts led to system collapse, especially at $W_0 < 10$. Thus, CaCl₂ had to be introduced before alginate addition.

Thus, to prepare the nanoparticles, an aqueous solution of sodium alginate (9 × 10^{-8} M) was added to the micellar solution, followed by stirring for 5 minutes. To crosslink the alginate trapped in the RMs, an aqueous solution of CaCl₂ (9 × 10^{-4} M) was rapidly added. For the BHDC-based RMs, the order of reactant addition was reversed, with CaCl₂ being added before the alginate solution. The mixture was stirred at room temperature for 2 hours to allow nanoparticle formation. The ALG-NPs within the RMs were stabilized overnight. The following day, acetone was added to disrupt the micellar system and remove the surfactant. The resulting ALG-NPs were precipitated through ultracentrifugation (12,000 rpm for 25 minutes) at room temperature and washed with acetone four times to ensure surfactant removal. The nanoparticles were air-dried at room temperature for 12 hours before their hydrodynamic apparent diameters (D_{app}) were measured. Before use in the different studies, fresh ALG-NPs aqueous solutions were prepared in pure water at pH 6.5 by adjusting with hydrochloric acid.



Figure S1. Optimal synthesis procedure used to obtain ALG-NPs from RMs. [ALG]= $9x10^{-8}$ M, [CaCl₂] = $9x10^{-4}$ M.

DLS

Dynamic light scattering (DLS) measurements were performed using a Malvern 4700 system with a goniometer and an OBIS 488 nm solid-state laser source (Coherent Inc.). Before the experiments, the samples were filtered using a 0.45 μ m nylon membrane filter (Acrodisc, Sigma). To ensure statistical reliability, 30 independent measurements were conducted for each sample. The scattering angle was set at 90°.

The particle size distribution was analyzed by calculating the autocorrelation function, which assesses the temporal fluctuations in the intensity of scattered light and determines the diffusion coefficient of the particles. The decay rate of the autocorrelation function, known as G, is described by Equation S1:

$$G = \int_{0}^{\infty} I(t)I\left(t+\tau\right)dt = B + Ae^{-2q^{2D\tau}}$$
(S1)

where *B* is the baseline, *A* is the amplitude, and *D* is the translational diffusion coefficient. The scattering vector (q) is determined using Equation S2:

$$q = \frac{4\pi n}{\lambda_0} \sin \frac{\theta}{2}$$
 (S2)

Here *n* represents the solvent refractive index, λ_0 is the laser wavelength in a vacuum, and θ is the scattering angle, usually 90 degrees. The translational diffusion coefficient *D* determined through Brownian motion, was converted to the apparent hydrodynamic diameter using the Stokes-Einstein equation (S3)²:

$$d_{app} = \frac{kT}{3\pi\eta D}$$
(S3)

where k is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity. The data were processed with the CONTIN algorithm included in the DLS instrument's software.

The polydispersity index (PDI) for all solutions analyzed was consistently below 5%, indicating high uniformity in particle size.

Particle Charge

The zeta potential (ζ) of the ALG-NPs was determined from electrophoretic light scattering (ELS) measurements. The values of electrophoretic mobility (U_E) were collected using a the Zetasizer Advance Series trademark Malvern Panalytical using the software ZS XPLORER. and further converted to ζ values (mV) through the Henry's equation.

$$U_E = \frac{2\varepsilon\zeta f(ka)}{3\eta} \tag{S4}$$

where ε is the dielectric constant of the medium. The f(ka) = 1.5 is Henry's function calculated through the Smoluchowski approximation.

TEM Experiment

To assess the morphology of the synthesized ALG-NPs, transmission electron microscopy (TEM) images were acquired using a JEM-1400 Flash TEM (JEOL, Tokyo, Japan) operating at 120 kV.

Encapsulation studies in ALG-NPs

Encapsulation of PRODAN

Given that PRODAN undergoes aggregation in water³ at concentrations above 1.0×10^{-5} M, 3.0×10^{-6} M was used for encapsulation experiments. A stock solution of PRODAN in acetonitrile (2.7x10⁻³ M) was prepared, and the appropriate amount was transferred to a flask to achieve a final concentration of 3.0×10^{-6} M. The acetonitrile was evaporated by bubbling dry nitrogen, and the residue was diluted with HPLC-grade water. The resulting solution was used to resuspend the ALG-NPs. An absorption spectrum of PRODAN in water was recorded to determine the maximum absorption wavelength (λ_{max} =351 nm). Subsequently, the samples were excited at this wavelength to obtain fluorescence emission spectra.

Encapsulation of Curcumin

A stock solution of curcumin in acetone $(3.5 \times 10^{-3} \text{ M})$ was prepared. In a 5 mL flask, 8 mg of dry ALG-NPs were mixed with 50 µL of the stock solution. The acetone was evaporated at T = 25° C and the residue was diluted with HPLC-grade water. The resulting solution was stirred, and fluorescence emission spectra of curcumin in the ALG-NPs in water were recorded.

A UV-Vis absorption spectrum of curcumin in water was also obtained to identify its maximum absorption wavelength ($\lambda_{max} = 423$ nm). Samples and the blank (curcumin/water) were excited at $\lambda_{exc} = 423$ nm to collect fluorescence emission spectra.

Absorption, Emission and TSCP Experiments.

Absorption spectra were obtained using a Shimadzu 2401 spectrophotometer, maintaining a controlled temperature of 25° C. The measurements were conducted using a quartz cell with a 1 cm optical path length.

Fluorescence emission spectra were recorded with a Spex Fluoromax spectrofluorometer equipped with a thermostatic cell holder, also set at 25° C. The emission spectra were measured using a 1 cm path-length quartz fluorescence cell. Corrected spectra were obtained by applying the correction factor file provided by the manufacturer.

The spectra were collected under controlled conditions, with appropriate baseline corrections and normalization to minimize instrumental fluctuations. The analysis of shifts and intensity variations is based on well-established photophysical principles.

PRODAN Fluorescence decay data were measured with the time-correlated single photon counting technique on Edinburgh Instruments FL-9000 equipment. Fluctuations in the pulse and intensity were corrected by making alternate collections of scattering and sample emissions. The quality of the fits was determined according to the chi-squared parameter (χ^2), which must be approximately 1.⁴

The decay curves were fitted using established models, and fitting parameters were obtained with high statistical confidence. The goodness of fit was assessed through chi-squared (χ^2) values and residual analysis.

Table S1. D_{app} and zeta potential (ζ) values of ALG-NPs obtained in AOT, TX-100, and BHDC RMs at $W_0 = 5$. [ALG] = 9x10⁻⁸ M and [CaCl₂] = 9x10⁻⁴ M. Resuspended in water at pH = 6.5. T = 25 °C. PDI = Polydispersity index values.

Nanoreactor	D _{app} (nm) ^a	PDI	D _{app} (nm) ^b	PDI	D _{app} (nm) ^c	PDI	ζ (mV) ^a
ΑΟΤ	3.2±0.2	0.36±0.02	4.0±0.2	0.27±0.01	155 ± 4^1	0.07 ±0.01	-48 ± 3
BHDC	6.1±0.3	0.33±0.01	6.8±0.3	0.40±0.01	220 ± 6	$0.19\pm\!0.01$	-23 ± 2
TX-100	13.0±0.7	0.12±0.01	13.6±0.7	0.14±0.01	180 ± 5	0.25 ± 0.04	-19 ± 2

^a Data corresponding to the reagent-free RMs. ^b Data corresponding to the RMs containing

the reagents. ^c Data corresponding to the NPs dissolved in water after the removal of RMs.



Figure S2. TEM images of ALG-NPs obtained using (A) TX-100 RMs, (B) AOT RMs and, (C) BHDC RMs.



Figure S3. Emission spectra of empty ALG-NPs dissolved in water after recovery from the different RMs used as nanoreactors. The emission spectra of the RMs without reactants are also shown. $\lambda_{exc} = 351$ nm.

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