Supporting Information

DoE-assisted Green Synthesis of Silver Nanoparticles using peel extract from *Nephelium lappaceum*: Systematic Size Optimization Enabling Antibacterial and Antioxidant Activity

Luis Castillo-Henríquez[1,2*], Pablo Agüero-Hidalgo[2,†], Juan Miguel Zúñiga-Umaña[2,†], Gabriela Montes de Oca-Vásquez[3], Fátima Arce-Vásquez[1], Zacarías Pereira-Vega[1], Badr Bahloul[4], Yohann Corvis[5], José Roberto Vega-Baudrit[2]

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Preliminary considerations for DoE

Table S1. Special considerations for the factors under study

Factor	Impact on particle size (hydrodynamic diameter)	Comments
Extract concentration	The exploratory study demonstrated an increase in size with more concentrated extract solutions.	The literature usually reports that 10 g of the vegetal material is needed to prepare 100 mL of extract. Therefore, a 0.05 g mL^{-1} extract solution was evaluated to determine the feasibility of the green synthesis using a lower concentration. On the other hand, solutions with concentrations higher than 0.15 g mL^{-1} of the vegetal material represented a challenge for the filtration step.

Extract/$AgNO_3$ volume ratio	An increased ratio would have the same impact described for extract concentration, provided that more bioactive compounds are available during the synthesis and could lead to stability issues (*e.g.*, Ostwald ripening).	Solvent-antisolvent protocols described in the literature usually inject 1 mL of the active compound solution into 10 mL of the antisolvent. To maintain symmetric distance to the previous condition, 0.5 and 1.5 mL injection volumes were defined as the low and high levels, respectively.
Agitation speed	Insufficient agitation can create local concentration gradients in which particle growth governs nucleation. On the contrary, high shear stress might also induce aggregation.	Given the magnetic bar and stirrer under use, the minimum speed to provide suitable agitation conditions in a 10 mL volume was 250 rpm. On the other hand, excessive turbulence is seen above 750 rpm, introducing many bubbles in the bulk of the liquid and causing splashing of the solution.
P188 concentration	The lack of a stabilizing agent can result in short colloidal stability of the formed AgNPs. However, the presence of P188 under agitation may induce their aggregation.	The idea behind using P188 relies on two important aspects: i) To determine if the capping property of the bioactive reducing compounds is enough to provide colloidal stability to the AgNPs, and ii) To evaluate the possibility of incorporating a PEG copolymer into the protocol to confer stealth property to the NPs for a potential parenteral administration. Our group has successfully employed P188 solutions close to 1 mg mL^{-1} to rehydrate excipient-free dry nanocrystalline powder.

DoE experimental plan

Table S2. Experimental uncoded plan for the Plackett-Burman design and collected data

Std. Order	X_1: Extract concentration (g mL^{-1})	X_2: Extract/$AgNO_3$ volume ratio	X_3: Agitation speed (rpm)	X_4: P188 concentration (g mL^{-1})	Y_1: Hydrodynamic diameter (nm)	Y_2: PDI
12	0.05	0.05	250	0	62.1	0.377
9	0.05	0.05	250	0.001	87.2	0.349
7	0.05	0.15	750	0.001	66.8	0.916
2	0.15	0.15	250	0.001	203.7	0.523
6	0.15	0.15	750	0	179.2	0.388
1	0.15	0.05	750	0	159.1	1.000
10	0.15	0.05	250	0	154.1	0.308
11	0.05	0.15	250	0	82.7	0.349
8	0.05	0.05	750	0.001	66.0	0.371
4	0.15	0.05	750	0.001	235.7	0.367
3	0.05	0.15	750	0	81.6	0.330
5	0.15	0.15	250	0.001	216.1	0.523

DoE statistical analysis

Table S3. Factorial regression for hydrodynamic diameter at t_{24h}

Term	Y1: Hydrodynamic diameter at t_{24h}	
	Coefficient	*p*-value
X_1	58.46	0.000
X_2	5.49	0.419
X_3	-1.46	0.826
X_4	13.06	0.081
R^2 %	92.7	
R^2 adj %	88.5	
Predictive linear equation	$Y_1 = 132.86 + 58.46 * X_1 + 5.49 * X_2 - 1.46 * X_3 + 13.06 * X_4 + 6.40$	

Optimization and DoE validation

Table S4. Optimized green-synthesized AgNPs data and validation of the predictive model at (-1,+1,+1,-1)

Sample	Hydrodynamic diameter (nm)	PDI	ZP (mV)
1	85 ± 1	0.243 ± 0.019	-14.8 ± 9.1
2	79 ± 3	0.275 ± 0.043	-13.7 ± 4.2
3	77 ± 1	0.236 ± 0.025	-14.9 ± 2.1
Average	80 ± 4	0.260 ± 0.016	-14.5 ± 0.7
Predicted value	81 ± 10		
Error %	1.2		

By substituting the coded level of only significant terms in the polynomial equation from the model we obtained the predicted value:

$$Y_1 = 132.86 + 58.46 * X_1 + 6.40$$

$$Y_1 = 132.86 + 58.46 * -1 + 6.40$$

$$Y1 = \ 81 \text{ nm}$$

To validate the model, we calculated the relative prediction error of the model with respect to the experimental value, as follows:

$$Relative\ prediction\ error = \frac{|Experimental\ value - Predicted\ value|}{Predicted\ value} * 100$$

$$Relative\ prediction\ error = \frac{|80 - 81|}{81} * 100$$

$$Relative\ prediction\ error = 1.2\%$$

The previous relative prediction error means that the experimental result validates the model in 98.8%.

Aggregation-driven antimicrobial paradox

To experimentally validate the proposed aggregation-driven antimicrobial paradox, which arises from AgNPs concentration-dependent physicochemical state in biological media, additional DLS measurements were performed after incubating in MH culture medium for 24 h. Contrarily to measurements performed after the synthesis, the hydrodynamic diameter distributions obtained in culture medium followed a multimodal, time-evolving, and non-Gaussian mode. Due to the previous nature of these distributions, a Monte Carlo approach was applied to assess statistically the changes in hydrodynamic diameter (**Figure S1**).

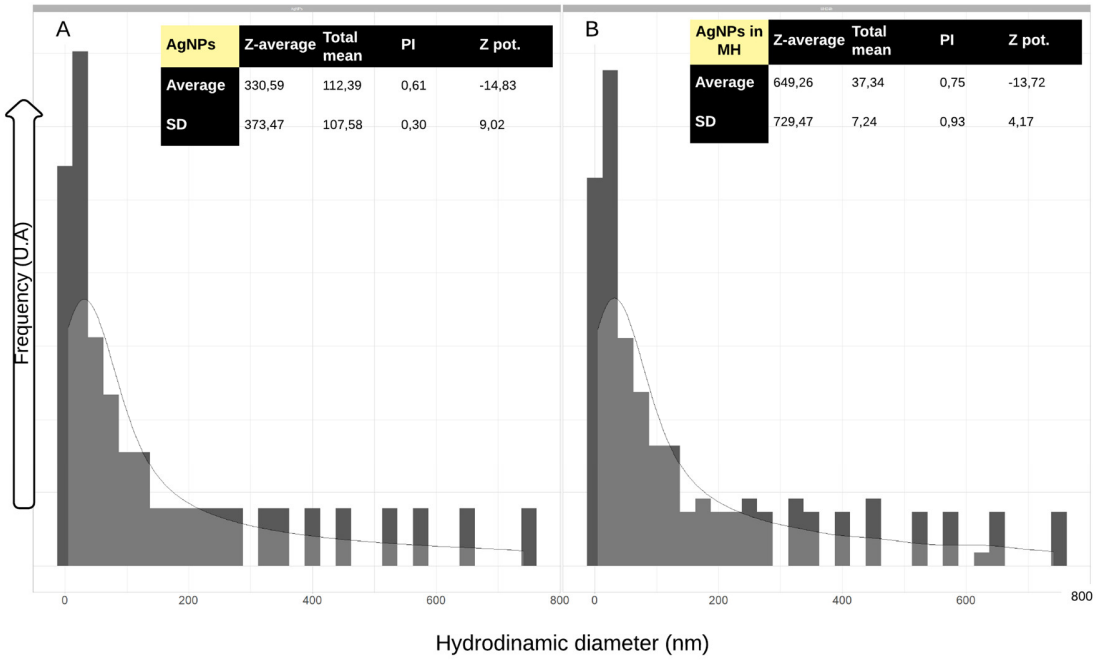


Figure S1. DLS analysis of rambutan AgNPs samples A) after dissolved in MH medium at 1x concentration and B) incubated in MH medium at 1x for 24 h. The size logs in terms of hydrodynamic diameter (Z-average) differ empirically and the frequency distributions differ significantly (KS test, D = 0.08, simulated *p*-value = 0.003 based on 1x106 simulations)

The analysis revealed a clear shift toward larger hydrodynamic diameters after incubation in culture medium, confirming aggregation and soft-corona formation under biologically relevant conditions. The latter reduce the effective surface area available for interaction with bacterial membranes, leading to a reduction in antimicrobial efficacy.