Supplementary information for

Is centrifugal ultrafiltration a robust method for determining encapsulation efficiency of pesticide nanoformulations?

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Figure S1. Centrifugal ultrafiltration devices used in this work. In the figure the length of the devices, the maximum sample volume (V_{max}) and the active area of their membranes are indicated.
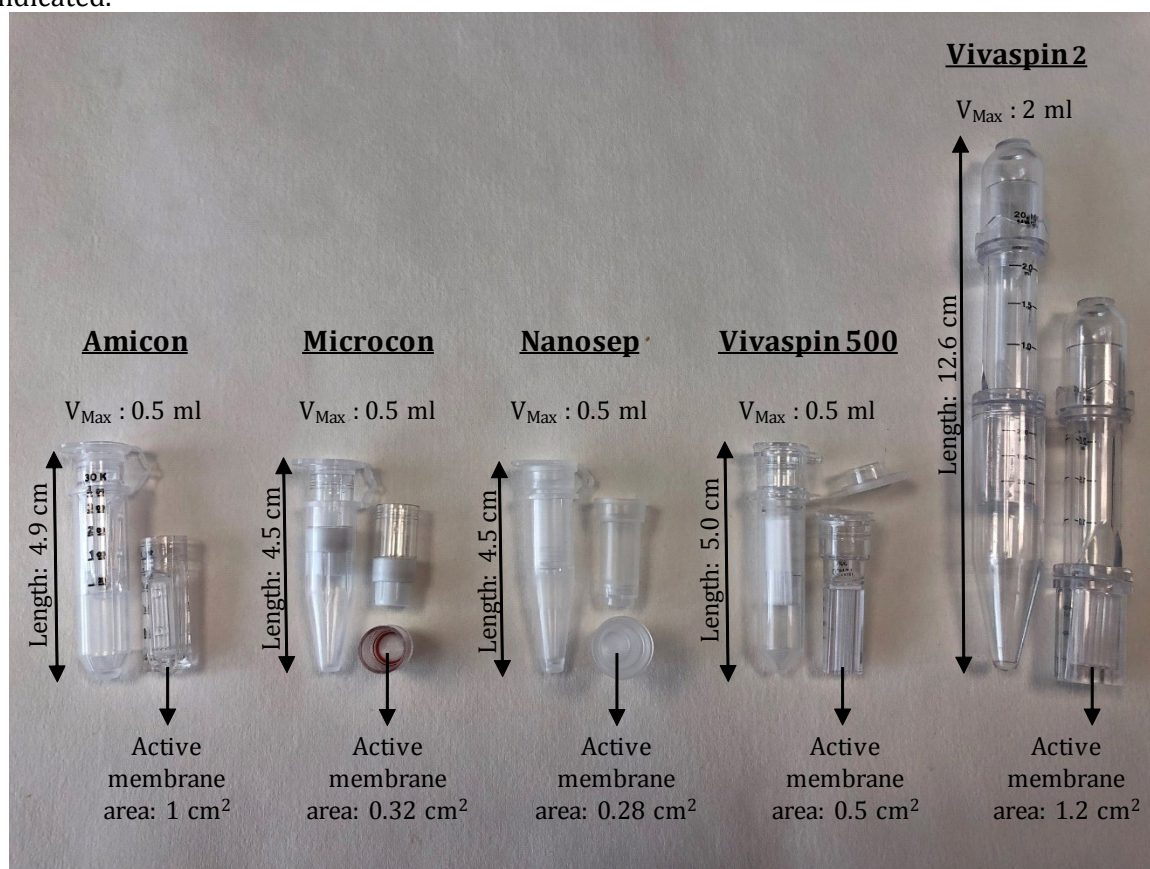

Amicon

V_{Max} : 0.5 ml

Length: 4.9 cm

Active membrane area: 1 cm^2

Microcon

V_{Max} : 0.5 ml

Length: 4.5 cm

Active membrane area: 0.32 cm^2

Nanosep

V_{Max} : 0.5 ml

Length: 4.5 cm

Active membrane area: 0.28 cm^2

Vivaspin 500

V_{Max} : 0.5 ml

Length: 5.0 cm

Active membrane area: 0.5 cm^2

Vivaspin 2

V_{Max} : 2 ml

Length: 12.6 cm
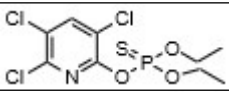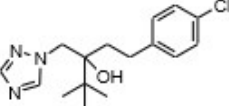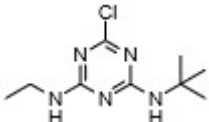
Active membrane area: 1.2 cm^2

Table S1. Main physicochemical properties and chemical structure of chlorpyrifos (CLP), tebuconzole (TBZ) and terbuthilazine (TBA) from Pesticides Properties DataBase.[1]

	M_W[a] (g mol^{-1})	S_W[b] (mg l^{-1})	Log K_{OW}[c]	P_v[d] (mPa)	H[e] (Pa m^3 mol^{-1})	Chemical structure
CLP	350.6	1.0	4.7	1.43	0.478	
TBZ	307.8	36.0	3.7	0.0013	1×10^{-5}	
TBA	229.7	6.6	3.4	0.152	2.3×10^{-3}	

[a] M_w : molecular mass
[b] S_W : solubility in water at 20 °C
[c] K_{OW}: octanol-water partition coefficient at pH 7, 20 °C
[d] P_v: vapor pressure at 20 °C
[e] H: Henry's law constant at 25 °C

Table S2. Physicochemical characterization of the nanoformulations poly(ε-caprolactone) loaded with tebuconazole (PCL-TBZ) and with terbuthylazine (PCL-TBA).

	Total concentration (μg/ml)	Particle size (nm)	Polydispersity index	Zeta potential (mV)
PCL-TBZ	449.3 ± 10.9	238.0 ± 1.9	0.173 ± 0.021	-41.4 ± 0.7
PCL-TBA	258.8 ± 18.75	282.4 ± 3.1	0.201 ± 0.004	-38.4 ± 0.4

Table S3. Centrifugation conditions for each centrifugal ultrafiltration device tested.

Device	Rotor type	Spin Speed x g	Time (min)
Microcon Amicon Nanosep-O Nanosep-B Nanosep-G Vivaspin 500	Fixed angle	14000	30
Vivaspin 2-PES Vivaspin 2-CTA Vivaspin 2-HY	Swing bucket	3100	30

Table S4. Concentration and mass of chlorpyrifos (CLP) in the filtrate, water rinse, tube rinse and filter rinse. The CLP mass in tube rinse was calculated without considering (Tube rinse) and considering (Tube rinse + non-desorbed) the desorption of AI from tube material after extraction with methanol (Table 3).

Device		Concentration (µg/ml)	Mass (µg)
	CLP unfiltered	0.50 ± 0.02*	0.25 ± 0.01
Microcon	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	0.005 ± 0.001	0.002 ± 0.000
	Tube rinse + non-desorbed		0.004 ± 0.001
	Filter rinse	0.34 ± 0.05	0.17 ± 0.02
Amicon	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	<LOQ	<LOQ
	Filter rinse	0.30 ± 0.01	0.15 ± 0.01
Nanosep-O	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	<LOQ	<LOQ
	Filter rinse	0.44 ± 0.02	0.22 ± 0.01
Nanosep-B	Filtrate	<LOQ	<LOQ
	Water rinse	0.001 ± 0.001	0.003 ± 0.000
	Tube rinse	0.009 ± 0.002	0.005 ± 0.001
	Tube rinse + non-desorbed		0.007 ± 0.001
	Filter rinse	0.329 ± 0.005	0.164 ± 0.003
Nanosep-G	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	<LOQ	<LOQ
	Filter rinse	0.390 ± 0.030	0.195 ± 0.015
	CLP unfiltered	0.48 ± 0.01	0.24 ± 0.01
Vivaspin 500	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	<LOQ	<LOQ
	Filter rinse	0.41 ± 0.01	0.20 ± 0.00
Vivaspin 2-PES	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	<LOQ	<LOQ
	Filter rinse	0.44 ± 0.01	0.22 ± 0.00
Vivaspin 2-CTA	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	<LOQ	<LOQ
	Filter rinse	0.34 ± 0.02	0.17 ± 0.01
Vivaspin 2-HY	Filtrate	<LOQ	<LOQ
	Water rinse	<LOQ	<LOQ
	Tube rinse	0.004 ± 0.000	0.002 ± 0.000
	Filter rinse	0.28 ± 0.03	0.14 ± 0.02

Device		Concentration (µg/ml)	Mass (µg)
	TBZ unfiltered	0.46 ± 0.00[*]	0.23 ± 0.00

[*]Value ± Standard deviation

Table S5. Concentration and mass of tebuconazole (TBZ) in the filtrate, water rinse, tube rinse and filter rinse. The TBZ mass in tube rinse was calculated without considering (Tube rinse) and considering (Tube rinse + non-desorbed) the desorption of AI from tube material after extraction with methanol (Table 3).

Device		Concentration (µg/ml)	Mass (µg)
Microcon	Filtrate	0.348 ± 0.019	0.174 ± 0.010
	Water rinse	0.036 ± 0.003	0.018 ± 0.002
	Tube rinse	0.005 ± 0.000	0.002 ± 0.000
	Tube rinse + non-desorbed		0.016 ± 0.001
	Filter rinse	0.0?? ± 0.001	0.020 ± 0.000
	TBA unfiltered	0.46 ± 0.00*	0.23 ± 0.00
Amicon / Microcon	Filtrate	0.388 ± 0.010	0.194 ± 0.005
	Water rinse	0.008 ± 0.001	0.004 ± 0.001
	Tube rinse	0.015 ± 0.000	0.008 ± 0.000
	Filter rinse	0.454 ± 0.006	0.227 ± 0.003
Nanosep-O	Filtrate	0.044 ± 0.005	0.022 ± 0.002
	Water rinse	0.028 ± 0.001	0.014 ± 0.001
	Tube rinse	0.003 ± 0.000	0.001 ± 0.000
	Tube rinse + non-desorbed		0.005 ± 0.000
	Filter rinse	0.422 ± 0.013	0.211 ± 0.006
Nanosep-B	Filtrate	0.267 ± 0.009	0.134 ± 0.004
	Water rinse	0.194 ± 0.021	0.097 ± 0.011
	Tube rinse	0.002 ± 0.000	0.001 ± 0.000
	Tube rinse + non-desorbed		0.003 ± 0.001
	Filter rinse	0.045 ± 0.007	0.211 ± 0.006
Nanosep-G	Filtrate	0.265 ± 0.023	0.133 ± 0.012
	Water rinse	0.227 ± 0.017	0.113 ± 0.009
	Tube rinse	0.006 ± 0.001	0.003 ± 0.000
	Tube rinse + non-desorbed		0.009 ± 0.002
	Filter rinse	0.029 ± 0.010	0.015 ± 0.005
Vivasprin 500	Filtrate	0.004 ± 0.003	0.002 ± 0.001
	Water rinse	0.004 ± 0.002	0.002 ± 0.001
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.434 ± 0.025	0.217 ± 0.013
	TBZ unfiltered	0.429 ± 0.006	0.214 ± 0.003
Vivaspin 2-PES	Filtrate	< LOQ	< LOQ
	Water rinse	< LOQ	< LOQ
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.396 ± 0.015	0.198 ± 0.007
Vivaspin 2-CTA	Filtrate	< LOQ	< LOQ
	Water rinse	< LOQ	< LOQ
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.494 ± 0.012	0.247 ± 0.006
Vivaspin 2-HY	Filtrate	0.329 ± 0.007	0.165 ± 0.004
	Water rinse	0.054 ± 0.002	0.027 ± 0.001
	Tube rinse	0.005 ± 0.000	0.003 ± 0.000
	Tube rinse + non-desorbed		0.010 ± 0.001
	Filter rinse	0.040 ± 0.003	0.020 ± 0.001

*Value ± Standard deviation

Table S6. Concentration and mass of terbuthylazine (TBA) in the filtrate, water rinse, tube rinse and filter rinse.

	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.052 ± 0.003	0.026 ± 0.002
Amicon	Filtrate	0.063 ± 0.006	0.032 ± 0.003
	Water rinse	0.049 ± 0.002	0.024 ± 0.001
	Tube rinse	< LOQ	<LOQ
	Filter rinse	0.343 ± 0.032	0.172 ± 0.016
Nanosep-O	Filtrate	0.160 ± 0.002	0.080 ± 0.001
	Water rinse	0.016 ± 0.002	0.008 ± 0.001
	Tube rinse	< LOQ	<LOQ
	Filter rinse	0.287 ± 0.012	0.144 ± 0.006
Nanosep-B	Filtrate	0.333 ± 0.006	0.167 ± 0.003
	Water rinse	0.094 ± 0.002	0.047 ± 0.001
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.026 ± 0.003	0.013 ± 0.001
Nanosep-G	Filtrate	0.384 ± 0.012	0.192 ± 0.006
	Water rinse	0.058 ± 0.002	0.029 ± 0.001
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.010 ± 0.001	0.005 ± 0.000
Vivaspin 500	Filtrate	0.028 ± 0.014	0.014 ± 0.007
	Water rinse	0.006 ± 0.002	0.003 ± 0.001
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.394 ± 0.023	0.197 ± 0.012
	TBA unfiltered	0.389 ± 0.006	0.195 ± 0.003
Vivaspin 2-PES	Filtrate	< LOQ	< LOQ
	Water rinse	< LOQ	< LOQ
	Tube rinse	< LOQ	< LOQ
	Filter rinse	0.371 ± 0.005	0.185 ± 0.003
Vivaspin 2-CTA	Filtrate	0.005 ± 0.001	0.003 ± 0.000
	Water rinse	0.114 ± 0.005	0.057 ± 0.003
	Tube rinse	0.002 ± 0.000	0.001 ± 0.000
	Filter rinse	0.299 ± 0.004	0.149 ± 0.002
Vivaspin 2-HY	Filtrate	0.318 ± 0.005	0.159 ± 0.003
	Water rinse	0.033 ± 0.002	0.016 ± 0.001
	Tube rinse	0.003 ± 0.000	0.001 ± 0.000
	Filter rinse	0.042 ± 0.003	0.021 ± 0.002

[*]Value ± Standard deviation

Table S7. Mass of AI in the filtrate, water rinse, tube rinse and filter rinse after centrifugal ultrafiltration of undiluted and diluted (10 and 100 times) samples of PCL-TBZ and PCL-TBA using Microcon device. Mass of the AI free in the supernatant (m_{free}) was calculated as the sum of the mass of AI in the filtrate, in the tube and in the filter. The TBZ mass in tube rinse was calculated without considering (Tube rinse) and considering (Tube rinse + non-desorbed) the desorption with methanol (Table 3). Encapsulation efficiency (EE) was determined according to Eq. (4) from the m_{free} given in this table

		Mass (µg)	m_{free} (µg)	EE (%)
PCL-TBZ	Filtrate	$4.402 \pm 0.081^{*}$	5.80 ± 0.28	97.2 ± 4.6
	Water rinse	0.356 ± 0.091		
	Tube rinse	0.008 ± 0.001		
	Tube rinse + non-desorbed	0.049 ± 0.004		
	Filter rinse	1.345 ± 0.320		
PCL-TBZ d10	Filtrate	3.201 ± 0.045	4.31 ± 0.10	80.5 ± 0.4
	Water rinse	0.289 ± 0.009		
	Tube rinse	0.011 ± 0.005		
	Tube rinse + non-desorbed	0.071 ± 0.031		
	Filter rinse	1.079 ± 0.055		
PCL-TBZ d100	Filtrate	1.254 ± 0.028	1.57 ± 0.07	26.7 ± 4.9
	Water rinse	0.237 ± 0.036		
	Tube rinse	0.006 ± 0.001		
	Tube rinse + non-desorbed	0.020 ± 0.003		
	Filter rinse	0.299 ± 0.043		
PCL-TBA	Filtrate	2.189 ± 0.063	13.43 ± 2.20	91.2 ± 15.3
	Water rinse	0.962 ± 0.121		
	Tube rinse	0.009 ± 0.002		
	Filter rinse	11.24 ± 2.24		
PCL-TBA d10	Filtrate	1.704 ± 0.037	2.702 ± 0.04	73.4 ± 5.3
	Water rinse	0.221 ± 0.021		
	Tube rinse	0.005 ± 0.001		
	Filter rinse	0.985 ± 0.075		
PCL-TBA d100	Filtrate	0.471 ± 0.018	0.731 ± 0.05	40.8 ± 9.2
	Water rinse	0.092 ± 0.009		
	Tube rinse	0.002 ± 0.000		
	Filter rinse	0.258 ± 0.035		

Table S8. Mass of AI in the filtrate, water rinse, tube rinse and filter rinse after centrifugal ultrafiltration of undiluted and diluted (10 and 100 times) samples of PCL-TBZ and PCL-TBA using Vivaspin 2-HY device. Mass of the AI free in the supernatant (m_{free}) was calculated as the sum of the mass of AI in the filtrate, in the tube and in the filter. The TBZ mass in tube rinse was calculated without considering (Tube rinse) and considering (Tube rinse + non-desorbed) the

		Mass (µg)	m_{free} (µg)	EE (%)
PCL-TBZ	Filtrate	4.323 ± 0.182*	8.953 ± 1.15	96.0 ± 3.4
	Water rinse	1.096 ± 0.120		
	Tube rinse	0.030 ± 0.010		
	Tube rinse + non-desorbed	0.114 ± 0.015		
	Filter rinse	4.516 ± 1.073		
PCL-TBZ d10	Filtrate	3.001± 0.042	5.512 ± 0.31	74.8 ± 2.9
	Water rinse	1.340 ± 0.179		
	Tube rinse	0.040 ± 0.008		
	Tube rinse + non-desorbed	0.151 ± 0.030		
	Filter rinse	2.359 ± 0.241		
PCL-TBZ d100	Filtrate	1.176 ± 0.011	1.420 ± 0.01	32.8 ± 2.3
	Water rinse	0.190 ± 0.012		
	Tube rinse	0.013 ± 0.001		
	Tube rinse + non-desorbed	0.049 ± 0.003		
	Filter rinse	0.195 ± 0.007		
PCL-TBA	Filtrate	1.792 ± 0.033	7.212 ± 2.21	94.4 ± 10.1
	Water rinse	0.392 ± 0.078		
	Tube rinse	0.005 ± 0.001		
	Filter rinse	5.415 ± 2.291		
PCL-TBA d10	Filtrate	1.677 ± 0.015	2.758 ± 0.21	86.5 ± 18.1
	Water rinse	0.412 ± 0.231		
	Tube rinse	0.005 ± 0.001		
	Filter rinse	1.072 ± 0.192		
PCL-TBA d100	Filtrate	0.603 ± 0.004	0.732 ± 0.02	33.8 ± 3.1
	Water rinse	0.050 ± 0.008		
	Tube rinse	0.002 ± 0.000		
	Filter rinse	0.127 ± 0.015		

desorption of AI from tube material after extraction with methanol (Table 3). Encapsulation efficiency (EE) was determined according to Eq. (4) from the m_{free} given in this table.

*Value ± Standard deviation

References

1   K. A. Lewis, J. Tzilivakis, D. J. Warner and A. Green, *Hum. Ecol. Risk Assess.*, 2016, **22**, 1050–1064.