Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Electronic Supporting Information

Ellagic Acid micro and nano formulations with amazingly increased water solubility by its entrapment in pectin or non-PAMAM dendrimers eligible for clinical applications

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Section S1 FTIR, ^{1}H NMR and ^{13}C NMR of Ellagic Acid **1** and FTIR of pectin.

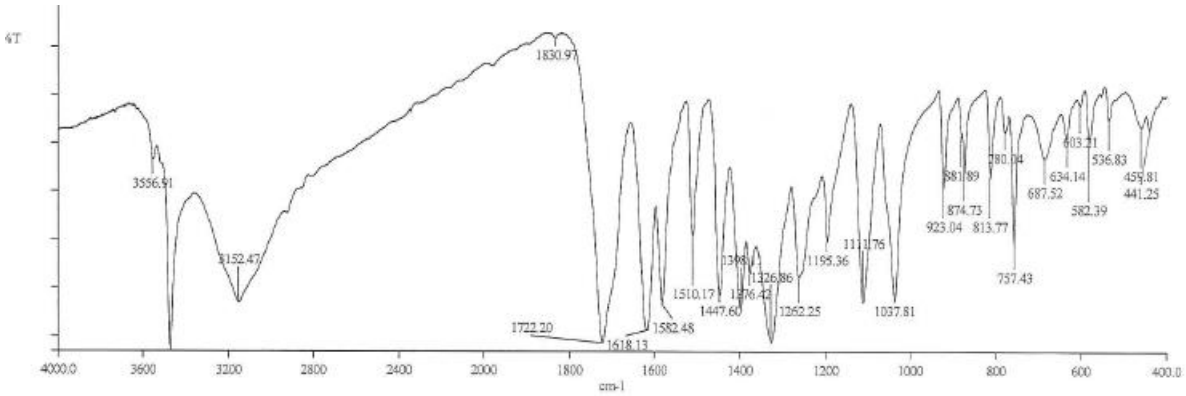


Figure S1. FTIR (KBr) of Ellagic Acid (**1**)

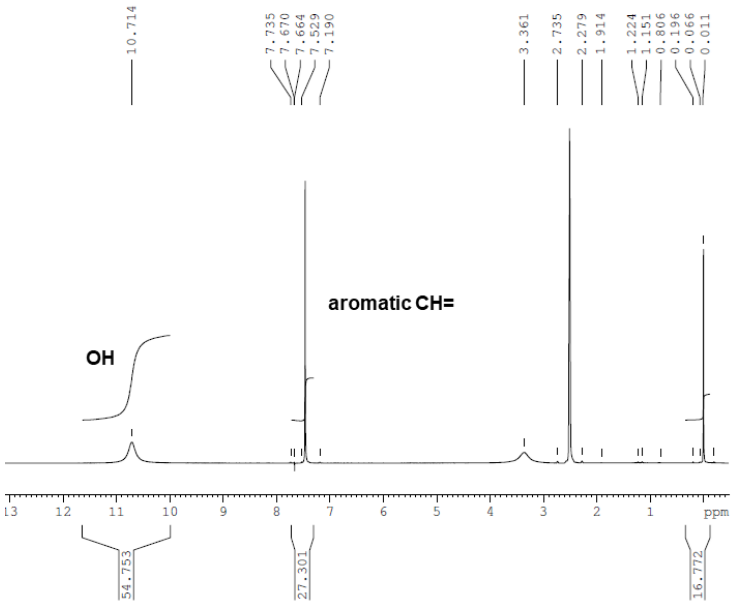


Figure S2. ^{1}H NMR (DMSO-d_6, 300 MHz) of EA (**1**)

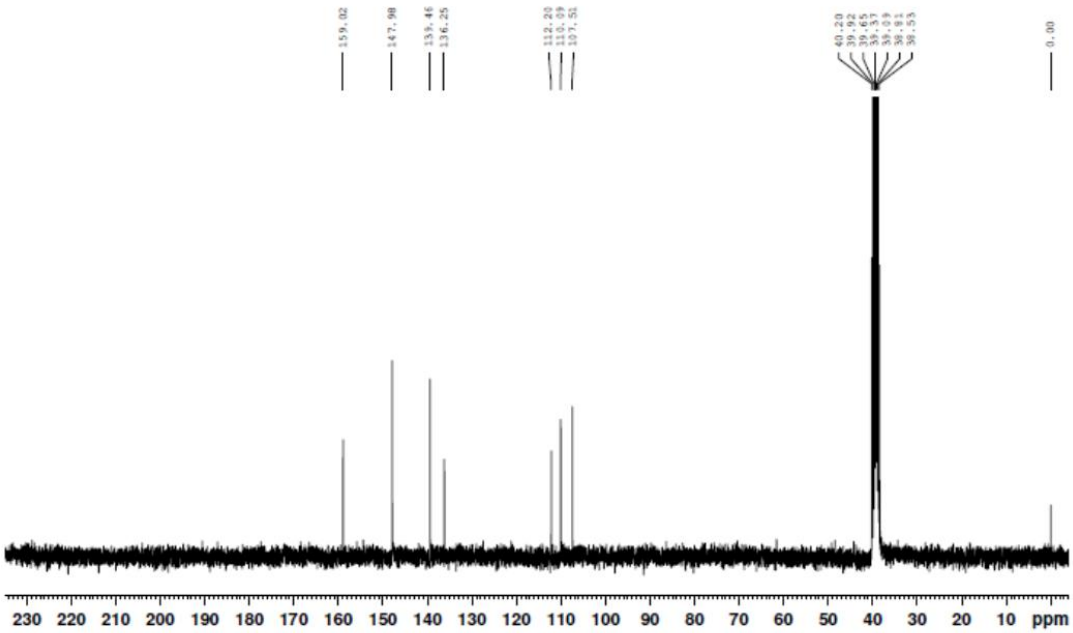


Figure S3. ^{13}C NMR (DMSO-d_6, 75.5 MHz) of EA (**1**)

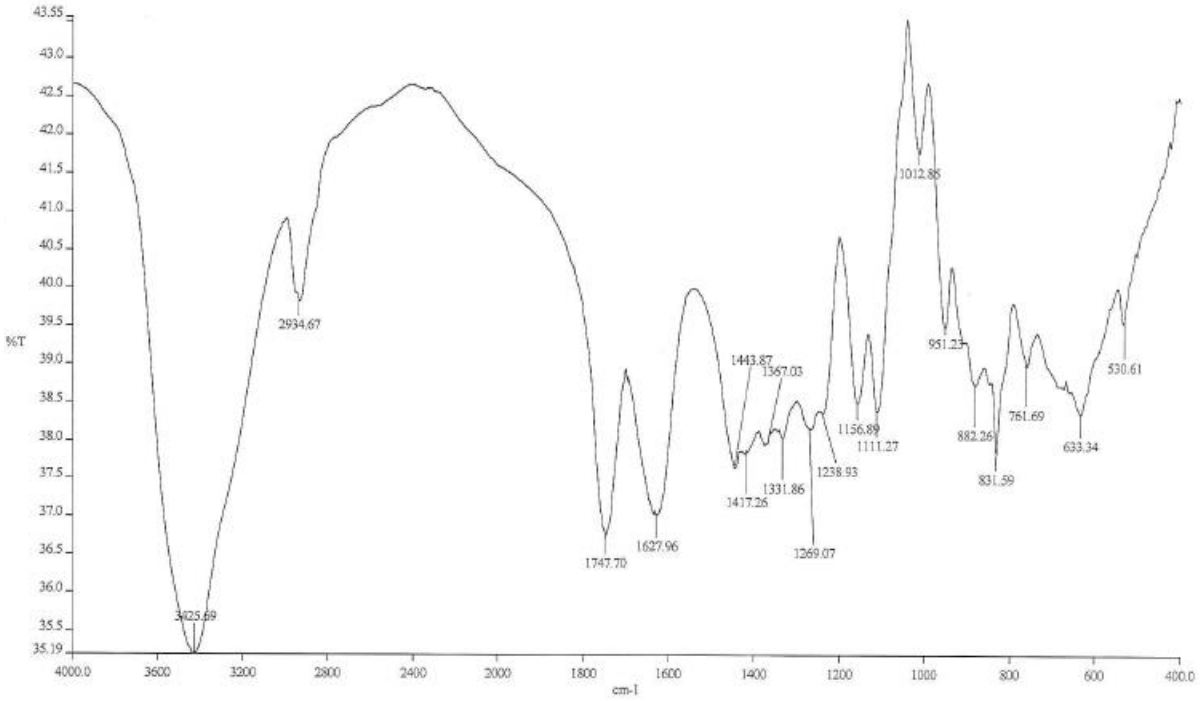


Figure S4. FTIR of LM pectin

Section S2 Data about dendrimers **2** and **3**

AD S1 Characterization data of dendrimers **2** and **3**.[1,2]

Dendrimer 2 (79 HCl). [1] Slightly Hygroscopic, off white spongy solid (250.7 mg, 0.01798 mmol, 92.1 % overall yield).

^{1}H NMR (300 MHz, DMSO-d_6, 25° C, TMS): δ = 1.00-2.00 [more broad signals, 298H, (138H, CH_3 G4 + 96H, CH_2CH_2CH_2 Lys + 64H, CH_2CH_2 Arg)], 2.76 (m, 32H, C$H_2^{\varepsilon}NH_3^+$ Lys), 3.10-3.30 (m, 32H, CH_2^{δ}NH Arg), 3.47 (br s, 24H, C$H_3NH_2^+$ sarcosine), 3.50 (br s, 2H, CH_2OH), 3.76 (s, 42H, (CH_3)$_2$NH$^+$ DMG), 4.01 (m, 32H, CHNH$_3^+$ Arg + Lys), 4.10-4.50 [m, 215H (14H, CH_2NH$^+$ DMG + 16H, C$H_2NN_2^+$ sarcosine + 184H, CH_2O G4 + 1H, OH)], 8.08, 8.23, 8.81 [three broad signals, 247H (48H, NH_3^+Arg + 32H, $^{\omega'}NH_2^+$ Arg + 32H, $^{\omega}NH_2$ Arg + 16H $^{\delta}NH$ Arg + 48H, $^{\alpha}NH_3^+$ Lys + 48H $^{\varepsilon}NH_3^+$ Lys + 7H, NH$^+$ DMG + 16H, NH_2^+ sarcosine)]. FTIR (KBr, cm^{-1}): 3431 (NH_3^+ + OH), 2934, 1741 (C=O esters), 1630 (NH); elemental analysis calcd (%) for $C_{474}H_{924}N_{111}Cl_{79}O_{185}$: C, 40.84; H, 6.68; Cl, 20.09; N, 11.15%. Found: C, 41.20; H 6.86; Cl, 20.08; N, 10.96.

Dendrimer 3 (37HCl). [2] Hygroscopic, pale yellow glassy solid (372.6 mg, 0.054 mmol, 88.8%, overall yield: 70.1%).

^{1}H NMR (300 MHz, DMSO-d_6, 25° C, TMS): δ = 0.85 (t, 3H, J = 7.0 Hz, CH_3 stearate), 1.00, 1.03, 1.08 and 1.10 (four signals, 63H, CH_3 D1, D2, D3), 1.26 (s, 28H, CH_2 stearate), 1.00-2.00 [m, 72H (28H, CH_2CH_2 Arg + 42H, CH_2CH_2CH_2 Lys + 2H, one CH_2 stearate)], 2.30 (s, 2H, CH_2C=O stearate), 2.76 (m, 14H, C$H_2^{\varepsilon}NH_3^+$ Lys), 3.10-3.30 (very broad signal, 14H, CH_2^{δ}NH Arg), 3.52 (br s, 2H, CH_2OH), 3.75 [s, 42H, (30H, CH_3NH$^+$CH_3 DMG + 12 H, C$H_3NN_2^+$ sarcosine)], 4.00 (m, 14H, CHNH$_3^+$ Arg + Lys), 4.10-4.50 [m, 108H (10H, CH_2NH$^+$ DMG + 8H, C$H_2NN_2^+$ sarcosine + 90H, CH_2O D1, D2, D3 + CH_2O *core*)], 8.08, 8.21, 8.76 [three broad signals, 111H (21H, NH_3^+Arg + 14H, $^{\omega'}NH_2^+$ Arg + 14H, $^{\omega}NH_2$ Arg + 7H $^{\delta}NH$ Arg + 21H, $^{\alpha}NH_3^+$ Lys + 21H $^{\varepsilon}NH_3^+$ Lys + 5H, NH$^+$ DMG + 8H, NH_2^+ sarcosine)], 1H, OH, not detected. FTIR (KBr, cm^{-1}): 3600-2400 (NH_3^+ + OH), 1742 (C=O esters), 1626 (NH).

References

[1] S. Alfei, S. Catena. Synthesis and characterization of fourth generation polyester-based dendrimers with cationic amino acids-modified crown as promising water soluble biomedical devices. Polym. Adv. Technol. **2018**, *29*, 2735-2749.

[2] S. Alfei, S. Catena. Synthesis and characterization of versatile amphiphilic dendrimers peripherally decorated with positive charged amino acids, Polym. Int. **2018**, *67*, 1572-1584.

Table S1
Cell viability values for dendrimers **2** and **3**

		Cell viability (%)	
Cpd	**μg/mL**	**B14**	**BRL**
2	20.7	69.9±3.1	84.2±1.7
3	11.2	109.2±8.4	105.8±3.3

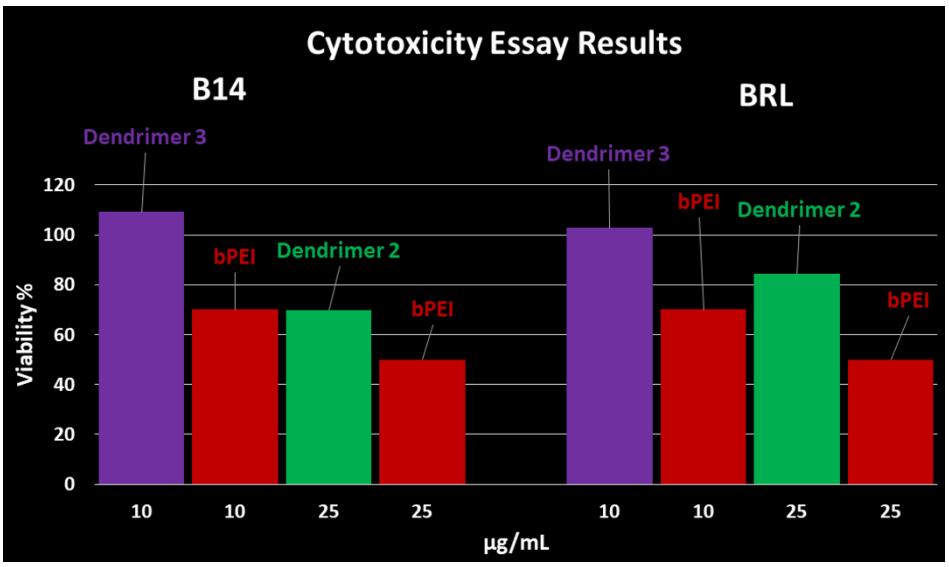


Figure S5. Comparison between cytotoxicity data of dendrimers **2** and **3** and *b*-PEI taken as reference

Section S3 FTIR and ^{1}H NMR spectra of microspheres (EAMSs).

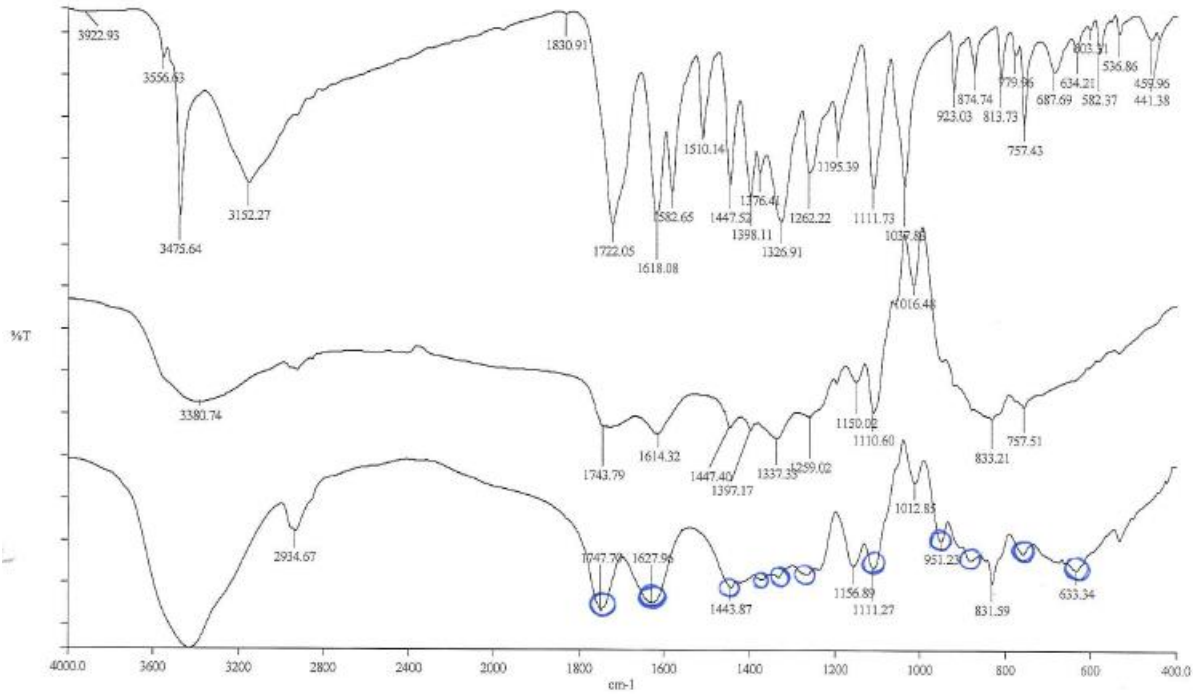


Figure S6. FTIR spectrum of EAMSs (bottom panel) with in evidence the signals derived from **1** compared to FTIR of EA (top panel) and LM pectin (middle panel). FTIR spectrum of EAMSs was very similar to pectin one but more articulated in the area between 1050 and 1500 cm^{-1} and below 1000 cm^{-1}. Then the bands around 1700 and 1600 cm^{-1} appeared much more intense thanks to the contribution of EA in the microspheres.

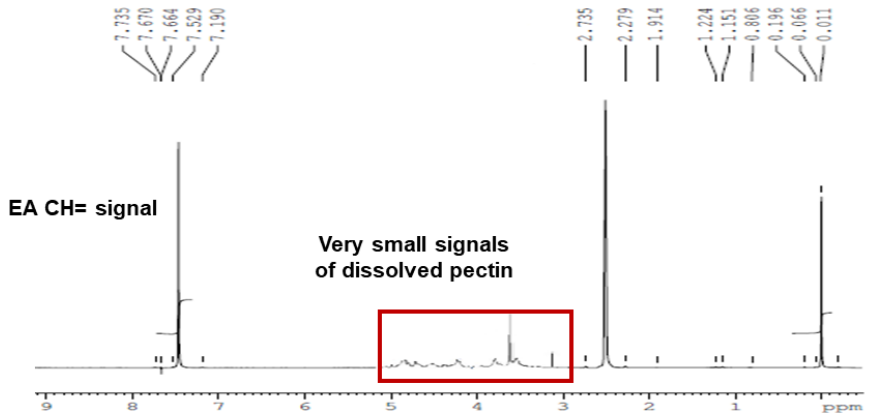


Figure S7. ^{1}H NMR spectrum of EAMSs (soluble fraction): the peak at 7.53 ppm relative to the only non-exchangeable aromatic protons of EA, further confirmed that it had been successfully loaded into pectin matrix

Section S4 Physicochemical and spectroscopic data and FTIR spectra of DPXs **4** and **5** with in evidence the signals derived from **1** compared to FTIR of EA and parent dendrimers **2** and **3**.

AD S2.

For a better understanding of the name attributed to each DPX, it should be noted that the amino acid composition has been indicated using the common three letter acronyms whenever possible (Arg = arginine, Lys = Lysine). DMG stands for dimethylglycine, MG for methylglycine, OH stands for eventually present hydroxyl group and **1** for EA. The numbers in parentheses indicate the number of units of that residual.

*DPX **4**: [Arg(16)Lys(16)DMG(7)MG(8)OH(1)**1**(39)]*

Slightly hygroscopic orange amorphous solid [38.6 equiv. of **1** per dendrimer mole (73.3 mg, 0.00286 mmol, yield: 99.9%). ^{1}H NMR (300 MHz, DMSO-d_6, 25° C, TMS): δ = 1.00-2.00 [more broad signals, 298H, (138H, CH_3 G4 + 96H, $CH_2CH_2CH_2$ Lys + 64H, CH_2CH_2 Arg)], 2.76 (m, 32H, $CH_2{}^{\varepsilon}NH_3^+$ Lys), 3.19 (br m, 32H, $CH_2{}^{\delta}NH$ Arg), 3.47 (s, 24H, $CH_3NH_2^+$ sarcosine), 3.50 (m, 2H, CH_2OH), 3.58 (s, 42H, $(CH_3)_2NH^+$ DMG), 3.80-4.40 [very broad signals, 247H (32H, $CHNH_3^+$ Arg + Lys + 14H, CH_2NH^+ DMG + 16H, $CH_2NN_2^+$ sarcosine + 184H, CH_2O G4 + 1H, OH)], 7.51, 7.54, 7.55, 7.60, 7.63 and 7.69 (more s signals, 78H, CH= aromatics of EA), 8.00-9.00 (very small signals of H atoms linked to N atoms of parent dendrimer **2**. FTIR (KBr): 3406 (OH and NH), 1736 (C=O ester), 1624 (NH and EA band), 1582, 1449, 1376, 1328, 1260, 1192, 1107, 1040, 755, 603, 574 (bands mainly derived from EA).

*DPX **5**:[Arg(7)Lys(7)DMG(5)MG(4)OH(1)**1**(25)]*

No hygroscopic yellowish amorphous solid [24.9 equiv. of **1** per dendrimer mole (87.6 mg, 0.0061 mmol, yield: 99.9%). ^{1}H NMR (300 MHz, DMSO-d_6, 25° C, TMS): δ = 0.85 (very small signals, m, 3H, CH_3 stearate), 1.00-2.00

[more signals, 163H (63H, CH_3 D1, D2, D3 + 30H, CH_2 stearate + 28H, CH_2CH_2 Arg + 42H, $CH_2CH_2CH_2$ Lys)], 2.30 (s, 2H, CH_2C=O stearate), 2.70-3.00 (m, 14H, $CH_2{}^{\varepsilon}NH_3^+$ Lys), 3.19 (12 H, $CH_3NN_2^+$ sarcosine), 3.30 (m, 14H, $CH_2{}^{\delta}NH$ Arg), 3.48 [two overlapped signals, 32H (2H, CH_2OH + 30H, $CH_3NH^+CH_3$ DMG)], 3.90-5.00 [very broad signal, H (14H, $CHNH_3^+$ Arg and Lys + 10H, CH_2NH^+ DMG + 8H, $CH_2NN_2^+$ sarcosine + 90H, CH_2O dendrimer scaffold)], 5.22 (s, 1H, OH), 7.48, 7.51, 7.54 (three s signals, 50H, CH= aromatics of EA), 8.00-9.00 (very small signals of H atoms linked to N atoms of parent dendrimer **3**), 10.71 (very small br s of OH of EA). FTIR (KBr): 3411, 3336 (OH and NH), 1734 (C=O ester), 1627 (NH and EA band), 1579, 1508, 1449, 1400, 1376, 1125, 1045, 917, 892, 815, 755, 641 (bands derived from EA).

FTIR spectra of DPXs 4 and 5: Together with bands belonging to dendrimers [2929 (**2**), 2851 and 2929 (**3**) cm^{-1} (methyl and methylene groups) and 1736 (**2**), 1734 (**3**) cm^{-1} (C=O esters)] several bands belonging to EA were detectable.

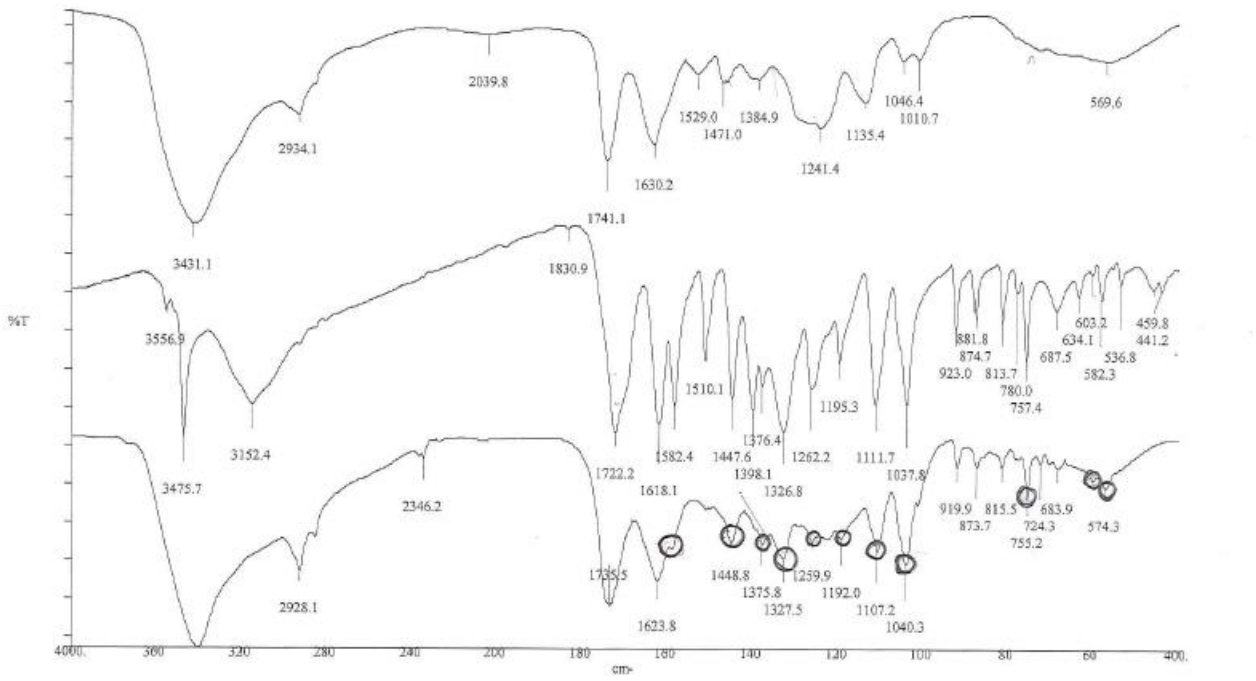


Figure S8. FTIR spectra of DPX **4** (bottom panel) with in evidence the signals derived from **1** compared to FTIR of EA (middle panel) and parent dendrimer **2** (top panel).

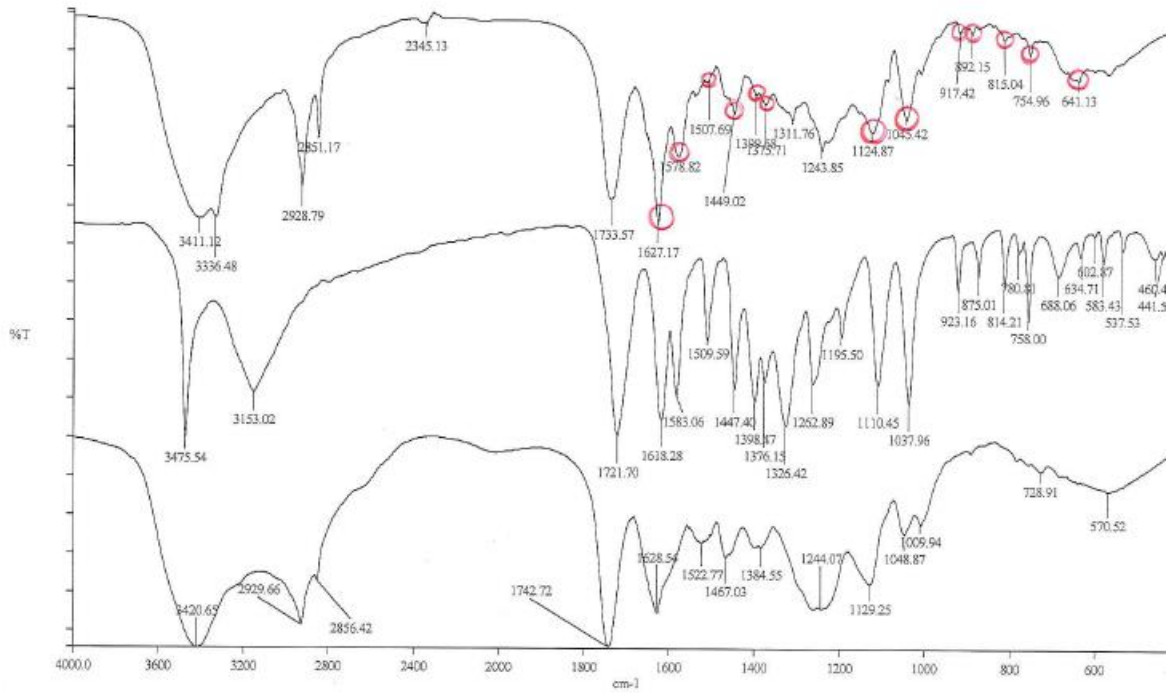


Figure S9. FTIR spectra of DPX **5** (top panel) with in evidence the signals derived from **1** compared to FTIR of EA (middle panel) and parent dendrimer **3** (bottom panel).

Section S5 Further characterizations of formulations.

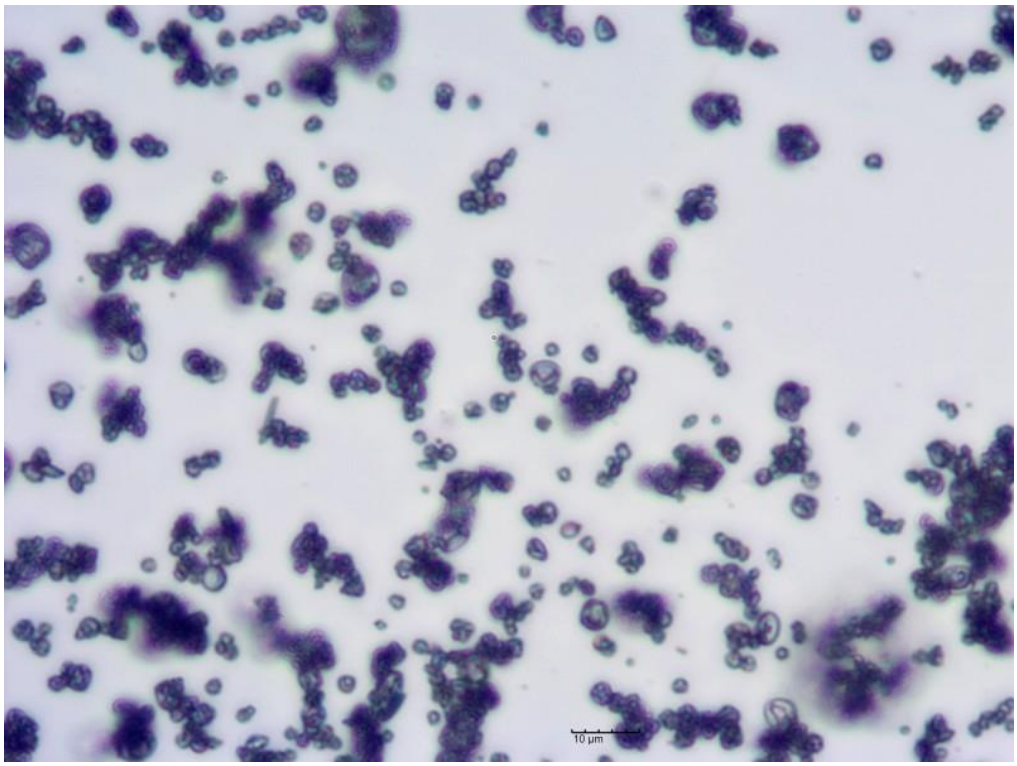
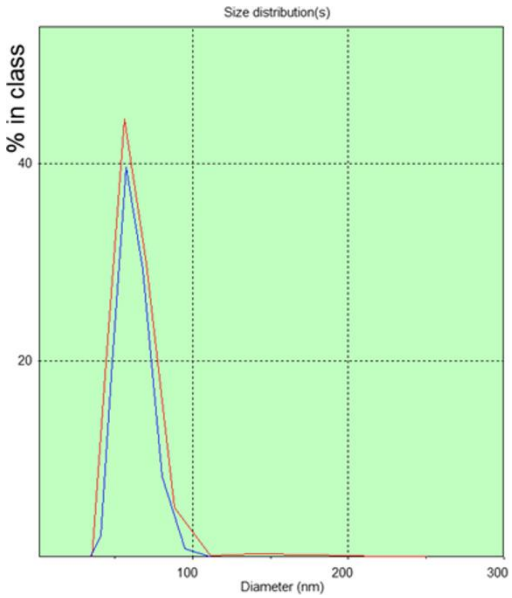


Figure S10. EAMSs images from Optical Microscopy Analysis.



Size(nm)	Intensity	Volume	Number
20.5	0.0	0.0	0.0
24.3	0.0	0.0	0.0
28.8	0.0	0.0	0.0
34.1	0.0	0.0	0.0
40.5	0.0	0.7	2.1
48.0	0.5	9.9	19.8
56.9	10.6	23.5	39.5
67.4	12.1	21.6	29.2
79.9	4.6	8.6	8.2
94.7	0.0	1.4	0.9
112.3	0.0	0.0	0.0
133.1	0.0	0.0	0.0
157.8	0.0	0.0	0.0
187.1	0.3	0.3	0.0
221.8	8.2	1.0	0.0
262.9	18.1	2.1	0.0
311.6	21.9	3.8	0.0
369.4	16.8	9.4	0.1
437.9	6.9	12.4	0.1
519.1	0.0	5.4	0.0
615.3	0.0	0.0	0.0
729.4	0.0	0.0	0.0
864.7	0.0	0.0	0.0
1025.0	0.0	0.0	0.0

Peak Analysis by intensity

Peak	Area	Mean	Width
1	27.8	65.1	24.7
2	72.2	314.2	176.5

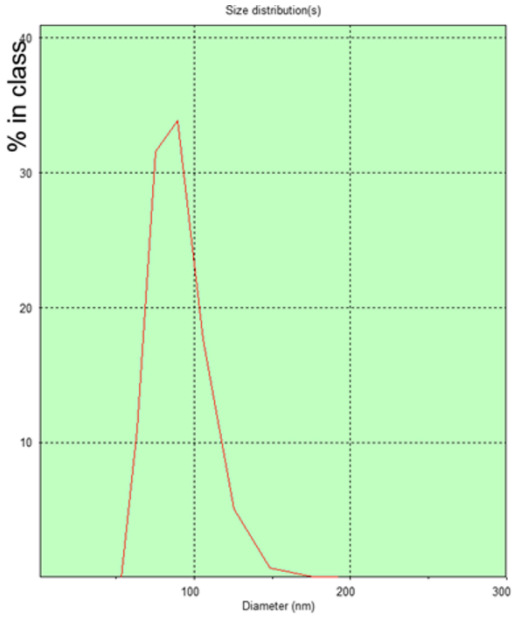
Peak Analysis by volume

Peak	Area	Mean	Width
1	65.6	62.6	27.6
2	34.4	399.2	173.3

Peak Analysis by number

Peak	Area	Mean	Width
1	99.8	60.1	25.1

Figure S11. Dynamic Light Scattering Analysis of **4**.



Size(nm)	Intensity	Volume	Number
13.1	0.0	0.0	0.0
16.5	0.0	0.0	0.0
20.8	0.0	0.0	0.0
26.2	0.0	0.0	0.0
32.9	0.0	0.0	0.0
41.5	0.0	0.0	0.0
52.2	0.0	9.3	22.9
65.7	16.5	20.2	47.7
82.8	5.6	12.6	26.9
104.2	0.0	1.6	2.0
131.2	0.0	0.2	0.0
165.2	4.3	1.0	0.1
208.0	18.5	2.0	0.2
261.8	19.3	2.2	0.1
329.7	7.3	1.3	0.0
415.1	0.0	0.4	0.0
522.6	0.0	0.0	0.0
658.0	0.0	0.2	0.0
828.4	2.7	5.1	0.0
1043.0	8.8	14.2	0.0
1313.2	10.8	16.9	0.0
1653.4	6.1	10.1	0.0
2081.7	0.1	2.6	0.0
2621.0	0.0	0.0	0.0

Peak Analysis by intensity

Peak	Area	Mean	Width
1	22.1	70.1	19.7
2	49.4	243.3	135.4
3	28.5	1259.3	779.8

Peak Analysis by volume

Peak	Area	Mean	Width
1	43.9	69.5	34.4
2	7.1	249.0	184.5
3	49.0	1293.3	840.8

Peak Analysis by number

Peak	Area	Mean	Width
1	99.5	68.0	32.6

Figure S12. Dynamic Light Scattering Analysis of **5**.

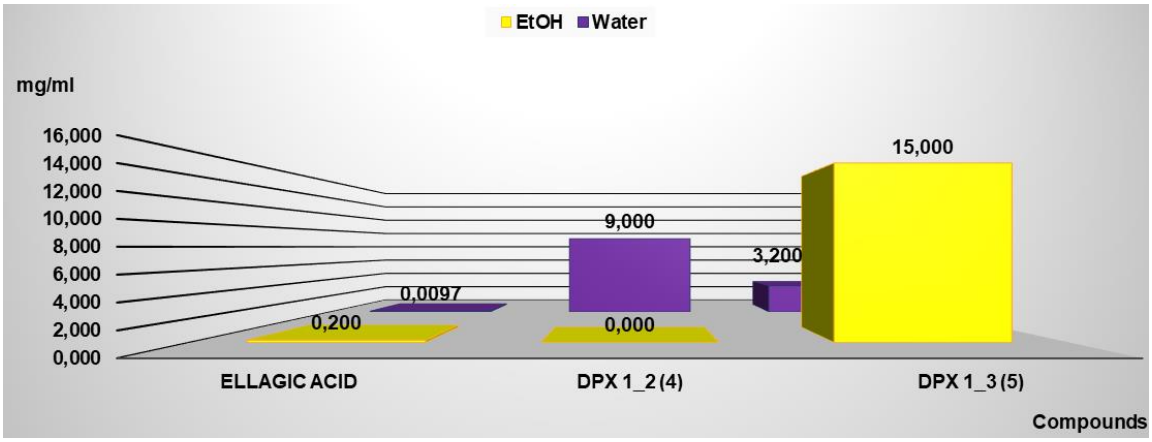


Figure S13. Solubility of DPXs **4** and **5** in biocompatible solvents (EtOH and water) compared to solubility of free EA **1**.[1]

Reference.

[1] I. Bala, V. Bhardwaj, S. Hariharan, M. N. V. R. Kumar, *J. Pharm. Biomed. Anal.* **2006**, *40*, 206–210.

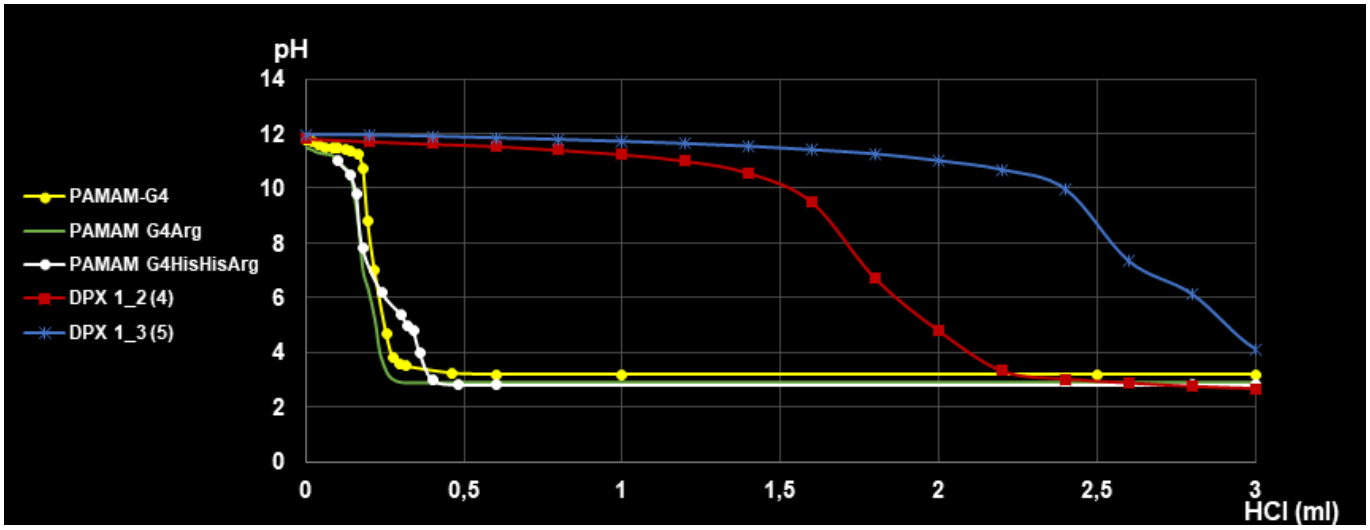


Figure S14. Potentiometric titration of DPXs **4** and **5** and of three G4-PAMAMs taken as reference

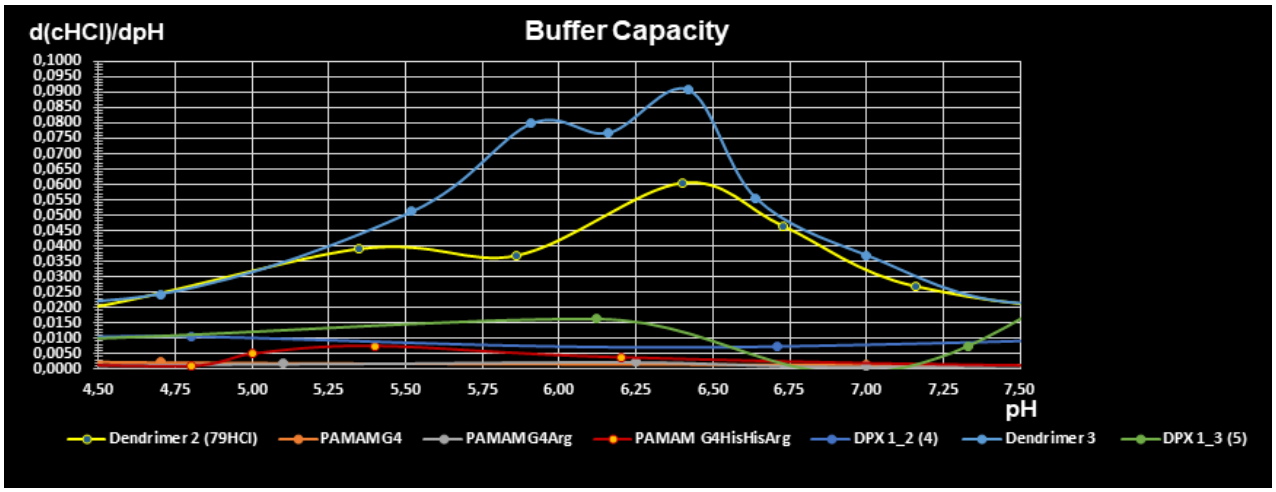


Figure S15. Buffer Capacity of DPXs **4** and **5**, parent dendrimers **2** and **3** and of three G4-PAMAMs taken as reference

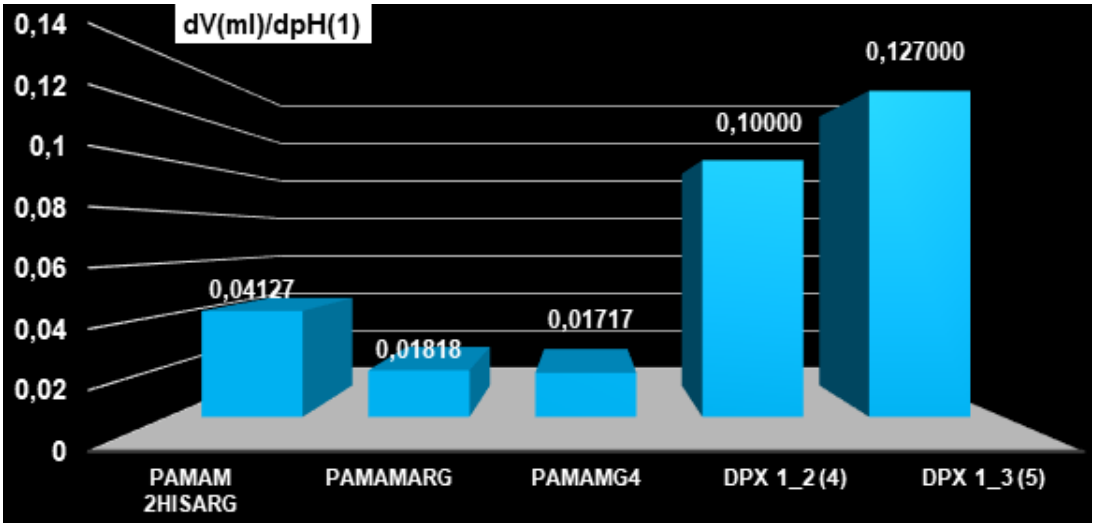


Figure S16. Average Buffer Capacity* of DPXs **4** and **5** and of three G4-PAMAMs taken as reference (pH range = 4.5-7.5)

*calculated for three degrees of freedom

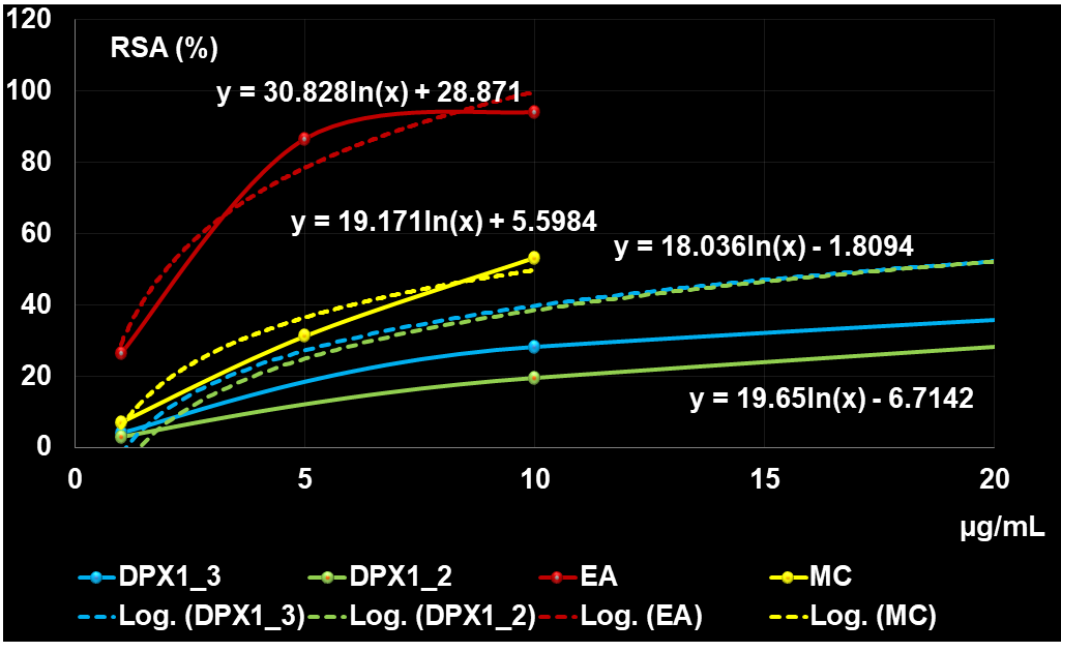


Figure S17. RSA (%) curves recorded at different EA, EAMS (MC) and DPXs concentrations in methanol or water solution with the corresponding exponential tendency curves and related equations used to derive the IC_{50} and IC_{90} values are available.

Table S2

Comparison between main properties of achieved EA-loaded formulations and available literature data about EA formulation previously achieved.

EA Formulation	DL‡ (%w/w)	Solubility mg/mL	In vitro antioxidant activity (RSA%) IC_{50} (µg/mL) IC_{90} (µg/mL)	Mean particle size	Average Buffer capacity
EAMSs	22	0.3 (Water)	10 81	Max 20 µm¶	Not evaluated
DPX **4**	46	9 (water)	18 134	62.6±2.0 nm[a]	0.100
DPX **5**	53	3.2 (water) 15 (ethanol)	18 164	69.2±0.9 nm[a]	0.127
EA/PLGA[1]	52-62§	Not evaluated	Not evaluated	125-293 nm§,[a]	Not evaluated
EA/PCL[1]	47-57§	Not evaluated	Not evaluated	128-281 nm§,[a]	Not evaluated
EA/PLGA[2]	42-67§	Not evaluated	Not evaluated	149-618 nm§,[a]	Not evaluated
EA/PL†,[3]	96	0.029 (water) 0.988 (*n*-octanol)	Not evaluated	Not evaluated	Not evaluated
EA/liposome[4]		Not evaluated	Not evaluated	387 nm§	Not evaluated
EA/SLNs[5]	89	Not evaluated	Not evaluated	96 nm§	Not evaluated
EA/β-CD[b, 6]		0.039 (water)	Not evaluated	10-100 µm[c]	Not evaluated

†PL=phospholipids; ‡DL = Drug Loading; §a range was given because values differ in function of stabilizer used for preparing the EA-loaded nanoparticles; ¶determined by Electronic Microscopy Analysis; [a]determined by dynamic light scattering using zetasizer Nano ZS (Malvern Instruments, UK); [b]Cyclodestrins; by SEM analysis.

[1] K. Sonaje, J. L. Italia, G. Sharma, V. Bhardwaj, K. Tikoo, M. N. Kumar, *Pharm. Res.* **2007**, *24*, 899-908.

[2] I. Bala et al., *Nanotechnol.* **2005**, *16*, 2819.

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