Electronic Supplementary Information

Polymer brush decorated nanoparticles immobilised on polymer monoliths for enhanced biopolymer elution

Marcello Iacono,[a] Damian Connolly,[b] Andreas Heise[a,c*]

[a]School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland. [b]Pharmaceutical and Molecular Biotechnology Research Centre (PMBRC), Department of Science, Waterford Institute of Technology, Waterford, Ireland. [c]Royal College of Surgeons in Ireland, Department of Pharmaceutical and Medicinal Chemistry, 123 St. Stephens Green, Dublin 2, Ireland; andreasheise@rcsi.ie; tel.: 00353 402 5198.

1 Experimental Section

Methods

A Perkin-Elmer Spectrum 100 was used for collecting attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra in the spectral region of 650–4000 cm^{-1}. The spectra were obtained from 2064 scans with a resolution of 2 cm^{-1}. A background measurement was taken before the sample was loaded onto the ATR unit for measurements. Spectrometric studies were carried out using a Perkin-Elmer Lambda 900 spectrometer (Foss, Ireland), Euthech Instruments PH510 pH meter, Hitachi S3400n SEM microscope, World Precision Instruments (WPI) SP120PZ syringe pump for laboratory use. The dynamic light scattering (DLS) experiments were performed at 25° C on a ZetasizerNano ZS particle analyzer (Malvern Instruments) using a detection angle of 173 degree and a 4 mW He–Ne laser operating at a wavelength of 633 nm.

Materials

All chemicals were purchased from Sigma-Aldrich and used without further purification unless noted otherwise. GMA-*co*-EDMA monoliths in polypropylene housings and poly(methacrylic acid) decorated SiNP were prepared as previously reported by us.[12]

Preparation of the tetra-methyl-ammonium (TMA) modified EDMA-co-GMA monolith

The monolith was modified using a static and a dynamic approach.

In the static approach a 15 mm monolith was washed with 5 mL deionized DI water using a syringe pump (flow rate: 2500 µL/hour). A solution of trimethylamine hydrochloride TMA HCl (0.5 g, 5.23 mmol) in 5 mL iPrOH/water (20/1 wt/wt) was pumped through the monolith at a flow rate of 2000 µL/hour and the eluates discarded. The formed reactant soacked monolith was then sealed with teflon tape and left standing in a beaker in an oven set to 100 °C for 24 hours. Afterwards the monolith was washed with 15 mL DI water at a flow rate of 2000 µL/hour. The last mL of eluate was tested to ensure a neutral pH. The monolith was left in a vacuum desiccator for 3 days and afterwards stored in a sealed N_2 purged vial at room temperature.

The dynamic approach was performed by flushing 15 mL of the reactant solution for 24 h through the monolith buried in a sand bath set to 100°C at a flow rate of 500 µL/hour followed by the same washing steps as in the static approach.

Both procedures produced the same results.

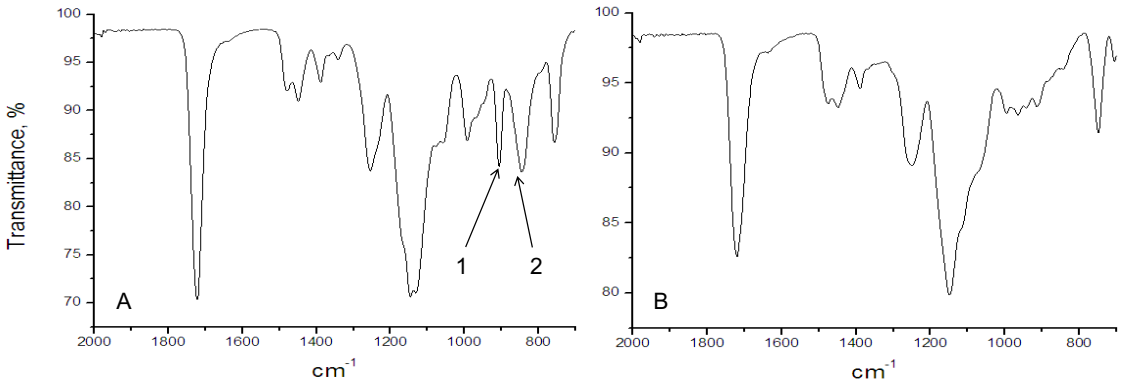

Figure S1: ATR-IR spectra of EDMA-co-GMA monolith before (A) and after (B) treatment with trimethylamine hydrochloride (TMA HCl) by the dynamic approach. Peak 1 (900 cm^{-1}) and 2 (850 cm^{-1}) are diagnostic for the epoxy ring.

PMA-SiNP decorated monolith

100 mL of a milky suspension of 0.5 mg/mL PMA-SiNP were flushed through a PBS buffer (0.1 M) pre-wetted 15 mm long TMA modified EDMA-co-GMA monolith using a syringe pump and a 1 mL syringe (bigger syringes generated high back pressure and consequently piston blocking). Initially, 5 mL of this suspension were flushed at 1000 µL/h, then 70 mL of the same suspension were flushed at 2000 µL/h. Each 5 mL the flux direction was reversed in order to obtain a uniform particle coverage. For the first 60 mL, the eluted is a clear solition, then the eluted becomes progressively cloudier. In this phase further 25 mL were flushed until the ingoing and outgoing solution had the same cloudiness. The monolith was washed with 15 mL DI water and dried for 5 days at room temperature (0.010 mBar) until a constant mass was obtained.

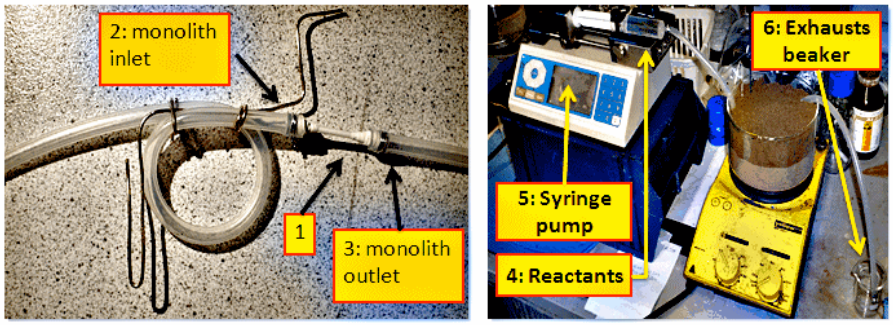

Figure S2: Experimental setup of the monolith surface modification by dynamic approach. The tubing connected cartridge (1) is placed on a dry sand bed on a heating plate and connected to a syringe pump (5). The upper sand layer (browner upper layer in the crystallizator) is wet (with water

or oil depending on desired temperatures/times) in order to assure stable and homogeneous heat transfer to the monolith positioned at the interfate of the dry/wet sand system. The monolith (1) ends are fixed to two PP tubing. The tubing loop at the monolith inlet assures more efficient pre-heating before entering the monolith. At the end of each treatment, the surface modified monoliths are gently flushed with 15 mL of acetone at 1000 μL/h, methanol and water and then stored at RT.

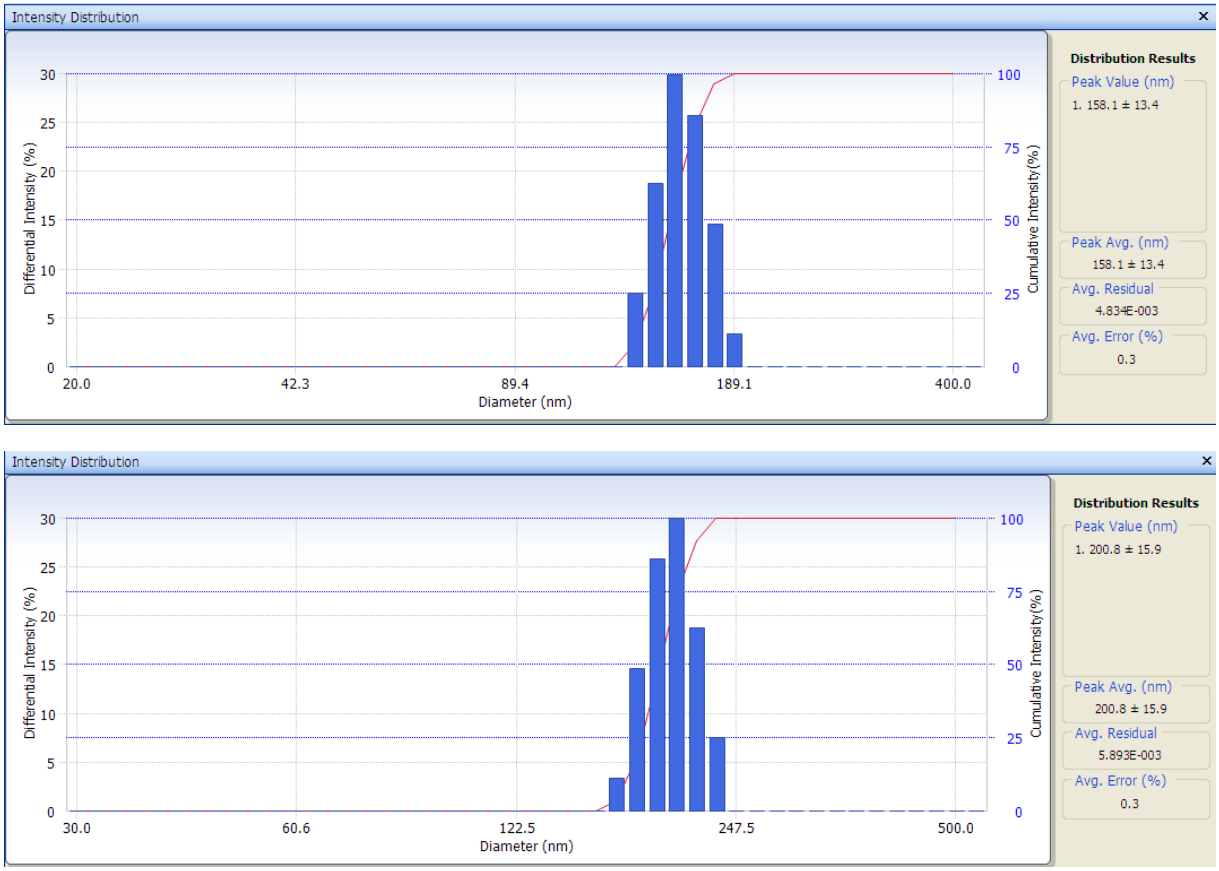

Figure S3: DLS Intensitity Distribution of SiNP (top) and PMA-SiNPs (bottom). Concentration 0.5 % wt/wt. Dispersant: 0.1 M PBS buffer at pH=7.4.

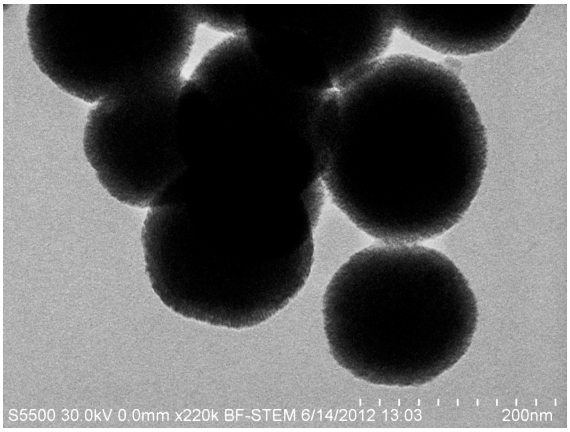

Figure S4: STEM image of PMA-SiNP.

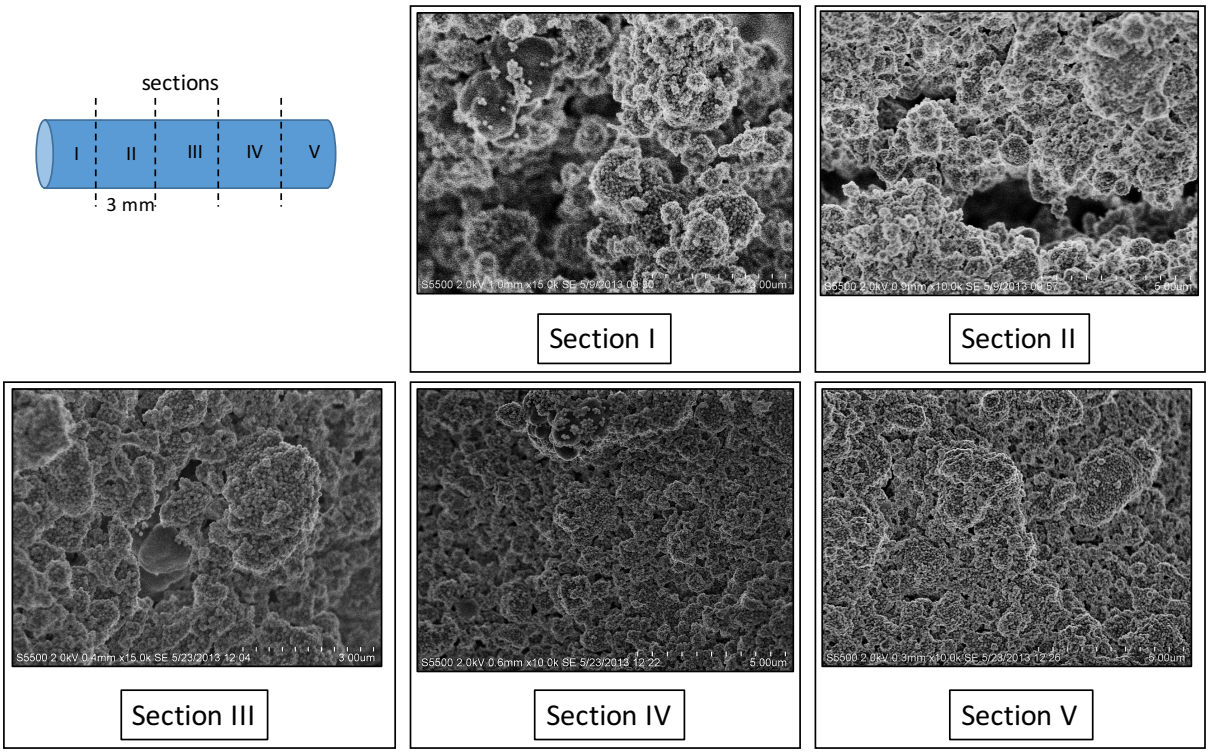

Figure S5: SEM images of deferent sections of the 15 mm monolith after PMA-SiNP modification.

Preparation of the carboxylated GMA-*co*-EDMA monolith

The monolith was modified using a two-step approach.

Step 1: In the first step the GMA-*co*-EDMA monolith was modified with azido groups using both a static and a dynamic approach. In the static approach a 15 mm GMA-*co*-EDMA monolith was connected to a syringe pump and flushed with 5 mL water at 2500 µL/hour followed by a 5 mL of a 10:1 ethanol/water solution containing 2.1 mmol sodium azide and 0.48 mmol ammonium chloride at 2000 µL/hour. The monolith was then sealed and incubated in an oil bath at 110 °C for 72 hours. The monolith was then rinsed with 15 mL water (2500 µL/hour) and stored under nitrogen until further use. In the dynamic approach the same sodium azide/ammonium chloride solution was flushed through the monolith at 2000 µL/hour using a sand bath set to 110 °C. Both approaches produced the same result.

Step 2: The azido-modified monoliths were carboxylated using both a "static" approach and a "dynamic" approach. A reaction solution of 4-pentynoic acid (0.174 g, 1.77 10^{-3} mol), CuI (61 mg, 3.20 10^{-4} mol) and PMDETA (1 mL, 0.83 g, 4.79 10^{-3} mol) in 5 mL DMF was stirred under N_2 for 15 min. Under an N_2 atmosphere (using an AtmosBag), the azido-modified monolith was flushed with N_2 - purged distilled water (15 mL) followed by the N_2 - purged reaction solution each at 500 µL/hour. The monolith was sealed and incubated at 85 °C for 72 hours followed by rinsing with 3 mL of 7 M NH_3 in MeOH (2000 µL/hour). The monolith was finally flushed with 15 mL water, 15 mL 0.1 M HCl and 15 mL water each at 2000 µL/hour prior to storage (sealed) at room temperature. In the dynamic approach the the N_2-purged reaction solution was flushed through the water-washed monolith at 500 µL/hour using a sand bath set to 85 °C. Both approaches produced the same result.

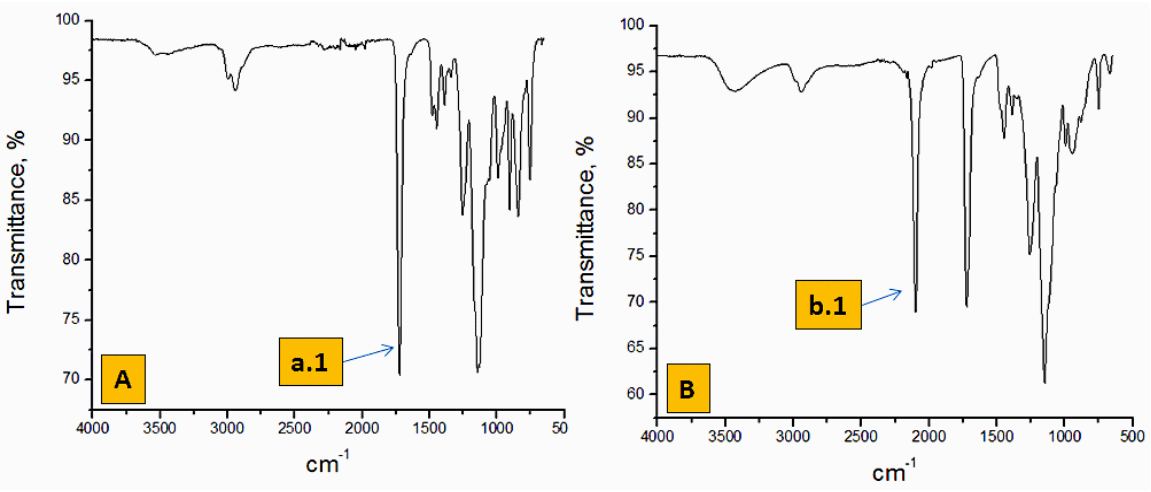

Figure S6: ATR-IR spectra of (A) bare EDMA-co-GMA monolith (carbonyl signal at 1730 cm^{-1}; peak a.1) and (B) after reaction with sodium azide, (azido signal at 2100 cm^{-1}; peak b.1).

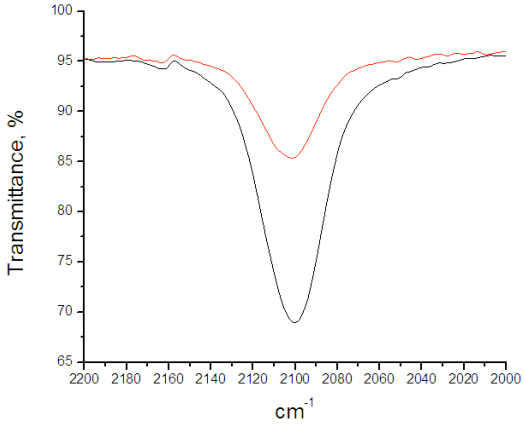

Figure S7: Black line: ATR-IR peak at 2100 cm-1 (azido signal) of the azido treated EDMA-co-GMA monolith. Red line: the ATR-IR peak at 2100 cm-1 (azido signal) of the EDMA-co-GMA monolith after click reaction for three days.

2 Solid phase extraction

Monolith conditioning: 5 mL of 0.1 M of phosphate buffer saline (PBS) solution at pH=7.4 was flushed trough the monolith at a flow rate of 1000 µL/hour.

Sample flushing: A solution of the analyte (C_0(M) = C_0(BD)= 2 mg/mL) in 0.1 M PBS at pH=7.4 (2 mg/mL) was flushed through the monolith at a flow rate of 1000 µL/hour.

Sample elution: A aqueous solution of HCl (pH=5.4) was flushed through the monolith at a flow rate of 1000 µL/hour. 50 samples of 0.5 mL were collected and the concentration of the analytes analysed by UV spectroscopy (λ_{MAX}= 409 nm for Myoglobin and λ_{MAX}= 619 nm for Blue Dextran) using a calibration curve. The 0.1 M of phosphate buffer saline (PBS) solution was shown not to interfere with analyte monitoring.

Data evaluation

Each UV derived value was normalized by dividing the respective absorbance of the Myoglobin and the Blue Dextran standard solutions at the relevant λ_{MAX}. ***Table S1*** shows the normalized experimental data of the four tests. The experimental data were then fitted in Origin 8.5 with a Boltzmann curve (***Table S2***) used for regression parameters A_1, A_2, x_0 and dx (***Table S3***). The reduced R^2 close to 1 and the close to zero chi-square values confirm the quality of the fit under the variance null hypothesis (data ruled by Gaussian distribution).

Table S1: Normalized UV data for the 4 sets of samples. S/M: Myoglobin on carboxylic acid surface functional monolith; S/BD: Blue Dextrane on carboxylic acid surface functional monolith; NP/M: Myoglobin on nanoparticle monolith; NP/BD: Blue Dextrane on nanoparticle monolith. (S)l_{MAX}=409 nm for Myoglobin; l_{MAX}=619 nm for Blue Dextran; C_0(M) = C_0(BD)= 2 mg/mL. Myoglobin UV sample dilution factor: 1/20 V/V. Blue Dextran UV sample dilution factor: 1/2 V/V. Dilutions, monoliths conditioning and UV blank are in 0.1 M Phosphate Buffer Saline solution, pH=7.40.

Sample no.	mL	S/M	NP/M	S/BD	NP/BD	Sample no.	mL	S/M	NP/M	S/BD	NP/BD
1	0.5	0.092	0.022	0.041	0.001	**26**	13.0	1.000	1.000	1.000	0.999
2	1.0	0.049	0.094	0.061	0.001	**27**	13.5	1.000	1.000	1.000	1.000
3	1.5	0.302	0.060	0.051	0.003	**28**	14.0	1.000	1.000	1.000	1.000
4	2.0	0.679	0.050	0.004	0.003	**29**	14.5	1.000	1.000	1.000	1.000
5	2.5	0.987	0.095	0.343	0.005	**30**	15.0	1.000	1.000	1.000	1.000
6	3.0	0.997	0.011	0.605	0.006	**31**	15.5	1.000	1.000	1.000	1.000
7	3.5	0.998	0.043	0.954	0.005	**32**	16.0	1.000	1.000	1.000	1.000
8	4.0	0.999	0.082	0.999	0.006	**33**	16.5	1.000	1.000	1.000	1.000
9	4.5	1.000	0.309	1.000	0.007	**34**	17.0	1.000	1.000	1.000	1.000
10	5.0	1.000	0.663	1.000	0.009	**35**	17.5	1.000	1.000	1.000	1.000
11	5.5	1.000	0.921	1.000	0.010	**36**	18.0	1.000	1.000	1.000	1.000
12	6.0	1.000	0.989	1.000	0.009	**37**	18.5	1.000	1.000	1.000	1.000
13	6.5	1.000	0.999	1.000	0.009	**38**	19.0	1.000	1.000	1.000	1.000
14	7.0	1.000	1.000	1.000	0.008	**39**	19.5	1.000	1.000	1.000	1.000
15	7.5	1.000	1.000	1.000	0.006	**40**	20.0	1.000	1.000	1.000	1.000
16	8.0	1.000	1.000	1.000	0.010	**41**	20.5	1.000	1.000	1.000	1.000
17	8.5	1.000	1.000	1.000	0.009	**42**	21.0	1.000	1.000	1.000	1.000
18	9.0	1.000	1.000	1.000	0.011	**43**	21.5	1.000	1.000	1.000	1.000
19	9.5	1.000	1.000	1.000	0.012	**44**	22.0	1.000	1.000	1.000	1.000
20	10.0	1.000	1.000	1.000	0.015	**45**	22.5	1.000	1.000	1.000	1.000
21	10.5	1.000	1.000	1.000	0.047	**46**	23.0	1.000	1.000	1.000	1.000
22	11.0	1.000	1.000	1.000	0.361	**47**	23.5	1.000	1.000	1.000	1.000
23	11.5	1.000	1.000	1.000	0.686	**48**	24.0	1.000	1.000	1.000	1.000
24	12.0	1.000	1.000	1.000	0.966	**49**	24.5	1.000	1.000	1.000	1.000
25	12.5	1.000	1.000	1.000	0.998	**50**	25.0	1.000	1.000	1.000	1.000

Table S2: A: The Boltzmann function and its four parameters A_1, A_2, x_0, xd (eq. 1); the re-arranged Boltzmann equation in the eq. 1, to calculate points on the x scale (to determine for example the values V_B, V_R and V_E). B: Equation for the determination of the number of theoretical plates N. C: The determination of the retention factor k; for the determination of the void volume of the SPE monoliths, see the experimental part.[3] D: Equation for the determination of the retention factor. In this study, the total monolith volume V_0 is in each test equal to the V_E value of each process being the residual volume (25 –V_E mL) not considered to determine the recovery factor.

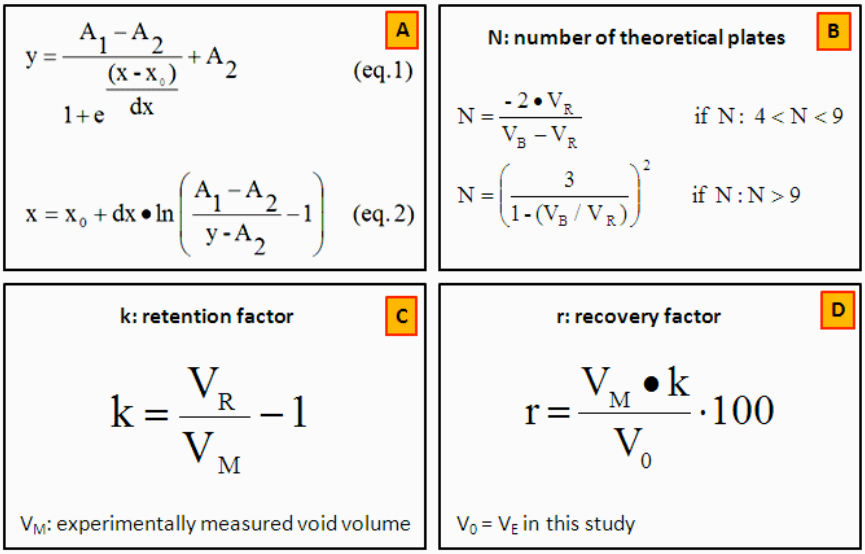

Table S3: Regression parameters A_1, A_2, x_0 and dx for the SPE 1, 2, 3 and 4 SPE tests. From the regression curves it is possible to calculate the values of V_B, V_R and V_E , in correspondence of y= y=0.159, 0.500 and 0.841 respectively (see **Table S2**).

Test		Value	Standard Error	Reduced Chi-Square	Adjusted R-Square
S/M	A1	0.00806	0.01478	1.55E-04	0.9964
	A2	1.00092	0.00187		
	x_0	1.73946	0.01974		
	dx	0.34482	0.01545		
NP/M	A1	0.03104	0.00656	3.06E-04	0.99768
	A2	1.00048	0.00280		
	x_0	4.75145	0.01709		
	dx	0.26061	0.01427		
S/BD	A1	0.03621	0.01133	3.51E-04	0.99524
	A2	1.00058	0.00287		
	x_0	2.73857	0.02176		
	dx	0.30786	0.01841		
NP/BD	A1	0.00581	0.00176	6.08E-05	0.99974
	A2	1.00109	0.00154		
	x_0	11.22856	0.00800		
	dx	0.31920	0.00693		

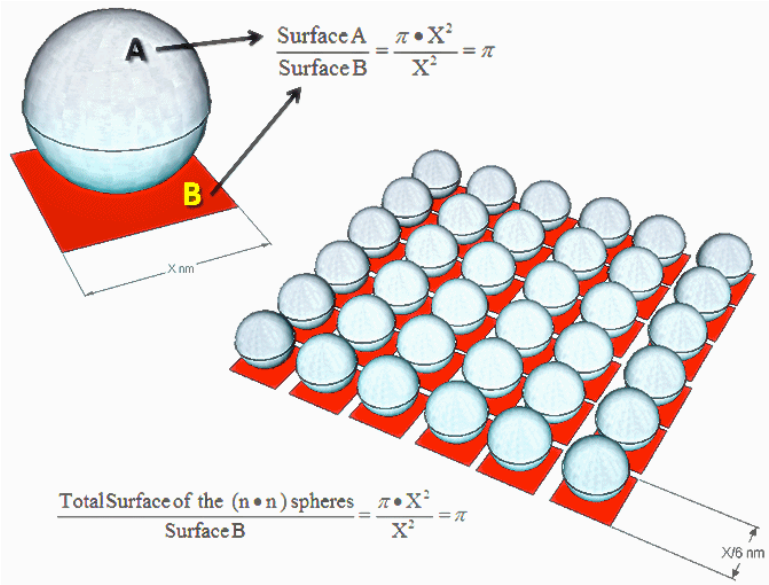

Figure S8: A sphere A of radius X/2 nm has a surface of πX^2 nm^2 resulting in a surface π times bigger than the square surface B of X^2 nm^2. This ratio B/A=π remains unmodified if the square surface B is covered with a (n x n) square collection of spheres of the same radius 1/2n. For the example of 6X6 spheres, the sum of these 36 spheres will have a surface π times bigger of the square surface B of X^2 nm^2).

[1] Iacono, M; Heise, A. Polymers 2015, 7, 1427.
[2] Iacono, M.; Connolly, D.; Heise, A. *Materials* **2016**, 9, 263.
[3] Malik, A.; Jinno, K. *Chromatographia* **1990**, *30*, 135.