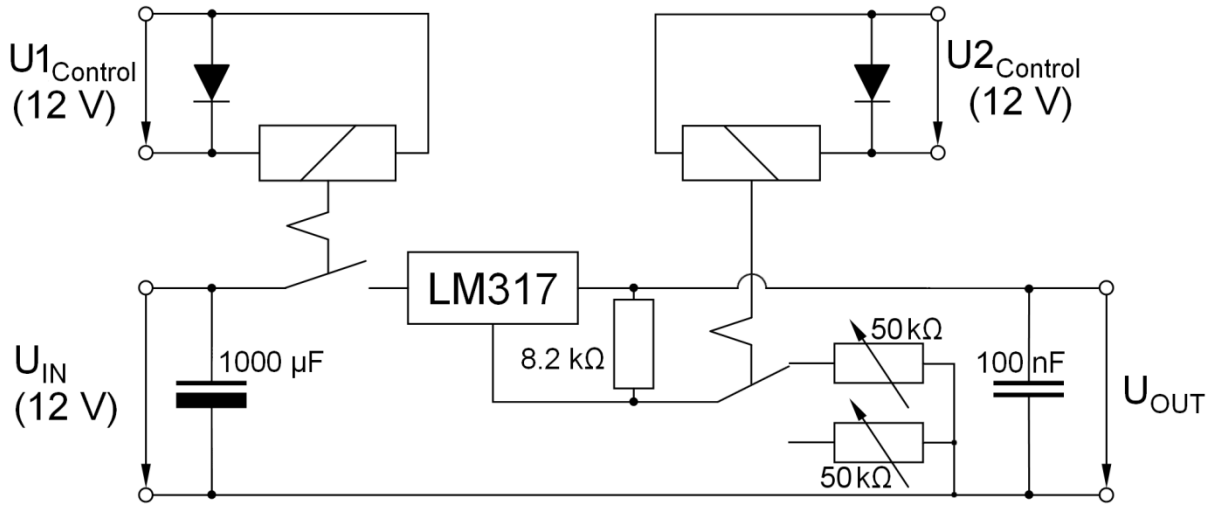
Electronic Supplementary Material (ESI) for Analytical Methods.



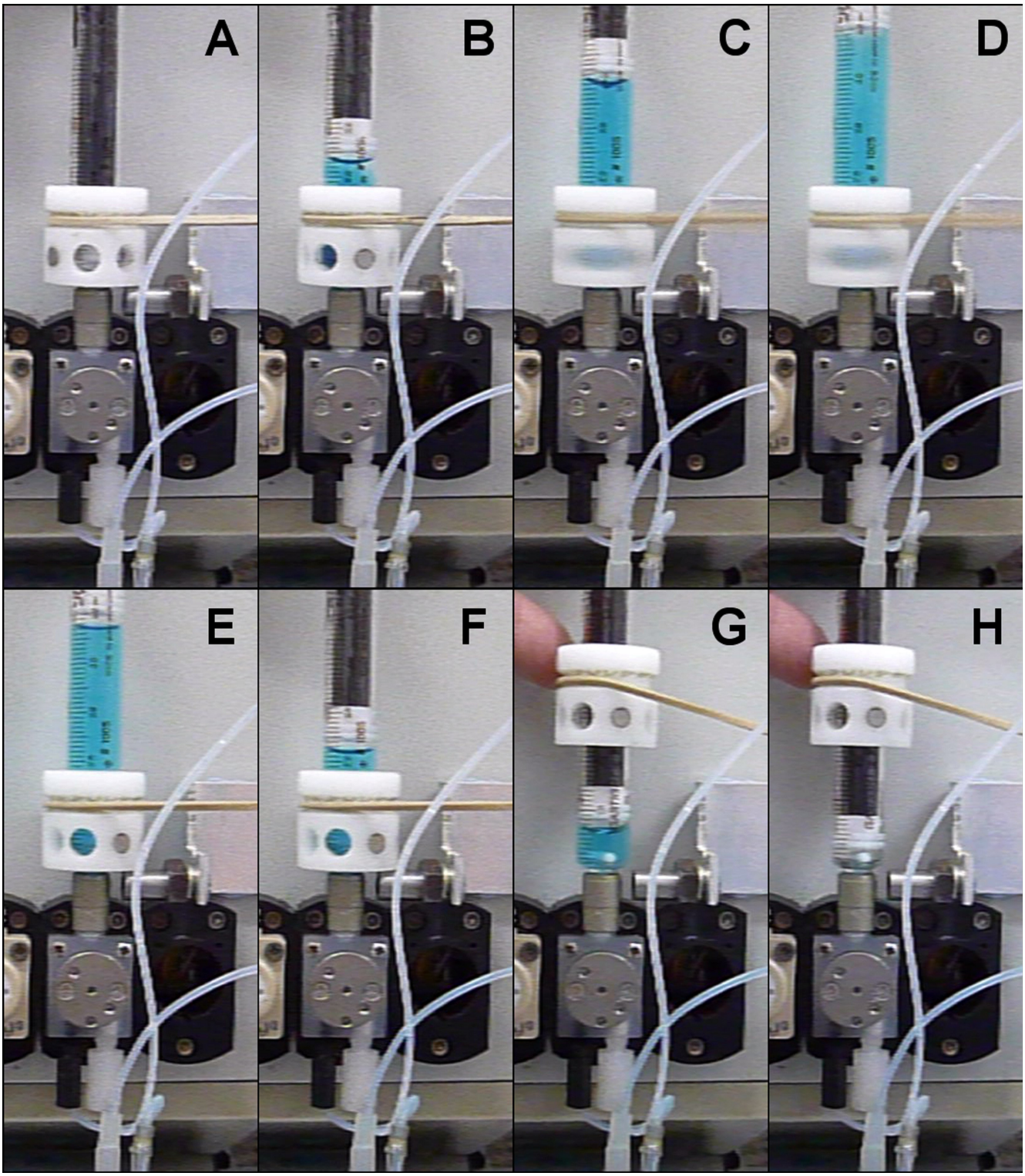
Supplementary material 1: Control circuit for the DC motor used for in-syringe stirring. By using two different auxiliary analog outputs on the multisyringe device, stirring enabling and selection of two different stirring velocities were achieved.

Supplementary material 2: Procedure 1 for automated in-syringe stirring-assisted DLLME of cationic surfactants without organic phase washing (simple extraction).

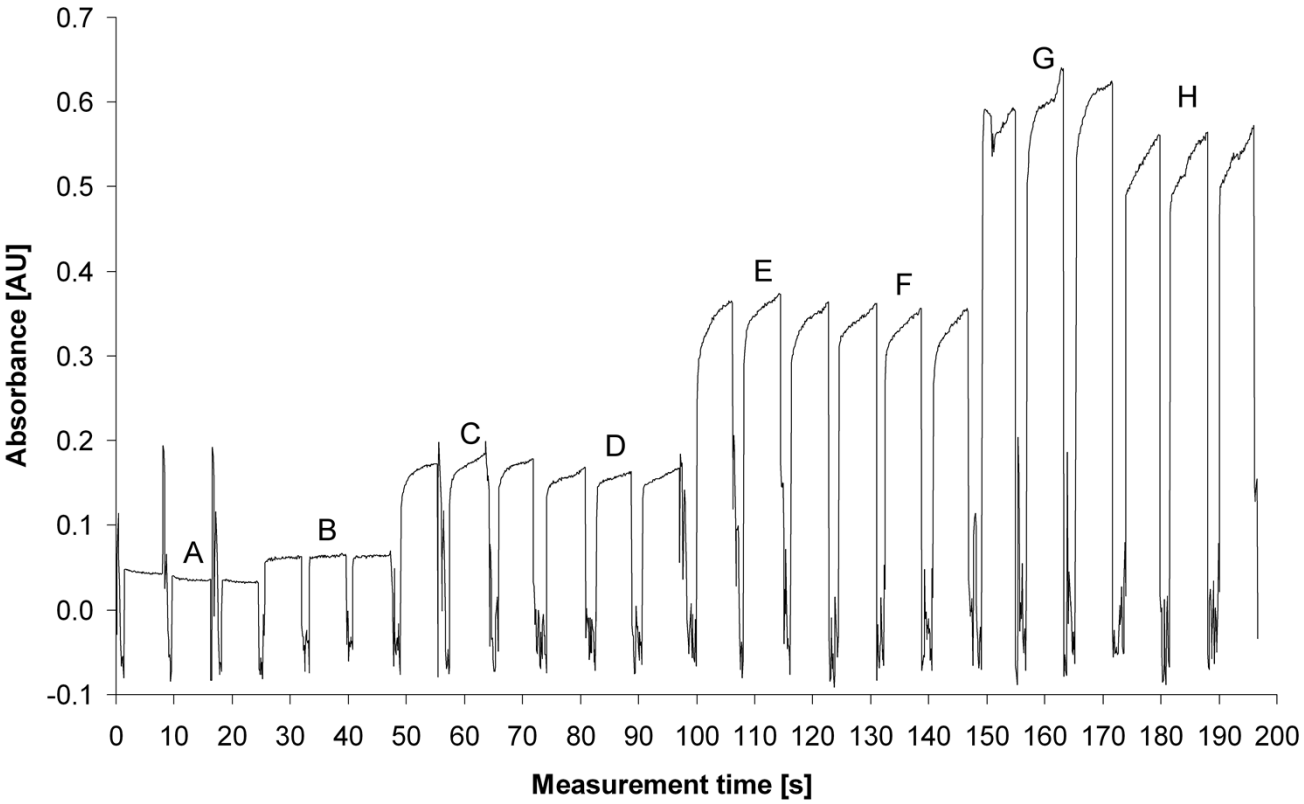
No	Instrument	Instruction	Comment
1a	SV	Move to position 4	Clean syringe with sample with stirring at high speed, 3x repetition
1b	MS	Pickup 0.60 ml at 7.5 mL/min [V in ON, U1 ON, U2 OFF], Wait 2 s	
1c	MS	Empty at 15 mL/min [V in OFF, U1 OFF, U2 OFF]	
2a	SV	Move to position 5	Aspiration of buffer
2b	MS	Pickup 0.25 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 2 s	
3a	SV	Move to position 6	Aspiration of dye
3b	MS	Pickup 0.15 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 2 s	
4a	SV	Move to position 4	Aspiration of sample with stirring at low speed
4b	MS	Pickup 4.00 ml at 7.5 mL/min [V in ON, U1 ON, U2 ON], Wait 2 s	
5	MS	No flow step [V in OFF, U1 OFF, U2 OFF]	Stop stirring
6a	SV	Move to position 7	Aspiration of organic phase
6b	MS	Pickup 0.22 ml at 2.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
7a	SV	Move to position 2	Aspiration of air with stirring at low speed
7b	MS	Pickup 0.22 ml at 2.5 mL/min [V in ON, U1 ON, U2 ON]	
8a	MS	No flow step [V in OFF, U1 ON, U2 OFF]	Stirring at high speed for DLLME
8b	Wait	Wait 35 s	
9a	MS	No flow step [V in OFF, U1 ON, U2 ON]	Decreasing to stirring at low speed
9b	Wait	Wait 5 seconds	
10a	MS	No flow step [V in OFF, U1 OFF, U2 OFF]	Stop stirring and phase separation
10b	Wait	Wait 35 seconds	
11a	D	Measurement at 638 nm against 550 nm	Dispense of organic phase to detection cell and measurement
11b	MS	Dispense 0.75 ml at 1.5 mL/min [V in OFF, U1 OFF, U2 OFF]	
11c	D	Stop measure	
12	MS	Priming in dispense at 15 mL/min [V in OFF, U1 OFF, U2 OFF]	Empty syringe to waste at high flow rate

Supplementary material 3: Procedures 2 for automated in-syringe stirring-assisted DLLME of cationic surfactants with double organic phase washing.

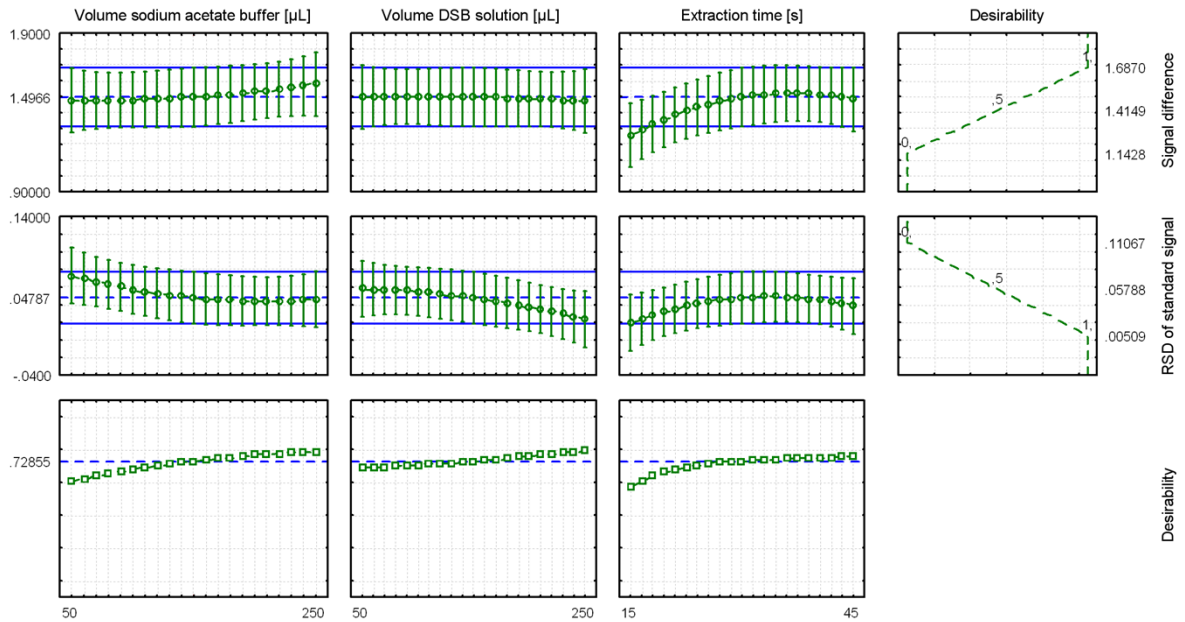
No	Instrument	Instruction	Comment
1a	SV	Move to position 4	Clean syringe with sample with stirring at high speed, 3x repetition
1b	MS	Pickup 0.60 ml at 7.5 mL/min [V in ON, U1 ON, U2 OFF], Wait 2 s	
1c	MS	Empty at 15 mL/min [V in OFF, U1 OFF, U2 OFF]	
2a	SV	Move to position 5	Aspiration of buffer
2b	MS	Pickup 0.25 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 2 s	
3a	SV	Move to position 6	Aspiration of dye
3b	MS	Pickup 0.15 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 2 s	
4a	SV	Move to position 4	Aspiration of sample with stirring at low speed
4b	MS	Pickup 4.00 ml at 7.5 mL/min [V in ON, U1 ON, U2 ON], Wait 2 s	
5	MS	No flow step [V in OFF, U1 OFF, U2 OFF]	Stop stirring
6a	SV	Move to position 7	Aspiration of organic phase
6b	MS	Pickup 0.26 ml at 2.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
7a	SV	Move to position 2	Aspiration of air with stirring at low speed
7b	MS	Pickup 0.22 ml at 2.5 mL/min [V in ON, U1 ON, U2 ON]	
8a	MS	No flow step [V in OFF, U1 ON, U2 OFF]	Stirring at high speed for DLLME
8b	Wait	Wait 35 s	
9a	MS	No flow step [V in OFF, U1 ON, U2 ON]	Decreasing to stirring at low speed
9b	Wait	Wait 5 seconds	
10a	MS	No flow step [V in OFF, U1 OFF, U2 OFF]	Stop stirring and phase separation
10b	Wait	Wait 35 seconds	
11a	SV	Move to position 2.	Dispense organic phase into holding coil
11b	MS	Dispense 0.28 ml at 2.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
12	MS	Priming in dispense at 15 mL/min [V in OFF, U1 OFF, U2 OFF], Wait 1 s	Dispense rest content of syringe to waste
13a	SV	Move to position 3.	Aspiration of water
13b	MS	Pickup 2.00 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
14a	SV	Move to position 2	Aspiration of air with stirring at low velocity
14b	MS	Pickup 0.22 ml at 7.5 mL/min [V in ON, U1 ON, U2 ON]	
15	Wait	Wait 20 s	Stirring at low speed for extract washing with water
16a	MS	No flow step [V in OFF, U1 OFF, U2 OFF]	Stop stirring and phase separation
16b	Wait	Wait 20 seconds	
17a	SV	Move to position 2.	Dispense solvent into HC
17b	MS	Dispense 0.28 ml at 2.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
18a	MS	Priming in dispense at 15 mL/min [V in OFF, U1 OFF, U2 OFF], Wait 1 s	Dispense rest content of syringe to waste
18b	SV	Move to position 3.	Aspiration of water
18c	MS	Pickup 2.00 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
19a	SV	Move to position 5.	Aspiration of barium acetate
19b	MS	Pickup 0.15 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1	
20a	SV	Move to position 6.	Aspiration of dye
20b	MS	Pickup 0.20 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF], Wait 1 s	
21a	SV	Move to position 2	Aspiration of air with stirring at low speed
21b	MS	Pickup 0.22 ml at 7.5 mL/min [V in ON, U1 OFF, U2 OFF]	
22a	MS	No flow step [V in OFF, U1 ON, U2 OFF]	Stirring at high speed for DLLME
22b	Wait	Wait 35 s	
22c	MS	No flow step [V in OFF, U1 ON, U2 ON]	Decreasing to stirring at low speed
23a	MS	No flow step [V in OFF, U1 OFF, U2 OFF]	Stop stirring and phase separation
23b	Wait	Wait 35 seconds	
24a	D	Measurement at 638 nm against 550 nm	Dispense of organic phase to detection cell and measurement
24b	MS	Dispense 0.75 ml at 1.5 mL/min [V in OFF, U1 OFF, U2 OFF]	
24c	D	Stop measure	
25	MS	Priming in dispense at 15 mL/min [V in OFF, U1 OFF, U2 OFF]	Empty syringe to waste at high flow rate



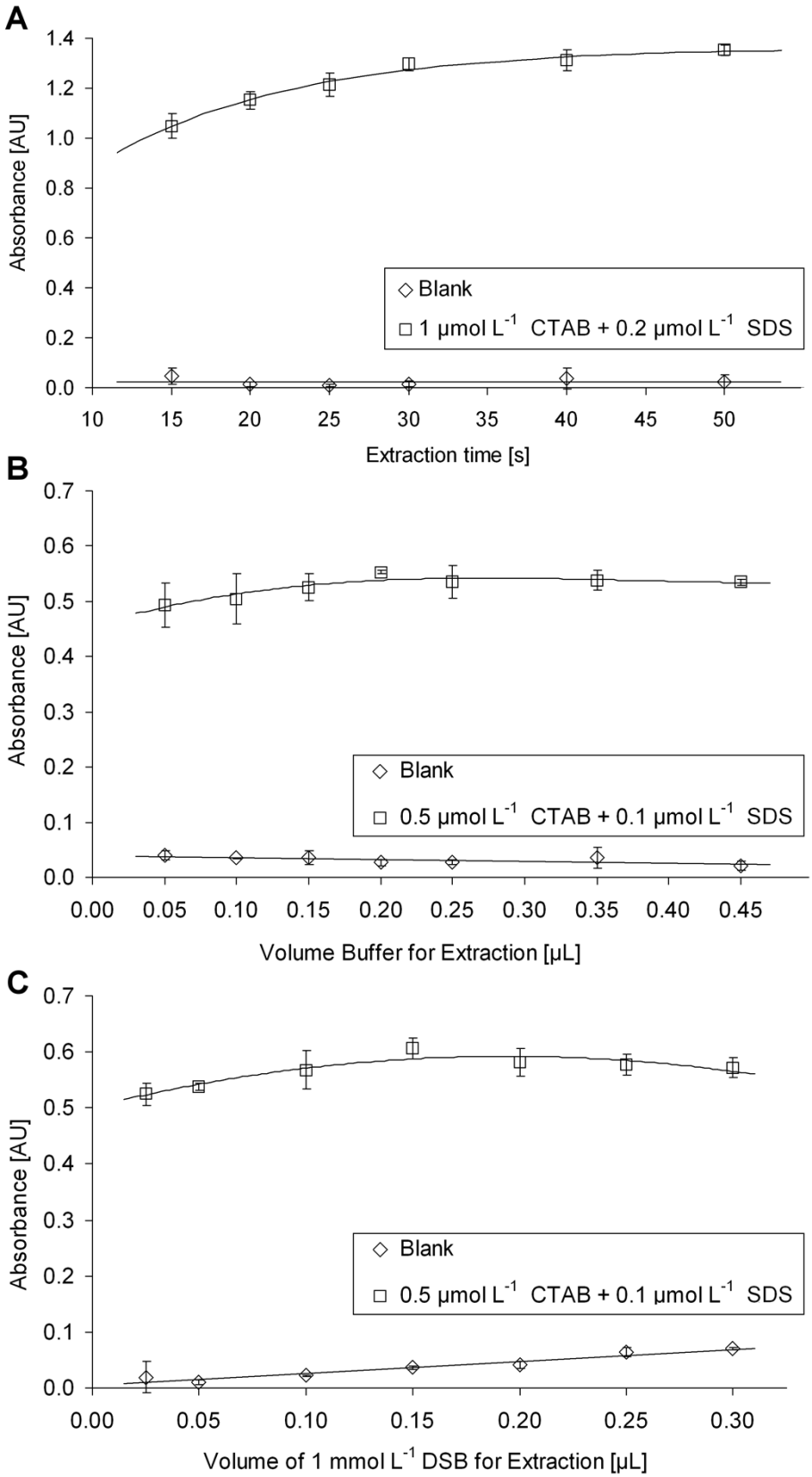
Supplementary material 4: Photo documentation of operation scheme of the simple extraction procedure 2. a: aspiration of dye, b: aspiration of buffer and sample, c: mixing of solutions at low stirring speed, d: DLLME at high stirring speed, e: phase separation, f+g: dispense of aqueous phase, h: final / initial piston position.



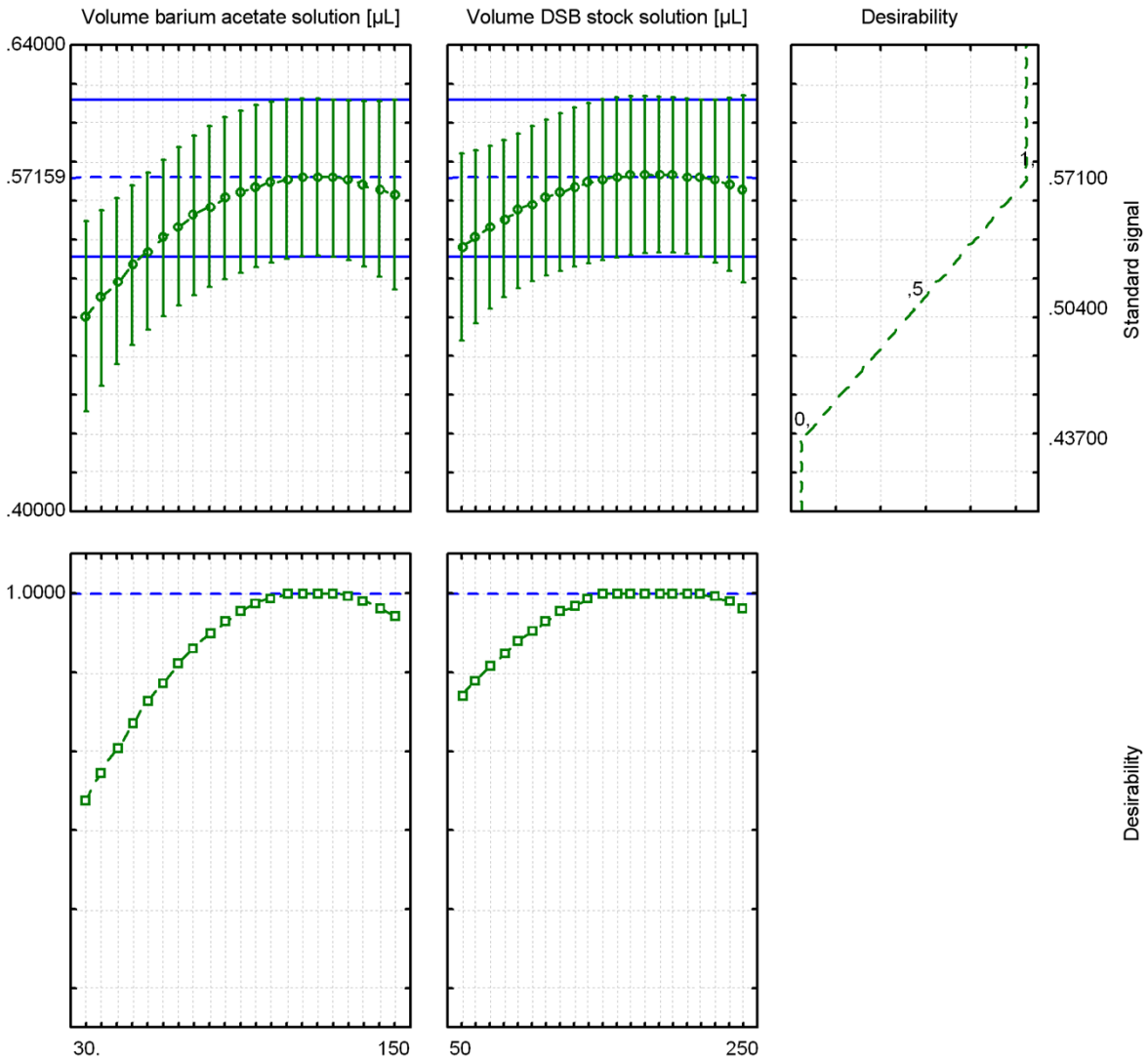
Supplementary material 5: Example of peak signals of calibration with water standards of blank, 0.25 µmol L^{-1}, 0.50 µmol L^{-1}, and 0.75 µmol L^{-1} using 3 mL of standard and 1 mL of water, performed with both procedure 1 (A, C, E, G) and 2 (B, D, F, H).



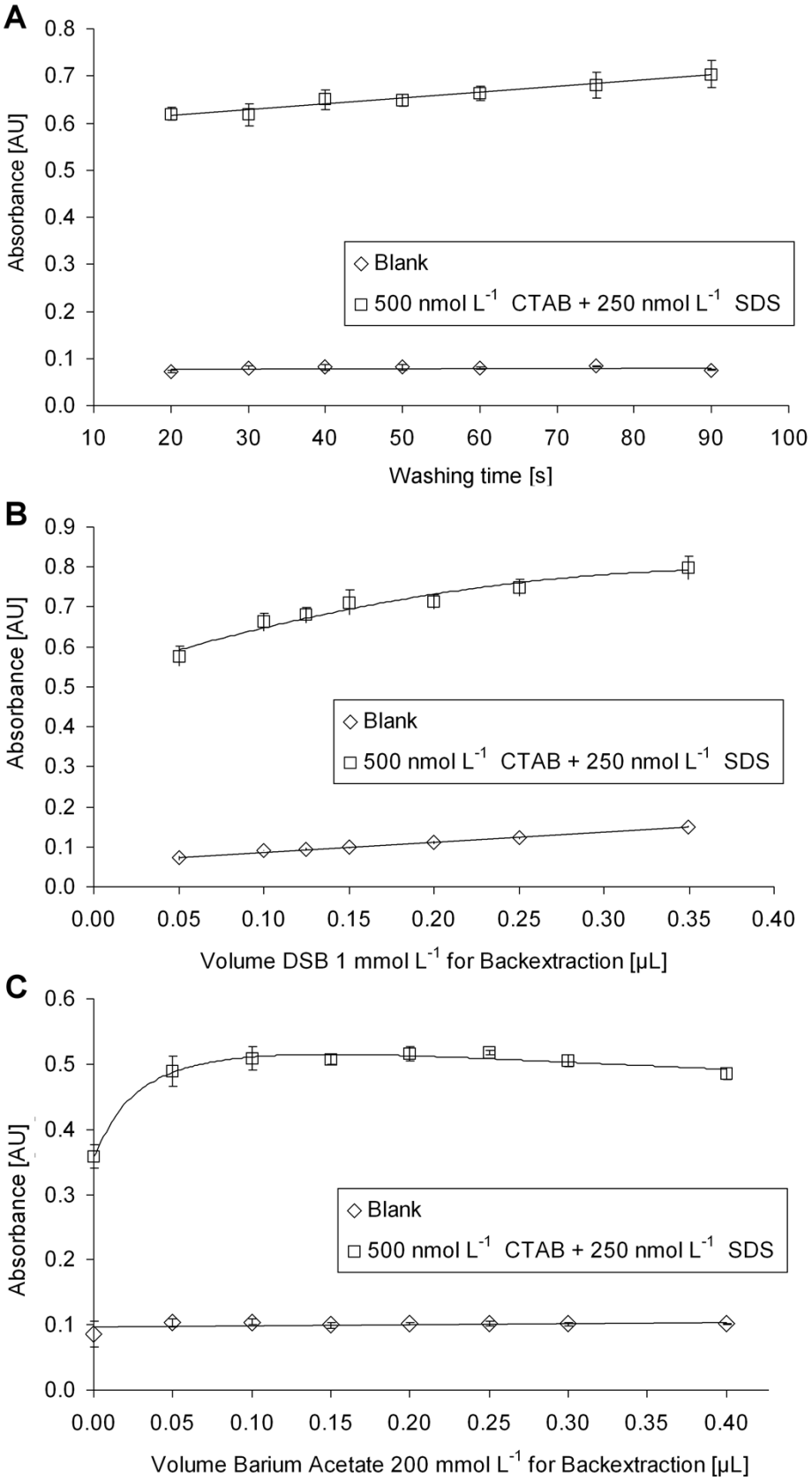
Supplementary material 6: Box-Behnken experimental design for the optimization of the volumes of buffer and DSB stock solutions and extraction time. Conditions: blank and 4.1 mL 1 µmol L^{-1} CTAB solution with the addition of 0.2 µmol L^{-1} SDS, phase separation time 35 s.



Supplementary material 7: Optimization of parameters for simple extraction being the stirring time (a), volume of acetate buffer solution (b), and the volume of DSB solution (c). Conditions (a) 200 µL 5 %v/v n-HexOH in CCl_3H, 4.1 mL STD 1 µmol L^{-1} CTAB + 0.2 µmol L-1 SDS, 250 µL acetate buffer pH 5.0, 150 µL 1 mmol L^{-1} DSB, separation time 35 s. (b) as in (a) but 250 µL organic solvent, 3.9 mL STD 0.5 µmol L^{-1} CTAB + 0.1 µmol L^{-1} SDS, extraction time 35 s (c) as in (b) but 3.7 mL STD, 250 µL of acetate buffer pH 5.0.



Supplementary material 8: Box-Behnken experimental design for the optimization of the volumes of barium acetate and DSB stock solutions for extraction solvent washing. Conditions as given in supplement material 7 C but using 150 µL of 1 mmol L-1 DSB for the first extraction and 35 s for the organic phase washing.



Supplementary material 9: Optimisation of parameters for extract washing being the stirring time (a), volume of DSB solution (b), and the volume of barium acetate solution (c). Conditions in a: 0.25 mL of 5 %v/v hexanol/chloroform, 4 mL of sample (STD 0.5 µmol L^{-1} CTAB + 0.1 µmol L^{-1} SDS), 0.25 mL of R1, 0.15 mL of R2, 35 s extraction time, 35 s separation time 1 and 2, washing with 2 mL of water, 0.075 mL of R2, and 0.2 mL of R3. b: as in (a) but applying 50 s washing time. c: as in (a) but applying 0.2 mL of 1 mmol L^{-1} DSB stock solution.