Polyacrylate Stir Bar Sorptive Extraction of 2,6-Dichlorobenzamide in Water followed by Liquid Chromatography-Tandem Mass Spectrometry

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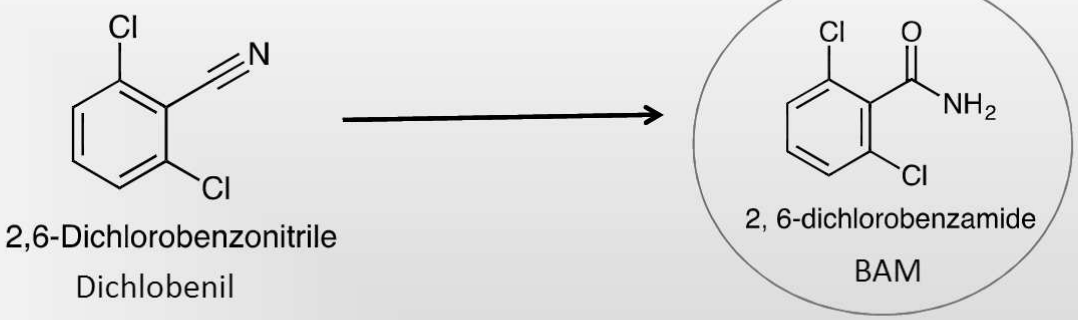
SBSE Technical Meeting, Paris, January 26- 27, 2015

- The study was carried out on the basis of a collaboration with SRA-Italia and a Water Company in North Italy
- The Water Company was involved in the environmental monitoring of sites presenting former contamination from dichlobenil, using 2,6-Dichlorobenzamide (BAM) as a tracer of this herbicide
- They required us to develop a fast and simple method for BAM

- They required us to develop a fast and simple method for BAM determination

…from soil …

… to water



2,6-Dichlorobenzonitrile

Dichlobenil

2, 6-dichlorobenzamide

BAM

- 2,6-Dichlorobenzamide (BAM), a metabolite of the herbicide dichlobenil is a prominent ground water contaminant in Europe
- Dichlobenil was banned in Europe in 2008, nevertheless because of its slow release from soil, its main metabolite BAM is still frequently detected in ground water reservoirs
- BAM is a highly polar molecule with a good water solubility

SBSE Technical Meeting, Paris, January 26- 27, 2015

Why this method?

- Stir Bar Sorptive Extraction (SBSE) is a suitable technique for pre-concentration of trace water contaminants
- Polar twisters are now available for polar compounds (low $\log K_{ow}$)
- LC-MS/MS analysis offers high sensitivity and specificity

- Analysis of BAM in water has been reported using only SPE *(Auersperger, et al. 2005; Jensen, et al. 2009)*: to propose an innovative method

Our previous experiences with SBSE : emerging pollutants

Talanta 85 (2011) 2375–2384

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid and selective determination of UV filters in seawater by liquid chromatography–tandem mass spectrometry combined with stir bar sorptive extraction

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Chromatographia (2012) 75:973–982
DOI 10.1007/s10337-012-2202-z

ORIGINAL

Stir Bar Sorptive Extraction and LC–MS/MS for Trace Analysis of UV Filters in Different Water Matrices

Emanuele Magi · Marina Di Carro · Carlo Scapolla · Kieu T. N. Nguyen

UV filters are non-polar or moderately polar molecules

Analytical Methods

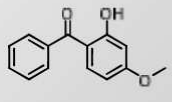
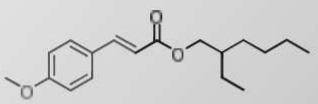
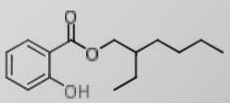
RSC Publishing

PAPER

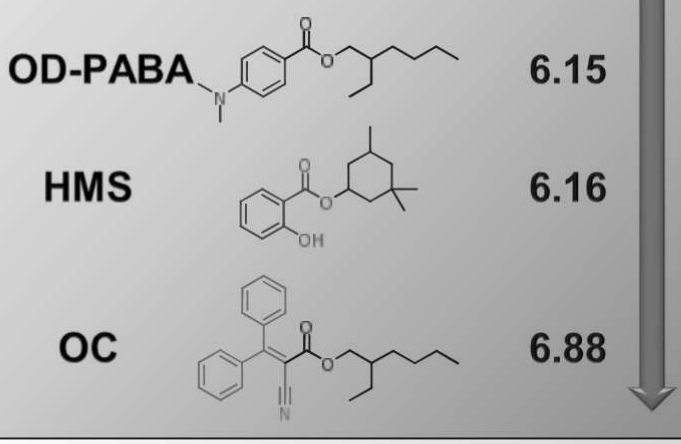
Emerging pollutants in aquatic environments: monitoring of UV filters in urban wastewater treatment plants†

Emanuele Magi,* Carlo Scapolla, Marina Di Carro, Paolo Rivaro and Khou Thi Ngoc Nguyen

SBSE & LC-MS: determination of UV filters in water

		$\log K_{ow}$
BP-3		3.39
EHMC		5.80
EHS		5.97

- R^2 calibration curves : > 0.997
- RSD% withinday : < 9%
  interday : <11%



- Analyte Recovery : close to 85%, except BP-3 (64%)
- Matrix effect: negligible
- LODs : 0.9 – 3.3 ng/L , except HMS, EHS (94, 114)

Trends in Analytical Chemistry 54 (2014) 11–23

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Review

New coatings for stir-bar sorptive extraction of polar emerging organic contaminants

Núria Gilart, Rosa Maria Marcé, Francesc Borrull *, Núria Fontanals

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

"…there has been growing interest in developing more polar in-house coatings for SBSE and, therefore, extend the applicability of this sorptive extraction technique…"

"…very recently, SBSE stir bars with polar coatings were marketed by Gerstel…"

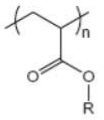
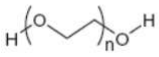
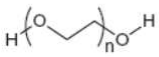
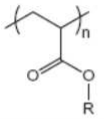
Two commercial new coatings: Acrylate and EG Silicone

- both Twisters are PDMS-based phases
- enhancement of polarity is due to hydroxyl (PEG) and ester groups (PA)

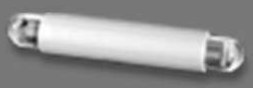
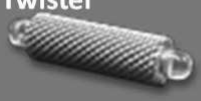
Structures and applications of commercial new coatings

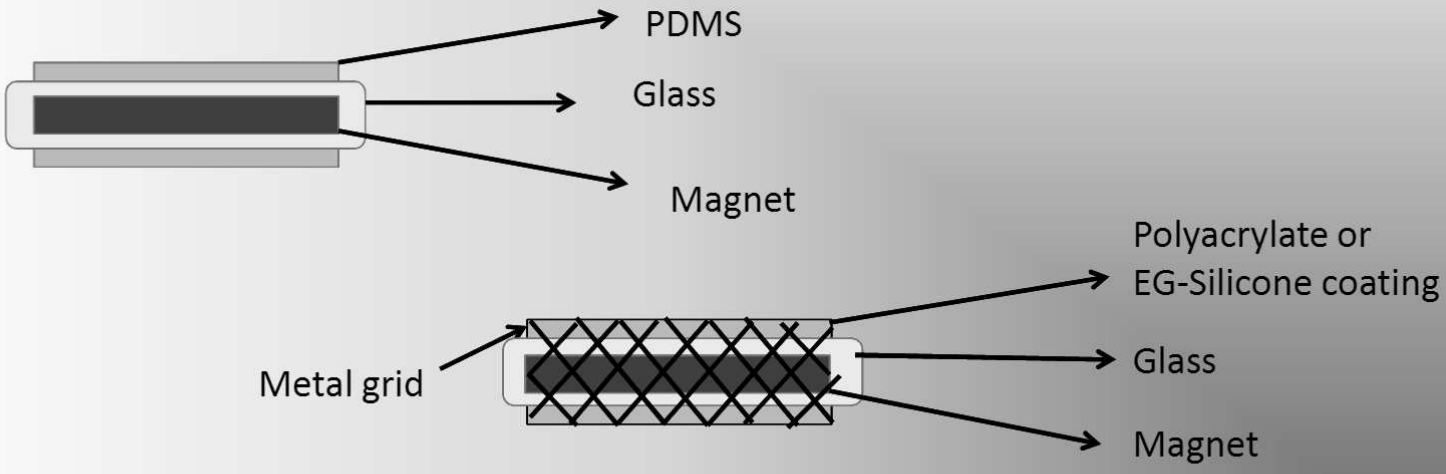
Table 1
Structures and application of novel commercially available coatings for stir-bar sorptive extraction (SBSE)

Coating Phase*	Structure	Analyte	Matrix	Sampling Mode	Desorption	Analysis	Ref.
PDMS (Twister®)	PDMS (Twister®)		Food and	Immersion/			

PA (Acrylate Twister®)		VOCs	Food and cosmetic	Immersion/ HS	TD	GC-MS	[18]
PEG (EG Silicone Twister®)							
PDMS (Twister®)	 PA and PDMS-based (Acrylate Twister®)						
PA (Acrylate Twister®)		PPCPs	Wastewater	Immersion	LD	LC-MS/MS	[19]
PEG (EG Silicone Twister®)	 PEG and PDMS-based (EG Silicone Twister®)						
PEG (EG Silicone Twister®)	 PEG and PDMS-based (EG Silicone Twister®)	Bisphenols	PCPs	Immersion	TD	GC-MS	[20]
PA (Acrylate Twister®)	 PA and PDMS-based (Acrylate Twister®)	Benzothiazole	Untreated wastewater	Immersion	TD	GC-MS	[17]

GC–MS, Gas chromatography-mass spectrometry; HS, Headspace; LD, Liquid desorption; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; PA, Polyacrylate; PCP, Personal-care product; PEG, Poly(ethylene)glycol; PPCP, Pharmaceuticals and personal-care product; TD, Thermal desorption; VOC, Volatile organic compound
*Commercial name in brackets

GERSTEL	PDMS Twister 	EG Silicone Twister 
Phase	Polydimethylsiloxane (PDMS)	PDMS / Ethylene glycol (EG) - copolymer on an inert metal grid for mechanical stabilization
Enrichment	For apolar compounds $\log(K_{ow}) > 4$	For apolar compounds and polar compounds presenting low $\log(K_{ow}) < 4$ and form H-bonding



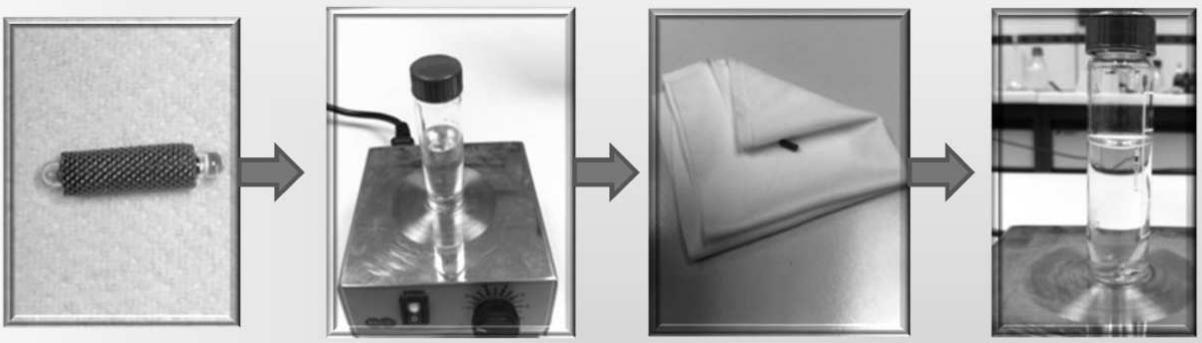
Gerstel and SRA Italia provided some polyacrylate and ethylene glycol-silicone Twisters to test

(10 mm long, 0.5 mm phase thickness)

BAM has a log $K_{o/w}$ = 0.77

A polar phase is required to extract BAM from water

Twister conditioning and extraction

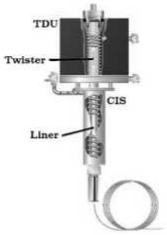


Polar Twister | Stir in solvent | Wipe out | Stir in water sample

… and now ?

SBSE: TD and LD, two ways to recover the analytes

Thermal desorption



GC-MS TDU-CIS

Liquid desorption *(back-extraction)*

GC-MS

HPLC-MS

Advantages:
- Fully automated
- Solvent free
- High sensitivity

Shortcomings:
- One-shot
- GC only

Advantages:
- Replicates
- GC, LC and …
- Cheap

Shortcomings:
- manual step
- time

Instruments & conditions

HPLC Agilent 1200 SL

ZORBAX Agilent Technologies

XDB-C_{18} : 50x4.6mm **1.8µm**

MS Agilent 6430 QQQ

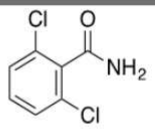
Injection: **10 µL**
Flow: **0.4 mL/min**
Temperature : **30 °C**

Mobile phase: A = 5 mM ammonium acetate buffer, pH 2.4; B = Acetonitrile
Isocratic elution: 50% A - 50% B

MS experimental conditions

- **Ionization: ESI+**
- **MS/MS acquisition mode: MRM**

Table 1 Physical properties, structure, analytical relevant data of BAM and optimized LC-ESI-MS conditions

Name	Abbreviation	Empirical formula	Mw (g/mol)	Structure	Log $K_{o/w}$
2,6-Dichlorobenzamide	BAM	$C_7H_5NOCl_2$	190.03		0.77

MS parameters							
RT[a] (min)	IM[b]	$[M+H]^+$	PI[c]	DT[d]	FV[e]	CE[f]	CAV[g]
1.72	Positive	190	173, 145	500	135	30	7

[a]Retention time; [b]Ionization mode; [c]Product ions; [d]Dwell time (ms); [e]Fragmentor voltage; [f]Collision energy; [g]Cell acceleration voltage

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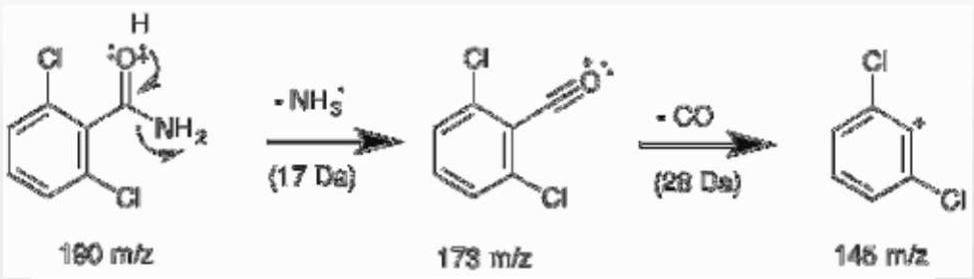
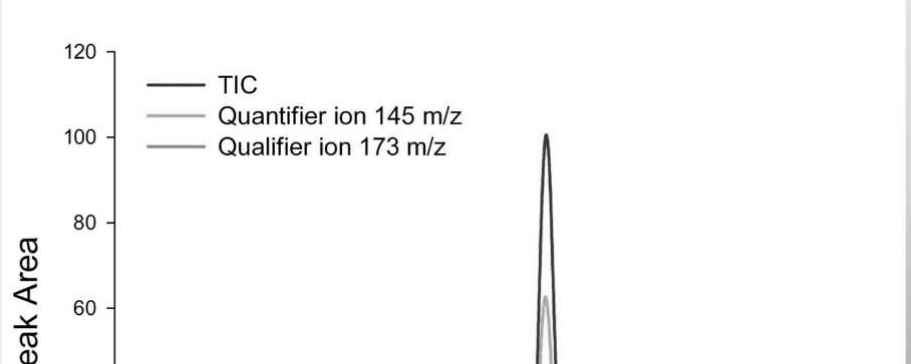
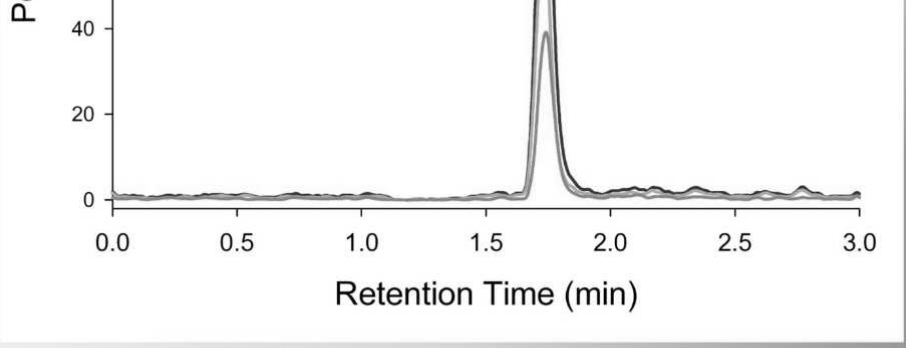
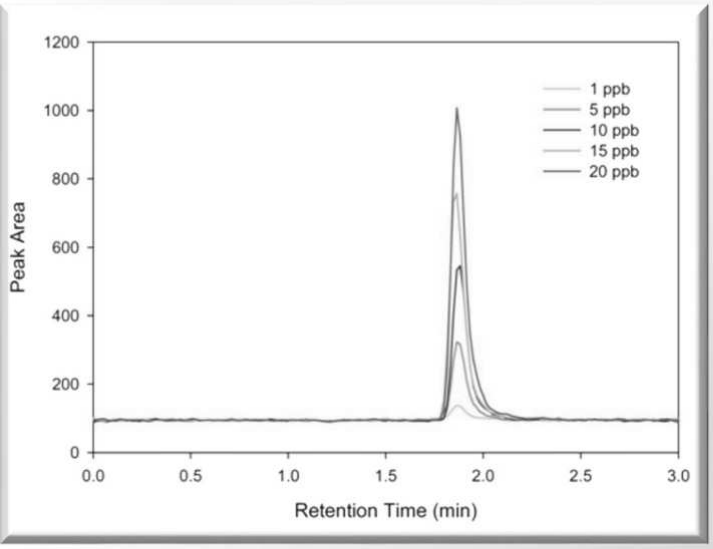
Parent ion Fragments

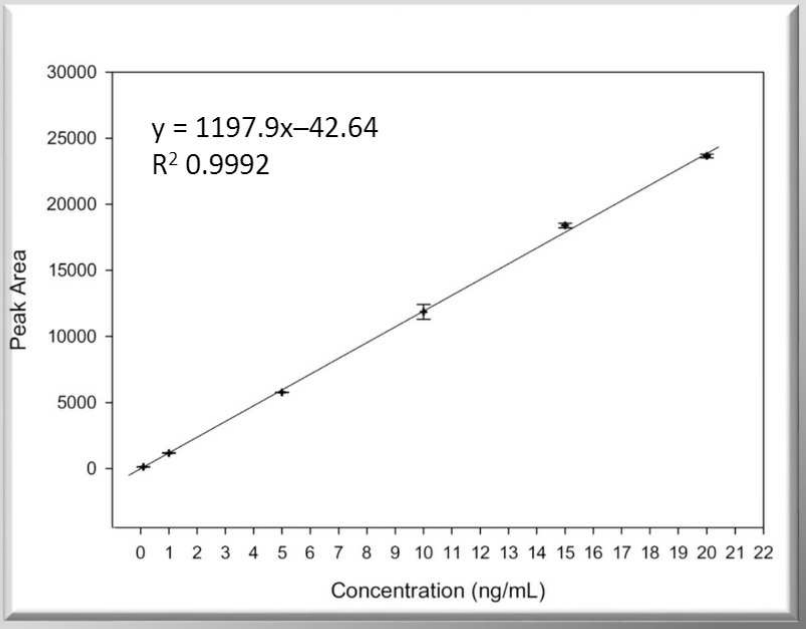






MS: quantitative analysis





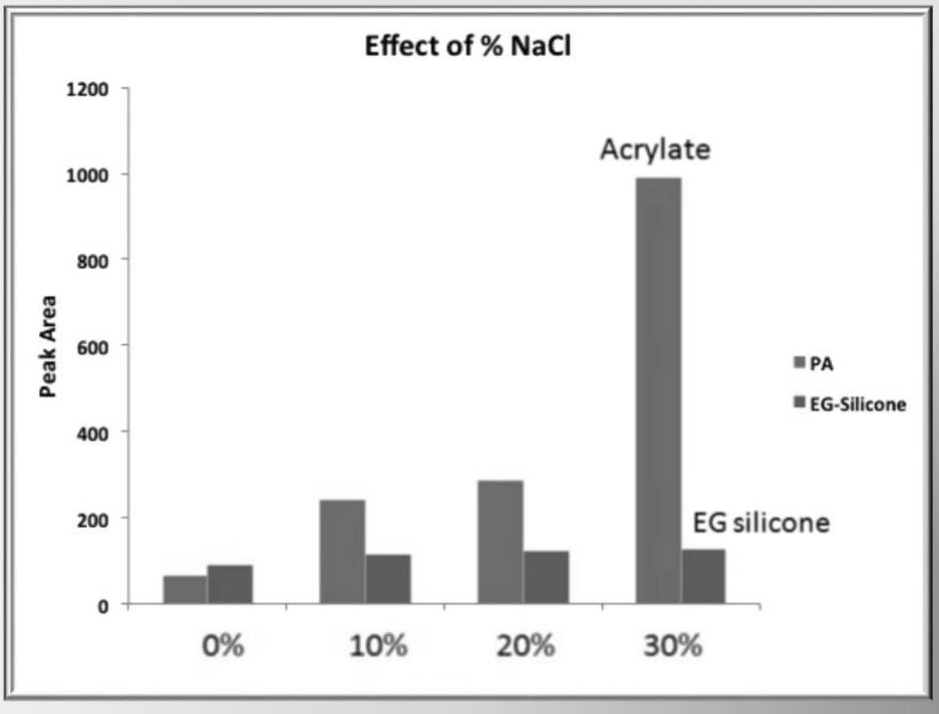
SBSE-LD: main parameters to be optimized

- ionic strength (and pH)
- stirring speed and time

- **sample volume**
- **liquid desorption solvent**
- **LD method** (stirring or sonication) **and time**

SBSE-LD: ionic strength effect

- **Both the polar PA and EG silicone Twisters tested, spiking a water sample (analyte-free) with 1 ng mL^{-1} of BAM.**
- **Four concentration levels of NaCl (0, 10, 20 and 30% w/v), other variables constant** *(sample volume 5 mL, stirring speed 500 rpm, and stirring time 4 h)*



SBSE-LD: main parameters to be optimized

EG silicone was abandoned *(extraction of BAM < 15%)* :

a thorough optimization of the extraction method was carried out using polyacrylate Twister

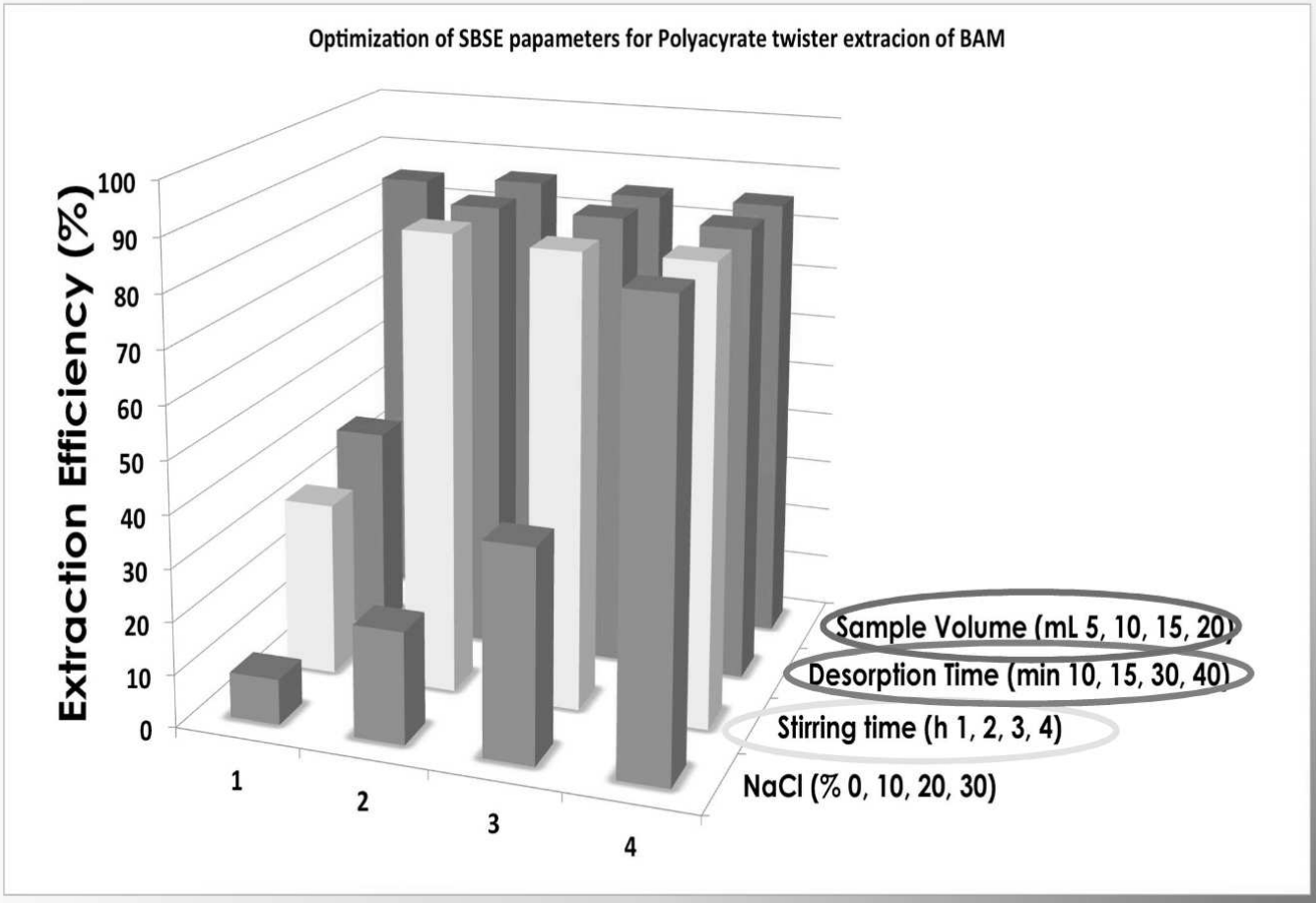
- ionic strength (and pH)
- stirring speed and time
- sample volume
- liquid desorption solvent
- **LD method** (stirring or sonication) **and time**

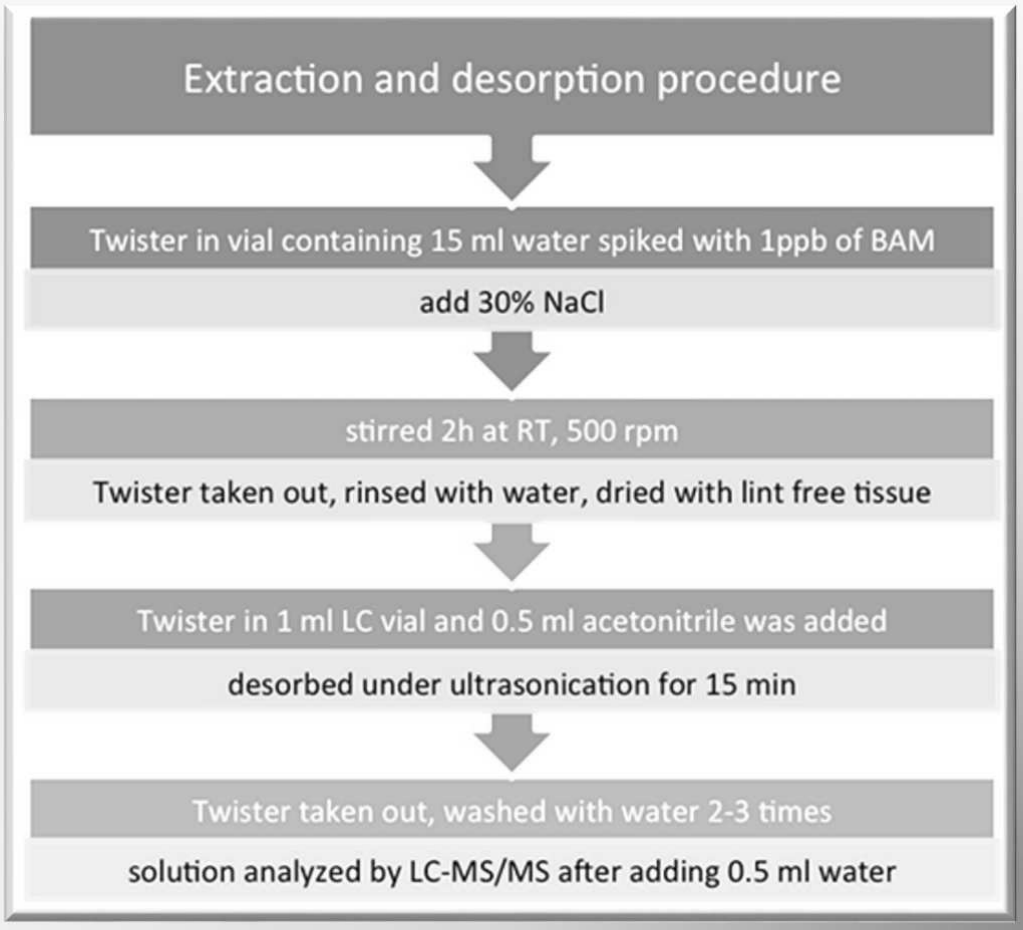
Liquid desorption

- Literature data suggested that LD by ultra sonication accelerates analyte desorption from the stir bar (*)
- In our experiments, stirring was extended up to 4 hours but provided a back-extraction efficiency close to 20%, so it was discarded
- LD was then performed by sonication

(*) GIORDANO A., FERNANDEZ-FRANZON M., RUIZ M.J., FONT G., and PICO Y., Pesticide residue determination in surface waters by stir bar sorptive extraction and liquid chromatography/tandem mass spectrometry, *Anal. Bioanal. Chem.*, 2009, 393, 1733-1743.

SBSE-LD: optimization of PA


Optimization of SBSE papameters for Polyacyrate twister extracion of BAM
Extraction Efficiency (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Sample Volume (mL 5, 10, 15, 20)
Desorption Time (min 10, 15, 30, 40)
Stirring time (h 1, 2, 3, 4)
NaCl (% 0, 10, 20, 30)


Extraction and desorption procedure
Twister in vial containing 15 ml water spiked with 1ppb of BAM
add 30% NaCl
stirred 2h at RT, 500 rpm
Twister taken out, rinsed with water, dried with lint free tissue
Twister in 1 ml LC vial and 0.5 ml acetonitrile was added
desorbed under ultrasonication for 15 min
Twister taken out, washed with water 2-3 times
solution analyzed by LC-MS/MS after adding 0.5 ml water

Repeatability
(n= 5)

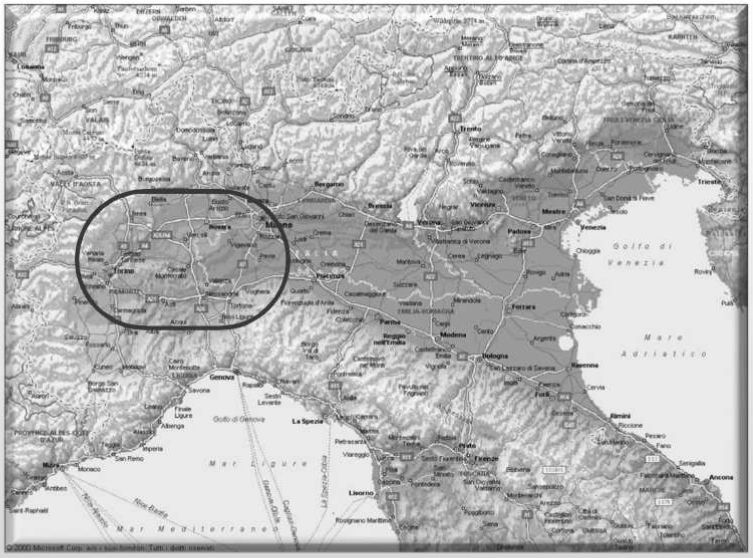
BAM conc. (µg L^{-1})	RSD (%)	
	Intra-day	Inter-day
0.1	1.5	5.6
1	2.6	7.1
5	3.3	9.7
10	1.9	9.6
15	1.3	9.6
20	0.9	2.7

Figures of merit

BAM	SBSE-LD LC-MS/MS
Recovery	86 %
Preconcentration factor	15:1
LOD	2.0 ng L^{-1}
LOQ	6.6 ng L^{-1}
RSD intra day	< 5 %
inter day	< 10 %
Linear range	0.1 – 20 µg L^{-1}

Linear range	0.1 – 20 µg L

Groundwater samples



Water samples	BAM conc. (µg L^{-1})	RSD (%)
1	0.161	4.0
2	0.282	6.1
3	0.103	13.8
4	0.168	4.3
5	0.070	2.5

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Preliminary tests to extend method to other polar pesticides

Nine pesticides chosen from a priority list of the most widespread pesticides in Northern Italy:

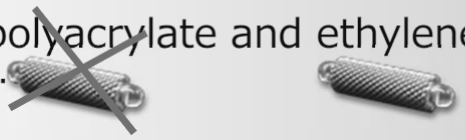
metolachlor, penconazol, alachlor, linuron, prometryn, terbutylazine, propazine, ametryn, atrazine

Range of $\log K_{o/w}$ of selected pesticides: **2.6 - 4.7**

Quantitative analysis was performed by LC–MS/MS using the method previously developed in our lab (*)

method previously developed in our lab (*)

Both polyacrylate and ethylene glycol-silicone twisters were tested … but



(*) Bono L., Magi E., *"Fast and Selective Determination of Pesticides in Water by Automated on-Line Solid Phase Extraction Liquid Chromatography Tandem Mass Spectrometry"*, Analytical Letters, 2013, 46, 1467-1476

Preliminary tests to extend method to other polar pesticides

Similar optimization of EG-silicone Twister to that previously described for BAM

In optimized conditions (*stirring speed 500 rpm, time 2 h, sample volume 20 mL, ultrasonic desorption 30 min with methanol*):

- Extraction efficiency in the range 50 - 80%
- Linearity in the range 5-250 ng L^{-1} , R^2 0.993-0.998
- LOD 1.6-25.5 ng L^{-1}, LOQ 5.2-43.7 ng L^{-1}
- Inter-day RSD below 10%

Altough preliminary, results indicate that EG-silicone can be successfully employed for trace level determination of polar pesticides ($logK_{o/w}$ 2.6 - 4.7) in water

Pesticides detected in groundwater samples

Real Samples	Concentration in µg L^{-1}					
	MET	PEN	ALA	TER	ATR	RSD%
1	<LOD	<LOD	<LOD	<LOD	<LOD	-

2	0.049	0.052	0.056	<LOD	<LOD	4.4-8.1
3	0.033	0.040	<LOD	0.059	0.075	3.8-11.2
4	0.032	0.033	<LOD	0.053	0.092	4.5-9.8
5	<LOD	<LOD	<LOD	<LOD	0.061	5.7

MET: metolachlor, PEN: penconazol, ALA: alachlor, TER: terbutylazine, ATR: atrazine

G Model
PBA-9778; No. of Pages 7
ARTICLE IN PRESS
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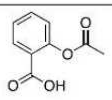
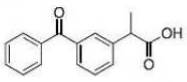
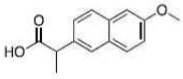
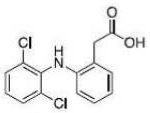
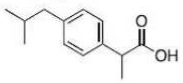
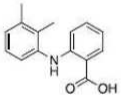
journal homepage: www.elsevier.com/locate/jpba

New Methods

Innovative sampling and extraction methods for the determination of nonsteroidal anti-inflammatory drugs in water

Shivani Tanwar, Marina Di Carro, Emanuele Magi*
Department of Chemistry and Industrial Chemistry, University of Genoa, Via Dodecaneso 31, 16146 Genoa, Italy

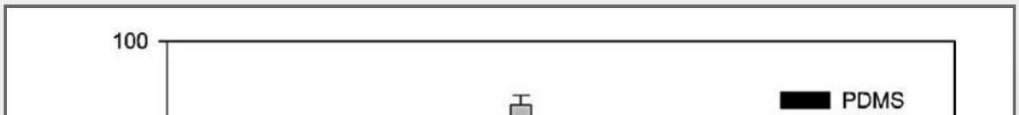
Table 1
Analyte structures and LC–ESI–MS/MS conditions in MRM mode. Negative ionization and cell acceleration voltage – 7 were used for all analytes).

Analyte	Structure	Log $K_{o/w}$	RT[a] (min)	Precursor ion	Product ion		DT[b]	FV[c]	CE[d]
					Quantifier	Qualifier			
ASA		1.19	1.2	179	137	–	80	70	0
KET		3.12	1.8	253	209	197	70	70	2
NAP		3.18	1.9	229	185	169	100	79	5
DIC		4.51	3.1	294	250	214	100	80	5
IBU		3.97	3.5	205	161	159	100	75	2
MEF		5.12	4.6	240	196	192	100	91	5

[a] Retention time.
[b] Dwell time (ms).
[c] Fragmentor voltage.
[d] Collision energy.

Extraction efficiency of NSAIDs

PDMS vs. EG-silicone



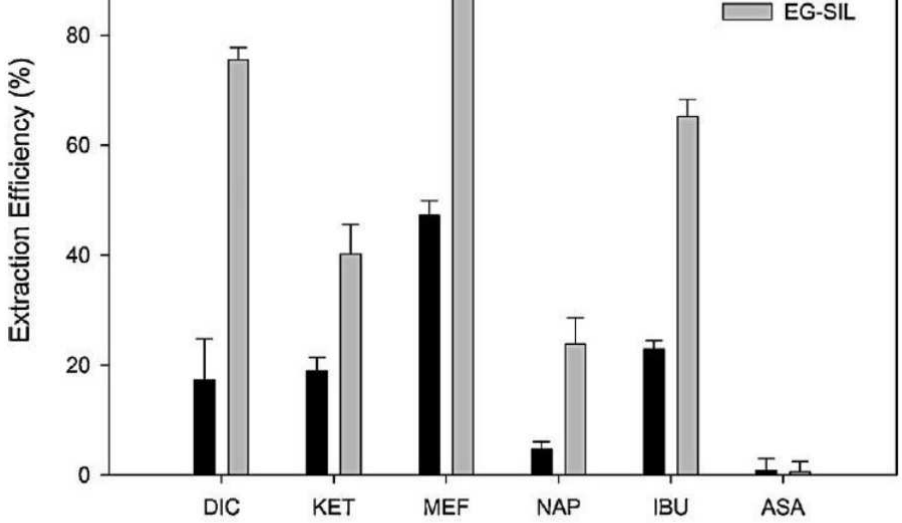


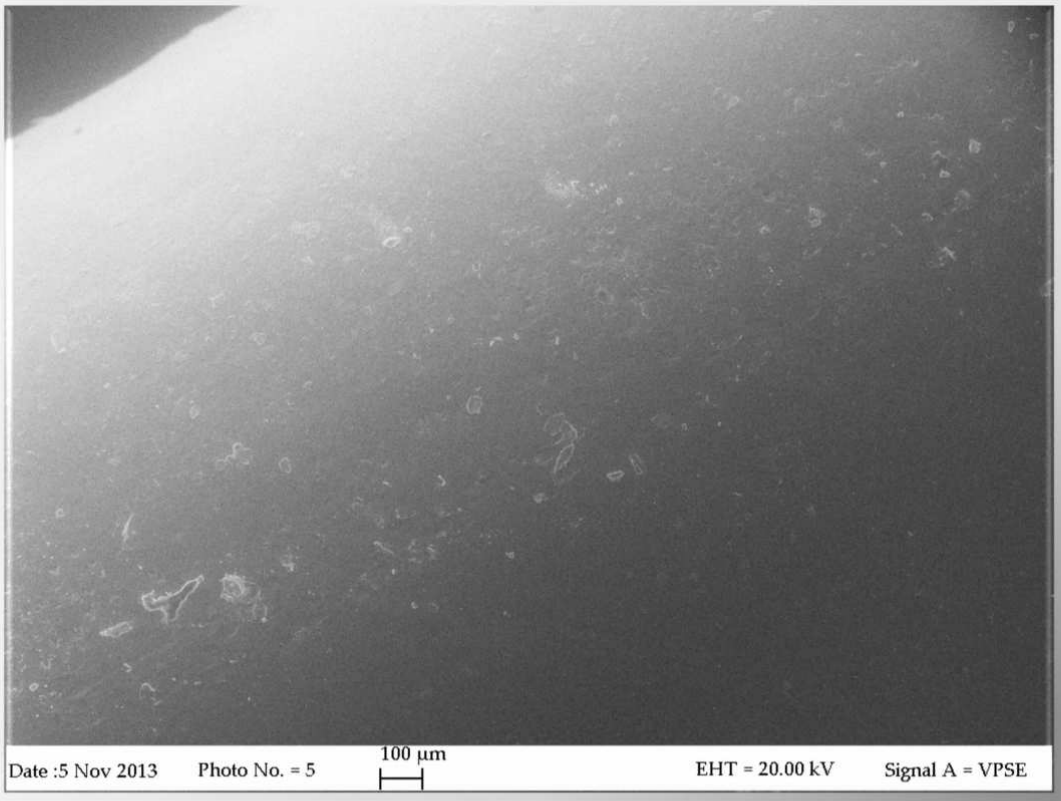
Fig. 2. Extraction efficiencies of NSAIDs obtained with EG-Silicone and PDMS (optimized methods).

Twister Robustness

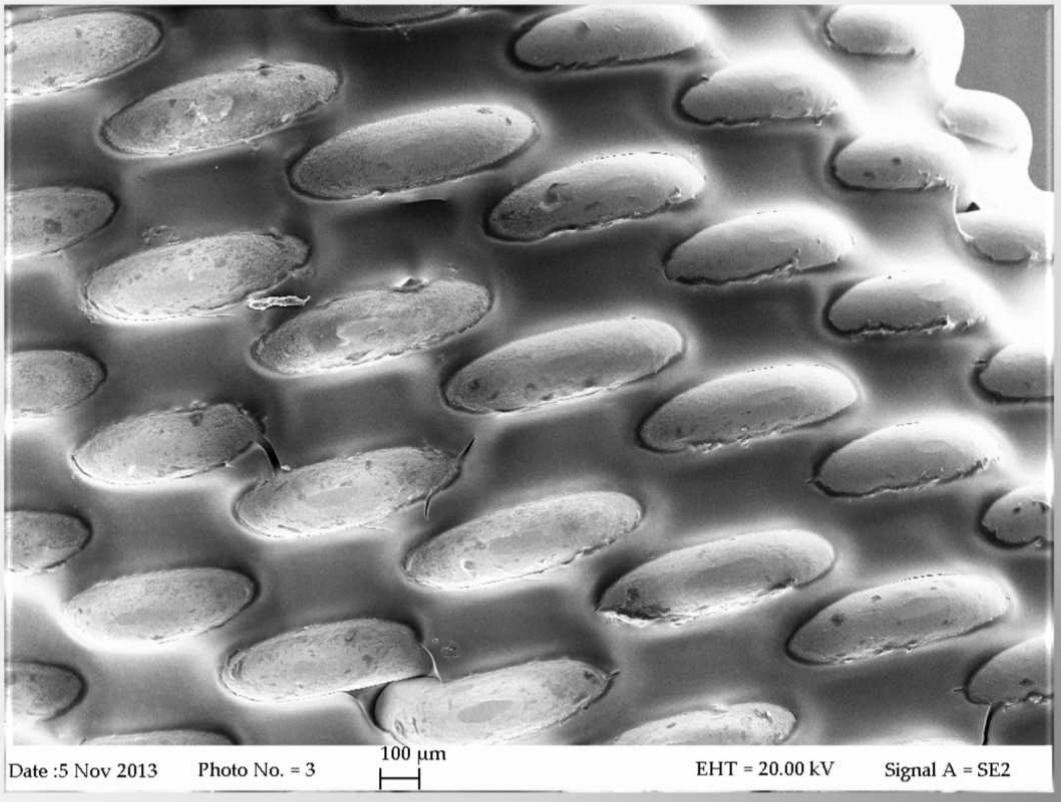
- PDMS Twister lifetime is about 50 cycles (extraction & desorption)
- In our experiments, polar stir bars exhibited an average lifetime of about 25 cycles
- Probably the stirring is not as smooth as for PDMS, thus affecting negatively the robustness of polar stir bars

Twister Robustness

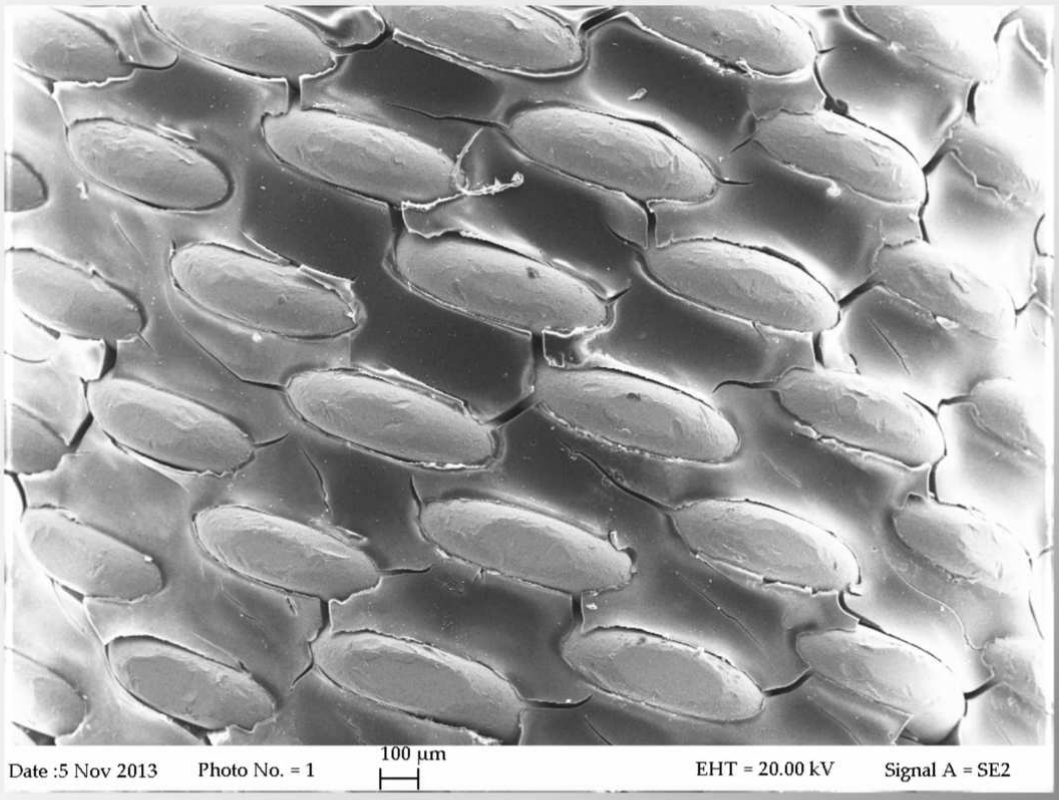
PDMS Twister after 20-25 cycles



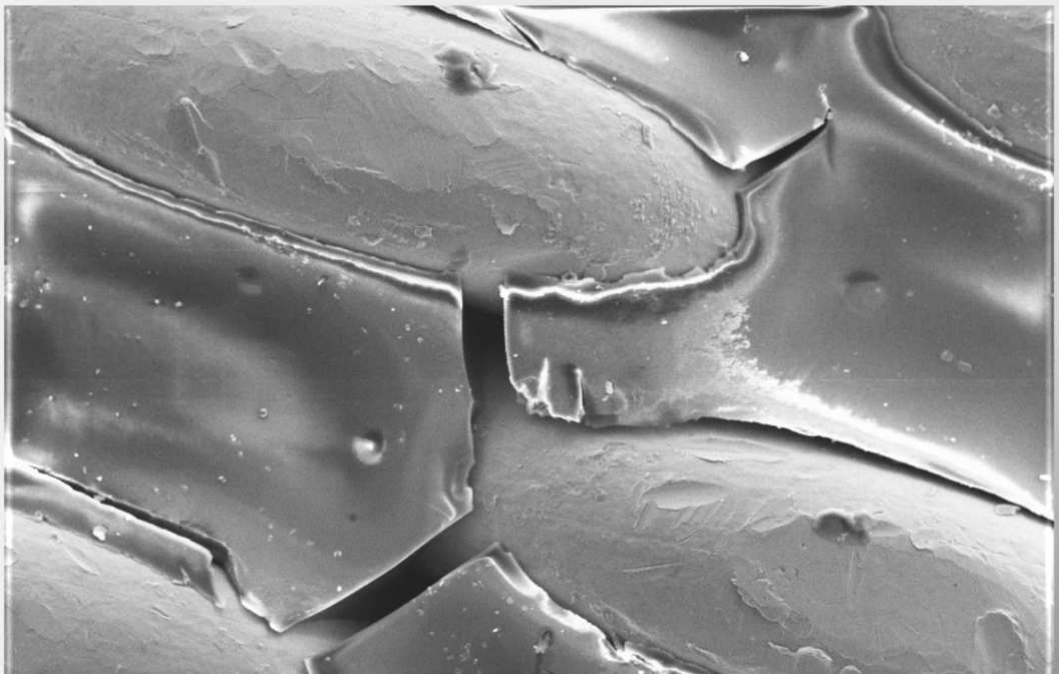
PA almost new



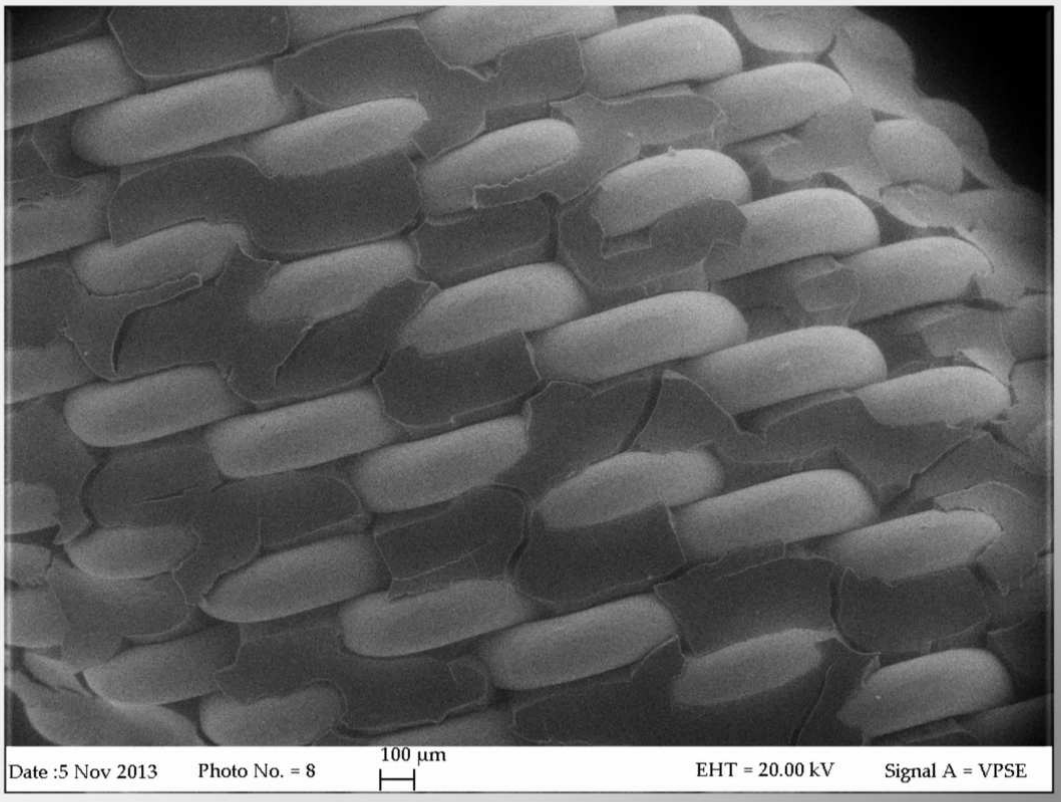
PA after 25 cycles



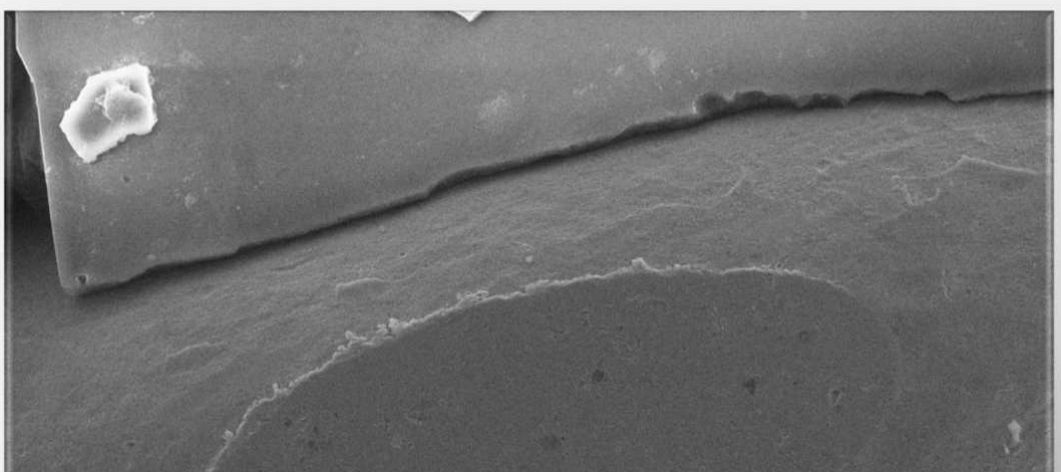
PA after 25 cycles

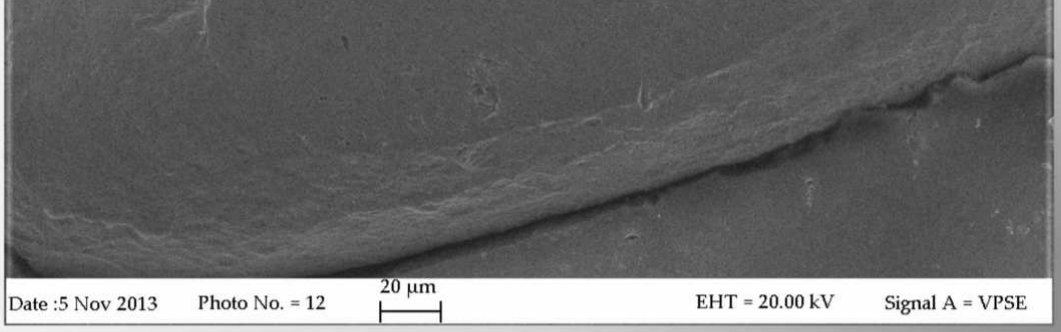


EG Silicone after 20-25 cycles



EG Silicone after 20-25 cycles

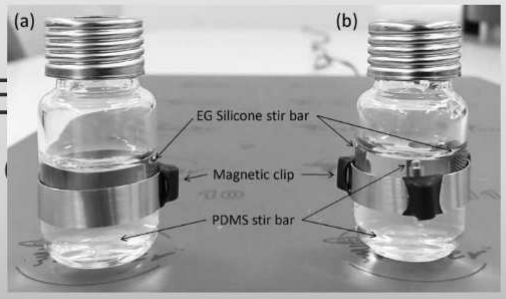




Twister Robustness

- Stirring is responsible for mechanical stress of coating, we don't know how much it compromise the extracting power of the Twister and its lifetime

- Ochiai, et al. 2013 (*) reported for E silicone stir bar a lifetime of 30 cycl using a "non-stirring" Twister



- Further studies are necessary for a better understanding and to enhance the robustness of polar Twister ... a challenging task for material scientists and manufacturers

(*) Ochiai, N., K. Sasamoto, T. Ieda, F. David and P. Sandra. 2013. Multi-stir bar sorptive extraction for analysis of odor compounds in aqueous samples. *J. Chromatogr. A* 1315: 70-79.

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