A novel improvement on biocompatibility and reusability of solid phase microextraction coatings for direct extraction from raw blood

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Overview

✓The study reports on improvement of biocompatibility and reusability of a SPME coating for extraction from whole blood.

✓C18-PAN 96-blade SPME coating was modified using various biocompatible polymers including polyacrylonitrile (PAN), 2-methacryloyloxy ethyl phosphorylcholine (MPC) and glycidol- derivatized polymer

✓In order to improve the reusability a new washing strategy (20s agitating) was used after extraction.

✓The modified coatings was evaluated in term of reusability, reproducibility and extraction efficiency.

✓A automated 96-autosampler was used for evaluation of coating for direct immersion whole blood extraction method.

✓The study showed that PAN modified C18-PAN coating (UV dried) resulted in best biocompatibility and reusability.

✓The modified PAN-C18-PAN coating was used for both direct immersion whole blood extraction and extracted blood spot (EBS) analysis.

✓The extracted blood spot (EBS) analysis using SPME coating was coupled with LC-MS/MS and DART-MS/MS systems.

Introduction

The surface chemistry and topography of the material in contact with biological matrices are important parameters that may impact protein adsorption and blood cell interaction. As a result, one common solution for minimizing the adsorption of proteins is to fabricate well-solvated polymer brushes as a high activating barrier on the surface and to protect the surface against proteins adsorption [1].

Up to date, several studies have been reported on development and evaluation of biocompatible SPME coatings for in vivo and in vitro applications using biocompatible polymers such as polyethylene glycol polyacrylonitrile (PAN), polydimethylsiloxane and polypyrrole (PPY). The development of biocompatible SPME coating can also be achieved through application of biocompatible extractive phases or modification of the conventional SPME coating with biocompatible polymers [2].

The phenomena of protein adsorption and cellular adhesion are two different issues which should be resolved using biocompatible coating. Not necessarily all biocompatible coatings which minimize the protein adsorption are also cable of effective prevention of the blood cell attachment. This will cause some limitation for utilization of biocompatible coatings in whole blood matrices contacting large volume of blood cells. Our studies showed that available lab-made or commercial biocompatible SPME coatings mostly suffers from lack of reusability in whole blood due to irreversible blood cell attachment on the surface of the coating. This may cause adverse influence on the kinetics of extraction and loss of efficiency of the coating in whole blood analysis.

Therefore, the current study focuses on modification of SPME coating to improve biocompatibility and to prevent blood cell attachment in whole blood matrix for long term use. For this purpose, this study reports on modification of C18-PAN coating using various biocompatible polymers including polyacrylonitrile (PAN), 2-methacryloyloxy ethyl phosphorylcholine (MPC) and glycidol- derivatized polymer along with new washing strategy after extraction. The biocompatibility of the optimized coating (PAN modified-C18-PAN) was evaluated for direct immersion whole blood analysis coupled with LC-MS/MS and *Extracted Blood (EBS) Spot* analysis coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and Direct Analysis in Real Time (DART)-MS/MS.

Experimental

Coating preparation procedure

The procedure of the preparation of polyacrylonitrile glue and C18-PAN SPME coating has been already reported in details [3]. The original C18-PAN were modified with the following procedures:

1.Polyacrylonitrile (PAN) modified C18-PAN:

1.1. Sprayed PAN-dried at 180 °C: C18-PAN coatings were sprayed with two layers of polyacrylonitrile glue followed by drying at 180 °C for 2 min

1.2. Dipped PAN-dried at 180 °C: C18-PAN coatings were dipped in polyacrylonitrile glue for (20 s) followed by drying at 180 °C for 2 min

1.3. Dipped PAN-dried at 70 °C: two sets of C18-PAN coatings were dipped in polyacrylonitrile glue for 20 and 60 s followed by drying at 70 °C for 4 h

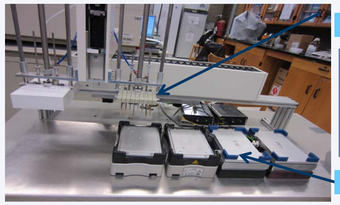
1.4. Dipped PAN-dried under UV light: two sets of C18-PAN coatings were dipped in polyacrylonitrile glue for 10 s and 60s followed by drying under UV light for 30 min (each side)

2. *Methacryloyloxy ethyl phosphorylcholine (MPC) modified C18-PAN:* C18-PAN coatings were dipped in 2-methacryloyloxy ethyl phosphorylcholine (MCP) solution in ethanol (5% w/v) for 60 s, followed by drying under 50 °C, coating and drying repeated for 3 times in order to get full coverage.

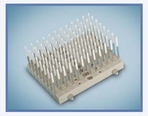
3. Glycidol derivatized -modified C18-PAN: PAN-modified C18-PAN coating (UV dried) was used for preparation of the glycidol derivatized-modified C18-PAN coating. The coatings were first dipped in n-butyl lithium (15s).The residue of n-butyl lithium was quickly washed out by dipping the coatings in toluene (2-3s). Then, the coatings immediately immersed in glycidol monomer (15 min reaction). Later, deprotonation of the coatings were perfomed by dipping them in an acidified DMF solution (DMF/concentrated HCl 47:3 v/v) while stirring for 1H.The excess of acid on the coatings were then neutralized by dipping in saturated NaHCO3 followed by rinsing with water and methanol.

New wash strategy on automated 96-thin-film SPME system

In order to improve washing the blood cells from the surface, an agitator was designed to be used for the wash step, and a 20s wash with agitation was optimized for complete removal of blood from the coating.



96-blade SPME



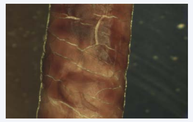
Wash agitator

Optimized SPME Condition	
Preconditioning	30 min in methanol/water 50:50 (v:v) at 850 rpm
Extraction	60 min at 1000 rpm
Washing	20 s in water at 850 rpm
Desorption	40 min in acetonitrile/water 50:50 (v:v) at 1500 rpm
Carry over	30 min in acetonitrile/water 50:50 (v:v) at 1500 rpm to remove <1.5% carry over from previous extraction

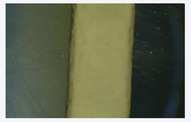
Results and discussion

The optimized modification method to increase biocompatibility and reusability for whole blood analysis

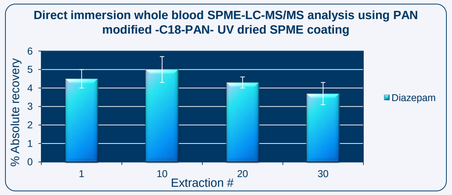
All modified biocompatible coatings were evaluated in term of extent of blood cell attachment, reproducible extraction efficiency and reusability in contact with whole blood in long term use. The study showed that thin dipped PAN-modified C18-PAN-dried under UV light (10 s dipping) presented the best results. This coating showed no blood cell attachment for 30 extraction, along with reproducible extraction recovery in long term use.



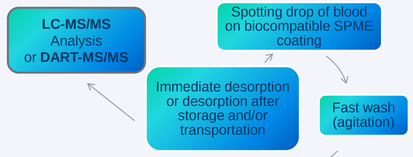
Unmodified C18-PAN after 5 extractions



Thin PAN modified -C18-PAN- UV dried SPME coating after 30 extractions



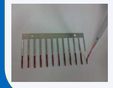
Extracted Blood Spot (EBS)



Main advantages of extracted blood spot (SPME coating) versus dried blood spot (filter paper)

1) Preserving the analytes against any variation including oxidation (because of extraction of the analytes into the coating).
2) Preventing the adverse matrix effect of the filter paper and blood matrix
3) Simple sample preparation technique with less experimental steps
4) Taking advantage of automation and high throughput analysis

New geometry for EBS-LC-MS/MS analysis

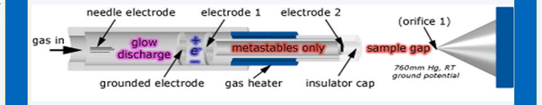


Advantages
✓2-fold larger surface area
✓Even distribution of blood drop
✓Matching with individual and high throughput 24-well format

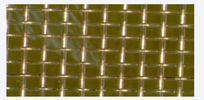
Results and discussion

Direct Analysis in Real Time (DART)

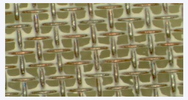
The DART source works by directing a flow of heated gas containing metastable (electronic or vibronic excited-state) atoms of helium and nitrogen to the sample. It heats the sample and desorbs the molecules of interest into the gas state where ionization happens. [4].



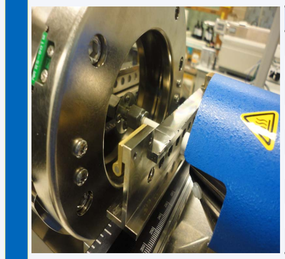
EBS-DART-MS/MS



Bare mesh

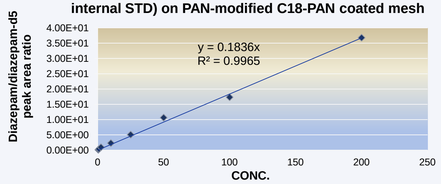


PAN- modified-C18-PAN coated mesh



Optimized EBS-DART-MS/MS	
Blood spot volume	5µL
Extraction time	5 min
Wash time (water)	5 s
MS	API 4000, Positive MRM
DART	SVP, Ionsense, Inc.
Source gases	H_2 &N_2
Ionization temperature	250ºC
Grid voltage	350 v
Sampling time	30s

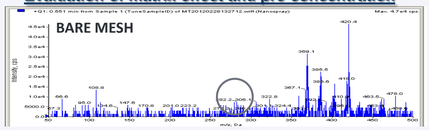
Human blood calibration for diazepam (diazeam-d5 as internal STD) on PAN-modified C18-PAN coated mesh

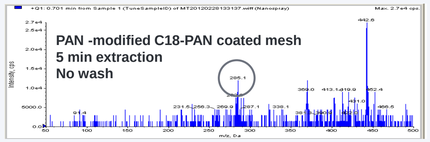


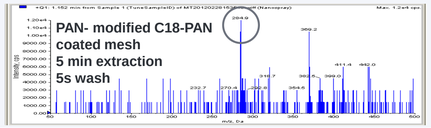
PAN-modified C18-PAN coated mesh	Recovery%	RSD% n=4	LOD mg/L	LOQ mg/L
EBS-DART-MS/MS blood analysis	97	3	0.3	1

Results and discussion

Evaluation of matrix effect and pre-concentration







Conclusion

Thin dipped PAN-modified C18-PAN-UV dried SPME coating demonstrated good biocompatibility and reusability for 30 extractions from whole blood without any blood cell attachment. This coating could be used for both direct immersion whole blood extraction and extracted blood spot sampling. Extracted blood spot sampling (EBS) can provide considerable advantages including preservation and pre-concentration of analytes, and minimizing the matrix effect. It can be applied as an alternative for dried blood spot sampling and can be coupled with LC-MS/MS or DART-MS/MS for analysis. DART is a fast method of analysis which provides direct detection of chemicals on surfaces without requiring sample pre-treatment, and it is applicable to a wide range of analytes. The combination of EBS with DART present significant advantages including minimizing the matrix effect which leads to more reliable quantitative results.

References

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4. J. Hajslova, T. Cajka, L. Vaclavik, Trends in Analytical Chemistry, 30, (2011) 204 -218.

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