

L. Kujovská Krčmová^{1,2}, Ch. Suwanvecho^{1,2}, D. Turoňová^{1,2}, K. Matušová¹, M. Matysová², F. Švec²

¹Department of Clinical Biochemistry and Diagnostics, University Hospital, Sokolská 581, Hradec Králové, 500 05, Czech Republic

²Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Akademika Heyrovského 1203/8, 500 05, Hradec Králové, Czech Republic

E-mail: Lenka.Kujovska@fnhk.cz.



Introduction:

Clinical research is a branch of medical science, that determines its safety and effectiveness of medications devices, diagnostic products and treatment regimens intended for human use. The requirements for biomedical analyses are continuously increasing. The groups of patients for statistical surveys have to be larger, which means that clinical research laboratories must process a larger number of samples. Timesaving and effective methods are used more often. The consumption of only small amounts of the sample and small volumes of the chemicals used is necessary.

In the development of new methods in bioanalysis, the pre-analytical phase is crucial and has a major impact on the final determination.

Laboratory practices have been increasingly focused on the miniaturization of the sample preparation process because it provides a solution for the requirements such as high performance, rapid analysis with a low running cost, and minimal environmental pollution. Microsampling is the process of collecting these small samples of blood, urine, or other with numerous advantages such as minute sample size, patient friendliness and ease of shipment.

Miniaturized sampling approaches have emerged as promising tools for sample collection.

Pipette tip extraction is based on the same principles and presents some similar steps than the traditional SPE. The several advantages as rapid extraction times, minimal amount of solvent, high extraction efficiencies and recoveries make this technique auspicious. In addition, different types of sorbents are commercially available, increasing the application area of this technique for several types of analytes.

Vitamin A and E

The vitamins A (retinol) and E (α -tocopherol) serve as reducing agents being able to inactivate the toxic effects of free radicals and protect the organism against oxidative stress.

Retinol plays an essential role in vision, normal bone and tooth development, reproduction, and the health of skin and mucous membranes. Vitamin A also acts in the body as an antioxidant, a protective chemical that may reduce the risk of certain types of cancer.

Tocopherol is a major antioxidant in serum. It serves also in protection of the cell membranes and regulation of platelet aggregation and protein kinase C activation. Its role is not only in antioxidant functions, but also pro-oxidant, cell signaling and gene regulatory functions.



Figure 1. Retinol and alpha-tocopherol formulas

Tip format	Name	Sorbent chemistry	Sorbent weight (mg)	Particle size (μ m)	Tip volume (mL)
DPX ^a	C18 XTR	C18	30	60	1
	RP XTR	Poly(styrene-co-divinylbenzene)	30	75	1
	WAX-S XTR+ 20 SALT	WAX ^b and SALLE ^c	5	60	1
	WAX-S XTR+ 40 SALT		5	60	1
μ -SPE-PT ^d	HybridSPE	Silica coated with zirconia	30	N.A. ^e	1
	C18 SiliaPrep	C18	0.4	N.A. ^e	0.2
	HLB SiliaPrep	HLB ^f	0.4	N.A. ^e	0.2
	HILIC SiliaPrep	HILIC ^g	0.075	N.A. ^e	0.2
	HILIC Hypersep	HILIC ^h	N.A. ^e	N.A. ^e	0.2

Table 1. Characteristics of pipette tips used in the study

^aDPX – disposable pipette extraction, ^bWAX – weak anion extraction, ^cSALLE – salting-out assisted liquid-liquid extraction, ^dN.A. – Not available, ^{e μ -SPE-PT – micro-solid-phase extraction using pipette tips, ^fHLB – hydrophilic-lipophilic balance, ^gHILIC – Hydrophilic interaction chromatography}

Pipette tip microextraction - results

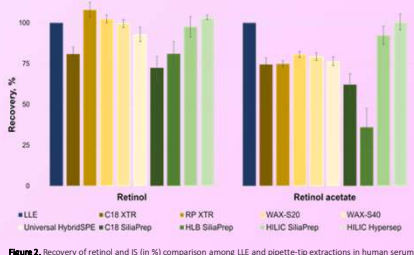


Figure 2. Recovery of retinol and IS (in %) comparison among LLE and pipette-tip extractions in human serum

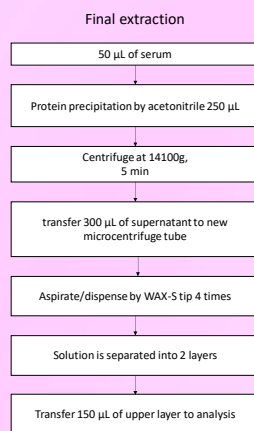


Figure 3. Final extraction procedure

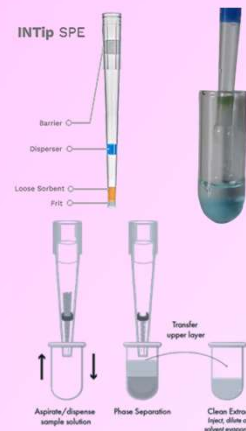
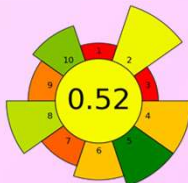


Figure 4. Principle of SALLE in tip extraction



Figure 5. Examples of pipette tips with sorbents

Figure 6. Greenness of sample preparation diagram of WAX-S. Overall greenness score and the corresponding color were presented in the center circle (0 with red color = the worst overall greenness performance, 1 with green color = the best overall greenness performance). Each performance criterion was represented by element around the inner circle which was identified with the number. The length of the trapezoid bar related to the assigned weight of that criterion, and the color demonstrated its performance (red = the worst greenness performance of that criterion, green = the best greenness performance of that criterion).



1. Favor in situ sample preparation
2. Use safer solvents and reagents
3. Target sustainable, reusable, and renewable materials
4. Minimize waste
5. Minimize sample, chemical and material amounts
6. Maximize sample throughput
7. Integrate steps and promote automation
8. Minimize energy consumption
9. Choose the greenest possible post-sample preparation configuration for analysis
10. Ensure safe procedures for the operator

Research in progress: Microsampling method development-pilot study

Name	Materials	Chemistry	Sample volume (μ L)
Hemaxis	Standard filter card Whatman 903	Cotton Linters (> 98% alpha-cellulose)	10
Captainer B	Ahlstrom grade 222	Cotton Linters	10
Nanofiber	50:50 PP-PHB	Polypropylene and polyhydroxybutyrate	10
	PCL	Polycaprolactone	10
	PCL micro/nano	Polycaprolactone	10
	PAN	Polyacrylonitrile	10

Table 2. Characteristics of DBS materials tested in the study.

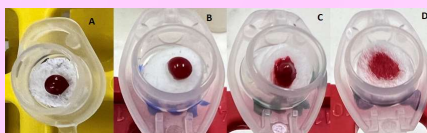


Figure 7. Blood spot on different types of nanofibers including (A) 50:50 PP-PHB, (B) PCL, (C) PCL micro/nano, and (D) PAN.

Based on the characterization of blood absorption onto different types of tested nanofibers, it is evident that PAN (Polyacrylonitrile) exhibited the highest level of absorption, rendering it a viable candidate for the advancement of dried blood spot (DBS) devices. The immediate and efficient absorption of blood spot into the material is crucial for practical usability. Failure in achieving prompt absorption could potentially lead to impracticality for users, and it might also contribute to sample loss during transportation.

PP-PHB and PCL materials were excluded from the experimental testing.

The key step in the extraction of whole blood from DBS involves the requisite wetting with water.

LL employing hexane demonstrates efficacy in reducing hydrophilic interferences that could otherwise compromise the integrity of the retinol peak. However, it is noteworthy that this approach reveals a limitation, as the tocopherol levels remain relatively subdued.

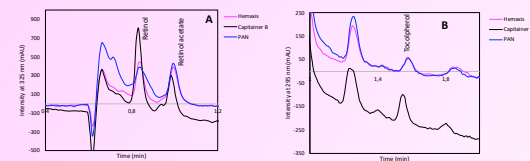


Figure 8. Chromatograms of retinol and tocopherol after DBS PAN sampling and LLE extraction

Future work: Microsampling devices and nanofibres

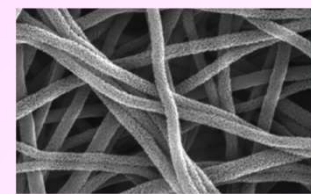
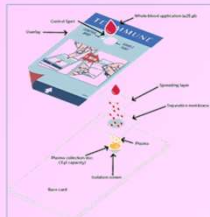


Figure 9. Examples of microsampling devices and cards

Figure 10. PAN nanofibers

Conclusion:

Modern analytical methods have increasingly reached clinical research and practice in recent years. The use of pipette tip extraction or modern microsampling methods is one possible tool to make the pre-analytical phase more efficient, faster and easier. The main benefit of these techniques is not only short processing time and greenness, but they bring also the benefit for patients (especially children and patients with mental disorders), who usually suffer from common venepuncture.

Acknowledgements:

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