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EXPERT OPINION

Making biosamples compatible with instrumental analysis

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Introduction

Bioanalysis is an exciting discipline, focused on the obtaining of chemical information from biosamples, which has a real and positive impact in society (e.g. better and rapid diagnosis). However, bioanalysis is also a challenging field due to its inherent characteristics which are particularly related to the type of samples. Biosamples are by far the most complex matrices, with a few exceptions, that can be analyzed in a laboratory. This complexity usually avoids the direct analysis of those samples making necessary a previous treatment. The aim of this treatment is to make the biosample compatible with the selected instrumental technique preventing the introduction of a large number of potential interferents in the equipment and preconcentrating the target analytes, if possible, in order to enhance the method sensitivity [1]. This is essential even when very selective and sensitive instrumental techniques like LC-MS are applied. The ion suppression produced by matrix components, compounds that can also deteriorate and dirty the instrumental systems, demands a previous sample clean-up. In addition, preconcentration is in some cases desirable as the sensitivity requirements/thresholds are in constant evolution (e.g doping control, toxicology), being also below the instrumental quantification limits.

The need for selectivity and sensitivity enhancement is especially critical in bioanalysis but it is also important in other fields like food analysis. Biosamples present also other special characteristics like the limited volume available of some fluids (in some cases re-sampling is not possible), the heterogeneity of some tissues, the dynamic evolution of in vivo systems, the instability of the samples when they are not stored properly or the rapid degradation of samples in fields like post-mortem toxicology.

Protein precipitation, liquid-liquid and solid phase extraction are classic techniques in bioanalysis. Although they have a clear and positive impact in the quality of the final results, these techniques are usually considered as "obstacles" between the system under study and the instrumental analysis. There are several reasons behind this statement, the tediousness and non-automated nature of some of these techniques being the principal limitations. As researcher in this field, I do not completely share this view, but I recognized in it what the market/professionals demand in this context.

Microextraction techniques: origins and impact in bioanalysis

These demands have been the driving force of the evolution of sample treatment in the last decades when an intensive research has been developed towards the simplification, automation and miniaturization of these procedures. In this constant evolution, microextraction techniques appeared in the 1990´s, both in the solid [2, 3] and the liquid phase format [4-6], and have become in a reference tool in many bioanalytical laboratories. Since then, an exponential development of those techniques has been taken place mainly on the basis of the synthesis of new extraction phases and the proposal of new methodologies. Let's focus the attention on the microextraction-bioanalysis binomial. On the one hand, bioanalysis is a demanding field for any sample treatment technique due

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to the previously described characteristics and therefore this binomial is very attractive for basic researchers. On the other hand, microextraction techniques are specially indicated for bioanalysis for several reasons. They are miniaturized techniques that can process low sample volumes that are unaffordable by classical techniques. They can be automated allowing the processing of a large number of samples which is critical for example in clinical testing. They are simple techniques requiring fewer resources (solvents, reagents) and steps [7]. Selectivity is an important property in extraction [8] but it is a central issue in bioanalysis since the sample matrices are complex and contain innumerable chemical compounds [9]. The use of separation (e.g chromatography) and instrumental (e.g MS) techniques partially mitigate this limitation but sample treatment still results key in this scenario. We may ponder how we can obtain an additional improvement of selectivity in microextraction. The answer is simple: giving priority to selective sorbents over non-selective ones even when the latter present a higher capacity. In short, non-selective sorbents can efficiently extract the target compounds from a biofluid but the final extract will content, also preconcentrated, a large number of matrix components. If non-selective sorbents are selected, a very carefully optimization of the chromatographic separation will be necessary and the use of very selective instruments (MS^n) is unavoidable. In addition, this strategy brings with the risk of the reduction of the lifetime of equipments [9]. Therefore, selective phases are the best option in bioanalysis. They can be divided in synthetic or natural based materials depending on their origin. Among the synthetic ones, molecularly imprinted polymers (MIPs) [10] and restricted access materials (RAMs) [11] can be highlighted. However, natural based materials comprising biomolecules are gaining special interest due to their enhanced selectivity (close to specificity) towards the target analytes [12]. Some of the biomolecules, like antibodies and enzymes, present selective cavities towards specific targets. Other biomolecules, like aptamers, can be in-vitro selected to bind the target in a very selective way [13].

The ideal microextraction technique in bioanalysis

The ideal microextraction technique should fulfill some requirements [14].

It should be:

- Efficient and selective enough allowing the isolation/preconcentration of the target analyte with a minimal influence of the sample matrix.
- Rapid and automated to improve the precision and to increase the sample throughput.
- As miniaturized as possible to reduce the sample volume (which is usually restricted) and the reagents and solvents volume (reducing the cost of the analysis).
- Safe to operators and environment
- Based on disposable units to avoid carry-over effect.

There are several techniques that accomplish some of these requirements but in the majority of cases the bioanalyst must select the appropriate one considering the inherent characteristics of the analytical problem to be solved. The description and enumeration of all the microextraction techniques that can be used in the bioanalytical field is out of the scope of this article but several strategies, some of them already consolidated, can be highlighted. Among the solid-based techniques the potential of SPME (Solid Phase Microextraction) and the derived technique thin film microextraction [15] is beyond any doubt. In addition, microextraction in packed sorbent [16] and pipette tip extraction are especially interesting as they can process low sample volumes and they can be automated and integrated to chromatographic equipments. In the liquid phase format, hollow fiber protected liquid phase microextraction [17] and derived techniques like electromembrane extraction [18] or parallel artificial liquid membrane extraction [19] present also a great potential.

Although these techniques are usually employed in the bioanalytical context, there is an intensive research in the development/application of new extraction techniques and new materials in this field.

In-vivo microextraction

Sampling is an unavoidable step in any analytical procedure and involves the obtaining of a representative sample that has similar properties than the system under study. After sampling, the sample is transported and stored in the laboratory for the final analysis. These steps are usually the source of innumerable errors associated to analytes losses or sample contaminations. In environmental science, these limitations have been overcome by the so-called on site analysis that integrates sampling and measurement (e.g water oxygen sensor).

In bioanalysis, on-site measurements are also possible and there are several examples like pulse oximetry that allows the determination of oxygen in arterial blood in a non-invasive way. On-site measurement cannot be applied to every target analyte due to selectivity and sensitivity issues. Prof. Pawliszyn`s group is the pioneer of in-vivo microextraction that solves this shortcoming [20]. As the name well indicates, this approach consists on the integration of sampling and microextraction and presents the following advantages: a) is faster than con-

ventional approaches; b) minimizes error associated to sample treatment and c) minimizes the error associated to sample storage since the analysis is performed after the in-vivo extraction [21]. In addition, the microextraction does not change the system equilibrium since the amount of extracted analyte is very low. Although this approach has been mainly used with animals [22, 23] probably due to ethical reasons, it has a great potential in humans as it has been outlined in a recent communication [24].

Extraction without elution

The title of this section is intentionally vague and responds to a personal vision about extraction techniques. For a chemist, extraction techniques are an exciting and stimulating field because they are based on chemical interactions between the target compounds and the extractant phases. The design of new phases, with enhanced capacity and selectivity, requires a deep understanding of the chemical nature of the target and its chemical environment. In the usual workflow, the sample is extracted and the analytes are isolated thanks to defined chemical interactions and finally transferred to a new liquid phase (a gas phase is employed in thermal desorption) where the final instrumental analysis takes place.

However, special designs can be developed avoiding the elution step. It sounds weird but the reader may agree with me that this is the basics of a sensor. Shortly, in (bio) chemical sensors [25] the analytes are isolated from the sample matrix into the receptor due to chemical interactions producing a change that is transduced into a signal. In this case, the elution is called regeneration step and it is necessary to reuse the sensor.

This personal view may be useful since all the advances in microextraction techniques, specially the synthesis of selective phases, can be exploited in sensor development that has a clear impact in the rapid diagnosis of some diseases.

Final consideration

This article provides a brief overview of sample treatment in the bioanalytical context giving special attention to microextraction techniques that play an important role in routine laboratories nowadays. The article does not aim to be an exhaustive revision of the field but a personal view of the author based on his own experience. Many different techniques are outlined and different research trends are highlighted. Anyway, a final quote should remain: the best sample treatment is the one that efficiently solve a given bioanalytical problem and in this scenario the bioanalyst has the final decision.

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