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作品名稱 Modification of silica surface with
supercritical water as a tool indicating new
possibilities of existing separation methods

得獎獎項 一等獎

國 家 Czech Republic

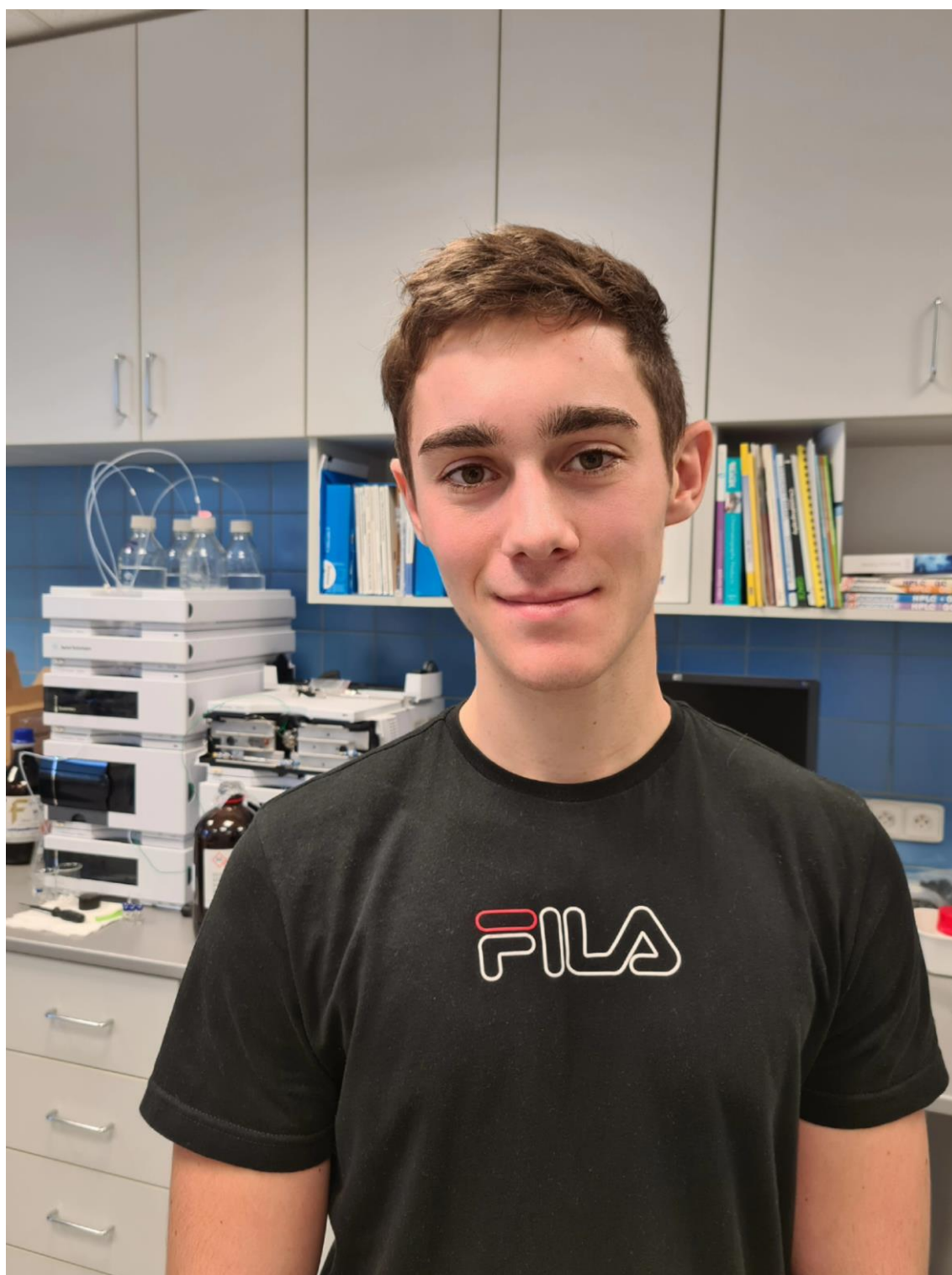
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關鍵詞 supercritical water 、 surface morphology 、
bacteria separation

作者照片



Abstract

Silica capillaries have been an integral part of the instrumentation used in many areas of analytical chemistry for decades, especially in analytical separations. In most cases, they are used without treatment, occasionally forceless chemical surface treatments are made to suppress or enhance the activity of silanol groups.

The aim of this work was to disrupt the inner surface of the capillary, perfectly smooth from manufactory, so that relatively coarse and various structures would be created, and to study their influence on the separation efficiency. The uniqueness of the used solution is based on the use of special properties of water exposed to high temperatures and pressures (supercritical water), which is able to disrupt this chemically inert material because of its aggressivity. In total, over 2000 experiments were carried out in order to define conditions suitable for the formation of various types of surface structures. Due to the high amount of resulting data, our own database application was created, allowing not only to save the picture of the structure and experimental conditions information, but also to clearly sort them out and create image reports according to the specified parameters.

Samples representing individual types of structures were then selected from this database and a number of silica capillaries with a configuration suitable for electromigration analyzes were prepared. The creation of a structured surface in the input part of the separation capillary enabled the separation of some classes of substances and biosamples, which cannot be analyzed on standard capillaries with a smooth surface.

An example is the complete separation of two strains of *Staphylococcus aureus* bacteria (MRSA and MSSA), or the use of the absorbing capabilities of a structured surface to study the interactions of these bacteria with bacteriophages. This ability was also used in the determination of *Aspergillus* fungus in a sample taken directly from the patient's lungs, where there was achieved a significant increase in the sensitivity of the analysis. Structured capillaries can also be used in the analysis of food samples, i.e., for the separation of β -lactoglobulins A and B in cow's milk, which belong to its main allergens.

Introduction

While "genuinely new" principles and trends such as chromatography, mass spectrometry, electromigration methods or spectroscopy have been discovered and massively introduced at the time, current research seems to be less energetic and driven primarily by economic and environmental factors. The size of analytical equipment is constantly decreasing and methods are being accelerated and cheapened. The emphasis on environmental aspects eventually led to the emergence of a new field of chemistry called "Green chemistry" [1].

Extraction with compressed CO₂ is one of the first successful attempts to replace toxic solvents [2], more modern research has afterwards yielded "ionic liquids" [3,4]. It is clear that in terms of toxicity, the most environmentally friendly solvent is water, which is also widely used due to its availability and price. However, a less-known fact remains that water can be brought to special states under high pressures and temperatures, where its physico-chemical properties change completely. Unlike other substances, the properties of water can be greatly changed by temperature and pressure; when its critical point is exceeded, supercritical water arises [5-8].

Gradual development and miniaturization of equipment for analytical separations (GC, HPLC, SFC, electrophoresis) has led to the introduction of thin silica capillaries [9], produced by fusing at high temperatures, so that their inner surface is perfectly smooth.

Increasing demands on the quality of analyses first led to attempts to modify their surface from a chemical point of view, later attempts were made to deepen the surface roughening. For this purpose, a number of very aggressive toxic solvents have been tested [10-14], the disadvantage of which has been the undesirable contamination of the etched surface by the reaction products.

The use of supercritical water to etch the inner surface of these capillaries paves the way for a more environmentally friendly and chemically clean process, which in combination with the original design of the apparatus allows the creation of such structures and their combinations that are not feasible by other methods.

Research background

Phase states of water and properties of its supercritical state

Besides the well-known phases of water - ice, liquid and gas (Fig. 1), there are also other states that can be considered as a combination of these phases. The different behavior and properties of individual H₂O states is caused by the continuous rearrangement or destruction of hydrogen bonds under the pressure of thermal and compressive forces.

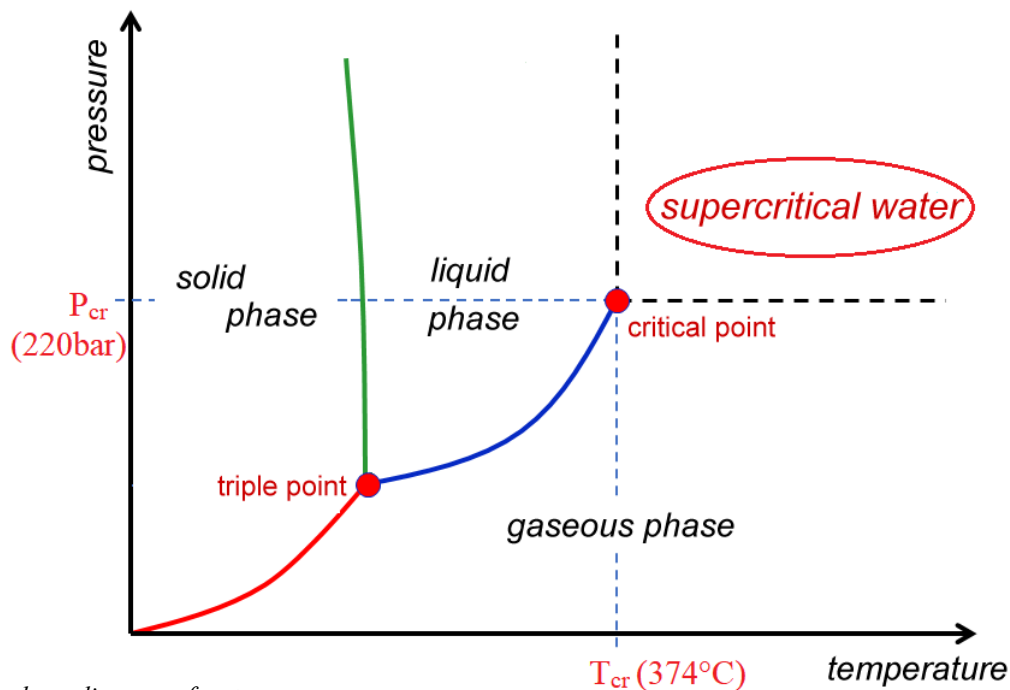


Fig. 1 - phase diagram of water

The less known state of water is the supercritical state. This occurs when the critical point of water is exceeded (374 °C, 220 bar), the hydrogen bonds are already very weak or cease to exist completely [15]. This results in a reduction in the solubility of polar substances and, on the contrary, the ability to dissolve non-polar substances arises. In addition, water becomes a solvent so aggressive that it is able to dissolve even such tough materials as glass or silica. The use of SCW in research is a relatively new direction, the commercial use of this technology is even rarer. Probably the most common use of SCW is the destruction of organic substances in an SCW reactor with simultaneous oxygen supply. Extreme conditions cause the total decomposition and oxidation of chemicals to their basic elements - carbon, nitrogen, oxygen, hydrogen and their oxides (Fig. 2).

Oxidation reactions in supercritical water

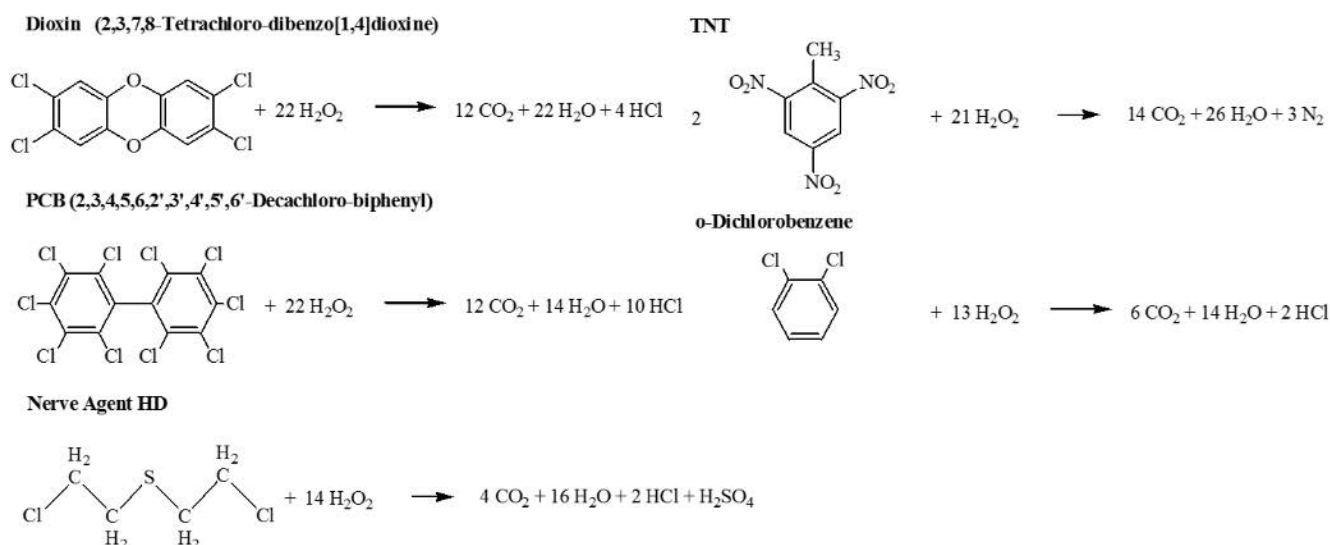


Fig. 2 - breakdown of warfare agents and pollutants with supercritical water

Glass and quartz surface treatment methods

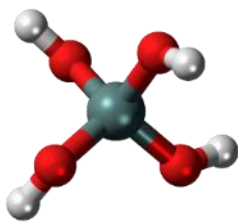
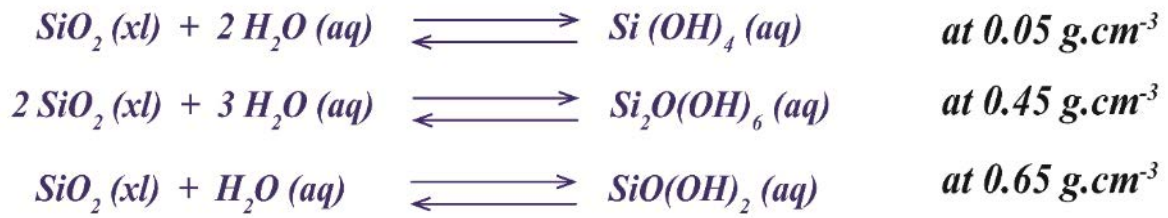
Surface treatment of glass can be in principle performed by mechanical tools or by a chemical method. If a chemical approach is used, sodium hydroxide can be used to roughen the glass surface at elevated temperatures, but one of the most common methods has been the use of hydrofluoric acid. Its downside is the high risk of handling it and the huge impact on the environment.

Chemically, this process can be written as follows:

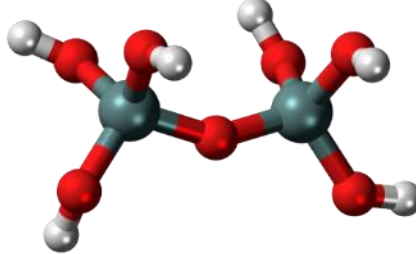


The new approach described in this work uses SCW as an environment-friendly substitute for the above reagents. The silica dissolution equation can be very simply (depending on the SCW density) described by the following equations:

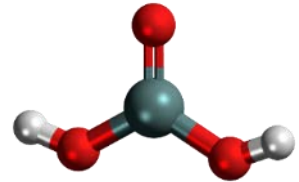
Wendlandt and Glemser, Brady 1963 and 1964



$\text{Si}(\text{OH})_4$
Orthosilicic acid



$\text{Si}_2\text{O}(\text{OH})_6$
parasilicic acid



$\text{SiO}(\text{OH})_2$
metasilicic acid

This property was used in our laboratory, i.e., for etching microchips in a static way (neither the substrate nor the SCW was moving) and for modification of the inner diameter of silica capillaries performed in a semi-dynamic way (the SCW moved, the capillary did not move). The aim of this work was to create a homogeneous structure of the entire surface of the capillary, and therefore it was necessary to use a fully dynamic process that involved not only movement of the SCW, but also movement of the capillary itself.

Experimental and Methodology

Lab made apparatus and devices

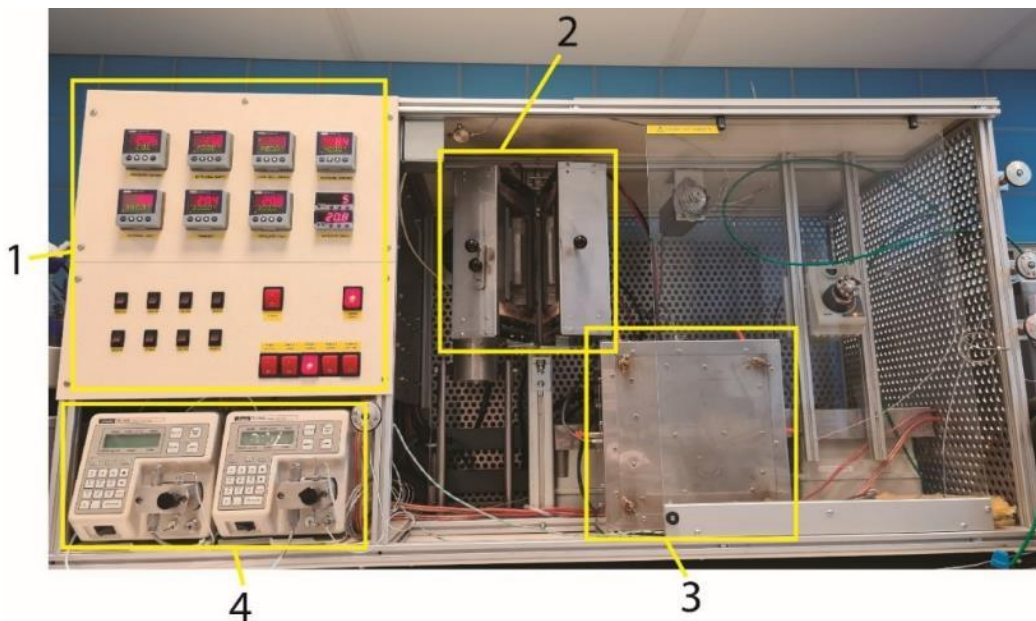


Fig. 3 - original version of apparatus for experiments with supercritical water

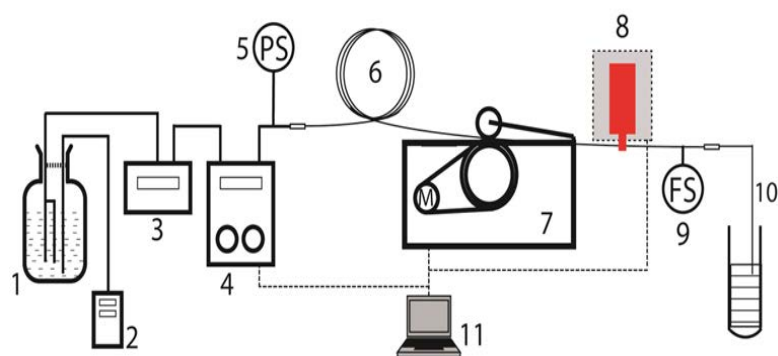


Fig. 4 - schematic view of a new hardware enabling capillary movement

In order to create homogeneous structured surfaces, the original apparatus (Fig. 3) had to be expanded with a new device, allowing continuous capillary movement through the heating zone. In this original approach, the SCW is not introduced to the inlet of the capillary, but it is created directly at the etching point, only in a small section defined by the size of the heating unit (Fig. 4_8).

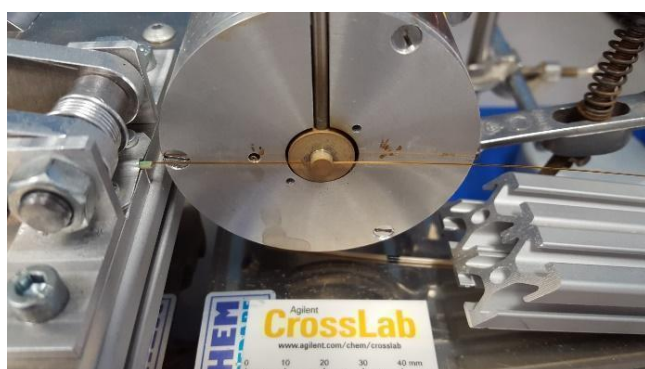


Fig. 5a – heating unit



Fig. 5b – inserts with different lenght of heating zone

Because the size of the hole in the heating unit is only a few tens of micrometers larger than the outer diameter of the capillary, it was necessary to ensure its precise passage through the heating unit. Part of the modifications was also the assembly of a new guide track adjustable in all axes (XYZ), which ensured this passing "in line" (Fig 5d).

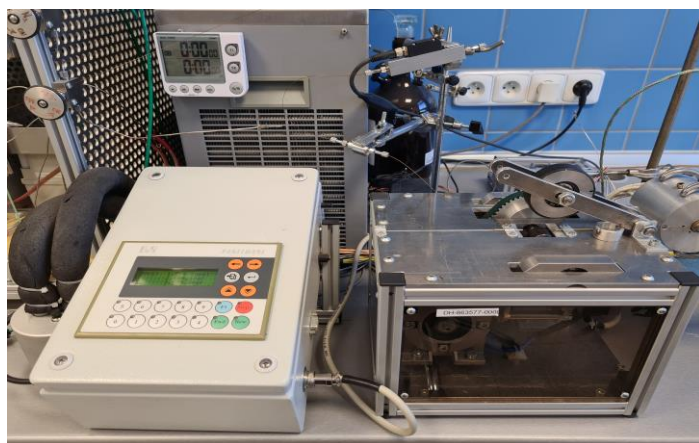


Fig. 5c – design of capillary movement device



Fig. 5d – guide track finely adjustable in all axes

Sample preparation and data processing

The use of SEM has proven to be the most suitable and easiest method to obtain information on the surface structure. Each etched capillary involved 4 sections corresponding to four capillary movement rates. To examine the influence of individual parameters on the inner surface of the capillary, each section was cut with a ceramic knife into pieces with a length of about 5-10 mm (Fig. 6a). To verify reproducibility and to exclude possible deviations, three samples were prepared from each experiment. A total of 12 samples were created from each capillary, which were then glued vertically to the duralumin block using a special double-sided conductive adhesive tape.

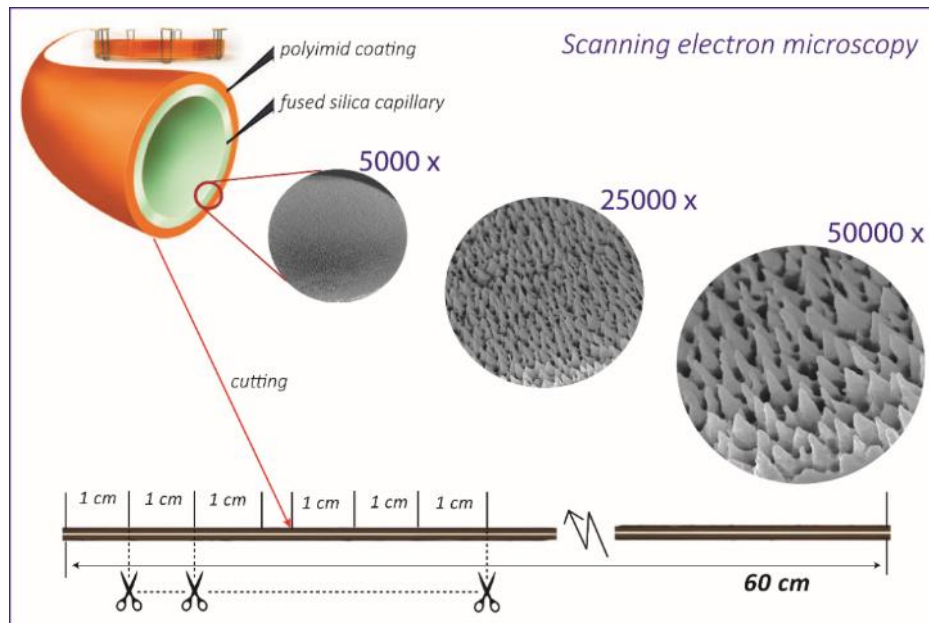


Fig. 6a – schematic view of capillary cutting and the SEM point

To examine the structural changes of the capillary surface, electron microscope TESCAN, Mira3 (Fig. 6b) was used. Because SEM requires electrically conductive sample surfaces, the entire block, including the capillaries, had to be gilded. For this purpose, a Baltec / SCD 500 Sputter Coater was employed, which applied a thin layer (12-20 nm) of gold to the samples using a plasma discharge in a vacuum environment (Fig. 6c).



Fig. 6b – SEM Tescan MIRA3

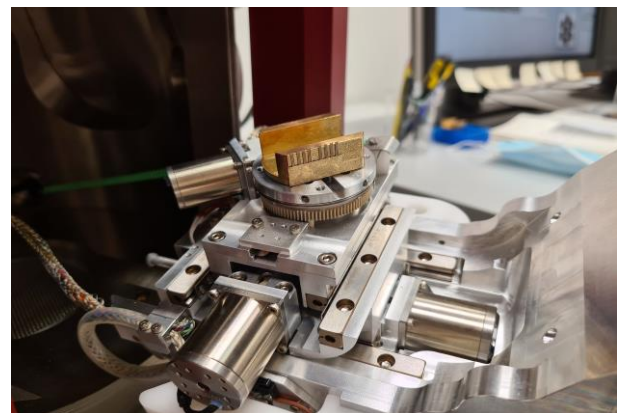


Fig. 6c – gilded samples placed in the SEM

The specificity of this work is that one of the most important outputs are the images of the created structure. It was already clear from the estimated number of experiments that a large number of photographs would be acquired, in which it would be impossible to orient oneself without a suitable system. For this reason, our own database application was developed, into which all available experimental data and the corresponding image were inserted for each experiment. In the database, it was possible to search for images, sort them and assemble print reports based on parameter queries.

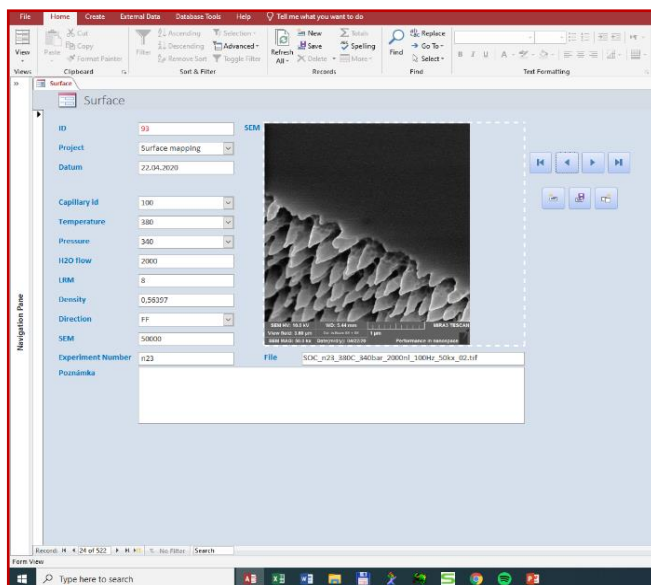


Fig. 7a – database input form

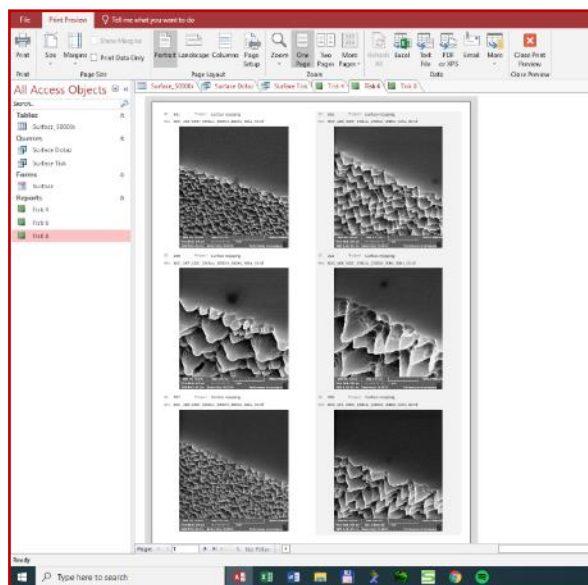
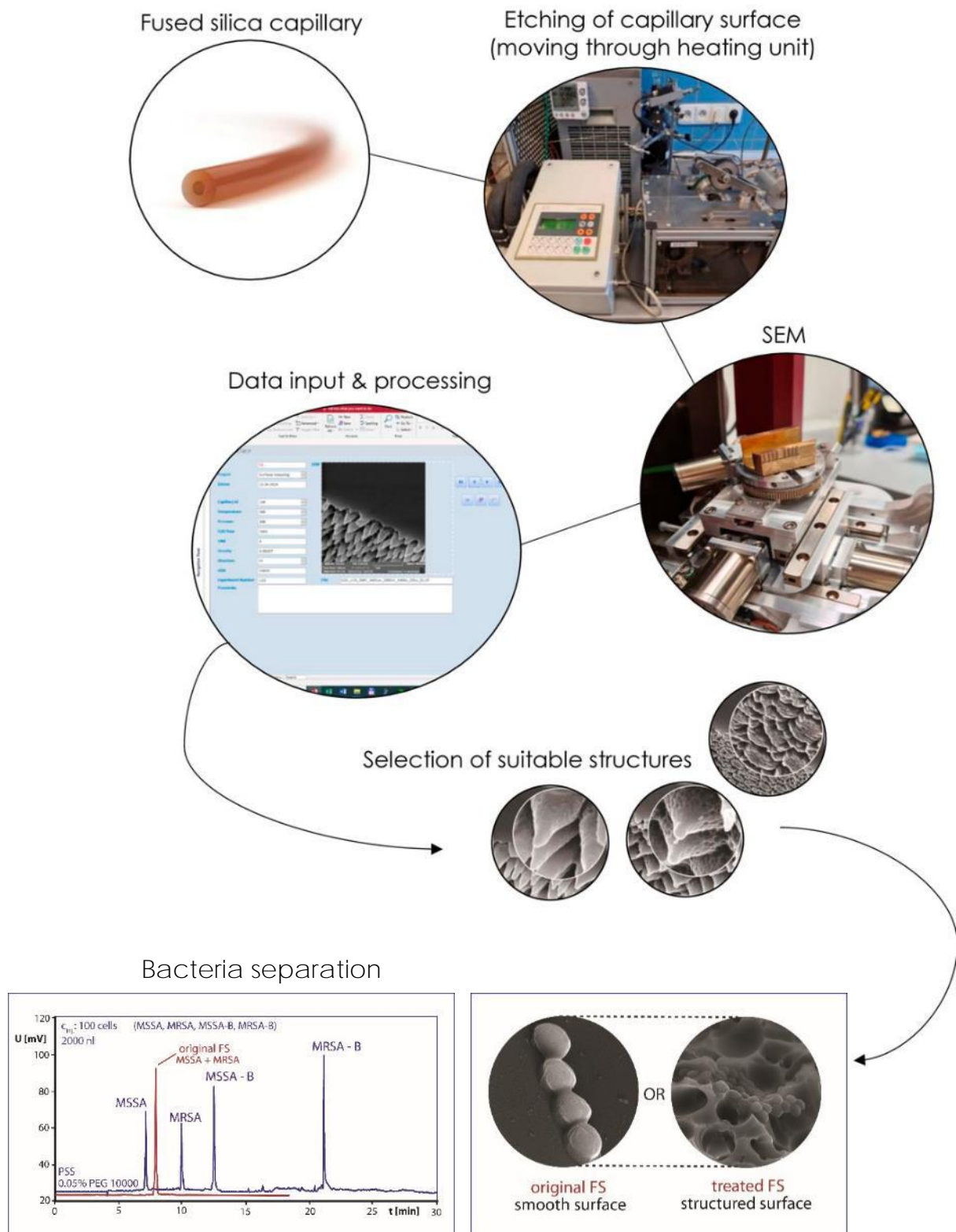


Fig. 7b – example of print output on base database query

Workflow diagram

The entire process from the creation of the surface structure to the demonstration of its use in practice can be simply illustrated by the following diagram:



Results and discussion

Dependence on SCW density controlled by pressure or temperature

An illustration of the effect of pressure on the morphology of the inner surface of the capillaries can be seen in Figure 8. In the first column, there are the structures achieved during etching below the critical water temperature (320°C) and at pressures from 200 to 660 bar. In this range, water has a relatively high density ($0.697\text{--}0.766\text{ g.cm}^{-3}$) and forms rather low conical formations with a wide base. If the density exceeds 0.720 g.cm^{-3} , the formation of this structure disappears, but the dissolution of the surface continues, as evidenced by the increased inner diameter of the capillary. The middle column (400°C) maps the behavior of the system in the region where even a small change in pressure initiates a large change in density of $0.101\text{--}0.629\text{ g.cm}^{-3}$. That results in various types of structures, from a fine "spherical" surface through etched "dimples" to tall, narrow "brush alike" structure type. In the last column at 460°C and 200 bar, deep surface etching can be observed, which

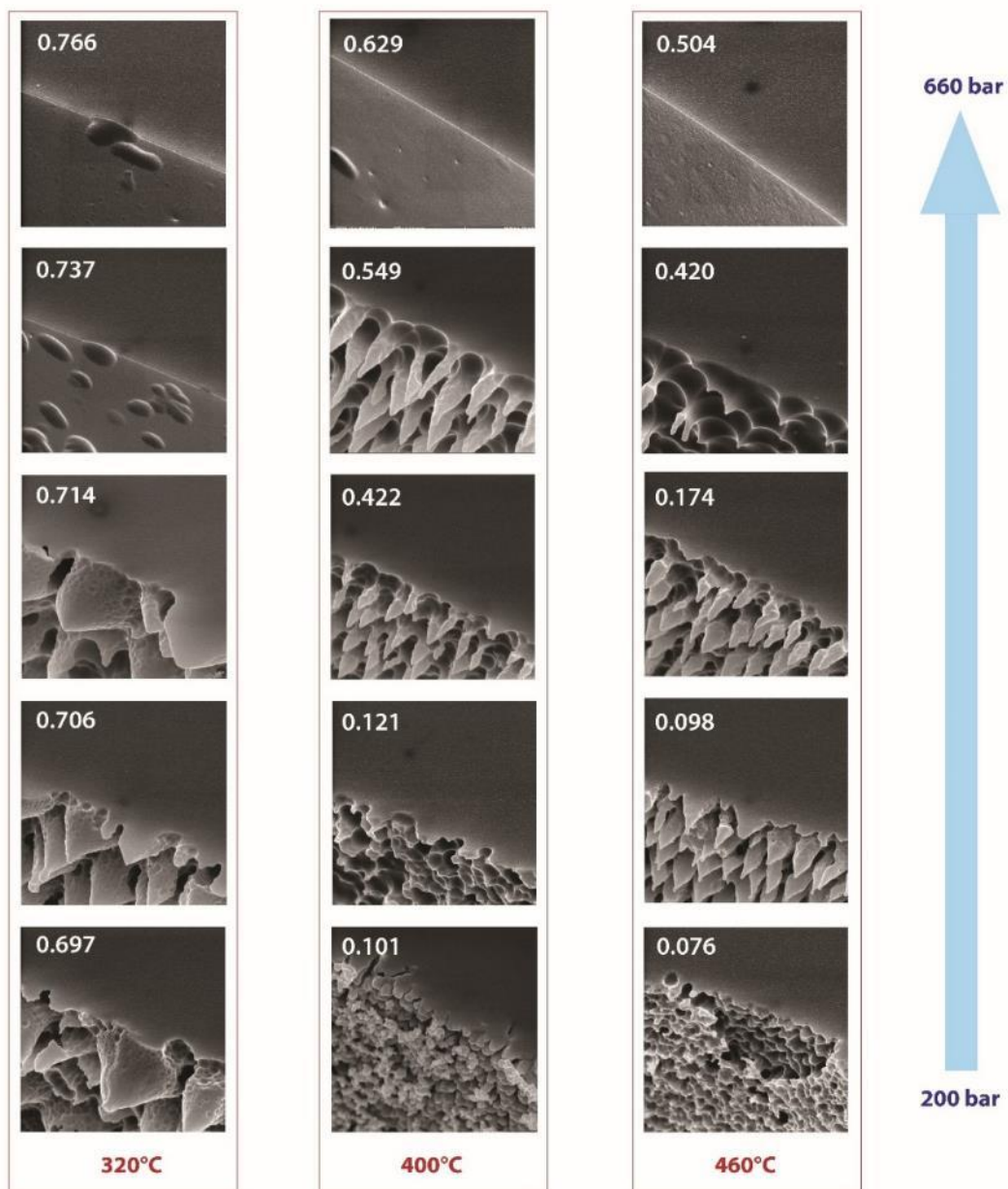


Fig. 8 – isothermal measurements – density controlled by pressure

is probably due to the extremely low density of water (0.076 g.cm^{-3}) allowing its very easy penetration into the micropores of the surface. As in the previous case, the increasing pressure produces "thorns" whose base gradually thins, and at 460 bar (0.420 g.cm^{-3}) it is so thin that the flowing water breaks off the thorns and the fragments are removed from the system.

Figure 9 shows the temperature dependence of the structure (320°C - 460°C) at four pressure levels 250, 310, 340 and 500 bar. At 500 bar and above no structures formed, the high-density water etched the surface of the capillary more or less with no apparent effect. The effect of increasing temperature on the type and size of the etched structure was the higher the lower the used pressure level, the most significant surface changes were achieved at 250 bar. Because pressure and temperature define density, it can appear at first glance that the two dependencies above can be summarized in

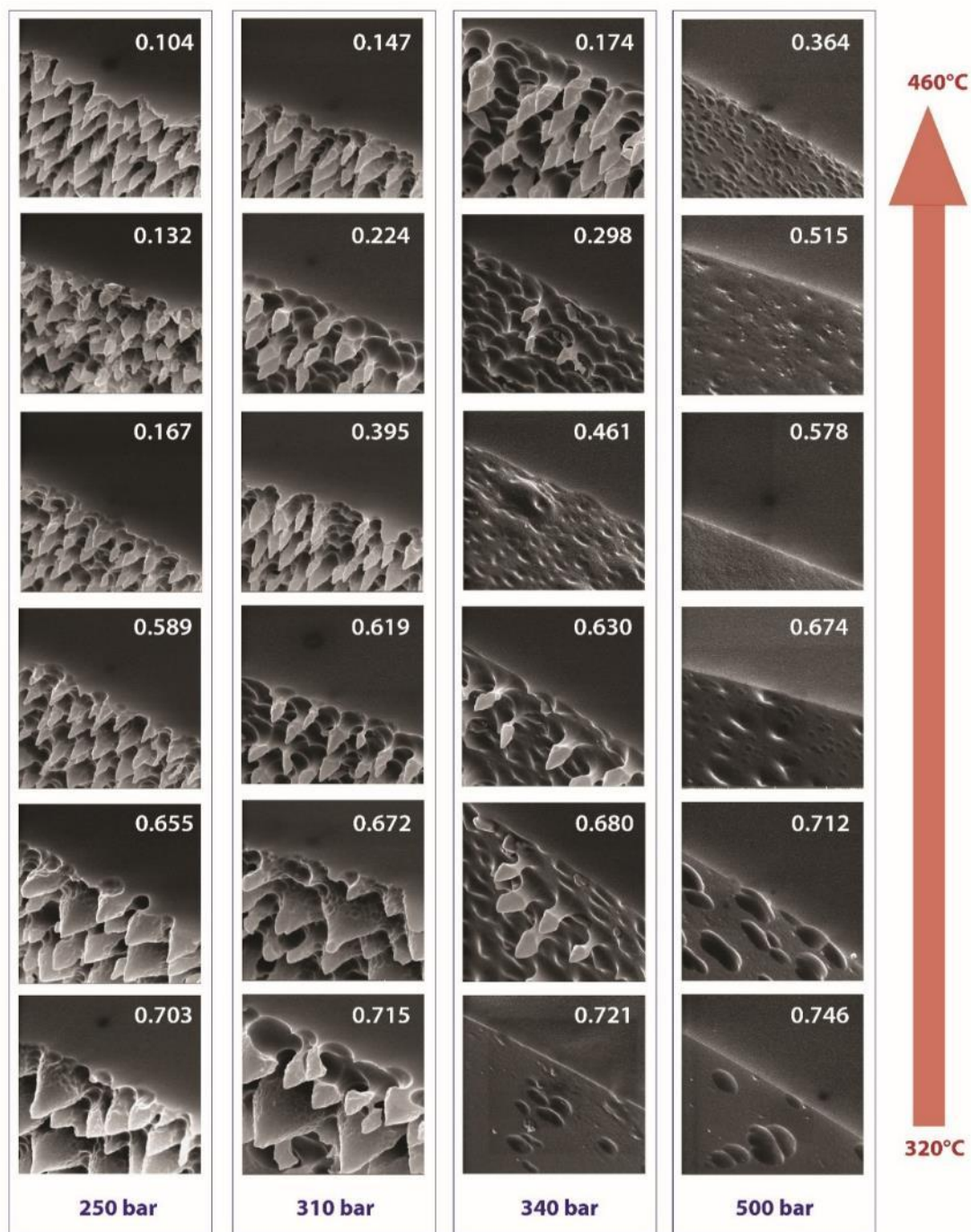


Fig. 9 – isobaric measurement – density controlled by temperature

a single one: density dependence. However, this cannot be simplified because the same water densities can be achieved at different combinations of pressures and temperatures. It is obvious that experiments with constant density, so-called isochoric measurements, would be very interesting and would bring further knowledge about the etching process, but due to the scope and complexity of measurements, experiments of this kind are not in competence of this work.

Dependence on SCW flow rate and capillary movement speed rate

The effect of water flow rate is somewhat simpler to describe. It plays a crucial role in surface formation, especially due to the effect on the dynamics of the process. At a flow rate of 1000 nl / min, the water had enough time to properly heat up and etch the surface and created a densely structured relief. At flows of 2000 and 3000 nl / min, structuring was still taking place, but with a reduced depth and frequency of protrusions. At higher flow rates, the surface was attacked very little, but even such surfaces have a practical utilization, for example, chemical anchoring, separation

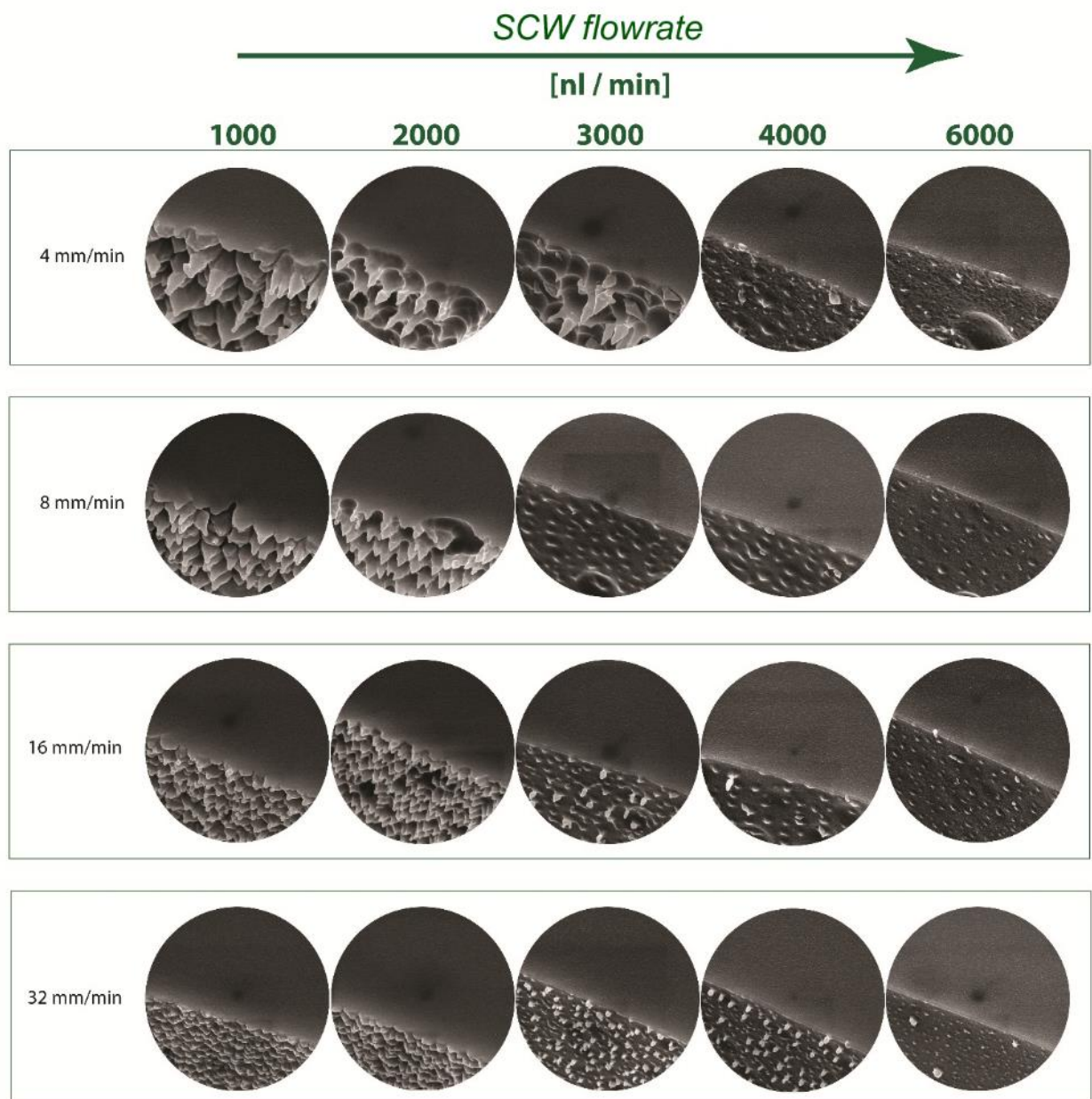


Fig. 10 – influence of supercritical water flow rate

phases or adjustment of electroosmotic flow. One of the parameters, the importance of which in affecting the surface of the resulting structure was assumed from the beginning, was the linear movement speed rate of the capillary through the heating unit. In general, it can be concluded that increasing the capillary movement speed rate reduces the depth of the structure and practically never changes its morphology. When choosing this parameter, we can predict the appearance of the resulting structure before the experiment, with high accuracy.

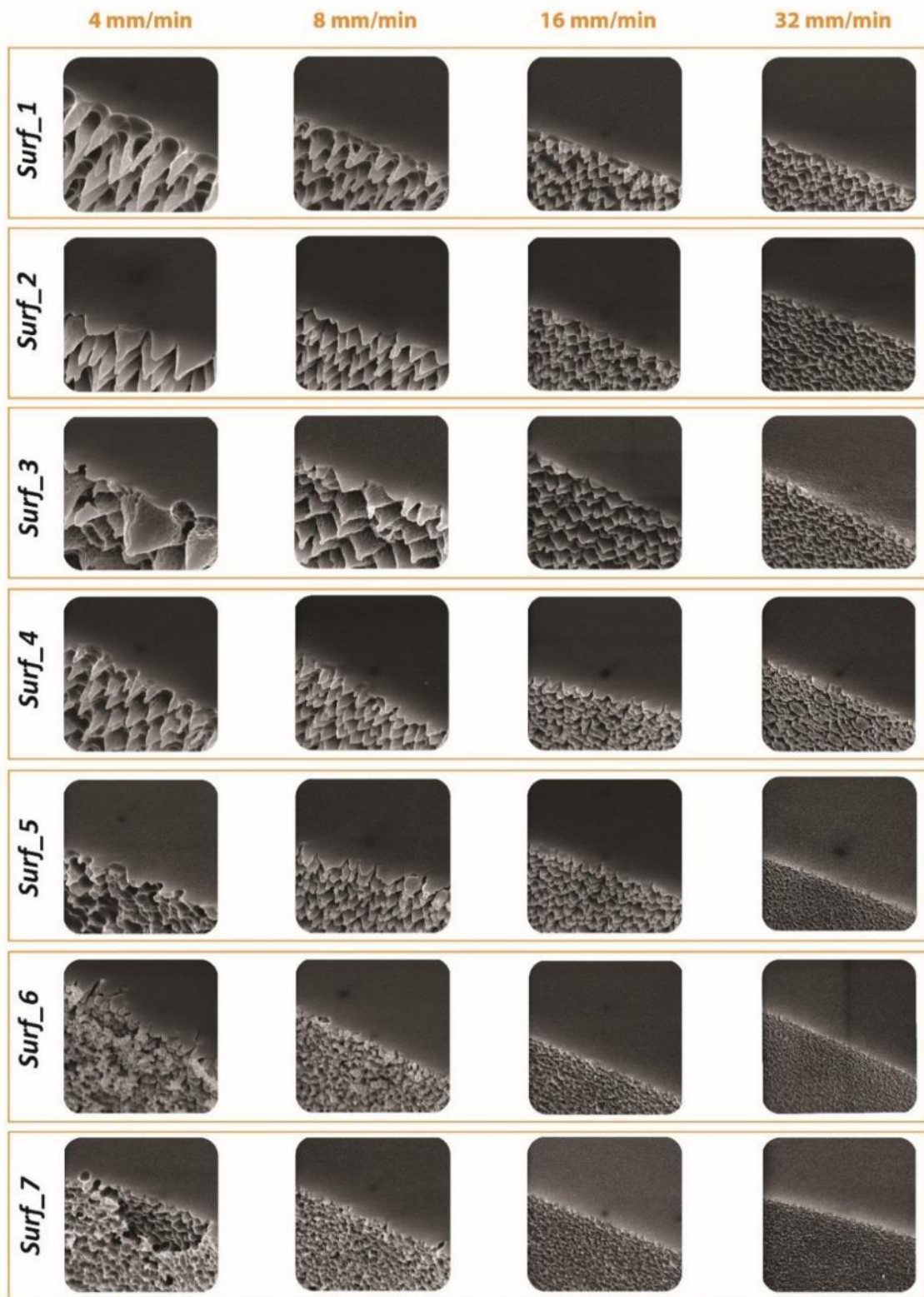


Fig. 11 – size of surface roughness versus capillary movement rate

Selected types of surface structure

However, if we are aiming for the application of etched capillaries in analytical practice, then the main selection criterion should be the variety of created surfaces. Fig. 12 shows a set of images that have been selected precisely on the basis of this criterion. Some of these capillaries were used

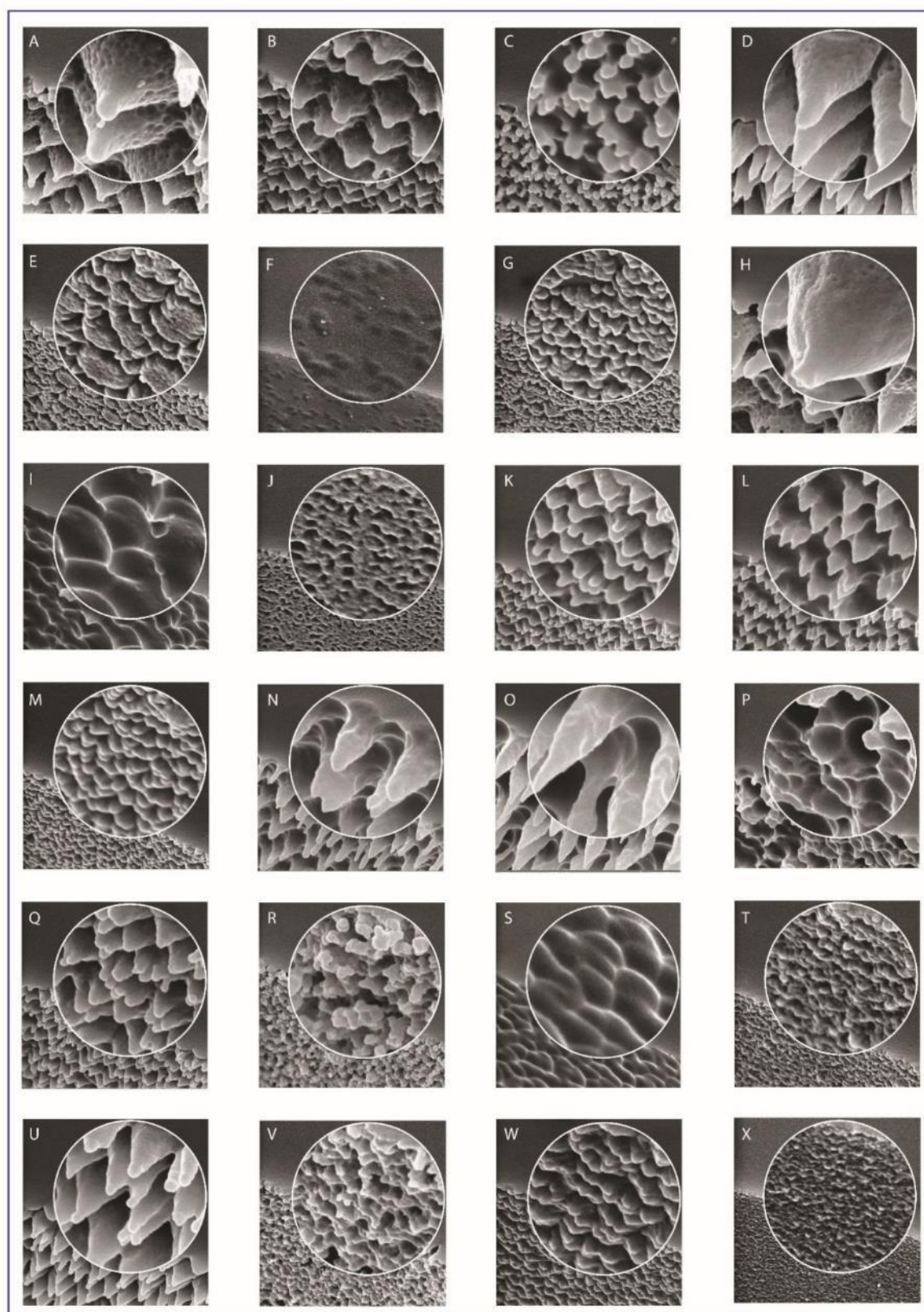


Fig. 12 – selected structure types

for practical measurements, where they achieved unique results, most of them will be subjected to a future research during 2022.

Use in practice

Although the unique properties of structured capillaries can be used in a wide range of analytical applications, the following examples demonstrate their usefulness in electromigration separations of biological samples. As the handling of viruses and bacteria and subsequent analyses requires highly trained personnel, I would like to thank the laboratory staff for this help, which has verified the "functionality" of the capillaries produced this way.

Separation of *Staphylococcus aureus* bacteria was one of the first applications. By introducing a structured surface into a part of the capillary (Fig. 13a), the course of electrophoretic analysis changed so much that we were able to separate not only MRSA and MSSA strains, but also to determine whether they are strains cultured in a laboratory on a dish ("in agar") or whether the bacteria proliferated directly in human blood (MSSA-B and MRSA-B, blue curve) (Fig. 13b).

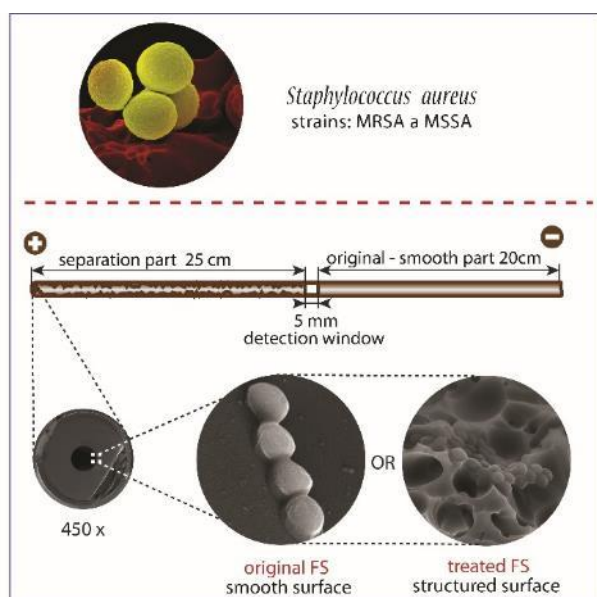


Fig. 13a – *S. aureus* on smooth and structured surface

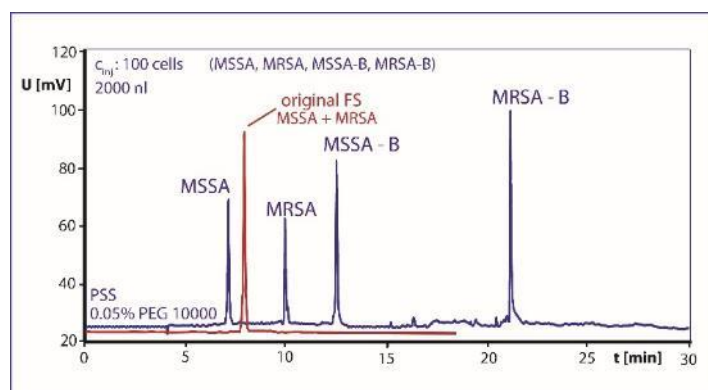


Fig. 13b – *MSSA*, *MRSA* mixture electrophoreogram

Because the structured capillary showed, besides its separation properties, a strong retention capacity of these bacteria, it could also be used to investigate bacteria interactions with bacteriophages (Fig. 14). This property made it possible to study the rate of the bacteriophage propagation process and the extinction rate of bacteria and to determine their number over time. This seems to have huge potential in the case of experimental therapy where currently available antibiotics are ineffective.

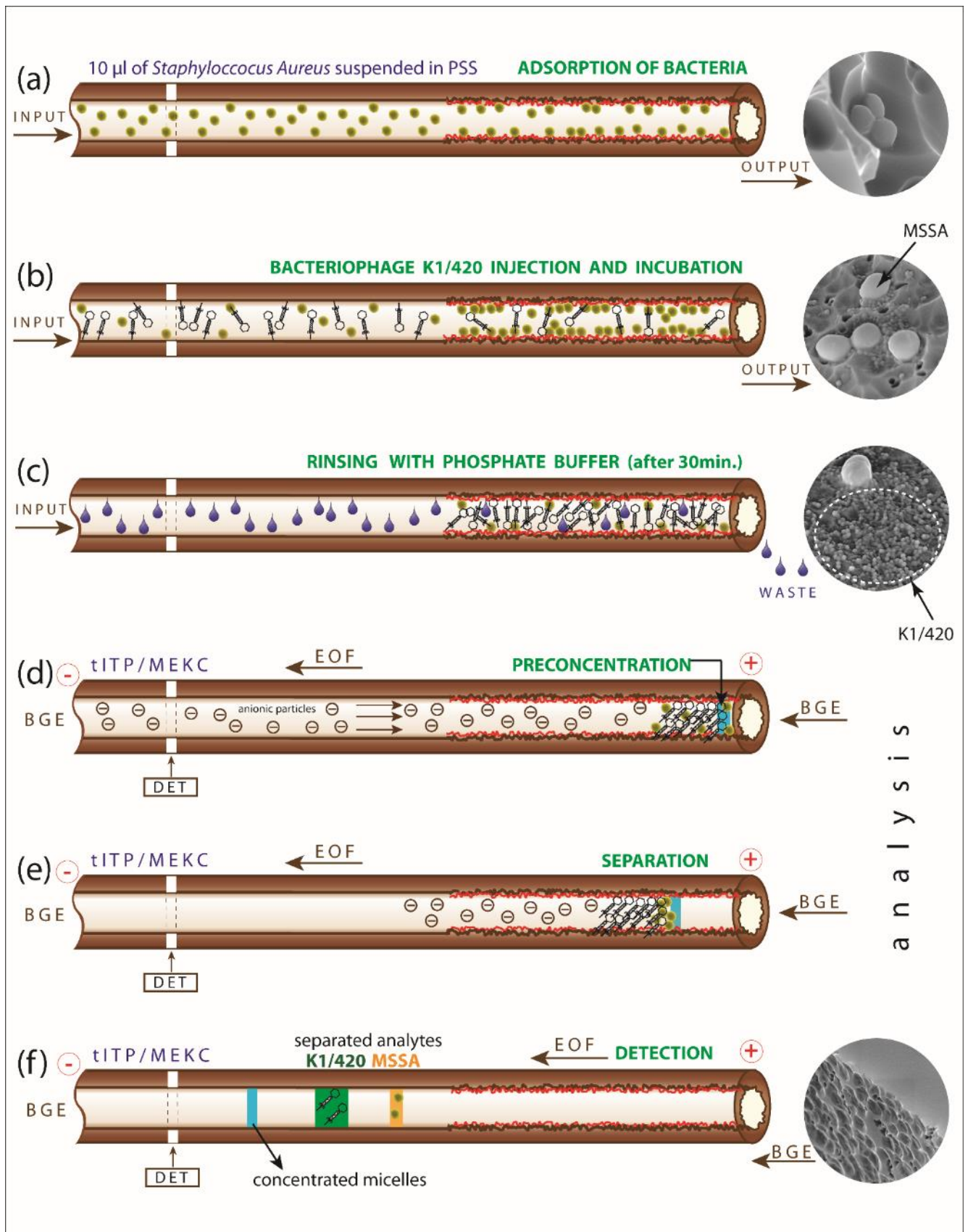


Fig. 14 – propagation and analysis of new growing bacteriophage K1/420

The third example is the determination of the pathogen *Aspergillus* in bronchoalveolar lavage from the patient's lungs. In this case, the structured capillary allowed us to increase the sensitivity of the analysis and perform it in one online step without losses. In this case, we were able to inject up to 100 times less concentrated sample and detect this pathogen much earlier than usual.

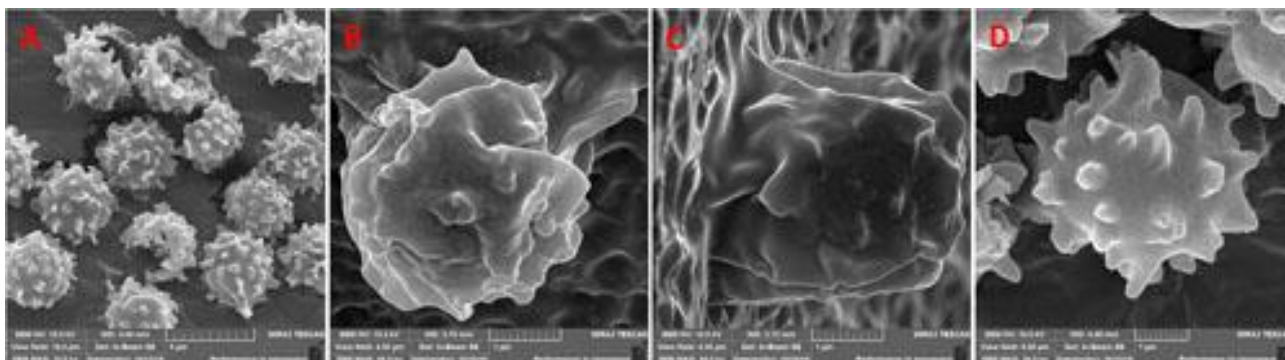


Fig. 15a – SEM of conidia (particles excreted into the air by *Aspergillus* spp.)

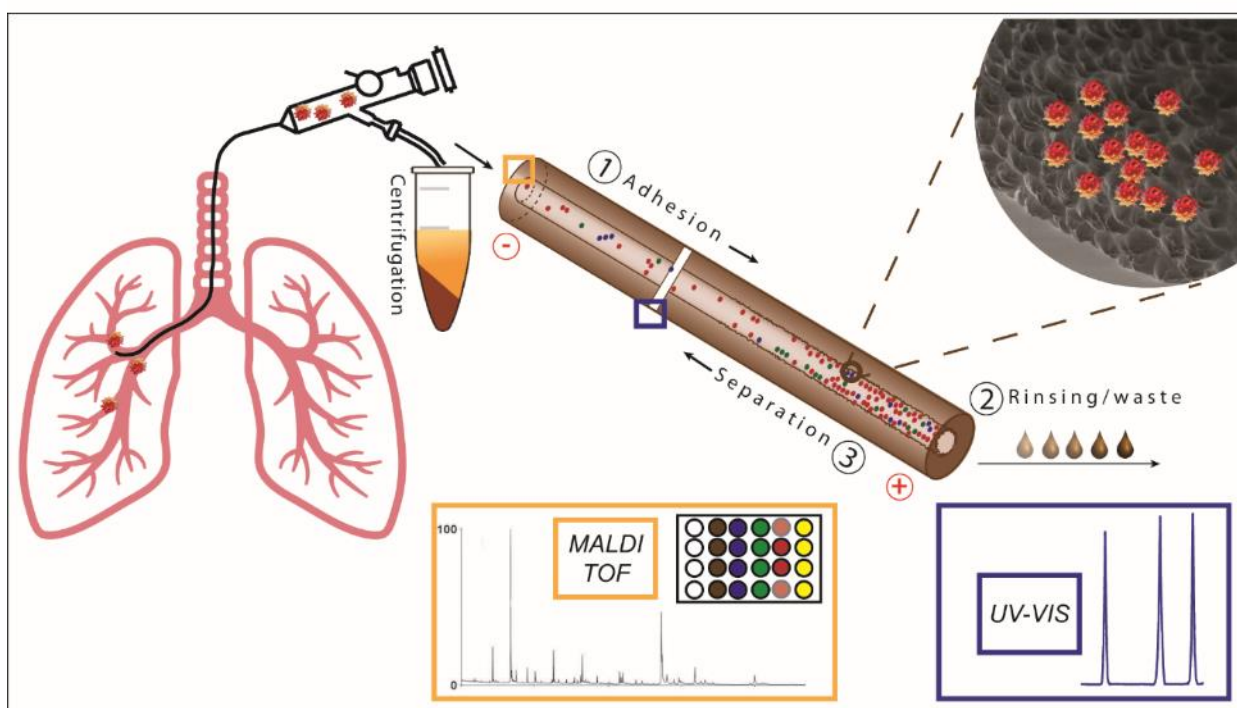


Fig. 15b – collection and analysis of *Aspergillus* spp. on surface-treated capillaries

The last example is the determination of the proteins' contents in cow's milk. It is important not only from the point of view of the milk quality control, but also from the medical one. The most commonly studied proteins found in milk include serum albumin (BSA), α -lactalbumin (α -LA) and β -lactoglobulin (β -LG] from which BSA is the weakest and β -LGs are major allergens in cow's milk. When using capillary electrophoresis with a standard smooth capillary without any modifications, these analytes cannot be separated and are eluted as a single peak.

Figure 16 then shows the result that can be achieved using a capillary with a surface etched with supercritical water, which will allow not only the identification of β -LG-A and β -LG-B in milk, but also the determination of the content of each protein separately.

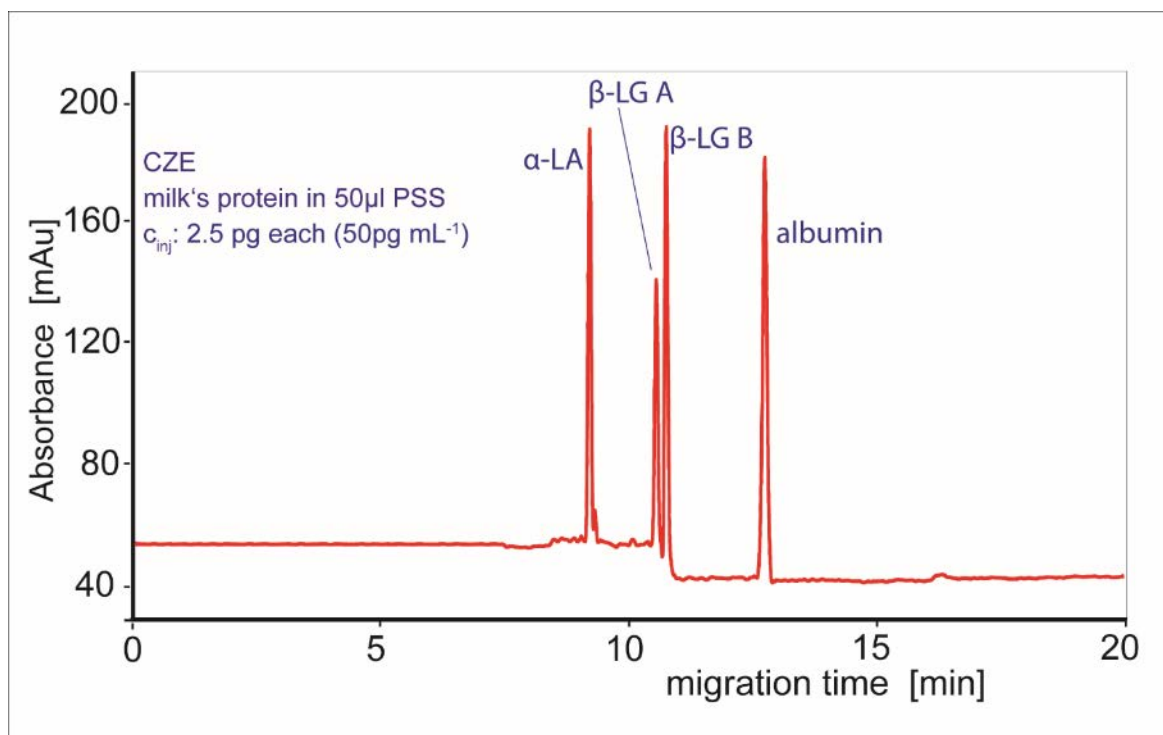


Fig. 16 – example of cow's milk allergen separation on etched capillary

Conclusion

The special properties of water exposed to high temperatures and pressures (SCW) were used to targeted structuring of the inner surface of silica capillaries. Over 2000 experiments were carried out in order to define the conditions suitable for the formation of different types of surface structures and to control their size. The inner surfaces of the capillaries were analyzed by electron microscopy and the obtained data processed by our own database application.

A total of 24 different morphological structures were selected and capillaries with a configuration suitable for electromigration measurements were manufactured. Practical measurements have shown that surface structuring makes it possible to separate analytes that cannot be separated on commercial capillaries, e.g. *Staphylococcal* strains MRSA and MSSA. Furthermore, they can also be distinguished on the basis of origin (agar, human blood). In addition to separation capabilities, the structured surfaces are suitable for capturing bacteria, which can then be infected with bacteriophages for subsequent study of bacteria-bacteriophage interaction. The determination of conidia, particles excreted into the air by *Aspergillus spp.*, in a sample taken directly from the patient's lungs can also be considered as up-to-date application.

In conclusion, although the surface of the capillary can be disturbed by traditional substances, such as NaOH or hydrogen fluoride-based mixtures, only a single type of morphological structure arises. Even more significant drawback is contamination of surface with foreign heteroatoms, which can be an obstacle, especially in the case of sensitive biological analyses. The advantages of using supercritical water are above all the purity of the final product, the ability to create segments with different structures on one capillary and a huge number of combinations of parameters, enabling the creation of other structures, not presented in this work.

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【評語】 030026

Excellent work and very impressive presentation.

Besides use the morphology to evaluate the etched products, you may also perform the gas absorption and desorption to quantitatively determine the surface area of the silica. This will also help on quality control in mass production.