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A New Approach to On-Site Liquid Analysis

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A mixer with an integrated analysis chamber for (bio-) chemical analysis is presented. This mixer as a stand-alone component provides the full functionality of an integrated multicomponent mixing system for one measurement, as both reactants are supplied by capillary forces instead of pumps. Mixing is done by diffusion. Therefore, no additional fluidic components are needed. To evaluate the properties of the mixing process and the suitability for optical sensing, pH values of different solutions have been measured using a universal indicator. The results agreed with macroscopic determination of pH values. An iodine-iodide-reaction was observed using a modified UV/VIS spectrometer and the results agreed with tests performed using cuvettes.

1. Introduction

A growing need of systems for low-cost on-site analysis in environmental and medical testing has encouraged some approaches to micro total analysis systems (μ-TAS).[1-3] However, for lower cost analysis, it may be worthwhile to develop completly new concepts for breakthroughs even in domestic fields.

Mixing plays a major role in typical analytical applications. Effective mixing is crucial for the reproducibility of results because, depending on the type of analysis, results may strongly depend on the concentration of the reagent in the sample. In addition, the economical use of expensive reagents is requested by potential users. Thus the minimum possible amount of reactants should be employed and mixed without loss of reagents inside system components. Surface sensing can substitute for the mixing of reagents in

some applications, but attention must be paid to adsorption to the active surface. Furthermore, the size and the two-dimensional structure of a sensor compared to the three-dimensional fluid distribution may cause inaccuracy.

Existing analytical devices are all based on the same principle: the reactants are stored inside their containers and are supplied by pumps. For reliable analysis, these pumps must provide the exact dosage within some tenths of a percent. Therefore, either principally defined pump volumes (some piston pumps, gear pumps, roll pumps or specially designed valves) are used, or the flow is controlled by a flow sensor/controller unit. The defined amounts of reactants are mixed and transported into the analysis chamber. An additional fluidic circuit is usually added to clean the tubes and chambers.

Miniaturisation seems logical to further reduce the volumes of reactants. All previously presented μ-TASs follow the principles of macro analysis devices. This leads to major drawbacks: unless reactants are carefully filtered, clogging by particles is always a concern. Even small air bubbles can knock out the system, until it is dried and refilled again (priming with carbon dioxide to prevent air bubbles has been proposed by Zengerle *et al.*[4]). In particular, pumps and valves, with gaps of some tens of microns, can easily be blocked and destroyed. Existing micropumps usually do not provide the required dosing accuracy and are in no way reliable for any long period. Therefore, modular systems have been developed to enable the simple exchange of broken parts. But the required fluidic connections in the microworld are difficult to make, usually leading to ballast volumes (volumes not needed for straight fluid transport) and hence increasing the overall system volume. Fully equipped μ-TAS can be very expensive and fragile.

2. New Approach to Mobile Analysis

Because analysis is often only a tool for everyday research tasks and not the final goal, steps of the analysis should ideally be reduced to the simple task of adding a reagent into the fluid to be analysed. Pre-analysis tasks such as filtration should be decreased to the minimum required. The system itself must ensure the right dosage and other requirements. However, to keep system costs low, extensive use of electronics and consumption of materials must be avoided.

Therefore, the crucial questions that must be addressed in developing μ-TAS are: what type of tool is required, and how much 'luxury' can be provided? The crucial devices for analysis systems are the mixer and the analysis chamber, because all other components serve only to enable the function of the former.

The analytical tool required for everyday analysis tasks varies with the application. For on-site analysis, the major considerations are weight and size reduction. Furthermore, every system must be robust. However, maximum system integration might be as redundant as maximum mixing speed, since every user of an on-site analysis could actuate a manual pump. Mixing times are not a significant issue when paperwork must be done in the interim for documentation. A μ-TAS based on flow injection analysis (FIA) is considered too complicated for mobile use, and cleaning facilities can be omitted when disposable parts are used. These measures can contribute to the reduction in the complexity of the system.

The most vulnerable microcomponents of fluidic systems are the membrane actuators (pumps and valves), but the fluidic actuation can be achieved through mere physics instead of components: the entire system could be filled by capillary force. Mixing, of course, is achieved by diffusion. However, the mixing of two reactants is difficult; how can one capillary be filled by two reactants using capillary force?

3 Capillary-Force Filled Mixer

A device fulfilling the above requirements is the mixer filled by capillary force presented in ref. (5). An enlarged view of a chip containing two capillary-force filled mixers is shown in Fig. 1. The silicon chip consists of two anisotropically etched capillaries per mixer, separated by a thin very long plate clamped on three sides (in the following called plate) made of silicon dioxide. It is covered by two glass plates, each containing reagent supply holes for one channel.

The working principle can be seen in Fig. 2. There is a small gap (20 μm to 43 μm) between the plate in the virgin state and the glass plate. When capillary 1 is filled by capillary force, the plate bends towards the glass plate because of the surface tension of the reagent. The gap is closed and hence the first reactant is kept inside capillary 1. Then, capillary 2 can be filled, also by capillary force. Due to force equilibrium, the plate bends back and the gap opens. Immediately, mixing by diffusion begins. The size of the capillaries defines the mixing ratio within the tolerances of microtechnology. Both

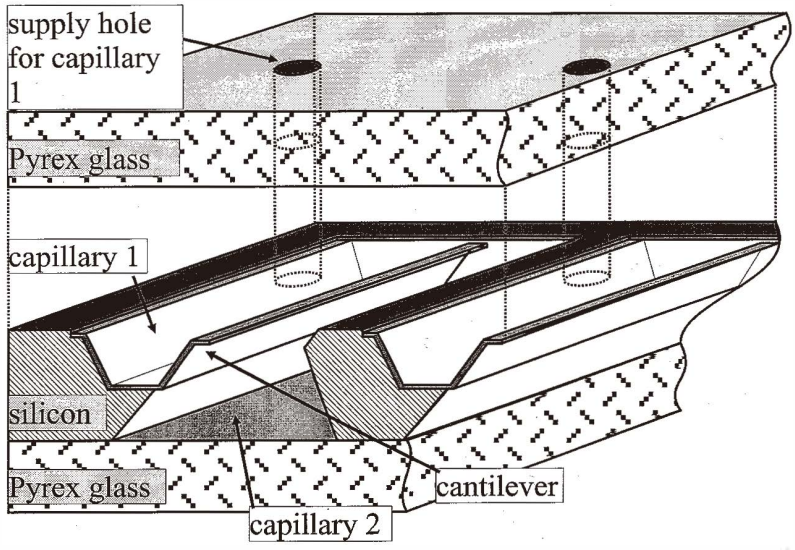

Fig. 1. Structure of the capillary-force filled mixer in silicon.

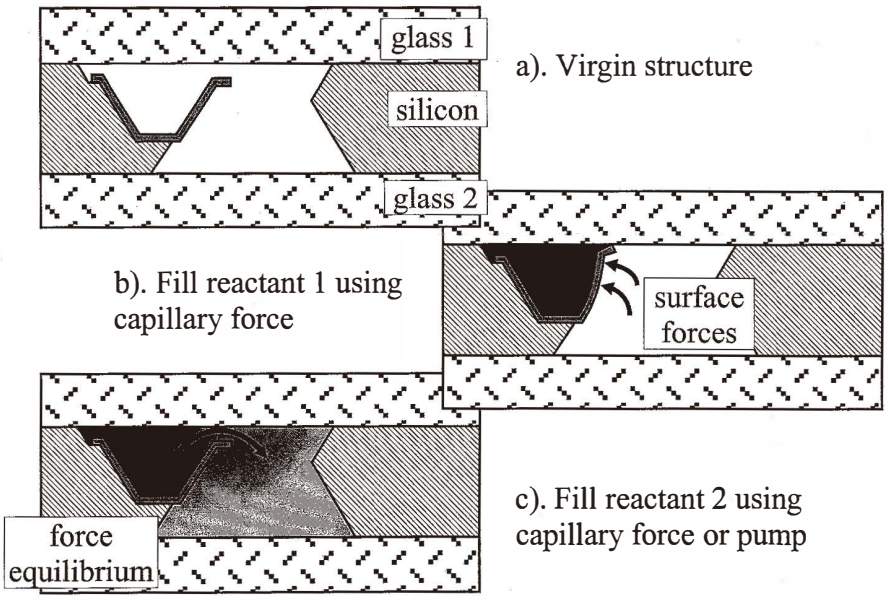

Fig. 2. Working principle of the capillary-force filled mixer.

capillaries must be hydrophilic for capillary force to work.

After both capillaries have been filled by one drop of reactant into one of the supply holes, the mixing chip can be inserted into a spectrometer or a microscope to enable observation of the mixing process. Provided electrodes are integrated on the glass plates, electrochemical reactions can also be observed *in situ*. Because of its closed design, the mixer suits all applications where evaporation during analysis is an issue. After all measurements have been completed, the mixture can be pumped or sucked out of the mixer. However, in many application fields, this is not necessary.

4. Theory

4.1 *Capillary forces*

The filling of both capillaries is achieved by capillary force. Therefore, the principles of capillary force will be shown here. The contact angle θ (Fig. 3) is determined by the competition between the liquid-liquid molecular forces and the liquid-solid forces and depends on the particular solid and liquid involved. It also depends on how smooth and clean the solid surface is. The shape of the meniscus inside a hydrophilic capillary is defined by the contact angle and the surface tension γ: the liquid always seeks the state of minimum energy. The force pulling the liquid into the capillary F_{up} and the pressure inside the capillary p_{cap} can be calculated as shown:

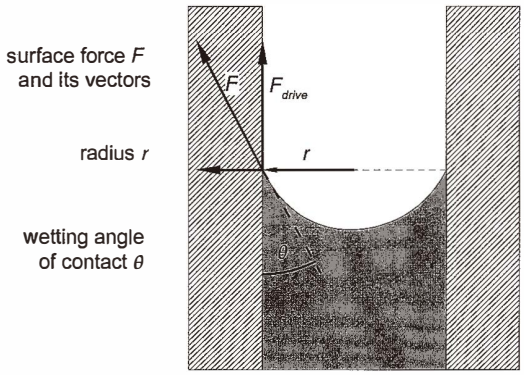

Fig. 3. Principles of wetting angle and capillary forces.

$$F_{up} = 2\pi r\gamma\cos(\theta)$$

$$p_{cap} = p_0 - 2\times\frac{\gamma}{r}.$$

With the contact angle $\theta_{water\text{-}glass} = 5°$ (between 0° and 5° depending on the cleanliness of the surface), the surface tension of water $\gamma = 7.1 \times 10^{-2}$ N/m and a radius of $r = 150$ μm, we obtain $F_{up} = 66.9$ μN and $p_{cap} = p_0 - 473.3$ N/m^2.

The rate of movement of the meniscus inside the capillary depends furthermore on the resistance of the capillary and the connected parts. In the case of the mixer, the supply holes are large due to their processing (sandblast hole drilling) and can be neglected. Therefore, filling is very rapid.

4.2 *Spectroscopy*

Here, only transmission spectroscopy will be discussed. This is for simplification since reflection spectroscopy follows the same principles: only the assembly of the latter is different and the optical path is doubled.

A light source is focussed on one side of the sample and detected on the opposite side. Incident light is reflected at phase transitions and scattered or absorbed inside the solution. Hence, the reflected and the scattered parts cannot be used for detection but a part of the incident light can be detected. Comparison between the initial and absorbed spectra reveals specific information about the sample.

The absorption is defined as the product of the concentration c, the optical path d and a spectral absorption coefficient κ (law of Bouguer-Lambert-Beer):

$$A(\lambda) = \kappa(\lambda)\times c\times d.$$

It can be seen that the reduction of the optical path reduces the absorption linearly. The

optical path of the capillary force mixer is d_{CFM} = 300 μm. Therefore, the expected absorption inside the capillary force mixer A_{CFM} is about 3% compared with the absorption inside standard cuvettes (d_0 = 10 mm, $A_{CFM} = A_0 \times d_{CFM}/d_0$). However, alignment of the focussed light source with the capillary is not easy and this might contribute to the losses, as do the long SiO_2 plate (crossed by the beam diagonally) and the cover glass plates, which are still very thick due to their processing. Clearly, reflection and scattering reduce the signal for detection and hence either a stronger light source or better detection devices is required. As the absorbed light is transformed into heat, the probe itself limits the intensity of the light source.

5. Experimental: Mixing Colours

A simple means of observing a mixing process is to watch two colours mingle, as presented in ref. (5). In all experiments therein, capillaries were filled by placing small drops of de-ionised water (DI water) mixed with food colouring into the reagent supply holes using a syringe. First, capillary 1 was filled with red, then the chip was turned round. Second, capillary 2 was filled with blue, and mixing was observed, taking photographs of each capillary after 30 s. In the case of the capillary-force filled mixer, both capillaries can be observed during mixing, and the mixing is complete when a uniform colour can be seen.

The human eye can resolve colours reliably, but for comparison of different mixing states, digital images were recorded. Using a photo-editor, JPEGs (photos *.jpg) can be transformed to such an extent that pixels of the colour of the final state on the last images are replaced by white on all images. Thus, a growing white zone indicates the progress of mixing. The colour transformation was performed with a tolerance of 8% (maximum concordance with subjective visual monitoring was between 8% and 10%). Figures 4(a) to 4(d) show diffusion after 0.2 min, 2 min, 3 min and 8 min. The ring shape, which can be seen on all images, is due to the circular light source of the microscope and bears no relation to the diffusion.

As presented in ref. (6), particles have been added to the mixed DI water to prove robustness against particles. Two different powders were used: Al_2O_3 with a particle size of 0.5 μm to 0.8 μm, and $ZrSi_2$, with a particle size from 1 μm to 100 μm (80% of the grains were smaller than 10 μm).

To obtain more detailed data for the mixing performance and to compare the results with those of macroscopic tests, the pH values of different buffer solutions (for calibration of pH sensors) were measured using liquid universal indicator with the mixer inside a beaker, with an indicator paper. Special attention was paid to the ability to distinguish colours because the optical path of the mixer is only 300 μm and the intensity of the colour is weak.

The measuring of spectra in microdimensions was tested using a modified UV/VIS spectrometer. Its (VIS-) light beam was focussed to about 1 mm diameter and coupled to the glass fibres. It was directed from the top of the mixer through the capillaries. Detection was performed from the bottom. The chemical reaction chosen for testing was that of starch with iodide (Fe(III)/starch-iodide). Iodide solution was added to Fe(III)/

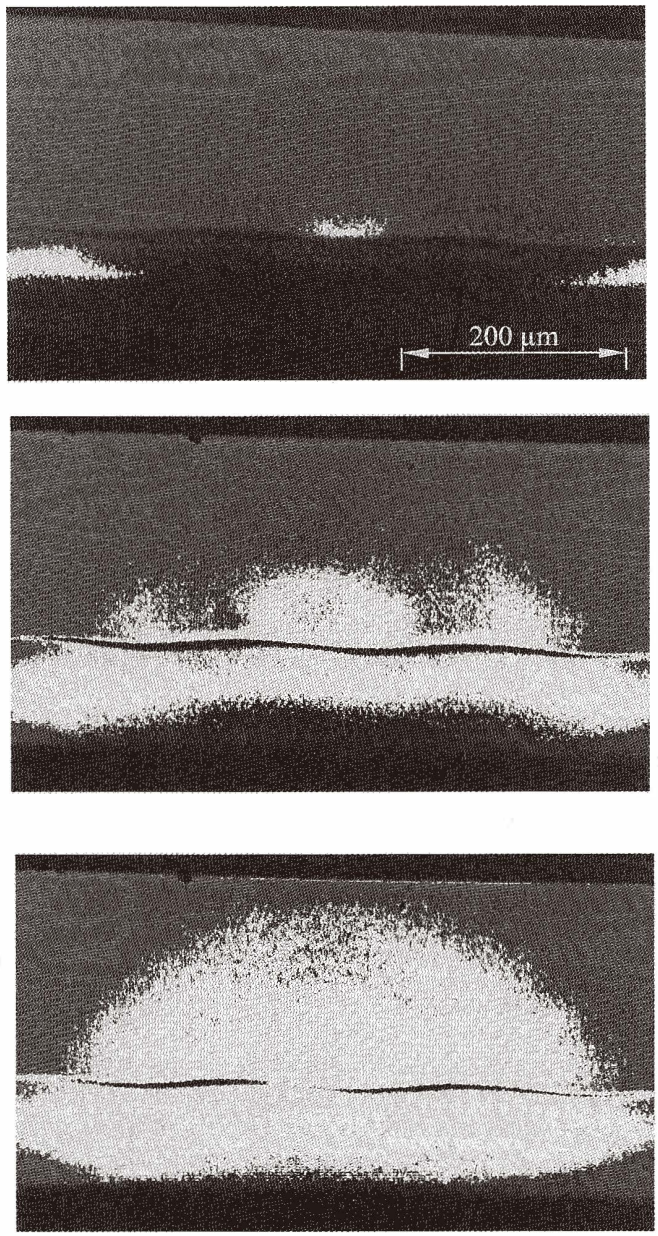

Fig. 4(a)–(c). Mixing profiles after (a) 0.2 min, (b) 2 min, and (c) 3 min. The white zones indicate completed mixing. Inside the capillary, the edge of the bending plate can be seen as a dark line. The ring-shape is due to the circular light source of the microscope and not to the diffusion process.

(d)

Fig. 4(d). (continued) Mixing profiles after 8 min. The white zones indicate completed mixing. Inside the capillary, the edge of the bending plate can be seen as a dark line. The ring-shape is due to the circular light source of the microscope and not to the diffusion process.

starch until a dark blue colour was observed. This colour derives from the embledding of the iodine atom into the helix of glukose. The iodine-iodide-reaction is a balanced reaction and fully reversible:

$$I_2 + 2\,e^- \leftrightarrow 2\,I^- \ .$$

Using the revamped UV/VIS-spectrometer, the colour change of the mixture was first detected using standard cuvettes and then the capillary force mixer, each with a frequency of $f = 10 \times 1/s$. All measurements were performed using 64 scans, an integration time of 0.025 s and wavelengths from 400 nm to 800 nm. At the end, the results were transferred into three-dimensional graphs.

6. Results and Discussion

A parameter variation has shown the effects of the size of the gap on the mixing times.[5,6] All capillaries have a length of 10 mm, and their depth and width are recorded in Table 1. Some mixing results can be seen in Figs. 4 (a) to 4 (d). Depending on the gap, the mixing times varied: 7 ~ 9.5 min for a 20 μm gap, 6.5 ~ 8.5 min for 30 μm, 6 ~ 6.5 min for 36 μm and 5.5 ~ 6 min for 43 μm. These times are long because the diffusion area is small compared with the diffusion distance (lateral dimensions of the capillaries). A new design to be published soon significantly reduces the ratio of diffusion distance to diffusion area.

The alcoholic universal indicator from Merck has a much lower surface tension than water. Hence, it was of interest to observe whether the capillaries could be filled separately. The mixers with gaps larger than 30 μm were difficult to fill. Clearly, the surface tension was too small to close the gap and the rapid wetting of the second capillary

due to the low wetting angle of contact caused the indicator to spill into capillary 2. Even with small gaps, problems occurred: the evaporation of alcohol at room temperature is rapid, so the filled capillaries cannot be stored for longer than before capillary 2 is moistened by condensation. This does not prevent capillary 2 from being filled, but it influences the longitudinal distribution of the mixing ratio.

The pH tests provide results almost immediately after reagent 2 has been supplied. This is expected, as the principle of pH measurement does not depend on diffusion and mixing ratio. However, the colour became much more intense. The concentration of the indicator had to be much higher than described in the manual. Low concentrations resulted in difficulties in evaluating the results using digital images and in comparing the colours with the colours of the same buffer solution as that tested inside the beaker. With the adjusted concentration, the optical path was sufficiently long to distinguish colours reliably. The pH values of various reagents measured using the new mixer are compared with values obtained using indicator paper in Table 2.

The absorption versus time of the iodine-iodide-reaction inside the standard cuvette is shown in Fig. 5. It has a peak at 580 nm and measures $A = 1.25$. Then, the same reactants were filled into both capillaries of the mixer. Figure 6 shows a spectrum of those measurements. The spectrum changes in the same way as before: the absorption has a peak at 580 nm and measures about $A_{CFM} = 0.015$. This is a decrease to about 3%, which is almost of the same dimension as the decrease of the optical path. The great difference between all miniature measurements is due to difficulties in aligning the lens with the glass fibres. At the beginning of each measurement, the lens was aligned to receive a strong signal but no saturation. Sometimes, however, saturation was reached during the

Table 1
Variation of the capillary geometry depending on safer thickness.

Dimensions of the mixer	Capillary 1		Capillary 2	
	Depth	Width	Depth	Width
Wafer thickness: 250 μm	135 μm	280 μm	250 μm	600 μm
Wafer thickness: 300 μm	175 μm	350 μm	300 μm	600 μm

Table 2

pH values of standard buffer solutions measured by different methods.

pH value of the buffer solution	Universal indicator inside μ-mixer	Universal indicator inside beaker	Paper indicator
pH=4.01	4	4	4
pH=7.0	7	7	7
pH=9.0	9	9	9

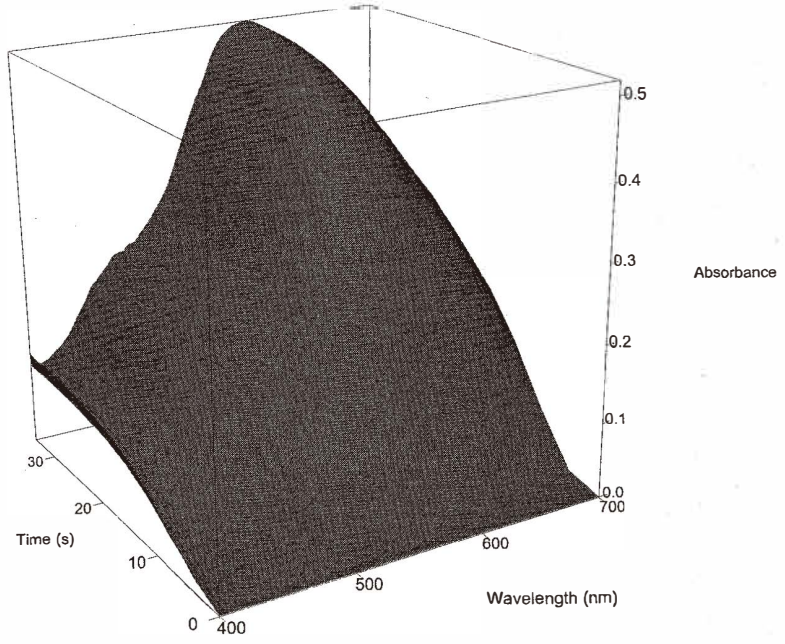

Fig. 5. Spectrum of the iodine-iodide-reaction inside a standard cuvette.

reaction. The overall system setup can still be improved further.

As expected, some additional losses occurred due to the reflection inside the structure of the mixer. Noise must also be considered, because the signal was small; however although it is clearly visible on the graphs, noise is not a significant concern.

7. Conclusion

The capillary-force filled mixer can be a powerful tool for on-site analysis. Mixing occurs in a few minutes; results of faster mixing with new designs will be published soon. The long mixing times compared with those of the μ-TASs published previously may not be a disadvantage. Most users are not able to handle a thousand samples per minute. The long reaction times offer the possibility of obtaining similar results to FIA, and even the long-time *in-situ* observation of very slow reactions is possible. After both capillaries have been filled, the reactants are enclosed, and evaporation cannot occur. Therefore, one of the reactants can be filled a long time before use.

The qualitative reliability of the mixing results has been previously demonstrated.[5-6] The new results show that the results of pH measurements using the capillary-force filled mixer are comparable to those of macroscopic tests. The results are obtained immediately, because the dosage only influences the colour depth, not the colour itself. However,

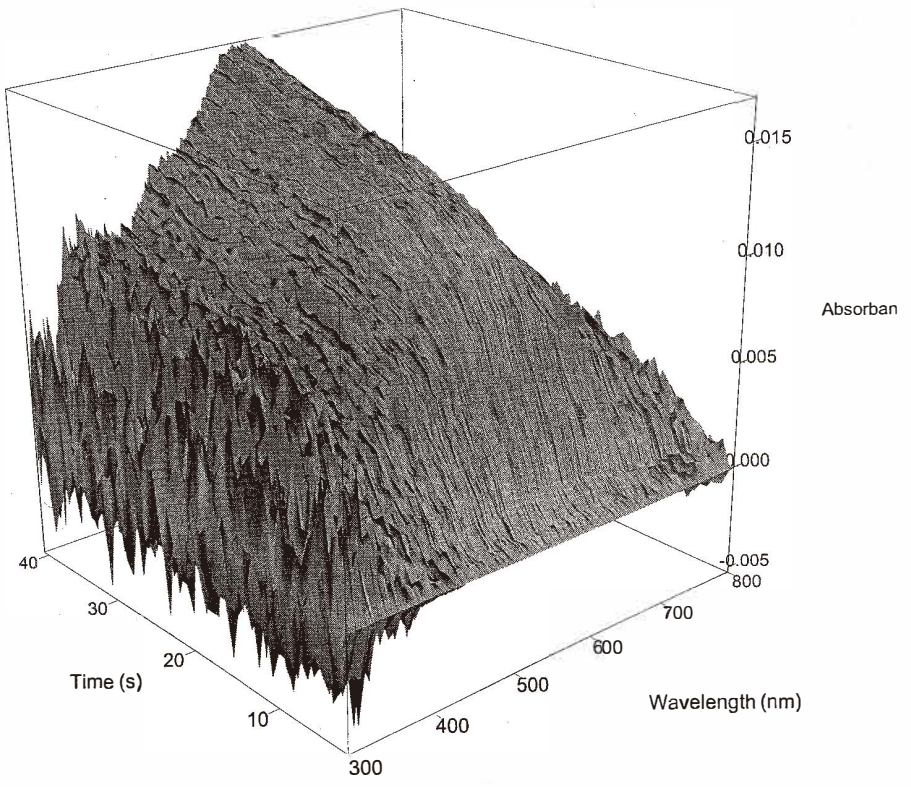

Fig. 6. Spectrum of the iodine-iodide-reaction inside the capillary force mixer.

alcohol did not prove to be an appropriate fluid because of its small surface tension and very small contact angle. A more suitable method was to fill the first capillary with an aqueous reagent and to add the indicator into the second capillary.

For on-site analysis, the mixer can be filled with a reactant during preparation inside the laboratory, sealed with tape and opened directly before use; the opposite may also be done. Given the low evaporation rate at the storage temperature, reactants could be filled into capillary 1 long before use. Capillary 2 is then filled on site. Hence, this procedure makes very simple μ-TAS possible, and the cost of the system as well as analysis costs should be low. Particles do not influence the mixing process.

A slightly modified UV/VIS spectrometer can be used to detect the spectra even with a very small optical path. A major part of the signal is lost because of reflection inside the structure of the mixer. Absorption is small, but a sufficient signal-to-noise ratio is achieved. The shapes of the spectra equal those of measurements using much larger optical paths; the mixer itself does not show specific absorption in the observed wave-

lengths.

The capillary-force filled mixer exhibits many new features which have the potential to make it a universal tool for everyday analysis.

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