One- and two-dimensional microtoxicological screenings in nanoliter-fluid segment sequences with fast micro photometric and bead-based fluorimetric read-out

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Motivation

- Over 100.000 chemical substances are commercial available in the European Union^{1,2}
- New EU chemical legislation REACH
- Need in research for determining the risk of the combined effect from two or more substances^{1, 3, 4}
- High complexity with high request to the conventional toxicological standard methods
- There is a strong demand for new efficient methods^{5, 6}
 - 1. REACH: Die neue Chemikalienpolitik in Europa, Bundesinstitut für Risikobewertung, 2008
 - 2. http://www.euronews.net/de/article/17/06/2008/chemicals-under-watch, 2008
 - 3. Fent, K.: Ökotoxikologie, 3. Auflage, Stuttgart: Gerog Thieme, 2007
 - 4. Witte, I et al.: BIS-Verlag, Oldenburg, 2007
 - 5. Grimme LH et al.: Human and Ecological Risk Assessment 2: 426-433, 1996
 - 6. Filser J.: Basic and Applied Ecology 9(4), 333-336, 2008







- Assembling and optimization of a micro fluid system by the application of the segmented-flow method for the characterization of combined effects of environmental pollutants
 - Integration of pH-sensitive sensor particles in microfluid segments
 - Assembling and optimization of fluorometric sensors for pH measurement in a micro fluid system
 - Integration of different detection units for the monitoring of varied toxicological endpoints such as cell density and segmentinternal pH
 - ➤ Application for biological studies → toxicological screenings with selected micro organisms



Benefit of micro fluidic method

Droplet flow against conventional methods:

- High throughput of small volumes
- Safe handling
- Smaller amount of chemicals required
- Reduced costs

Droplet flow against microtiterplates:

- Less evaporation
- Screening of multidimensional parameter fields
- Reduced wall contact
- Mixing by segment-internal convention
- Smallest volumes



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Principle of "segmented Flow"





Material and Methods



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Optical pH-sensitive particles

- HPTS/p-HEMA particles^{1,2} (8-<u>H</u>ydroxy-<u>p</u>yrene-1,3,6 <u>tris</u>ulfonic acid of amino-modified poly (hydroxyethyl methacrylate)
- Spectral properties
 - > Excitation : $\lambda ex = 468 \text{ nm}$
 - > Emission: $\lambda em = 530 \text{ nm}$

Pyren-Derivate



HPTS 8-Hydroxypyrene-1,3,6-trisulfonic acid



- 1. Funfak, A: Microchim Acta 164, 279-286, 2009
- 2. Source: Dipl.-Chem. P. Schrenkhammer, Institute of Analytical Chemistry, University Regensburg

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Material and Method

 Measurement of the parameter growth and pH inside microfluid segments





General Experimental-setup



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Slide 10

LabView applications for the continous segment generation

generation and analysis of fine graded concentration gradients



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LabView applications for the continous segment generation

generation of fine graded concentration gradients for the screening 2-dimensional parameter fields



LabView program for the investigation of combined effects (binary mixcures)



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Results and Discussion

Investigated substances:

2,4 Dinitrophenol (DNP)
2,4 Dichlorphenol (DCP)
Au - nano particles (Au-NP)



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Results & Discussion

Effects of 2,4-dinitrophenol – concentration resolved¹



1. Funfak, A: Sensors and Actuators B: Chemical 142 (1), 66-72, 2009



Results & Discussion

- Combined effects of 2,4-dichlorophenol and Au-NP
 - 2-D plot of the end point scattering (left) and for parameter normalized intensity ECFS (right)





Conclusion

- The growth of model organism *E. coli* had been determined within very low cultivation volumes (80 nL-400 nL)
- The application of a fluorometrical detector enables the simultaneous monitoring of the fluorescence signal of pH-sensitive micro beads inside microfluid segments
- Highly resolved dose response relationships can be determined for the effector DNP.
- Weak synergistic effects had been found for the binary mixture DCP and Au-NP on the growth and the autofluorescence of *E. coli* cultivated inside microfluid segments
- The potential of the introduced methods for both single and combinatorial toxicological studies had been shown

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Outlook

- Further optimization of sensors and analysis method, e.g. integration of pO₂-sensitive sensor particles
- Enlargement of 2-dimensional concentration fields for toxicological screenings
- Investigation of combinatorial effects on eukaryotic cells, human cells etc.



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Thank you for your attention

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Backup



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Isobologramm



Isobologramm für ein Effektniveau von 50 % (in Anlehnung an Loewe, 1953).

