Proceedings of the Estonian Academy of Sciences,
2012, **61**, 1, 52–57
doi: 10.3176/proc.2012.1.07
Available online at www.eap.ee/proceedings

CHEMISTRY

Ultrasonic on-line spectrophotometric system for reaction kinetic measurements

Sander Piiskop[*], Gerda-Johanna Raidaru, and Siim Salmar

Institute of Chemistry, University of Tartu, Ravila 14a, 50411 Tartu, Estonia

Received 25 May 2011, revised 22 September 2011, accepted 22 September 2011, available online 15 February 2012

Abstract. A unique ultrasonic online spectrophotometric system for kinetic measurements of homogeneous polar reactions under ultrasound was developed. The setup consists of an ultrasonic bath, an HPLC UV/Vis detector, an HPLC pump, a refrigerated heating circulator bath, and a data processing unit. An analogue–digital converter was constructed and a data processing program AD_com was written for registration of detector signal. The pH-independent hydrolysis of 4-nitrophenyl chloroacetate as a model reaction and also sonolytic degradation of 4-nitrophenol were used for the examination of the online spectrophotometric system. Preliminary kinetic studies proved that the system can be successfully applied for ultrasonic kinetic investigations of polar homogeneous reactions. This online system allows a sensitive and reproducible monitoring of various homogeneous processes, which can be detected spectrophotometrically under ultrasonic irradiation.

Key words: ultrasound, homogeneous polar reaction, kinetic measurements.

INTRODUCTION

Ultrasound has been used by chemists and chemical engineers for a variety of purposes in different areas, mainly because it can promote or accelerate a wide range of chemical and physical processes [1–4]. The nature of sonication effects on heterogeneous processes and free radical reactions is well understood by now. Cavitation induced by ultrasound can bring about mechanical effects and promote many reactions by generating free radicals that produce chain reactions in solutions [3,4].

Moreover, a number of homogeneous polar reactions that are not switchable to a radical pathway have been accelerated by ultrasound (for a review see refs [5,6]). Application of quantitative methods, including kinetic measurements and correlation analysis, to the study of homogeneous ionic reactions under sonication in aqueous and aqueous–organic binary solvents has brought to light specific solute–solvent interactions and hydrophobic effects that are not manifested in conventional kinetic investigations [7–10]. It has been concluded that the sonochemical effects are related to the perturbation of the molecular structure of the solvent and the destruction of hydrophobic solute–solvent interactions. In this regard, ultrasonic kinetic measurements as a method are a useful tool for physico–chemical investigations to obtain information not easily accessible by any other method [5,7–9].

Numerous studies ranging from the sonolysis of different organic compounds [11] to degradation of polymers and various materials [12] have been performed to characterize the rate of different degradation processes in homogeneous and/or heterogeneous systems under ultrasonic irradiation. There are also many studies on sonoelectrochemical kinetic processes [13]. However, only a few examples of homogeneous polar reactions, mostly solvolysis and hydrolysis reactions, have been kinetically investigated for sonication effects (for a review see refs [5,6]).

A great majority of all these kinetic studies were carried out with the sample withdrawal method, and aliquots were analysed with a variety of methods, e.g. GLC, HPLC, spectrophotometrically, etc. [5]. A significant disadvantage of withdrawing aliquots is the

---

[*] Corresponding author, sander.piiskop@ut.ee

permanent decrease in the volume of the reaction mixture during kinetic measurements, which affects the sonication effect. Furthermore, achieving accurate temperature control and a stable inert atmosphere causes problems. Low acceleration effects of only a few percentage points caused by ultrasound have been reported for some ester hydrolysis reactions in water–organic solvents, thus temperature control is decisive in correct kinetic investigations of sonication effects [5]. This means that it is difficult to make adequate comparisons between studies performed without and under ultrasound even in the same reaction system. Moreover, withdrawing aliquots for analysis could be time-consuming, which complicates the kinetic measurements in case of fast reactions.

However, withdrawing aliquots is generally preferred because continuous kinetic measurements directly in an ultrasonic field involve numerous complications. Mason and co-workers followed conductometrically the kinetics of 2-chloro-2-methylpropane solvolysis in aqueous ethanol under sonication employing bright Pt electrodes [14]. Nevertheless, cavitation causes erosion of various materials, damaging electrodes and reducing their lifetime. Metal traces from the immersed titanium sonication horn or other materials may also cause difficulties, contaminating the reaction mixture [7].

In the present study we developed a new online spectrophotometric system, which allows performing continuous ultrasonic kinetic measurements of reactions accurately and reproducibly.

ELABORATION OF THE ULTRASONIC ONLINE SPECTROPHOTOMETRIC SYSTEM

One of the basic problems for sonochemists is choosing an appropriate type of ultrasound source operating at required frequencies [1,2]. The generation of free radicals caused by cavitation is much more intense at higher frequencies of about 300 to 800 kHz than at 20 kHz [15,16]. Free radical and pyrolysis processes may disturb ultrasonic kinetic studies on polar homogeneous reactions by causing side-reactions. It is important to find conditions under which pyrolytic degradation and free radical processes would be relatively slow or entirely suppressed.

In general, on the laboratory scale there are three types of commercial sonicators that commonly operate at lower frequencies (e.g. 20, 25, 45 kHz): ultrasonic cleaning bath, ultrasonic probe system (directly immersed into solution), and cup-horn sonicator [1,2]. For sonochemists ultrasonic probe and cup-horn systems have several advantages compared to ultrasonic cleaning bath, mostly because of the supply of higher power into the reaction vessel. However, several problems such as difficult temperature control and well-known erosion of equipment materials caused by cavitation arise in kinetic measurements. For instance, metal particles dispersing from the sonication horn and contaminating the reaction mixture may interfere with various kinetic measurements or even catalyse the reactions under examination [7].

Hence we have concluded that the best ultrasonic device for kinetic studies is the classic ultrasonic bath, which has lower input power and allows quite easy temperature control. Our choice was ultrasonic bath Elma TI-H-5 MF2. Due to ultrasonic heating the temperature inside the reaction vessel is usually higher than in the ultrasonic bath [1]. Therefore both the ultrasonic bath and reaction vessel were thermostatically controlled. Under sonication (Elma TI-H-5 MF2, 25 kHz, 100 W, 20% of the electrical output energy) at 25 °C, the temperature stability in the reaction vessel was ±0.05 °C.

In our previous work we studied the kinetics of pH-independent hydrolysis of 4-nitrophenyl chloroacetate (4-NPCA) under ultrasound by using a spectrophotometric method [17]. We preferred a Shimadzu SPD-20A HPLC UV/Vis detector to follow the reaction kinetics in our ultrasonic online system. The HPLC UV/Vis detector has many advantages, including high sensitivity and direct electrical readout. Furthermore, an important feature of the HPLC detector is the small capacity of the flow cuvette, 12 μL in the case of Shimadzu SPD-20A. Thus only a small part of the total reaction solution that is needed for continuous detection is out of the ultrasonic field.

Online detection of the reaction kinetics requires circulating the reaction mixture continuously from the ultrasonic cell through the detector. Various ultrasonic flow cell systems have been used for continuous processing [1,2] but not for kinetic investigations. Moreover, combined circulating flow systems of irradiation by ultrasound and microwaves have been developed. These have been efficiently applied to perform various syntheses and processes that were still followed by withdrawing aliquots [18,19]. In these works peristaltic pumps were mostly used for circulation.

We tested some peristaltic pumps to circulate continuously the reaction solution through the HPLC detector; however, we encountered many disturbing factors such as a rise of pressure, leaks, and gas bubbles. In addition, in the case of these pumps only a minor part of the total reaction mixture was under sonication because it was necessary to use large-diameter tubes. Also temperature control was poor. All these circumstances reduce the reproducibility of kinetic measurements.

Examination of a Gilson 302 Piston HPLC pump gave excellent results. First of all, the capacity of the whole circulation system, consisting of tubing, a pump, and a detector, is considerably small, 0.4 mL. Thus, if there is 100 mL of reaction mixture in the sonication reactor, only 0.4% of the total amount will be continuously out of the ultrasonic field. At the maximum flow rate of the pump (5 mL/min), the dead time of the system is approximately 2.5 s. Thus it is possible to register continuously sonication effects that occur during a very short time of irradiation.

However, there were a few problems with this online system. The increase in the pressure in circulating tubes caused by the HPLC pump produces heat. Still, this temperature increase is constant for reactions under ultrasound and without sonication. Furthermore, it appeared that bubbles occurred in the reaction mixture, disturbing considerably the optical measurements when passing through the detector cuvette. Ultrasound-generated cavitational bubbles coagulate, forming larger aggregates, which can interfere optical detection. Intensive ultrasonic mixing and degassing similarly result in disturbing bubbling. To avoid this problem the tip of the inlet tube inside the reaction vessel was equipped with an HPLC solvent inlet PTFE filter. This filter helps also to hinder the contamination of the cuvette by the possible dispersed particles caused by cavitation.

Setup of the online system

The ultrasonic online spectrophotometric system for kinetic measurements consists of an ultrasonic bath, an HPLC UV/Vis detector, an HPLC pump, a thermostat, and a data processing unit (Fig. 1). A multi-frequency ultrasonic bath *TI-H-5 MF2* (max tank volume 4.7 L; 25/45 kHz, electrical output energy 100 W) was provided by Elma Hans Schmidbauer GmbH & Co. KG (Singen, Germany). A flat-bottomed reaction cell with a cooling jacket (100 mL) was made from Pyrex glass by an experienced glass-blower. Temperatures in both the ultrasonic bath and the reaction vessel were controlled and recorded with Refrigerated Heating Circulator Bath with an air-cooled cooling system *Ministat 125*, provided by Peter Huber Kältemaschinenbau GmbH (Offenburg, Germany). A mechanical stirrer *IKA*$^{®}$ *RW11 basic* was immersed into the reaction cell and a second stirrer, *IKA*$^{®}$ *RW14 basic*, into the ultrasonic bath. Both stirrers were provided by IKA® GmbH & Co. KG (Staufen, Germany). A *Gilson 302 Piston* pump (max flow rate 5 mL/min), provided by GILSON MEDICAL ELECTRONICS, Inc (Middelton, USA), and Shimadzu *SPD-20A* UV/Vis detector (cuvette 1.000 cm, capacity 12 μL), provided by SHIMADZU CORPORATION (Kyoto, Japan), were combined in series. The pump, the detector, and the reaction vessel were connected with the HPLC PTFE tubes. The inlet tube inside the reaction vessel was equipped with a PTFE solvent filter (diameter 10 mm, pore 2 μm).

An analogue–digital converter was constructed and a data processing program AD_com was written for the registration of detector signal (RS-232 output standard signal). The program allows selection of the registration time and the interval of signal (minimum interval 1 s) (Fig. 2). Data can be collected into a .txt file and processed in MS Excel or GraphPad Prism for further data analysis.

KINETIC MEASUREMENTS

The pH-independent hydrolysis of 4-nitrophenyl chloroacetate (4-NPCA) was used as a model reaction (Scheme 1) to test the online spectrophotometric system for ultrasonic kinetic measurements. The sonolytic degradation of 4-nitrophenol (4-NP) was also monitored in an identical manner because during the ester hydrolysis reaction under sonication this degradation can lead to defacement of the kinetic results [7].

Both 4-NPCA and 4-NP were purchased from commercial sources and were used without further purification. Reaction solutions were prepared by weighing Millipore Milli-Q water. The pH of the solutions was maintained at 3.65±0.05 with HCl.

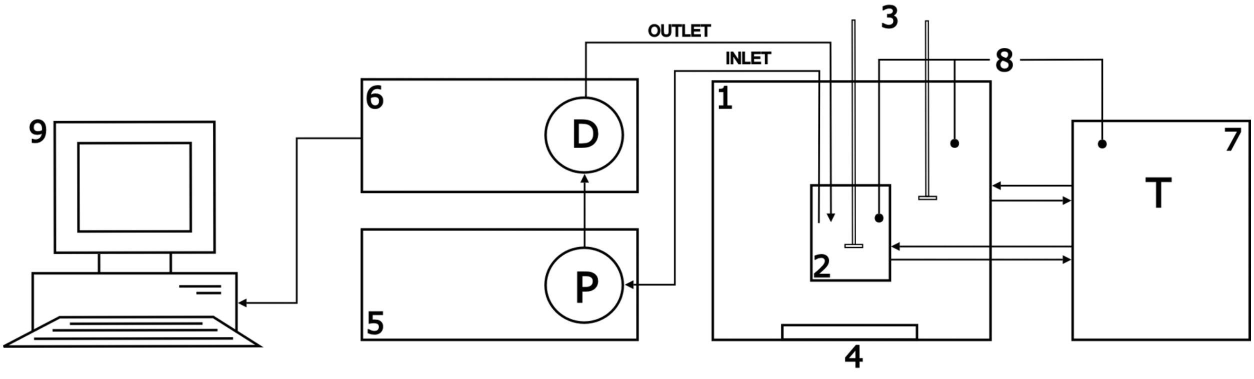
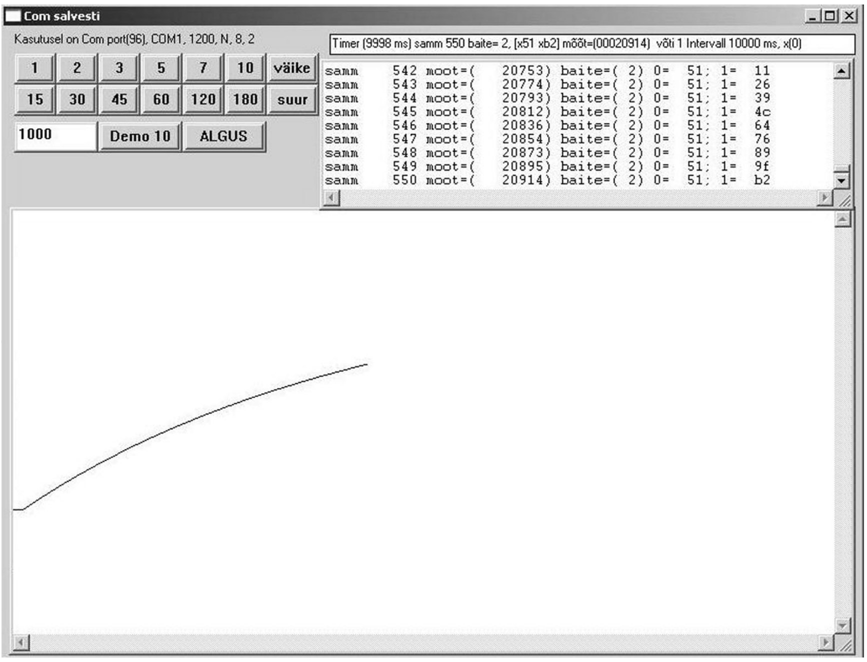


Fig. 1. The ultrasonic online spectrophotometric system: (1) ultrasonic bath, (2) reaction cell, (3) mechanical stirrers, (4) ultrasonic transmitter, (5) HPLC pump, (6) HPLC UV/Vis detector, (7) thermostat, (8) temperature control, (9) data processing unit.

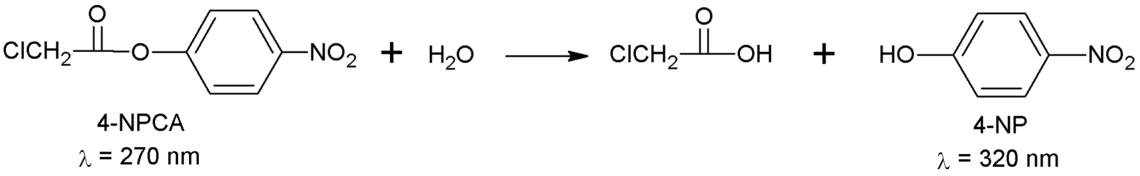


Kasutusel on Com port(96), COM1, 1200, N, 8, 2

Timer (9998 ms) samm 550 baite= 2, [x51 xb2] mõõt=(00020914) võti 1 Intervall 10000 ms, x(0)

```
samm     542 moot=(    20753) baite=( 2) 0=  51; 1=  11
samm     543 moot=(    20774) baite=( 2) 0=  51; 1=  26
samm     544 moot=(    20793) baite=( 2) 0=  51; 1=  39
samm     545 moot=(    20812) baite=( 2) 0=  51; 1=  4c
samm     546 moot=(    20836) baite=( 2) 0=  51; 1=  64
samm     547 moot=(    20854) baite=( 2) 0=  51; 1=  76
samm     548 moot=(    20873) baite=( 2) 0=  51; 1=  89
samm     549 moot=(    20895) baite=( 2) 0=  51; 1=  9f
samm     550 moot=(    20914) baite=( 2) 0=  51; 1=  b2
```

Fig. 2. Print-screen picture of a window of data processing program AD_com. The figure presents a UV/Vis detection of the formation of 4-nitrophenol during 4-NPCA hydrolysis without ultrasound.



Scheme 1. Hydrolysis of 4-NPCA.

Calculated amounts of 4-NPCA or 4-NP were dissolved in analytical grade acetonitrile. Of this solution 10 μL was injected into 100 mL of the aqueous solution in the reaction cell providing 5×10^{-5} M initial concentration of the reagents. Experiments were performed at 25.0±0.05 °C under argon atmosphere. The formation or the consumption of 4-NP was monitored at 320 nm.

Degradation of 4-nitrophenol

The sonolytic degradation of 4-NP was studied with an ultrasonic online spectrophotometric system operating at 25 kHz. Kinetic measurements were performed at different ultrasonic power of the cleaning bath at 50% and 20% of electrical output energy. The ultrasonic power entering the systems, 7.1±0.7 and 3.5±0.5 W/100 mL in water respectively, was determined calorimetrically.

This degradation was alternatively followed with a high-performance liquid chromatograph. Aliquots of 0.2–0.5 mL were withdrawn from the reacting mixtures at appropriate time intervals and analysed with an HPLC Shimadzu LC Solution (Prominance) system, connected with a series-diode array detector SPD M20A. The separation column was Gemini C18 5μ 250 × 4.60 mm (Phenomenex), run at a flow rate 1 mL/min. Elution was 0.1% TFA/MeCN (0.1% TFA) gradient solution.

The first-order rate constant of sonolytic degradation of 4-NP was found to be $k_I = 6.6 \times 10^{-6}$ s^{-1} in the case of 50% of the electrical output energy. No degradation of the 4-NP was detected during 1-h sonication with ultrasonic power adjusted at 20% of the electrical output energy. This experiment was repeated by following degradation of 4-NP with periodical withdrawal of aliquots for HPLC analysis. No degradation products were detected under these conditions.

Hydrolysis of 4-nitrophenyl chloroacetate

The kinetic measurements of pH-independent hydrolysis of 4-NPCA were carried out with the online

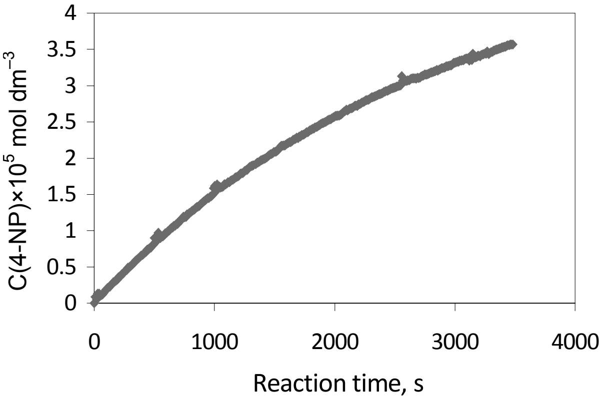


Fig. 3. Kinetic measurements of pH-independent hydrolysis of 4-NPCA under ultrasound carried out in the ultrasonic online spectrophotometric system.

spectrophotometric system without sonication and under ultrasound. The kinetic experiments under ultrasound were performed at 20% of electrical output energy of the ultrasonic bath. The duration of sonication was 1 h. A kinetic curve is presented in Fig. 3. The curve clearly shows that no significant signal interferences were found in experiments performed under ultrasound. The first-order rate constants for the pH-independent hydrolysis of 4-NPCA without sonication and under ultrasound were respectively $3.5 \pm 0.03 \times 10^{-4}$ s^{-1} and $3.62 \pm 0.04 \times 10^{-4}$ s^{-1} (values are averages from three parallel runs).

CONCLUSIONS

The kinetic measurements of homogeneous ionic reactions under ultrasonic irradiation are very complicated mostly because of the cavitation phenomena. In this work an ultrasonic online spectrophotometric system for kinetic measurements of homogeneous polar reactions under ultrasound was developed. The kinetic experiments of pH-independent hydrolysis of 4-NPCA and sonolysis of 4-NP were carried out without sonication and under ultrasound to test the online system. The results suggest that the ultrasonic online spectrophotometric system can be successfully applied for ultrasonic kinetic measurements of polar homogeneous reactions. This online system allows reproducible detection of small sonication effects on different homogeneous processes.

ACKNOWLEDGEMENTS

This work was financially supported by the Estonian Ministry of Education and Research (grant SF0180064s08) and by the Estonian Science Foundation (grant ETF7498). The technical assistance of Alo Raidaru is acknowledged.

REFERENCES

1. Mason, T. J. *Practical Sonochemistry: User's Guide to Applications in Chemistry and Chemical Engineering*. Ellis Horwood Limited, Chichester, 1991.
2. Mason, T. J. (ed.) *Sonochemistry: The Uses of Ultrasound in Chemistry*. The Royal Society of Chemistry, Cambridge, 1990.
3. Cravotto, G. and Cintas, P. Power ultrasound in organic synthesis: moving cavitational chemistry from academia to innovative and large-scale applications. *Chem. Soc. Rev.*, 2006, **35**(2), 180–196.
4. Luche, J. L. *Synthetic Organic Sonochemistry*. Plenum Press, New York, 1998.
5. Tuulmets, A., Cravotto, G., Salmar, S., and Järv, J. Sonochemistry of homogeneous ionic reactions. *Mini-Rev. Org. Chem.*, 2010, **7**(3), 204–211.
6. Tuulmets, A., Salmar, S., and Järv, J. *Sonochemistry in Water Organic Solutions*. Nova Science Publishers, New York, 2010.
7. Salmar, S., Cravotto, G., Tuulmets, A., and Hagu, H. Effect of ultrasound on the base-catalyzed hydrolysis of 4-nitrophenyl acetate in aqueous ethanol. *J. Phys. Chem. B*, 2006, **110**, 5817–5821.
8. Hagu, H., Salmar, S., and Tuulmets, A. Impact of ultrasound on hydrophobic interactions in solutions: ultrasonic retardation of benzoin condensation. *Ultrason. Sonochem*., 2007, **14**(4), 445–449.
9. Tuulmets, A., Järv, J., Salmar, S., and Cravotto, G. Ultrasonic detection of hydrophobic interactions: a quantitative approach. *J. Phys. Org. Chem.*, 2008, **21**, 1002–1006.
10. Tuulmets, A., Järv, J., Tenno, T., and Salmar, S. Significance of hydrophobic interactions in water-organic binary solvents. *J. Mol. Liq.*, 2009, **148**, 94–98.
11. Petrier, C. and Casadonte, D. The sonochemical degradation of aromatic and chloroaromatic contaminants. *Adv. Sonochem.*, 2001, **6**, 91–109.
12. Abedini, R. and Mousavi, S. M. Preparation and enhancing of materials using ultrasound technique: polymers, catalysts and nanostructure particles. *Petroleum Coal*, 2010, **52**(2), 81–98.
13. Cornpton, R. G., Eklund, J. C., and Marken, F. Sonoelectrochemical processes: a review. *Electroanalysis*, 1997, **9**(7), 509–522.
14. Mason, T. J., Lorimer, J. P., and Mistry, B. P. The effect of ultrasound on the solvolysis of 2-chloro-2-methylpropane in aqueous ethanol. *Tetrahedron*, 1985, **41**, 5201–5204.
15. Mark, G., Tauber, A., Laupert, R., Schuchmann, H.-P., Schulz, D., Mues, A., and von Sonntag, C. OH-radical formation by ultrasound in aqueous solution. Part II: Terephthalate and Fricke dosimetry and the influence of various conditions on the sonolytic yield. *Ultrason. Sonochem.*, 1998, **5**(2), 41–52.
16. Jiang, Y., Petrier, C., and Waite, T. D. Sonolysis of 4-chlorophenol in aqueous solution: effects of substrate

concentration, aqueous temperature and ultrasonic frequency. *Ultrason. Sonochem.*, 2006, **13**(5), 415–422.

17. Piiskop, S., Hagu, H., Järv, J., Salmar, S., and Tuulmets, A. Sonication effects on ester hydrolysis in alcohol–water mixtures. *Proc. Estonian. Acad. Sci. Chem.*, 2007, **56**, 199–206.
18. Cravotto, G. and Cintas, P. The combined use of microwaves and ultrasound: improved tools in process chemistry and organic synthesis. *Chem. Europ. J.*, 2007, **13**(7), 1902–1909.
19. Wu, Z.-L., Ondruschka, B., and Cravotto, G. Degradation of phenol under combined irradiation of microwaves and ultrasound. *Environ. Sci. Technol.*, 2008, **42**, 8083–8087.

Spektrofotomeetriline *on-line*-süsteem reaktsioonikineetika uurimiseks ultraheli all

Sander Piiskop, Gerda-Johanna Raidaru ja Siim Salmar

Homogeensete polaarsete reaktsioonide kineetika uuringud vesi-orgaanilistes solventsüsteemides ultraheli all on välja toonud spetsiifilised soluut-solventinteraktsioonid ja hüdrofoobsed efektid, mis jäävad märkamatuks konventsionaalse kineetika korral. On leitud, et ultraheli võib osutuda kasulikuks tööriistaks reaktsioonide füsikokeemilistes uuringutes. Samas on reaktsiooni kineetika mõõtmine ultraheli all osutunud komplitseerituks, mis on tingitud peamiselt kavitatsioonifenomenist.

Antud töös on välja arendatud ainulaadne spektrofotomeetriline *on-line*-ultrahelisüsteem homogeensete polaarsete reaktsioonide kineetika mõõtmisteks ultraheli all. Selle *on-line*-süsteemi testimiseks kasutati mudelreaktsioonina 4-nitrofenüülkloroatsetaadi neutraalset hüdrolüüsi ja samuti uuriti 4-nitrofenooli sonolüütilist degradatsiooni. Esmased kineetilised mõõtmised näitasid, et süsteemi saab edukalt rakendada reaktsioonikineetika ultraheliga uuringuteks. Arendatud süsteem võimaldab tundlikult ja reprodutseeritavalt jälgida erinevaid homogeenseid sonokeemilisi protsesse, mida on võimalik spektrofotomeetriliselt detekteerida.