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SIMULTANEOUS MEASUREMENTS OF HEAT PRODUCTION AND OPTICAL DENSITY IN OSCILLATING REACTIONS

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ABSTRACT

A device is presented which can be inserted into a calorimetric vessel to enable a simultaneous measurement of optical density and heat production. It consists mainly of a quartz rod and a photodiode which is sensitive from the near ultraviolet to the infrared. Specific wavelengths are selected by glass or interference filters placed between the light source and the rod. Homogeneity of the medium in the vessel can be achieved by a gentle but effective pneumatic stirring.

The applicability of the whole set-up is demonstrated for two oscillating chemical reactions (Belousov–Zhabotinskii and Briggs–Rauscher reactions) and for the oscillating glycolysis of a cell-free cytoplasmic extract from yeast.

INTRODUCTION

Although periodically changing phenomena such as the beating mercury heart [1] or the predator-prey system [2] have been well known for some time, interest in oscillating chemical reactions did not start before the middle of this century. When Belousov detected the now famous "Belousov–Zhabotinskii reaction" in 1958 [3] it attracted little attention since this bromate/bromide reaction was taken as a singularity. In the same way the temporal changes in substrates and products of the glycolytic breakdown of sugars in cells or in cell-free cytoplasmic extracts [4] were taken as a biologic specificity occurring only in living systems.

Ten years later a world-wide interest grew in the Belousov–Zhabotinskii reaction ("BZ reaction") as well as in other oscillating systems [5]. Many have been intensively studied and some conditions necessary for the appearance of oscillations are known [6] facilitating the search for new, e.g. bromine-free or halogen-free oscillating reactions [7]. This increasing interest originates from different scientific fields. Prigogine and his coworkers devel-

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oped the concept of open systems and the thermodynamics of irreversible processes [8] in which limit cycles, instabilities, bifurcations, spatial dissipative structures and oscillating reactions play an essential role. Theorists were fascinated by the challenge of complex non-linear partial differential equations [9–11] and biologists have been confronted for a long time with questions about pattern formation and periodic processes in organisms such as fibrillation of heart tissue, intestine peristaltics or circadian rhythms [12]. Many of the oscillating reactions investigated have frequencies of the order of minutes and thus do not seem to drive the internal clock of higher organisms, although recently reactions have been described with periods of several hours [13].

These oscillations were monitored experimentally in different ways, i.e. by spectophotometric or biochemical determination of the changing substrate or product concentrations in the system, by measurements with pH- or ion-sensitive electrodes and by manometric methods. Only a few calorimetric experiments have been performed on oscillating reactions, some on the Belousov–Zhabotinskii reaction [14–17], one on the metabolism of maintenance of yeast cells [18] and recently one on heat production during glycolysis [19]. This is due to a lack of appropriate instruments, to the long time constants of calorimeters or to the belief that calorimetry is a difficult and unspecific method. However, with oscillating reactions it is necessary to reveal the underlying energetical situation and to determine the phase relation between specific reaction steps and any indicators which reflect the overall status of the system.

Recently we investigated the energetics of glycolytic oscillations [19] and found correlations between the periodical changes in the metabolic level of NADH and the rate of heat production. The optical and calorimetric experiments were run separately and a phase relationship between both signals could not be established. The need for combining calorimetry with nephelometry for a true simultaneity was obvious.

In an earlier set of papers Berger described several semicalorimetric and calorimetric systems connected to spectrophotometric devices [20]. Heat production and optical density in a growing yeast culture were determined simultaneously by two optical fibres attached to a Calvet calorimeter [21]. Thermoluminescence was measured in a differential scanning calorimeter [22], light-induced processes in a batch system [23], and photon emission and corresponding heat production of luminescent bacteria in a microcalorimeter [24].

In this paper we wish to present a simple device for simultaneous optical and heat production measurements in a small batch calorimeter. The system consists essentially of a quartz rod for guiding the light into the calorimetric vessel and a photodiode to determine the light intensity. Moreover, the system allows the addition of substrates to start or to sustain the reaction and the stirring of the content of the vessel to obtain complete homogeneity.

Thus, the set-up can be used as a calorimetric CSTR (continuous flow stirred tank reactor) for the investigation of sustained oscillations.

METHODS AND MATERIALS

All measurements were performed isothermally with a twin microcalorimeter type TRIFLUX (Thermanalyse/Grenoble). The stainless steel vessels have a volume of 1.2 ml and a tightly fitting teflon cover. The sensitivity of the instrument amounts to 81.9 mV W^{-1}, the time constant to 117 s.

The calorimetric signal is fed to a microvolt amplifier (type 150B Mikrovolt Ammeter, Keithley/München) and then registered as 100 mV full scale deflection with a two channel recorder (type BD41, Kipp and Zonen/Kronberg). Depending on the experiment, sensitivities of 0.1 to 3 mV full scale deflection are chosen at the amplifier.

The light detecting system is described in Fig. 1. A support (1) made from Plexiglas fixes a quartz rod (2) of approximately 150 mm length and 3.1 mm diameter as a light guide for visible and near ultraviolet light. On the bottom of the support a photodiode (3) with an active area of 2.75 × 2.75 mm^2 faces the light guide. The diode (type BPX 91B, Siemens/Berlin) has its maximum sensitivity at 850 nm with a photo current of 0.47 A W^{-1}. At 360 nm it still responds by 25% of its maximum. The leads of the diode are soldered to connecting wires (4) embedded in small holes in the lowest part of the support which are closed with Perspex glue. The wires run inside the support so that the electric connections are completely isolated from the medium. The photodiode is connected directly to the second channel of the recorder with typical settings of 20 to 200 mV full scale depending on the light source and the filters used. Two stainless steel tubes (5,6) made from injection needles serve for the addition of substances to start the reaction, for continuous injections for sustained oscillations in reactor experiments or for pneumatic stirring if good homogeneity of the medium has to be achieved.

In experiments with gas production (for instance in the glycolytic oscillation or in the iodine clock, also called the Briggs–Rauscher reaction) small bubbles gather at the lower end of the quartz rod. These bubbles produce an irregular noise in the optical signal when they stick to the surface of the rod, move around or combine to larger ones. This problem was overcome by cutting the quartz under an angle of 45° so that the bubbles ascend in the medium without interference. The minimum distance between the light guide and the photodiode is 1.2 mm.

Depending on the experiment, two different light sources positioned directly above the calorimeter are applied, a luminescent lamp (type L8W/25 Universal-White, Osram/Berlin) for visible light and a Sylvania fluorescent lamp (type Blacklight Blue 8W, F8T5/BLB). Both lamps show no thermal interaction with the calorimetric signal and their intensities are high enough

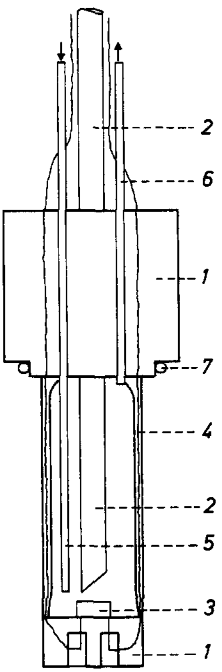

Fig. 1. Calorimetric device for the simultaneous determination of optical density and rate of heat production in a batch calorimeter. (1) Perspex (Plexiglas) support; (2) quartz rod with a 45 degree cutting; (3) photodiode; (4) connecting wires; (5) inlet capillary tube; (6) outlet capillary tube; (7) Viton O-ring. The original height of the support from the bottom to the O-ring is 24 mm.

to monitor extinction with more than 20 mV. In the experiments different glass or interference filters are used to select the desired wavelengths.

If chemicals have to be added during the experiment, e.g. to start an oscillating reaction, they must be in thermal equilibrium with the content of the calorimetric vessel. To achieved this the components pass a heat exchanger made from a stainless steel spiral of volume 0.3 ml, located in the cover just above the vessel. The liquid in the spiral is pushed into the vessel and mixed by means of 0.4 ml of air pressed out of a calibrated syringe.

A special stirring device was developed as the space in the calorimetric vessel was too small for an additional rotating or rocking stirrer as normally used in biological calorimetry [25]. The system consists of a small 24 V DC magnet connected to a pulse generator with variable frequencies. When attracted the piston of the magnet compresses a soft silicon tube of 4 mm inner diameter which is closed at one end and connected to the injection tube (5) protruding into the liquid. The action of the piston produces a gentle, but effective mixing of the liquid in the calorimetric vessel. Mixing results in a small but constant deviation from the calorimetric baseline.

RESULTS

In evaluating calorimetric and spectroscopic signals from oscillating reactions, the time constants of both signals have to be taken into account. Compared with the calorimetric response, optical devices are very fast and may be considered as operating instantaneously so that their output is smoothed only by diffusion processes in the vessel or by the recording system. Time constants of all three components, calorimeter, photodiode and recorder, can be measured separately. For the present purpose, it seemed more appropriate to perform an indirect determination. A fast reaction was chosen to obtain the time shift between optical and calorimetric response. 0.1 N HCl was neutralized with an excess of 0.3 N NaOH using a pH indicator (bromethymol blue). Figure 2 shows the traces and the time shift $\Delta\tau$ between the two signals. It is defined as time between the intersection of the steep decline at the beginning of the reaction with the nearly constant extinction after its completion and the maximum heat production. From the first decline a time constant of 3.7 s is calculated for the optical signal and the right slope of the calorimetric curve yields a time constant of 117 s for the calorimetric signal. In a number of experiments $\Delta\tau$ was evaluated as 34 s. It must be considered when calculating the phase shifts between heat production and optical changes.

The Belousov–Zhabotinskii reaction is the best known chemical oscillating system and the only one which has been investigated calorimetrically. It

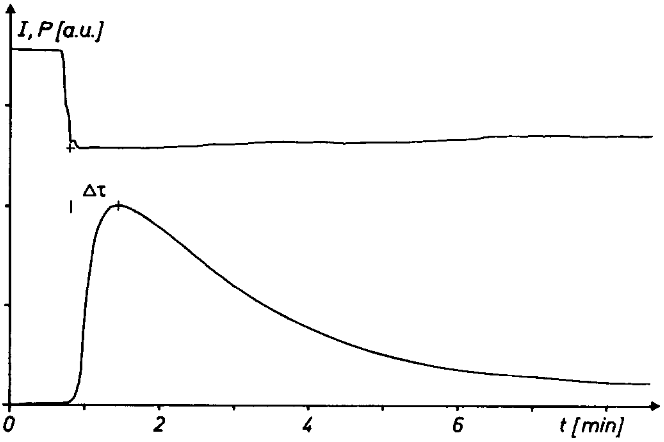

Fig. 2. Neutralization reaction of 400 μl of 0.1 M HCl with an excess of 150 μl of 0.3 M NaOH (temperature equilibrated in the heat exchanger spiral above the calorimetric vessel) in the presence of 60 μl 3.95 mM bromethymol blue as pH indicator. Upper trace, extinction at 530 nm versus time in arbitrary units; lower trace, exothermic power time curve of the neutralization in arbitrary units. $\Delta\tau$ indicates the time shift between the optical and the calorimetric signal.

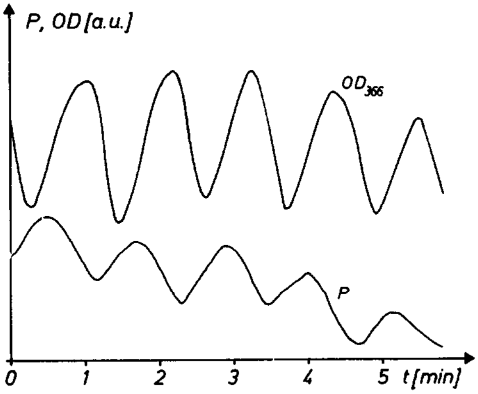

Fig. 3. Later part of the optical and heat production oscillations of the Belousov–Zhabotinskii reaction in a stirred vessel. Optical density OD increases downwards, rate of heat production P upwards. The retardation between both signals was not corrected for. Heat production rate and optical density are given in arbitrary units.

was worthwhile to demonstrate the applicability of our apparatus to this reaction. Figure 3 shows part of the oscillations after the first strongly exothermic reaction had finished. The chosen example comprises 0.063 M potassium bromate, 0.2 M malonic acid, both dissolved in 1 M sulfuric acid, with a 0.001 M aqueous ceric nitrate solution as a catalyst at 20°C. In this stirred mixture the modulation of the heat output amounts to approximately 10% of the mean heat flux. The period is 1 min. Taking into account the retardation between both signals, the maximum of the optical density, i.e. the highest concentration of Ce^{4+} ions, appears at the maximum of heat dissipation. This result is in good agreement with observations of Körös et al. [14] who found jumps in temperature and potential when the solution changed from colourless to light yellow.

Periodicity in both signals is best observed when the solution is stirred because homogeneity in the calorimetric vessel and simultaneous changes in the reaction are attained. In an unstirred solution inhomogeneities may occur which result in a deformation of the oscillations and a superposition of independent sinusoidal wavetrains. Such inhomogeneities are well-known as spatial structures and travelling bands in the Belousov–Zhabotinskii reaction [5,9]. On the other hand it has been shown that stirring at room temperature increases the amplitudes of the oscillations by up to a factor of 6 [15].

A second oscillating inorganic reaction, the so-called iodine clock, is presented in Fig. 4. The conditions for this reaction were adapted from refs. 26 and 27. A mixture of 3.6 M hydrogen peroxide, 0.201 M potassium iodate and 0.159 M perchloric acid was introduced into the calorimetric vessel and 0.150 M malonic acid, 0.0201 M manganese(II) sulfate and starch were kept in the heat exchanger spiral in the cover of the calorimeter. After equilibra-

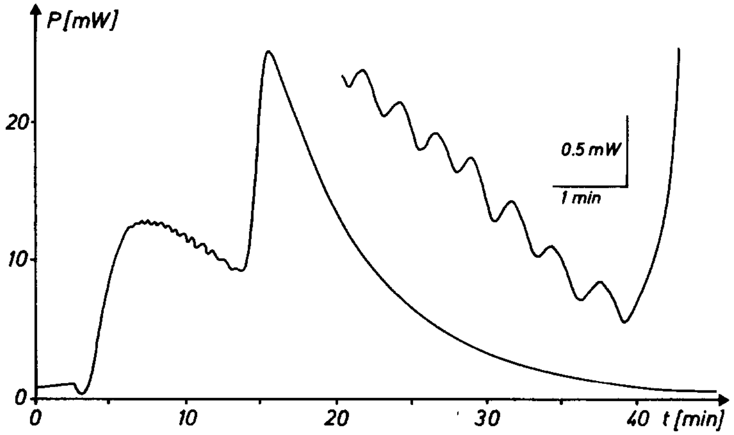

Fig. 4. Power time curve of the complete reaction of a "iodine clock" of potassium iodate, hydrogen peroxide, perchloric acid, malonic acid and manganese(II) sulfate. The insert shows the oscillations for the period shortly before the onset of the strong exotheric reaction in enlarged form.

tion the components were mixed. Following a short endothermic period an exothermic reaction occurred with small oscillations in the heat flux corresponding to the change from colourless to golden or even blue when observed by eye. Up to the moment when the black precipitate appears the strongly exothermic reactions appears in the power–time curve. The optical signal is omitted in Fig. 4 to simplify the graph. The insert shows the heat

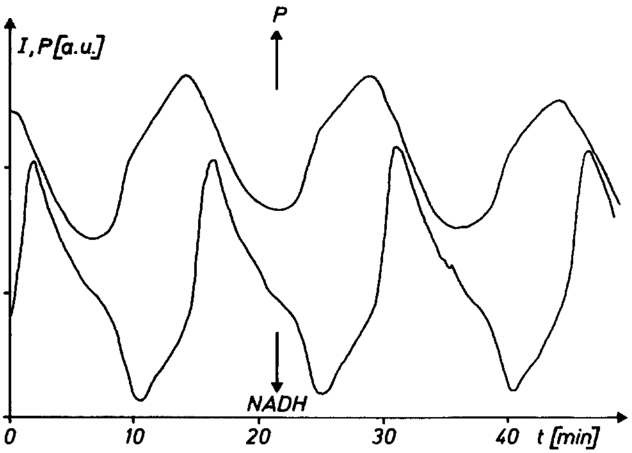

Fig. 5. Power time curve (upper trace) and changes in optical density (lower trace) in glycolytic oscillations at 21°C. Exothermic reactions are orientated upwards, the concentration of NADH increases downwards (see arrows). Both curves are given in arbitrary units with a bigger offset. The phase relation between both traces is not corrected for.

flux oscillations on an enlarged scale for that part of the reaction just before the strong increase in the heat production.

Figure 5 exhibits the periodic oscillations of glycolysis in a cell-free cytoplasmic medium extracted from yeast cells. The experimental conditions were as described in ref. 19. Briefly, the disaccharide trehalose is fermented by the extract giving rise to periodic concentration changes of several metabolic intermediates [28]. As an indicator of oscillations NADH (reduced nicotinamide–adenine–dinucleotide) was chosen which absorbs around 360 nm in the near ultraviolet. It is evident from the traces that both signals oscillate with the same period of 14.4 min with a slight increase when the oscillations advance. The maxima of both curves appear with a time difference of 2.2 min when no correction is made for the thermal inertia of the calorimeter (Fig. 2). Further details and results from heat production and phase relationships during glycolytic oscillations will be published elsewhere [29].

CONCLUSIONS

The few examples given show that the simple combination of a light guiding quartz rod and a UV sensitive photodiode is very well suited to monitoring changes in optical density connected with varying rates of heat flow. The sensitivity of the photodiode is high enough to use it with the usual luminescent or fluorescent lamps and to choose the appropriate wavelengths with glass or interference filters. Problems of inhomogeneity in the vessel content due to subdivision of the space by the optical set-up can be easily overcome by the proposed stirring device. It introduces marginal additional heat which in most experiments may be neglected in the energy balance of the reaction. In more critical cases it manifests itself merely by a minor deviation from the zero line.

With the small calorimeter used in these investigations a solid quartz rod sufficed as a light guide. Nowadays flexible light guides are available for the near ultraviolet and sophisticated constructions are possible for other types of calorimeters. Moreover, the proposed set-up can be used as a stirring and measuring device for reactions in larger calorimetric vessels. Such a construction could be similar to those used in calorimetric investigations of the growth of microorganisms [25].

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