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Properties of Wave Propagation in a Gel-type Belousov–Zhabothinsky Reaction under Micro-gravity

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Abstract The Belousov–Zhabothinsky (BZ) reaction is a chemical reaction which exhibits spatial as well as temporal pattern formation. Being an excitable medium, it can be influenced by even small external forces. One of these small forces which under ground conditions permanently is given is gravity. The gravity dependence of the BZ-reaction has been investigated in some detail up to now, and it has been found that especially the propagation velocity of waves in thin layers of fluid BZ-medium depends significantly on gravity-amplitude and -orientation. This finding has been mainly assigned to an interaction of gravity with diffusion and convection in the medium at the wave front, and consequently it has been stated that the propagation of waves in gels of BZ-medium is not significantly gravity dependent. We have now done more detailed experiments and have been able to show that also in gels the propagation velocity of BZ-waves is altered by gravity, but less than in fluid systems. Experiments have been performed in a lab centrifuge, a sounding rocket experiment and a parabolic flight mission.

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Introduction

Excitable media allow excitation waves to travel through them, which play an important role in a variety of natural processes, in physics, chemistry and live-sciences (Sagues and Epstein 2003; Tabony 2006). By far the best understood model system for all these processes is the Belousov–Zhabotinsky (BZ) reaction (Belousov 1959; Zaikin and Zhabotinsky 1970). This oscillating chemical reaction, among others, exhibits propagation of two-dimensional waves in thin layers (Luengviriya et al. 2006) of reaction solution or in gels (Yamaguchi et al. 1991), typically at velocities of some mm per minute.

According to the physical preconditions, excitable media are critically dependent in their behaviour on small external forces (Sagues and Epstein 2003; Tabony 2006), and accordingly wave propagation in BZ-systems has been studied depending on a variety of such forces, as there are for example small currents, electro-magnetic fields (Blank and Soo 2003; Miyakawa and Mizoguchi 1998; Sontag 2006) and others (Schmidt and Müller 1997; Ševčíková et al. 1996).

Gravity also is a small external force, which at ground conditions is permanently present with 1 g. Using different platforms, a variety of experiments have been performed at lower and higher gravity with different BZ-systems. In general it has been shown that waves in fluid systems significantly depend on gravity, both on the amplitude (Fujieda et al. 1997, 1999, 2002) and the direction of the gravity vector (Nagypal et al.

1986). This finding was at least partially explained by the interaction of convection at the wave front with gravity. Consequently in systems with reduced diffusion and convection, e.g. for example gel-systems, the velocity dependence on gravity should be significantly less than in fluid systems. In a first series of experiments it has been shown already that in a lab centrifuge at high gravity wave propagation changes in gels, depending on amplitude and direction of gravity. The propagation velocity of BZ-waves at higher gravity was usually slightly faster (Wiedemann et al. 2002) than under 1 g, but the effect is much smaller than in fluid systems.

We have now additionally performed experiments in microgravity, using a parabolic flight mission and a sounding rocket mission, from which the results are presented here.

Material and Methods

Set-Up

The basic principles of the set-up used for both missions, the Brazilian CUMA II sounding rocket and the ESA parabolic flight are depicted in Fig. 1. The set-up is an enhanced version of a set-up which has already been used in the CUMA I mission, but which unfortunately was lost on recovery (Fernandes de Lima et al. 2002; Piqueira et al. 2003). Four steel chambers (volume 75 ml) with poly-carbonate covers were used as reaction chambers, see Fig. 2, lower parts. These chambers were mounted on an aluminium drawer which could be inserted into the basic construction of the set-up, containing the illumination, the cameras, the power supply, and the video-multiplexer, as can be seen in Fig. 2 upper parts.

During the parabolic flight campaign the complete set-up was mounted in a closed box made from aluminium profiles and plastic (POM), see Fig. 3, upper part. In this box additionally a heating pad, and a video-recorder were mounted, as well as a video-monitor on top of the box. During the parabolic flight mission (ESA-student mission 2006), 2 days with 30 parabola each with a duration of about 22 s were done, and due to the profile of parabolic flights (Novespace 2007) recordings were obtained at 1.8, 1 g and μ-gravity During the parabolic flight, temperature effects were neglectable, as each evaluated data point was normalized to the 1 g phase directly before, thus due to the time scale of minutes, no temperature changes were observed by a build in temperature sensor.

For the CUMA II mission in 2007 the set-up was mounted inside the structure of the pay-load (Fig. 3, lower part). Here, the video-multiplexer was connected to a video-transmitter and the data were recorded on ground with a video-recorder. Data were collected before launch on ground at 1 g as reference, and after the acceleration phase of the rocket during the μ-gravity

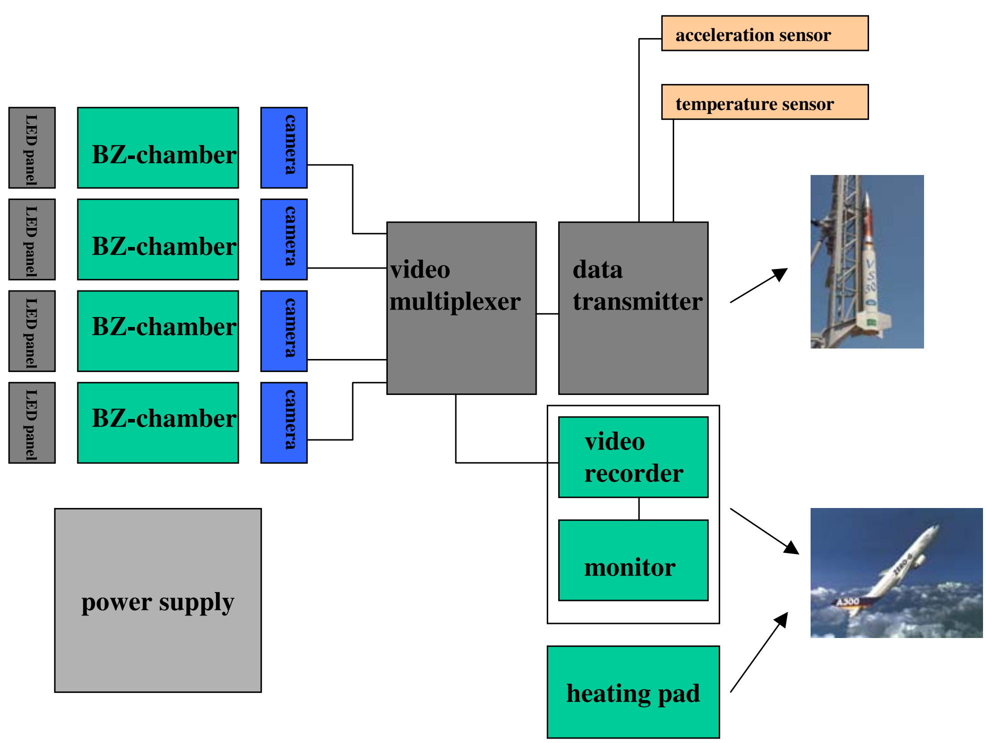

Fig. 1 Block diagram of the basic construction of the set-up as used in the parabolic flight campaign and the sounding-rocket. The data recording systems used were slightly different, and only in the airplane a heating system was installed. In the rocket, a data-transmission system was used to have the data available in the ground station by telemetry in order to visualize them on a monitor. The data were in parallel recorded on a video-recorder. In the airplane the data were directly recorded on a video-recorder and visualized on a monitor

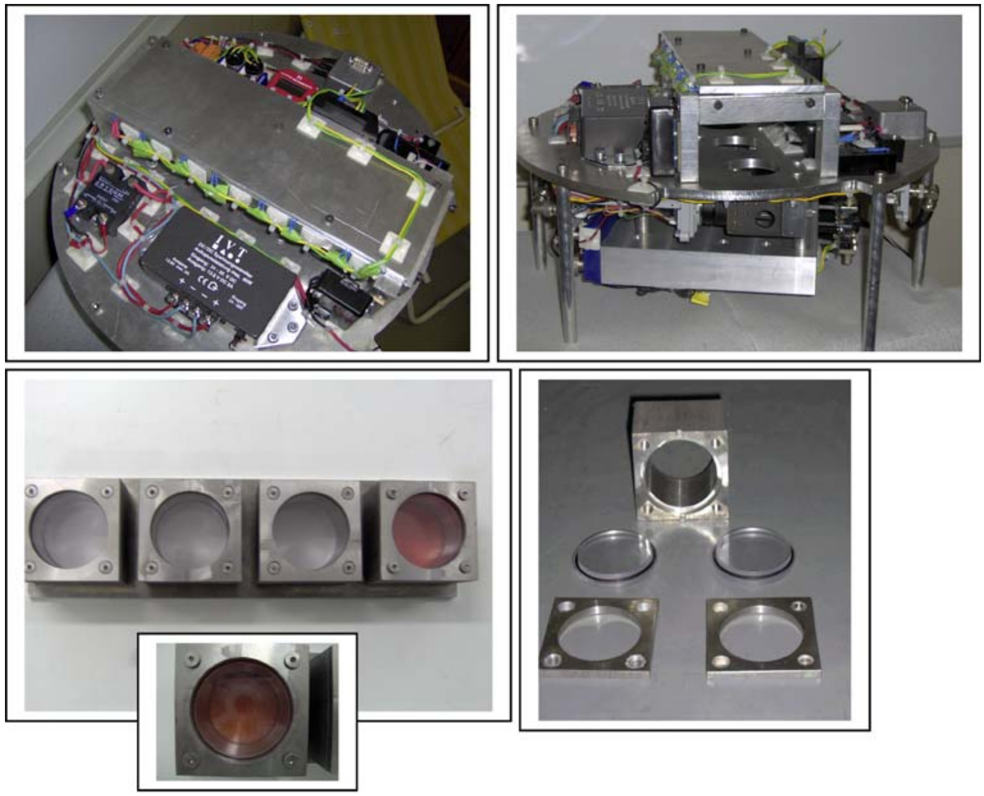

Fig. 2 Photos of details of the set-up. In the *upper left part*, the set-up itself can be seen, in the upper right part the set-up is shown as it was mounted in the CUMA II rocket. The *lower part show* the drawer with the four BZ-chambers and a more detailed view of one chamber, taken into parts

phase only. The CUMA II mission delivered a micro-g time ($<10^{-5}$ g) of about 6 min, but only part of this time could be recorded, due to problems with the telemetry ground station. The Brazilian CUMA system uses water recovery of the pay-load. Unfortunately, in both CUMA missions having been done to now, the pay-loads were lost after impact due to problems with the recovery. Data points were normalized for this mission to the velocities directly before launch, the temperature during the μ-g phase shifted ±2°C relative to that directly before launch, according to control measurements this has a smaller than the measured *g*-effect on the wave propagation velocity.

Preparation of Gels for the BZ-Reaction

The silica gels for the BZ-reaction were made slightly modified according to the procedure described by Yamaguchi et al. (1991). A gel-solution containing 9% $Na_2Si_2O_3$, 130 mM H_2SO_4 and 3.3 mM ferroin was made. A 5 mm thick layer of this solution was given into the steel chambers, which have been described above. After polymerisation of the gel, the surface of the gels was washed with 330 mM H_2SO_4, and then the steel-chambers were completely filled with a reaction solution containing 330 mM malonic acid, 330 mM $NaBrO_3$, and 330 mM H_2SO_4 and closed. The chamber were then immediately mounted in the set-up and used in both mission types.

Data Evaluation

In both missions, video tracks of all four chambers were finally (see above) multiplexed to a video-recorder at a switching rate of about 1 sec. The data after the mission were de-multiplexed and four separate traces in the avi-format were constructed, one for each gel, using the software VirtualDub®. Following that the avi-files were changed to stacks of jpg-files again by VirtualDub® and imported to the software ImageJ (freeware NIH). Using this software, the propagation velocity of the wave-fronts at different times was calculated. At the front of propagating waves, in regions marked in Fig. 6, the maximum brightness change was determined and then it was measured how far this front moved in a 20 s window for the μ-g phase by overlaying the frame at the beginning of the period and that at he end. This time interval was chosen as it best fits the μ-g phase of the parabolic flight protocol. In the parabolic flight situation this measurement was done for each parabola. in the CUMA II situation the measurement was done in a window directly before launch and then repeated throughout the 6 min μ-g time. In the 1.8 g phases of the parabolic flight, the time window was

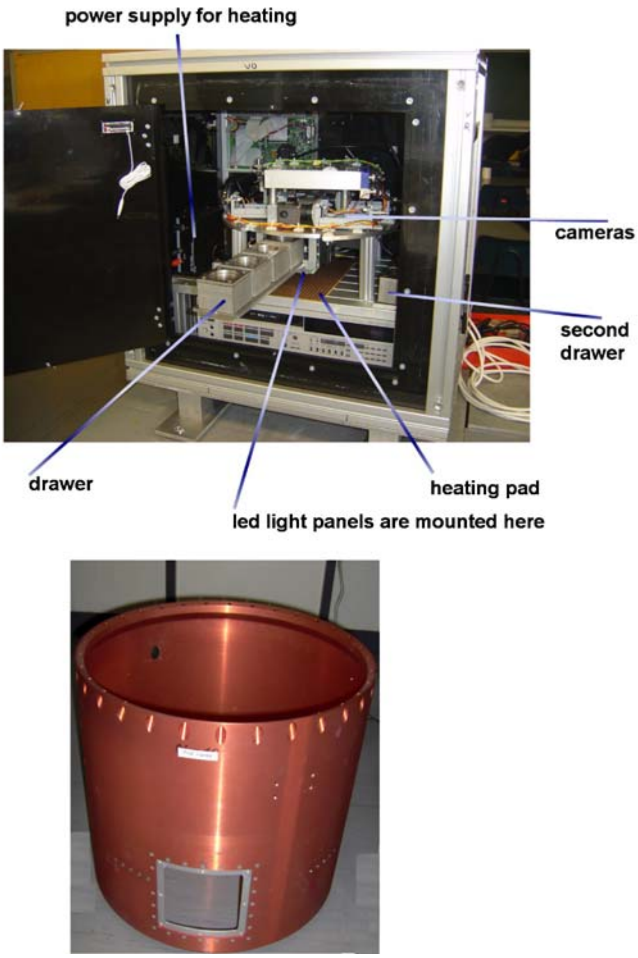

Fig. 3 *Upper photo* photo of the set-up as used in the parabolic flight campaign to record the propagation of waves in a gel-type Belousov–Zhabothinsky reaction. More details of the set-up are to be seen in Figs. 1 and 2. The set-up (Fig. 2) was directly mounted into the box made of aluminium profiles an plastic walls (POM). The video-recorder can be seen in the lower part of the set-up. Some additional parts used in the parabolic flight situation only can also be seen, the power supply for heating, a second exchange drawer with another four reaction chambers, a temperature sensor in the door and an additional data-recorder in the back of the black box. *Lower photo* Structure used for CUMA sounding rocket mission. The opening which can be used to insert the drawer at late access can be seen

sometimes chosen shorter due to the duration of these periods, this did not effect the overall results, but only resulted in a slightly bigger error-bar in the final data.

Additionally the sequences of photos were constructed and the tip movement of spiral in the observed gels was followed as far as possible.

Results

At first, in a series of laboratory controls the parameters of the gels we used were characterised. In Fig. 4 a montage of video frames from such an experiment is shown. The gels were illuminated from below with blue light (480 nm) for better contrast. Homogenous wave propagation with a velocity of about 4 mm/min can be extracted from such experiments. The BZ-chemistry in the gels is non-stationary, accordingly, due to the ongoing reaction, the colour of the gels on the time scale of hours is shifted towards blue colour. The propagation in these gels is stable over a time of 3–5 h.

In Fig. 5, upper part, the situation is depicted more quantitatively. A circular area of the gel with a diameter of 200 μm was recorded in brightness with a photomultiplier using blue illumination (480 nm) from below. The propagation of wave fronts then clearly can be observed by peaks of increasing brightness. Under these conditions uniform repetitive peaks with a period of some minutes can be observed. In the inset one of these peaks is shown in detail. In the lower part of Fig. 5 the amplitude of the peaks is plotted as a function of the number of the peak. As can be seen, the amplitude of the peaks taken relative to the baseline is about stable during the complete recording time (about 4 h). Again it can be seen that the baseline shifts to higher brightness in time. According to the blue illumination, this indicates the overall shift of the system from red to blue.

The propagation velocity in such gels is coupled strictly to the period of consecutive waves by the dispersion relation (Hanke 1999), thus the stability of the velocity of waves in time is also given, as the oscillation period is aproximately stable, see Fig. 5 upper part. However, the period of consecutive waves in identical gels as well as the coupled propagation velocity varies over a wide range (Hanke 1999).

Thus, in the gravity-related experiments, only waves in the same gel could be compared under different acceleration levels. All data were then normalized to the data obtained at 1 g, the propagation velocity at 1 g was set to 100%. This was done under three different experimental conditions. Data about centrifuge experiments have been published previously (Wiedemann et al. 2002) and showed that the propagation velocity of waves in gels of the BZ-reaction is slightly dependent on acceleration level in amplitude and direction. In case the *g*-vector was perpendicular to the gel, the velocity slightly decreased with increasing *g*-value. This configuration was used also in the micro-gravity experiments.

In the parabolic flight mission experiments at 1, 1.8 g and micro-gravity (about 10^{-3} g) according to the protocol of the flights (see materials and methods section) were carried out. The phases of each period are about 22 s. Two missions with 31 parabola each were

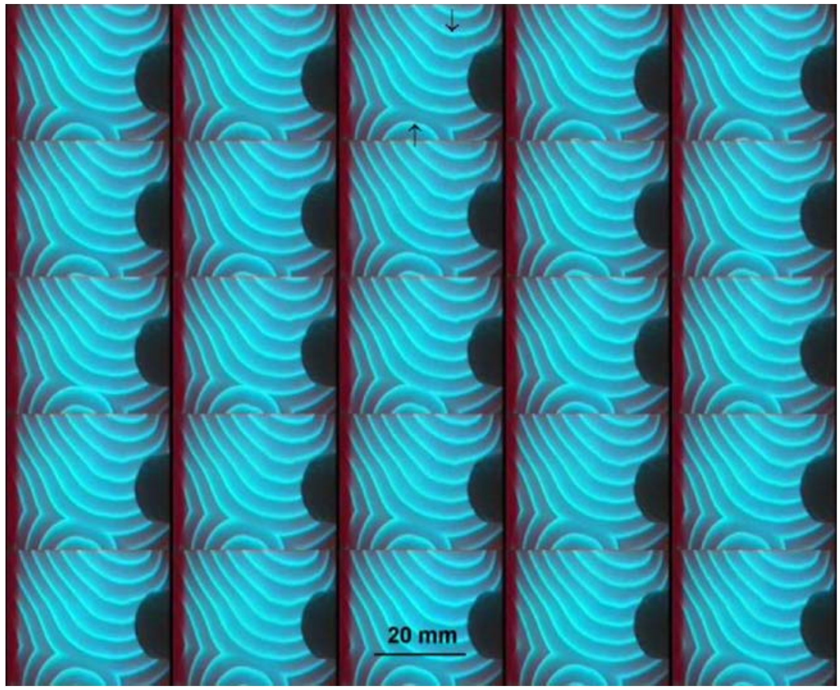

Fig. 4 A series of video frames from waves propagating in a BZ-gel is shown as measured in a laboratory set-up under control conditions. The time between two frames is 4 s. A scaling bar is in space given in the figure. The gel was illuminated with blue light from below. Wave propagation is in the direction of positive curvature of the wave fronts starting from two centres as marked by *arrows in middle frame of the upper row*

flown with four gels, from which finally three gels could be included in the data evaluation. In 20 s segments the wave propagation velocity was determined during the different *g*-phases (for details see the material and methods section). In Fig. 6 upper part, a series of video frames from such a gel is shown.

During the sounding rocket mission four gels were examined according to the protocol described in the materials and methods section. The velocity of waves was determined directly before launch in a 20 s time window, and from the beginning of the micro-g to its end in three 20 s time windows. During the acceleration phase and the landing period video was not available. In Fig. 6, lower part, video frames from the rocket mission are shown. The data for two gels could be evaluated, and the velocity of waves in these two gels at the beginning of the micro-gravity phase was a bit smaller than at its end, but according to the limited resolution of the data, this effect is not statistically significant.

In Fig. 7, the results from all 3 experiment types are summarized. A simple linear fit and a exponential fit of the data are given in the figure, both verifying a decrease of velocity with increasing *g*-value. The velocity of waves in BZ-gels thus slightly decreases with increasing *g*-value in case the vector is perpendicular to the gel. Whereas the data of the centrifuge and the sounding rocket mission are consistent, the slope of the decrease in the parabolic flight data is smaller, and the differences between the three values at μ–g, 1 and 1.8 g are not statistically significant. Nevertheless, the tendency, increasing propagation velocity at decreasing *g*-value, is statistically significant (see Fig. 7 and materials and methods section). This is as well true for the rocket and centrifuge data alone, as when including the parabolic flight data.

It has, additionally, to be taken into account that in this case the timescale of acceleration level changes is of the same order of magnitude as the intrinsic time scale of the oscillations in the gels (parabolic flight), thus the situation possibly better can be described as oscillating acceleration.

Significant changes in the overall pattern in the gels, as well as changes in spiral tip movement due to gravity influence could not be observed under the given conditions.

Discussion

Our results are clearly demonstrating two things, at first a technical aspect, and second data about the gravity dependence of propagating waves in excitable media.

It is obvious that in case of proper logistics, identical equipment can be used in different micro-gravity missions, in the given case in a sounding rocket and in a parabolic-flight mission. According to the limited material support in this field, this is a cost- and time-effective reduction of needed resources. In fact, according to the construction the same set-up even could have been used in a drop-tower mission, too.

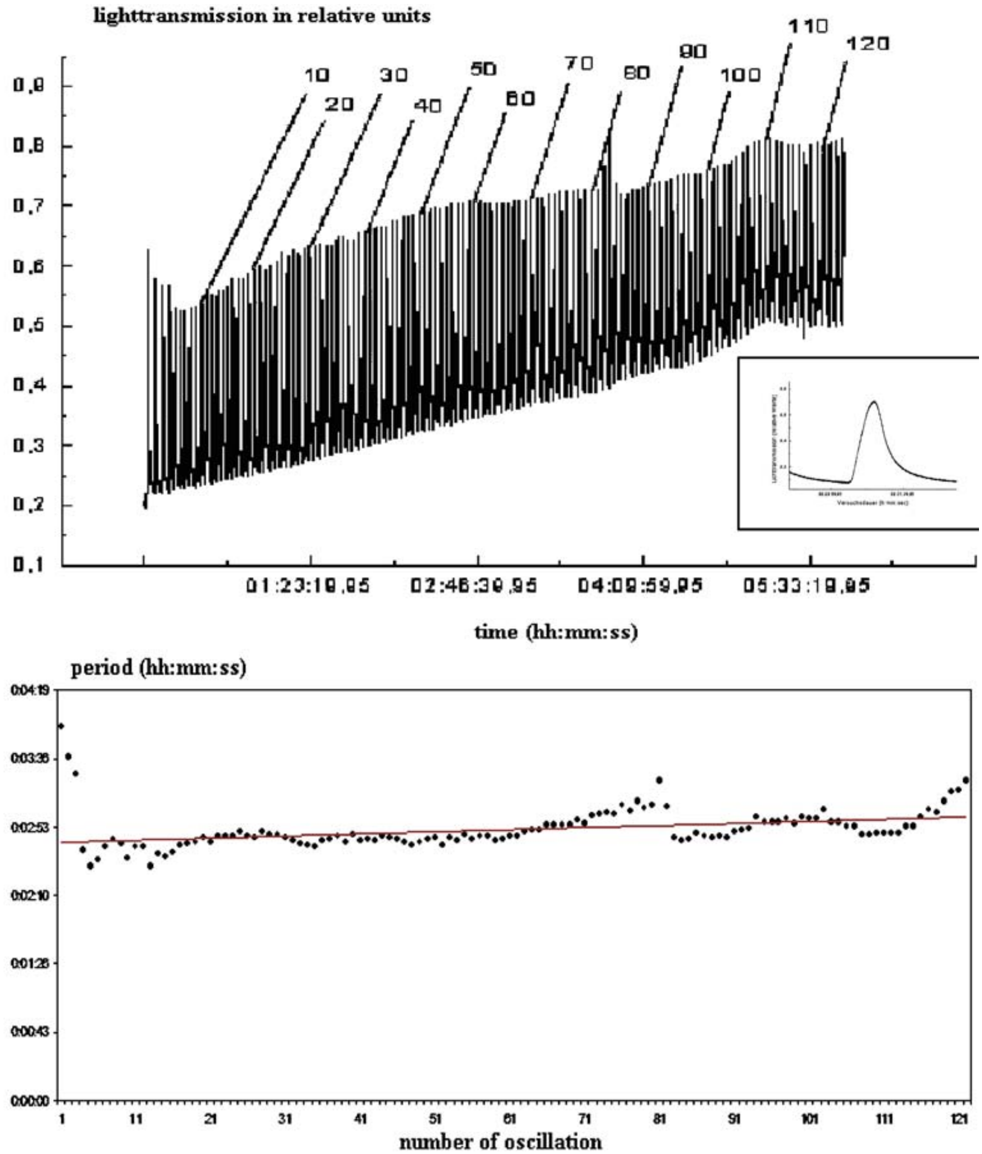

Fig. 5 In the *upper part* a photomultiplier recording of a small area of a BZ-gel is shown. As can be seen, upon the passage of waves a systematic oscillation of brightness occurs, which on a time scale of 1 to 2 h is relatively constant in period. On a longer time scale, in addition a slight increase of brightness can be seen as a consequence of the ongoing chemical reaction in the non stationary system. The insert shows the tenth peak in detail. In the *lower part of the figure* the oscillation period from the upper trace is plotted in time, and again it can be seen that the period is slightly increasing, but is about constant within a time scale of 1 to 2 h

As has been shown in the experiments, and is statistically verified in Fig. 7, waves in gels of the BZ-reaction are clearly gravity dependent. The magnitude of dependence is significantly smaller than for fluid systems, but this is in agreement with the reduced diffusion and convection in the gels compared to fluid systems (Fujieda et al. 1997, 1999, 2002; Pojman et al. 1997; Komlosi et al. 1998). In the centrifuge experiments at higher *g*-values, which have been published previously (Wiedemann et al. 2002), it has been shown that the *g*-effect is due to *g*-amplitude and direction relative to the medium in which the waves travel. This also has been demonstrated earlier in laboratory experiments by Nagypal et al. (1986). Whereas the direction effect could not be investigated in the micro-g experiments, the amplitude effect is present there, too. Towards lower gravity, usually wave propagation speeds up in case the *g*-vector is perpendicular to the gel. Even having in mind the different protocols in sounding rocket experiments (control at 1 g on ground, high acceleration and then 6 min of microgram) and parabolic flights (1, 1.8 g, microgram, 1.8, 1 g changing at a rate of minutes, thus in principle oscillating gravity), the results are in good agreement.

A possible mechanistical explanation of the data for experiments with the *g*-vector being perpendicular to the gel surface could be a fluid shift from the fluid phase on top of the gel in the gel at increasing gravity. However, additional experiments have to be designed to test this hypothesis.

As has been shown previously in centrifuge experiments (Wiedemann et al. 2002), acceleration level changes of up to 6 g, however, also changed wave propagation velocity in cases the *g*-vector was in the

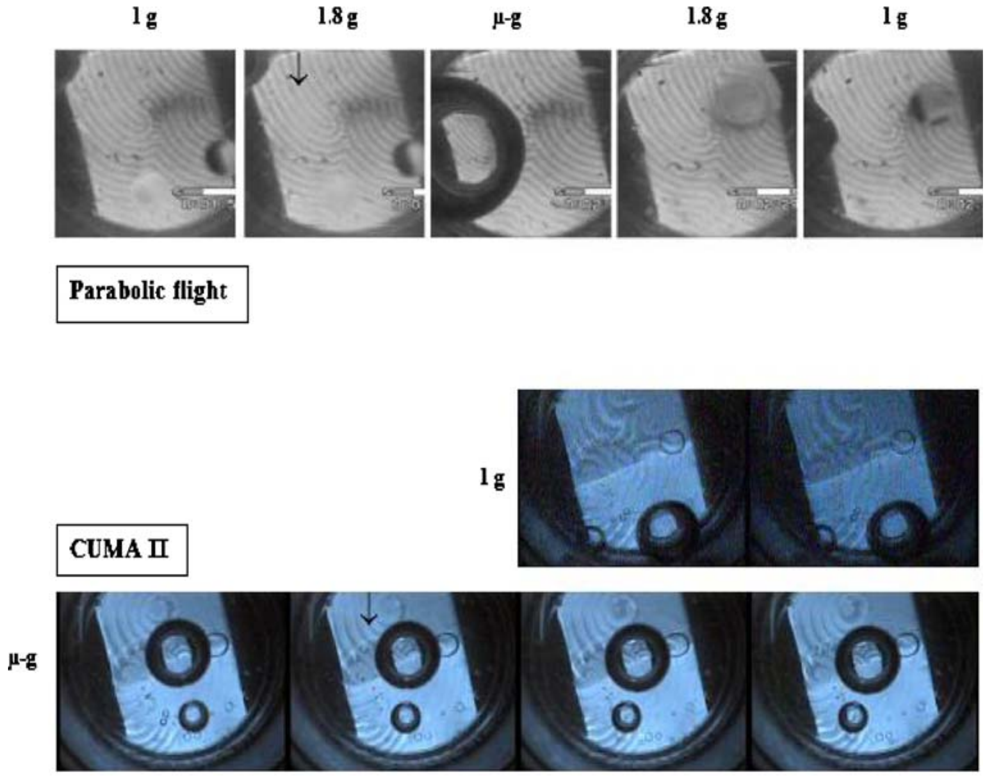

Fig. 6 In the upper row, a montage of a series of video frames from the parabolic flight campaign is shown at different *g*-values as marked above the frames. The size of each frame is about 50 × 50 mm. The *lower rows* shows some video frames from the CUMA II sounding rocket mission. In the *middle*, frames at 1 g before lift off are shown, in the *lower part*, frames are shown from the micro-g phase. The wave propagation in the frames is always in direction of positive curvature of the wave fronts, starting from different centres. The wave propagation velocity was determined in the area marked by *arrows*. The bubbles in the frames origin from CO_2 from the chemical reaction which remains in the closed chambers. In laboratory experiments such bubbles did not affect the behaviour of the waves, as long as the gels were not significantly damaged

plan of the gels. However, there was only a significant difference in case the *g*-vector was in the direction of wave propagation. In this cases, wave velocity slightly increased with increasing *g*-vector. In all other cases the changes were not statistically significant.

In parabolic flights the acceleration level is not constant, but oscillating, as waves in the BZ reaction are travelling with some mm per minute and the typical time between consecutive wave fronts is in the minute range as can be seen in the upper part of Fig. 5. Thus the time constants are similar, and possibly effects like resonance etc. could be expected. This might for example explain the smaller slope of the data measured during parabolic flights.

Within the resolution of our experiments, no changes in pattern formation and in the movement of spiral tips could be observed. As especially the movement of the tip of spirals is known to be very sensitive to external force, this could be a question of future experiments.

As a final consequence of our results the question must be asked, whether in a stirred bulk reaction oscillating in time there will be a gravity dependence of system parameters, as in this case diffusion and convection should no longer play a significant role. Furthermore, an oscillating BZ-system could be used as a model system to study the gravity dependence of biological rhythms, which are extremely important in sustaining life. Such experiments are in preparation in our lab and will be done in future missions.

The gel-type BZ-reaction by itself is a fascinating model system for other wave-propagation processes, especially in biological systems. As in this type of BZ-reaction, in most biological system diffusion is also limited. According to their gravity dependence some of these systems, such as the propagation of action potentials, and the propagation of spreading depression (SD) waves have already been investigated (Wiedemann et al. 2002, 2006; Meissner and Hanke 2005; Epstein and Pojman 1998), as they are, due to their theoretical description, quite similar to the BZ-reaction. Especially SD-waves, which even propagate with about the same velocity as BZ-waves. (Fernandes de Lima et al. 1999). Both, in AP's and SD-waves,

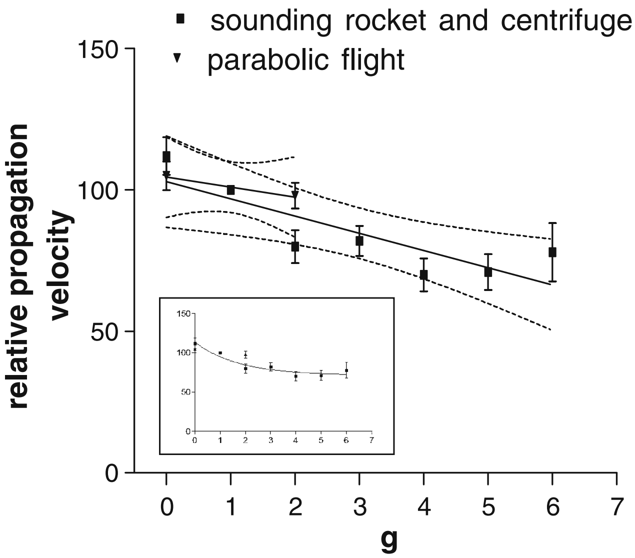

Fig. 7 Wave propagation velocity of waves in BZ-gels as measured during the parabolic flight campaign, at the CUMA II mission, and in a centrifuge, plotted in relative values. The propagation velocity at 1 g always was set to 100%. As can be seen, wave propagation velocity decreases slightly with increasing *g*-value. The centrifuge data are re-plotted from a previously published paper from our group (Wiedemann et al. 2002). Data are linearly fitted with a confidence interval of 95%. In total, the decrease of propagation velocity with increasing *g*-value is statistically significant (r^2 = 0.71; P = 0.018), for the parabolic flight data alone this does not hold. In case fitting all data with a single exponential decay, see inset, the fit is even more significant with $R^2 = 0.997$

gravity effects on the propagation velocity have been reported, and again usually these waves are faster at higher *g*-values on a scale comparable to the BZ-system.

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