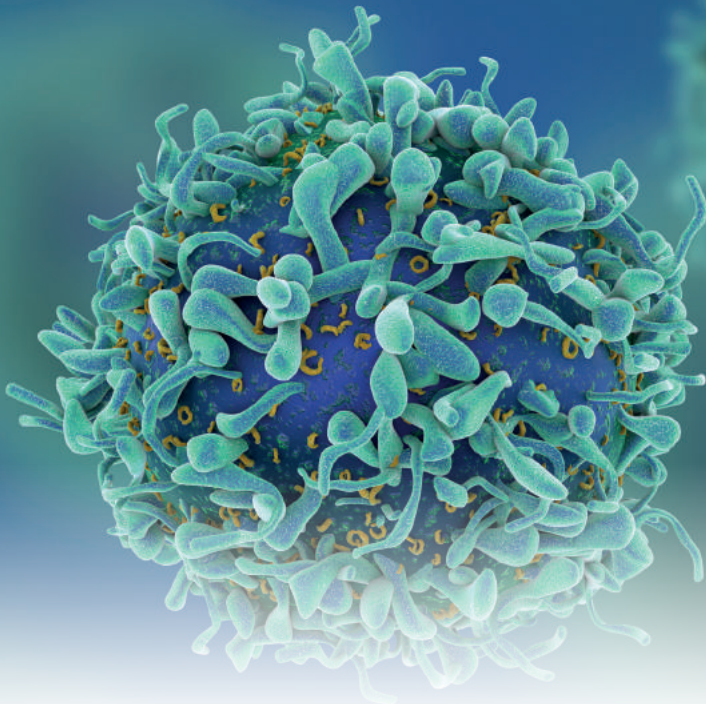


SONOPULS ultrasonic homogenisers

Instructions for the use and applications



- Homogenising
- Extracting
- Suspending
- Disagglomerating
- Degassing
- Emulsifying
- Disrupting cells and tissue
- Optimising processes
- Accelerating chemical reactions

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1964



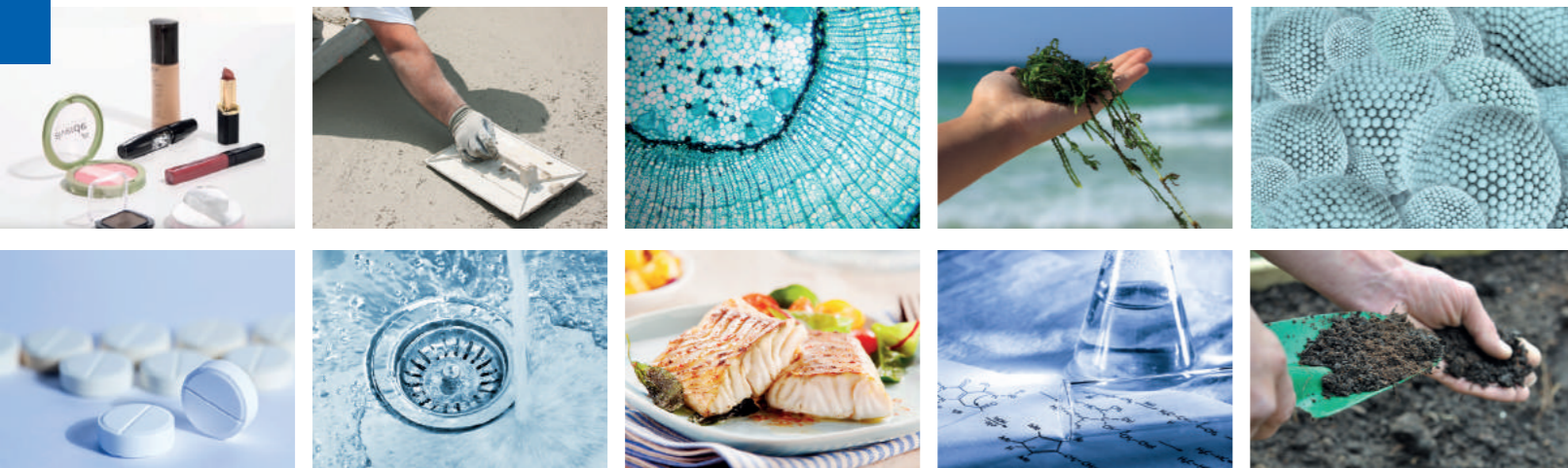
Production of the first ultrasonic homogenisers with tube technology
SONOREX HE 1

1 Introduction

Welcome to the third edition of the application guide for the SONOPULS ultrasonic homogeniser. Perhaps you are interested in its wide range of application options before purchasing or have just acquired the device and are now introducing it as the routine method for individual applications or several applications at the same time. Or maybe you have already been using the ultrasonic homogenising method for a specific application for a long time, and have now discovered how diversely it can be employed for further applications in your working environment.

On the following pages you can find a wealth of information for a wide range of fields and different purposes. And that is precisely our aim.

The application guide has been produced in response to our customers' suggestions and for the benefit of our existing and potential customers. And not only that: it has also been compiled **in cooperation with** our customers. Users report on their practical experiences and make available the method parameters that they have employed successfully. Last but not least, this third edition includes the experiences and knowledge collected in our ultrasound application seminars, in which we delved into the world of ultrasonics with theory and practice reports. The discussions and practical applications using the participants' samples resulted in an array of new experiences for the successful application of the devices. How can the devices be used successfully, how can they be optimally integrated into existing processes and what product features and information are important to users?



The ultrasonic homogeniser's method, in other words the direct application of ultrasonic power to the sample, has proven its worth as a complement to the old, familiar, laboratory ultrasonic baths, which have proven themselves in practice for decades. Foodstuffs, soil, waste, nanoparticles, materials, cosmetics, pharmaceuticals, biotechnology, microbiology, life sciences and chemistry are just some of the fields in which the ultrasonic homogeniser, manufactured by BANDELIN since 1964, is already in use.

Whenever the task involves

- homogenising, suspending, emulsifying
- sample preparation for analysis
- disagglomerating, extracting
- cell and tissue disruption, or
- sonochemistry,

the use of the ultrasonic homogeniser is of interest **as long as a liquid medium is available.**

2 Quick start for use of the device in laboratories

The following pages address a range of topics in detail with the aim of communicating a solid understanding of the methods themselves and their very diverse range of application possibilities. Below you will find the most important steps for a very quick start with the SONOPULS:

For which applications are actually possible with the ultrasonic homogeniser, please see page 23 onwards.

- 1. Structure**
 - a. See instructions for use
- 2. Selection of correct probe for respective application**
 - a. Already selected during sales consultation
 - b. or see page 14
- 3. Selection of vessel**
 - a. When the device and probe are selected appropriately, you can generally use the vessels of your choice, for example ones which are suitable for the activities before and / or after sonication.
 - b. Narrow, taller vessels are basically more suitable than wider, shallower vessels with the same volumes.
 - c. The vessel should not be filled more than 2/3 with liquid (risk of splashing).
 - d. If needed, further details can be found on page 21.
- 4. For further information on the application, tips and tricks, see page 17.**
- 5. For an explanation of how to select method parameters, see page 21.**
- 6. Overview of specific applications with information on all details of application, see page 29 onwards.**
- 7. Do you already use the soundproof box for considerable noise reduction during application? Find out more on our website at www.sonopuls.info or contact us directly!**

3 Basic principles

What is ultrasound and how does it work?

Oscillations with frequencies above 18 kHz (18,000 oscillations per second) are referred to as ultrasound.

Low-frequency ultrasound is used in laboratories whilst higher frequency ranges are used for medical diagnostics.

| Audible | Low-frequency ultrasound | Not currently employed in technical applications | High-frequency ultrasound (diagnostics) |
|---------|--------------------------|--|---|
| 18 Hz | 18,000 Hz 18 kHz | 50,000 Hz 50 kHz | 800,000 Hz 800 kHz 0.8 MHz |
| | | | 4,000,000 Hz 4,000 kHz 4.0 MHz |

The low-frequency ultrasonic oscillations result in the generation of millions of tiny vacuum bubbles in all liquids, which then implode immediately generating highly effective pressure surges. This process is called cavitation. Low frequencies of around 20 kHz create bubbles with larger diameters and more intensive pressure surges than compared with frequencies of around 35 kHz. Low-frequency ultrasound has been used in a wide range of ultrasound baths for decades. The cavitation process effectively and gently removes residual dirt from the surfaces of components immersed in the fluid as well as out of recesses and holes. In the case of sufficiently high sonication intensities, it is also possible to deactivate infectious agents in next to no time, in combination with disinfectants. Other applications include the degassing and mixing of liquids.

Ultrasonic baths versus ultrasonic homogenisers

Compared with the very widely employed ultrasonic baths, ultrasonic homogenisers can be used to apply a considerably higher power density in the liquid. The ultrasound power is released into the liquid via the tip (probe). The oscillations of the probe create at the tip the millions of minute vacuum bubbles described previously, which implode very quickly and trigger shockwaves above 1,000 bar, which can cause detachment of particles or mixing of solution components.

The following table illustrates more clearly the differences between ultrasonic baths and homogenisers.

| | Ultrasonic bath | Ultrasonic homogeniser |
|---|--|--|
| Sample volumes | Approx. 10 – 500 ml | Starting at 0.5 µl – 2 l (stationary) |
| Amplitude [µm] | Approx. 4 | Max. 300 |
| Intensity [W/l] | Up to 50 | Up to 3,000 |
| Frequency [kHz] | 35 Industry: 25 or 40 | 20 (30 / 40) |
| Sonic distribution | Broad | Focused |
| Contamination due to cavitation erosion | Insignificant, as sonication is usually indirect | Yes, low wear of probe tip in direct sonication → traces of minute titanium particles (TiAl6V4) observed in sample |

4 Devices and application parameters

Ultrasonic homogenisers are employed for a wide range of tasks in laboratories every day and the variety of devices on offer is just as varied. A sound understanding of

the basic structure of the homogenisers and the resulting application-specific selection of the individual components forms the basis for a successful application.

4.1 Basic structure of an ultrasonic homogeniser



Structure and function

Ultrasonic generator (control module)

Conversion of inputted low-frequency mains energy of 50/60 Hz into high-frequency voltage of 20 kHz. Control and display of all process parameters and workflows.

Ultrasonic converter

Conversion of the electrical voltage supplied by the generator into mechanical oscillations in the same frequency.

Standard and booster horns

They intensify the oscillations emitted by the ultrasonic converter. The degree of intensification of the amplitude depends on the design.

Probes

They transmit the mechanical oscillations to the sample. The oscillations are only emitted from the tip, not the sides. A high amplitude means particularly intensive sonication. The design of some probes allows them to generate multiple amplitude intensifications. Consequently, the probes attain the highest ultrasonic power densities in liquids.

4.2 Important characteristics of SONOPULS ultrasonic homogenisers

Understanding of the terms power and amplitude

The electrical power rating [W] is not the only decisive factor in the selection of an ultrasonic homogeniser. This value specifies the **power consumption** of the ultrasonic generator, **but not the power applied to the sample**. The **amplitude** of the probe in relation to the sample quantity is decisive for the efficiency and reproducibility of the sonication result. Compared with devices available on the market as standard, SONOPULS ultrasonic homogenisers deliver **higher probe amplitudes with the same electrical power consumption**.

What does that mean?

The higher the viscosity of the medium to be sonicated, the more power is required to reach the same amplitude. It can be compared with the speed of a car:

Objective: 40 km/h (= amplitude) – more power is required to maintain this speed when driving uphill.

The AMPLICHRON® procedure developed by BANDELIN guarantees a **constant amplitude**, independent of changing conditions in the sample to be sonicated and helps you achieve reproducible results. The relative amplitude in per cent is specified for BANDELIN devices and shown on the display. If the actual value for the amplitude does not conform with the set value, e.g., due to probe wear (see page 21) or the viscosity of the medium being too high, this is easily identified and allows conclusions to be drawn about the reproducibility of the results.

4.3 Choosing a device

4.3.1 Introduction – selection options

The wide range of devices and accessories on offer makes it easy to put together the optimal equipment for the respective application:

- Selection of SONOPULS series
- Type of probe
- Direct or indirect sonication
- Flow-through sonication
- Cooling during sonication

Even if a device is initially procured for individual first applications, a wide range of adaptation possibilities for further applications is available through the subsequent purchase of different accessories.

4.3.2 Overview of application fields and equipment of device series

There are currently three SONOPULS series available. The SONOPULS series HD 2000.2 and 4000 differ in their functions and equipment.

The following figures designate the power category of the respective device. HD 4050 = 50 W, HD 4100 = 100 W, HD 4200 = 200 W, HD 4400 = 400 W

The SONOPULS mini20 is a special design for the sonication of particularly small volumes, e.g., in ml reaction cups.

The following criteria apply as general guidelines:

Device series HD 4000

For volumes of approx. 0.5 ml to 3 l in stationary operation or up to 30 l/h in flow-through operation, advanced operation and setting of relevant parameters such as amplitude / power, time and pulsation with freely selectable time intervals, temperature measurement, RS 232 interface, **+ sequencing, different ultrasonic converter types can be connected**

Device series HD 2000.2

For volumes of approx. 2 ml to 1 l in stationary operation or up to 30 l/h in flow-through operation, simple operation and setting of basic parameters such as amplitude, time and pulsation with freely selectable time intervals.

mini20

Suitable for particularly small sample volumes of 0.1 – 25 ml in the µl range, for vessels such as Eppendorf cups, autosampler tubes, etc., preferably for manual handling.

The following table provides a more detailed overview of the application fields.

Further details on the series can be found as of page 27 in the “High-power ultrasound for laboratory and process engineering” brochure.

Please contact us for product recommendations for your application fields!

SONOPULS Series 4000, 2000.2 and mini20 ultrasound homogenisers in comparison



| | Series 4000 | Series 2000.2 | mini20 |
|--|---|---|---|
| | ADVANCED | BASIC | SPECIAL |
| Sample volumes | 0.5 – 3,000 ml | 2 – 1,000 ml | 0.1 – 25 ml |
| Batch | up to 30 l/h | up to 30 l/h | |
| Flow-through | | | |
| Possible probes | 2/3/4/6/9/13/16/19/25/32/38 | 2/3/6/13/19/25 | 1.5/2.0/2.5 |
| Ø [mm] | Possible configurations: GM 4100: – UW 50 or – UW 100 GM 4200: – UW 50 or – UW 200 GM 4400: – UW 400 or – UW 200 | GM 2070.2: – UW 2070 GM 2200.2: – UW 2200 | UW mini20 |
| Ultrasonic converter | 10 – 100% | 10 – 100% | 10 – 100% |
| Amplitude specification | Following insertion of attached probe | Following insertion of attached probe | Following insertion of attached probe |
| Automatic amplitude limitation | Working intervals 0.2 – 600 s Resting intervals 0.3 – 600 s | Working intervals 1 – 60 s Resting intervals 1 – 60 s | Working intervals 0.1 – 60 s Resting intervals 0.2 – 60 s |
| Pulsation | 9 h: 59 min: 59 s or continuous operation | 59 min: 59 s or continuous operation | 59 min: 59 s |
| Time setting | 9 h: 59 min: 59 s | 9 h: 59 min: 59 s | 59 min: 59 s |
| Safety shutdown | Alphanumeric LC display for amplitude, pulse level, time, energy input and temperature (optional) | Alphanumeric LC display for amplitude, pulse level and time | Alphanumeric LC display for amplitude, pulse level, time and energy input |
| Display elements | in kJ | – | in kJ |
| Power display | Optional, 0 – 120°C, temperature sensor required, optional acoustic signal or switch off | – | – |
| Temperature display and measurement | ✓ Multiple sequential batches | – | – |
| Batch operation | RS 232 (D-sub) | – | RS 232 infrared |
| Sequencing | ✓ | ✓ | ✓ |
| Remote control via PC | 20 kHz | 20 kHz | 30 kHz |
| Error diagnosis | 3, 9 | – | 3, 9 |
| Operating frequency | ✓ | – | ✓ |
| Programme storage | 230 V~ (± 10%), alternatively 115 V~ (±10%), 50/60 Hz | 230 V~ (± 10%), alternatively 115 V~ (±10%), 50/60 Hz | 100-240 V~, 50/60 Hz |
| Functional check | | | |
| Mains connection: | | | |

Additional accessories:

Soundproof boxes LS and stand HG



Glass sonication vessels



Stainless steel sonication vessel



Sleeve adapter

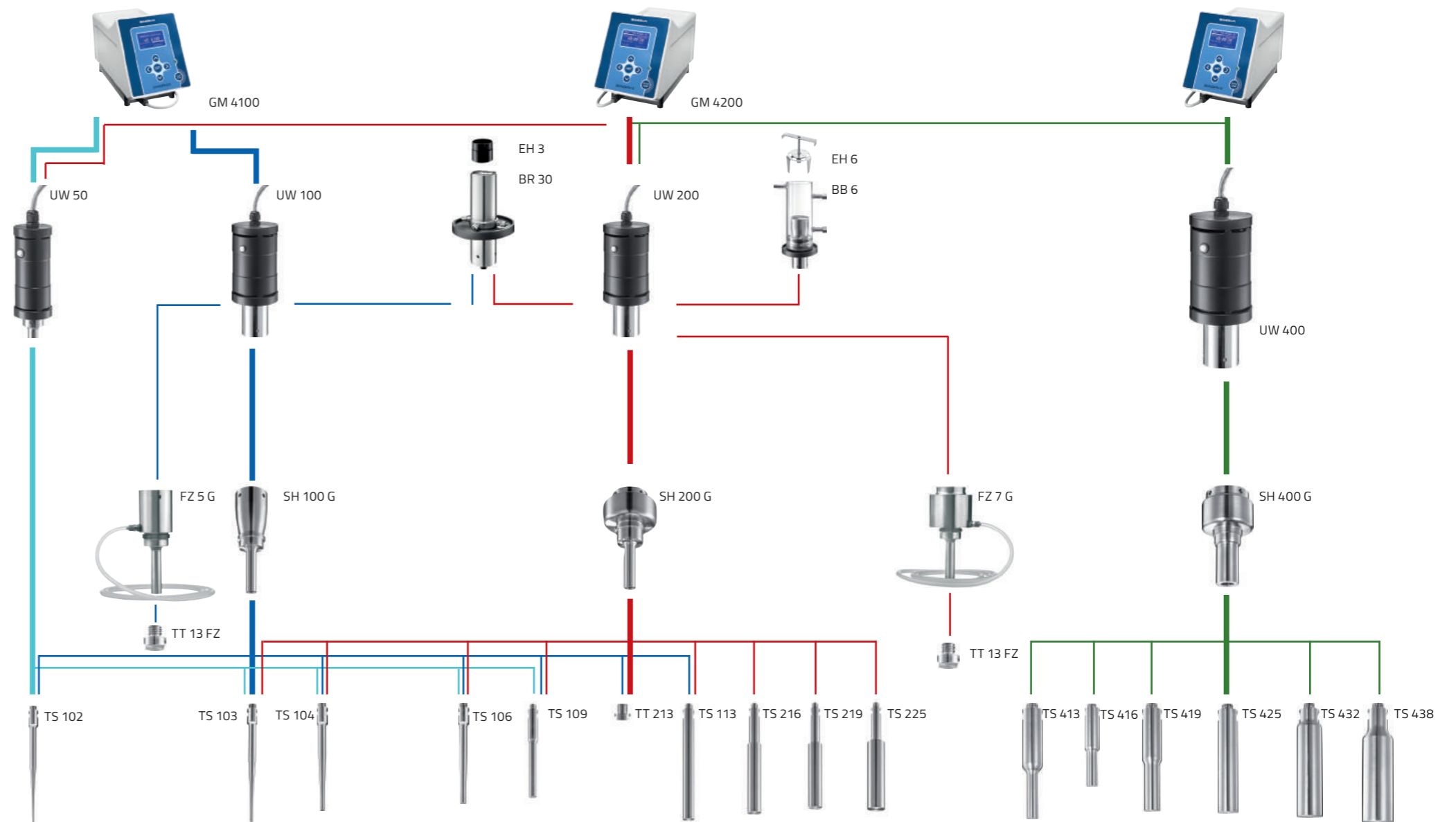


Foot switch TS 8

HD 4050 HD 4100

HD 4200

HD 4400



Stainless steel or glass sonication vessels and sleeve adapters depend on the ultrasonic homogeniser used and the probe.

Additional accessories:

Soundproof boxes LS and stand HG



Glass sonication vessels



Stainless steel sonication vessel



Flow-through vessel DG 4 G (stainless steel)

Sleeve adapter



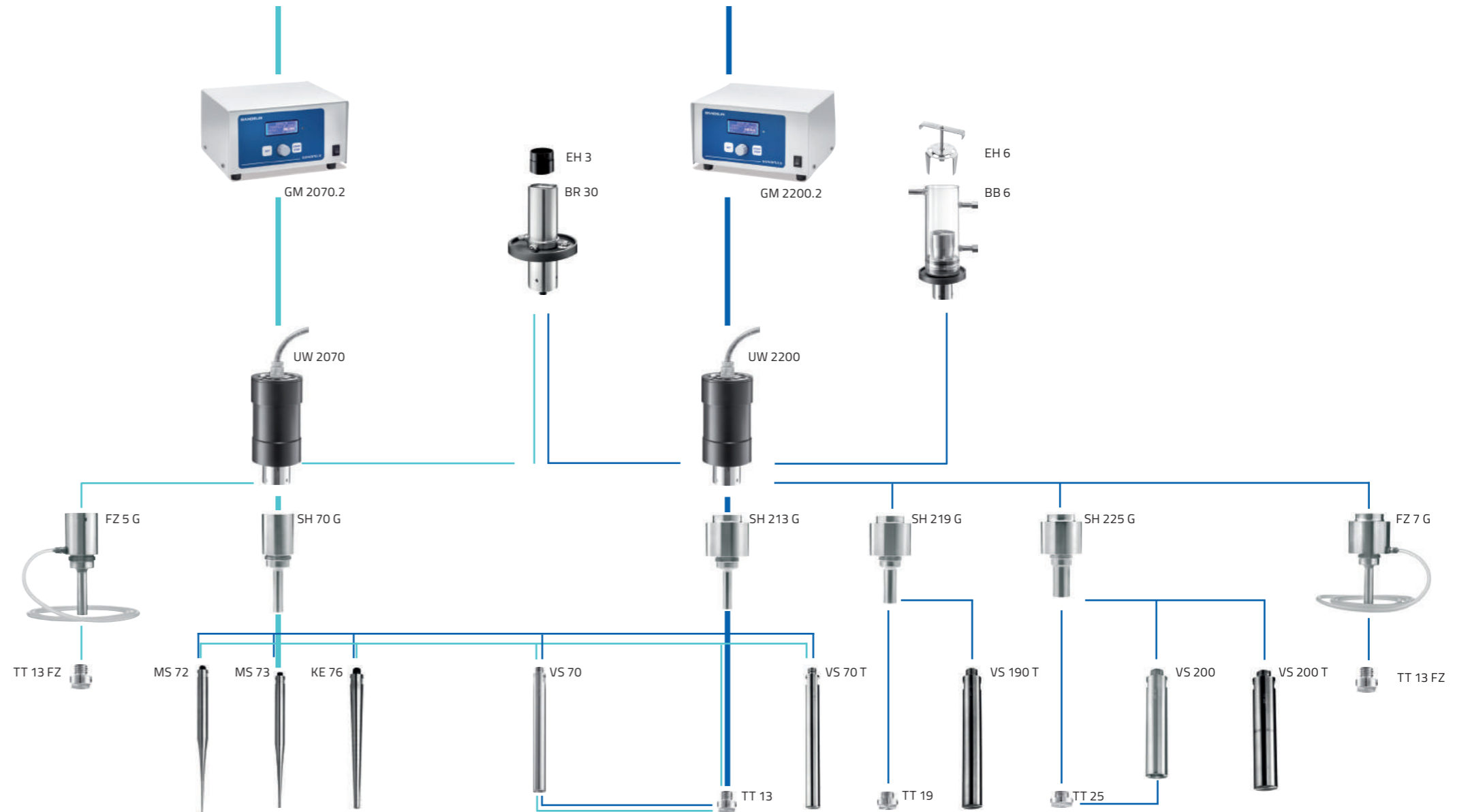
Sleeve adapter NA 29 G / NA 45 G (PTFE)



Foot switch TS 8

HD 2070.2

HD 2200.2



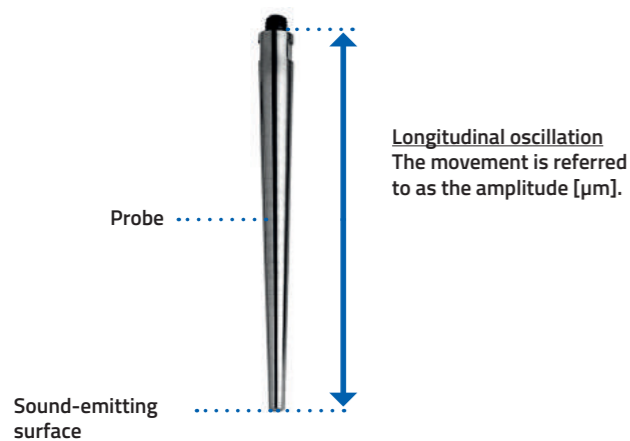
Stainless steel or glass sonication vessels and sleeve adapters depend on the ultrasonic homogeniser used and the probe.

4.3.3 Selection and use of the probes

The probes are thermally stable, autoclavable and resistant to practically all corrosive media. They are produced from a titanium alloy (TiAl6V4 / 3.7165).

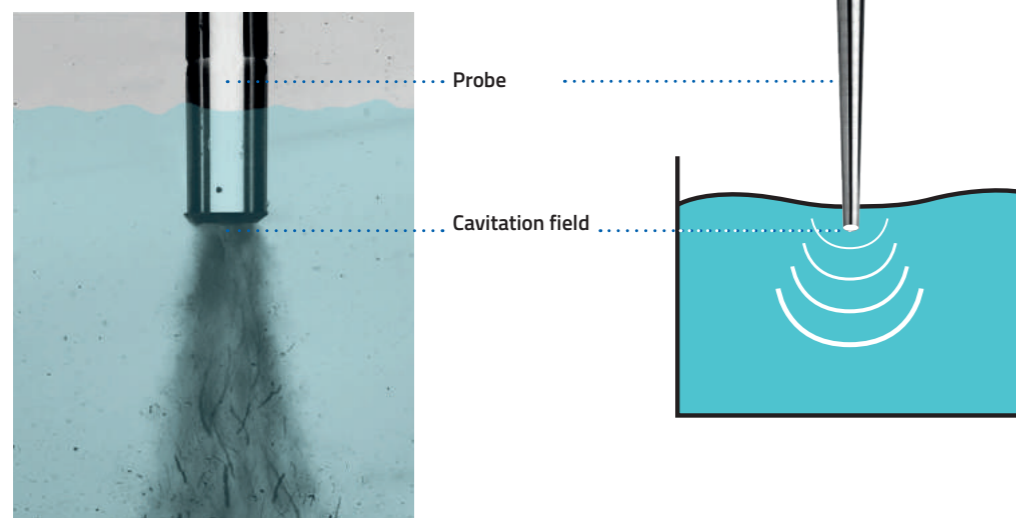
Which probe is best suited?

The probe selection is based on the sonication volume and the desired power density [W/l]. The larger the probe's diameter, the greater the volume that can be sonicated. However, the power density decreases. A very high power density is required for the disruption of yeast cells, for example. The sound is only emitted from the tip of the probe, not the sides!



The sonic distribution conforms to a row of "hemispherical shells" increasing in radius the further they are from the sound-emitting surface (see diagram below). The power density decreases at the same time.

The cavitation process is associated with erosive material abrasion on the probe tip. This becomes evident as a "pitted landscape" on the sound-emitting surface of the probe (see page 21) after a period of operation. The higher the amplitude, the higher in turn the material abrasion, with the service life becoming correspondingly shorter. In other words, the smaller the diameter, the shorter the service life at the same output. If used in continuous operation (100% amplitude, no pulsation), a thin probe can last approx. 6 hours. However, the majority of applications last but seconds or minutes. In some cases, this wear is undesirable as it always mixes with the medium to be sonicated (for example, in sample preparation for metal analysis or similar) – see "Indirect sonication".



The smaller the diameter of the probe tip, the higher the power density and cavitation power for the same electrical power consumption!

Basic probe designs and their application characteristics

In combination with the power of the ultrasonic generator, its design determines the maximum possible amplitude and the energy transferred to the medium. For this reason, the sound intensity transmitted to the medium is inversely proportional to the probe's sound-emitting surface. This means that probes with the smallest sound-emitting surfaces transmit the highest powers per surface [W/mm²] through high amplitudes, depending on the ultrasonic generator's electrical power consumption.

Micro tip (in combination with mini20 only)

Conical shape, used to process small volumes in reaction cups or centrifuge tubes volumes in small beakers



Example: MS 1.5:

Tapered tip

Conical shape, used to process medium-sized



Example: KE 76

Long probes

Rod-shaped, used to process larger volumes in beakers



Example: TS 113

Stepped probe

Broad range for smallest to larger volumes of approx. µl quantities up to 3 l



Example: TS 225

4.3.4 Sonication under special conditions Cooling

The sonication results in the conversion of mechanical power into heat through internal friction in the liquid, and thus to a more or less pronounced heating of the sample liquid. As such, cooling may be necessary for temperature-sensitive samples. For example, the vessels can be placed in an ice bath or vessels with a cooling jacket can be used.



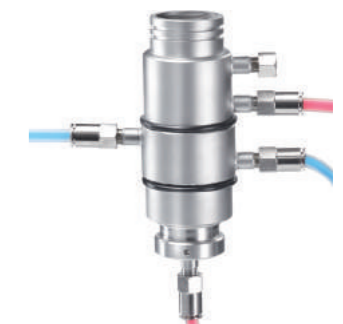
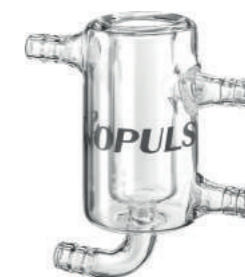
Cooling vessel KG

Flow-through sonication of sample liquid

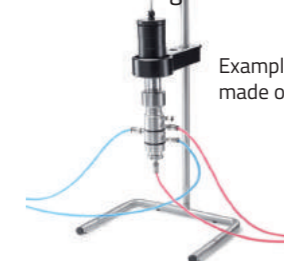
Flow-through volumes of up to 30 l/h are possible for the sonication of larger sample sizes. There are both glass and stainless steel vessels available. The solution can also be introduced into a circuit for intensification of the sonication process. The double wall of the vessels makes cooling possible.

DG 5 made of glass

DG 4 G made of stainless steel for a tight seal to the booster horn



The sonicated medium is introduced from below, in other words head-on to the probe's sound-emitting surface. The sonicated medium comes into contact with the cavitation field as a result of the short distance between the sound-emitting surface and the bottom of the vessel. It has to pass through the cavitation field in order to continue flowing.



Example setup with flow-through vessel made of stainless steel DG 4 G

Combination of two media (DG + FZ):

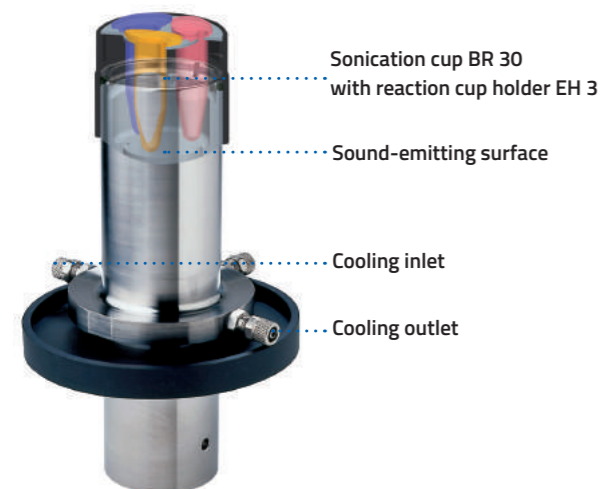
A flow-through horn FZ is used instead of a standard or booster horn. The first medium is introduced into the sonication chamber via the inlet of the flow-through cell, the second via the inlet of the flow-through horn. This medium enters the sonication chamber via the outlet in the sound-emitting surface. Both media can thus be well mixed, even in different ratios (see diagram).



Example setup with flow-through horn FZ 7 G and flow-through vessel DG 5

Indirect sonication

In order to avoid contamination resulting from probe wear, sonication can also be performed indirectly, e.g., in the cup booster BR 30, which also allows cooling.



It functions like a "mini high-performance ultrasonic bath". The sample material is enclosed in a sealed vessel and is sonicated from outside via a transfer medium (e.g., iced water). The transmitted power density [W/l] is approximately 150 times higher than in a "normal" ultrasonic bath, but lower than with direct sonication with a probe.

Connection of a SONOPULS ultrasonic homogeniser to special laboratory vessels

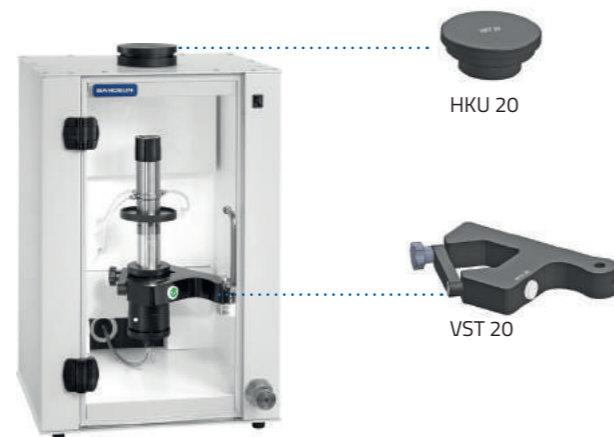
Vessels with the standard ground joint NS 29/32 or NS 45/40 are often used in laboratories for chemical reactions. There are standard ground joints made of PTFE available for ensuring a tight seal between the vessel and the homogeniser. They are screwed onto the external thread of the standard or booster horn and inserted in the vessel.

Figure on left NA 29 G, right NA 45 G



Noise reduction

The cavitation process is associated with noise development, which increases correspondingly with a higher amplitude / power, but also depends on other factors such as temperature and the sonicated medium. This can often be disruptive in the workplace. If there are many people working in the room, we recommended the use of a soundproof box. This can reduce noise by up to 20 dB(AU) (as per IEC 61012).



Example setup LS 20 for indirect sonication

Requisite accessories for indirect sonication

Optimised operation

In addition to using the START/STOP button on the ultrasonic generator, the device can also be operated using a foot switch or a button on the ultrasonic converter. The RS 232 interface can be used to read out data via the device or exchange commands, making it possible to control the device remotely or log process parameters, for example.

For further options, please consult the product brochure or contact us for a personal consultation.

4.4 Selection of method parameters

4.4.1 Introduction

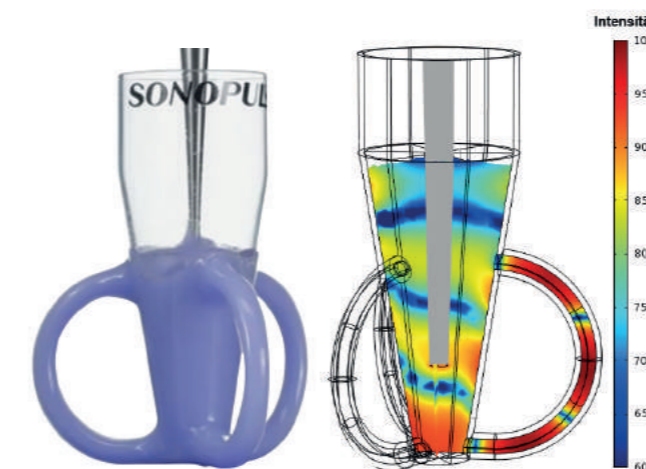
The success of sonication with the ultrasonic homogeniser is fundamentally dependent on the correct selection of the device and method parameters. On the basis of the versions outlined above and / or a consultation with BANDELIN employees, you have now selected the right device with the right probe and possible accessories.

The following chapter explains the parameters so as to allow you to identify the suitable method for your requirements and ensure the sonication is successful. As requirements can be very specific, the approach can be selected in such a way that a basic method is chosen on the basis of similar application scenarios, but needs to be modified in a range of initial tests in order to optimise conditions, using the basic knowledge communicated here so as to suit the individual requirements.

4.4.2 Basic information concerning use Selection of vessels

In theory, you can use any vessels made of any material (glass, plastic, etc.). However, better experiences tend to be gathered with narrow and conical (tapered) vessels. The power transmission is optimal and splashing is largely avoided.

A higher degree of swirling can be achieved with the **rosette cells** available as accessories. The ultrasound pressure forces the sample against the bottom of the vessel and then through the three side arms, so it is sonicated repeatedly. When placed in crushed ice, for example, the sample liquid is cooled very well and effectively thanks to the larger glass surface of the side arms and the uninterrupted circulation.



Fixation of the ultrasonic converter

The ultrasonic converters may only be held by the black housing, for example using a stand clamp. Failure to observe this condition can trigger malfunctions. For example, the set amplitude might not be reached and an error message emitted.

Immersion depth of the probe

The probe should generally be immersed approximately 1 cm into the medium to be sonicated. Too shallow immersion can result in foaming of the sample at the tip. In contrast, if too deep an immersion depth is selected, the result is lateral damping of the probe up to complete failure.

Sonication of a chunky sample in a liquid

In many cases, mechanical grinding of the sample is necessary in advance, as ultrasound is significantly more effective on smaller particle sizes. If chunky samples are to be sonicated, the probe should be positioned directly on the sample.

Probes with a "pitted" surface

The probe tip wears away with use (see Section 4.3.3). At the same time, the efficiency of the sonication deteriorates and the reproducibility of the sample sonication becomes poorer. The smoother the sound-emitting surface, the better the power output in the medium. Sand the probe while the pitting is still minimal (see instructions for use). If the pitting is deeper than approx. 1 mm, the probe should be reprocessed by BANDELIN or replaced.



Mounting of probes

Here it is imperative to ensure that a minimal torque [Nm] is always reached so that a stable mechanical connection between the probe and horn is always guaranteed. We recommend the use of a torque spanner to ensure a reliable mechanical contact and thus correct function (please consult the corresponding product information for the tightening torques). The same applies when changing the horn on the ultrasonic converter.



Further information

For minute volumes, we recommend immersing the probe as far as possible so as to avoid significant movements on the sample surface. If the sample still foams, try working with a lower amplitude, cooling the medium and / or selecting the pulse mode. If necessary, glass beads ($d = 0.5 \text{ mm}$) can also be added. These beads sink to the bottom following sonication and can be centrifuged out. Conical vessels and vessels with irregular interior surfaces are best suited for the sonication of minute volumes in order to prevent foaming.

4.5 Setting the sonication parameters

4.5.1 Amplitude

The amplitude is set to control the power input level and the extent of the cavitation strength. The value is selected as a percentage of the probe's maximum amplitude. The amplitude must be high enough to achieve a good sonication result. If the amplitude and sonication time, and therefore the power application, are too high, the result may be unnecessarily heavy heating, splashing or foaming of the sample liquid or possibly a destruction of the sample components. Settings guidelines can be taken from our application examples or determined in tests.

4.5.2 Pulsation

In the standard setting, the power is transferred to the sample continuously during sonication. In such cases, the device works in continuous operation (non-stop mode).

There are applications in which it can be practical to apply the energy in time intervals. Indications for pulsation include undesirable, rapid heating of the sample, desired settling of the sample on the bottom of the vessel, or allowing reactions to occur during the pauses.

4.5.3 Sonication time

In stationary operation, the sonication time is generally between 15 s and 5 min. Similarly to what applies for the selected amplitude, since too short a sonication period may be insufficient for the desired sonication result. Prolonged sonication, in contrast, may result in an unnecessary temperature increase of the sample or even affect the properties of the sample. Last but not least, it may result in an unnecessary increase in the processing efforts required. It is thus advisable to select a tendency for the sonication time based on the applications outlined in Section 6, and then to analyse in a series of small tests which duration is optimal for the actual application, as there is no 100% correct answer for each type of vessel, sample volume, concentration, etc.

4.5.4 Cooling

Depending on the conditions, the applied power is converted to heat and can thus result in considerable temperature increases in samples with small volumes. The heating can be influenced using the parameters described above: amplitude, pulsation and sonication time. It is necessary to check whether the heating still occurring has a negative effect on the sample. If so, cooling of the samples is recommended. This can be easily done by positioning the sample vessels in an ice bath or crushed ice. Alternatively, double-walled vessels which allow water cooling can also be procured from our range.

4.5.5 Use of beads

When using particularly solid material, it may be useful to add glass beads to the solution, as these intensify the effect of the ultrasonic cavitation. Depending on the application, glass beads in different sizes (up to 0.5 mm in diameter) and different quantities may be added. A ratio of 1/3 glass beads to 2/3 solution often delivers good results. The beads settle to the bottom of the vessel after sonication and can be centrifuged or filtered out. Higher probe wear also needs to be taken into account when using beads.

5 Overview of applications

The number of possible applications is very high and the range of application areas especially broad, with new ones being added all the time. The most important procedures and branches in which the ultrasonic homogeniser is used in laboratories or the sonoreactor at production level, are listed below. Regard it as inspiration for your own situation, as the ultrasonic homogeniser or sonoreactor might represent a viable solution.

5.1 Basic procedures

5.1.1 Dispersing; suspending, emulsifying

Dispersing is a procedure in which substances which do not or barely dissolve in one another, are mixed together optimally. A distinction is made between different types of dispersion depending on the dispersing medium and the dispersed phases.

Emulsion – liquid in liquid
(dispersed phase)

Suspension – solid in liquid

Ultrasonic homogenisers can achieve great results when emulsifying as well as suspending. Particles are disagglomerated and electrostatic attractive forces (Van der Waals forces) perturbed. The high forces (see basics of ultrasound) make it possible to achieve very finely dispersed emulsions / suspensions with very small droplet or particle sizes in the micrometre and nanometre range, which leads to very good stabilities of the resulting emulsions / suspensions. The clumping, agglutination, sedimentation and undesirable inclusion of air experienced with other methods do not occur. Application examples include the production of ink, paints, cosmetics, technical oils, etc.

A particular explosion of applications has been observed in the area of nanoparticles in recent years. Here, it is possible to achieve particularly good dispersion results with regard to the average particle size and particle size distribution, using ultrasound.

Ultrasonic sonication is possible in all size ranges, from μl right up to production levels via upscaling. The sonication can be performed discontinuously or in flow-through.

One example is the production of pharmaceutical preparations, especially minutely dispersed emulsions such as lotions and ointments. When mechanical homogenisers are used, excessively slow stirring often results

in separation of the liquid, and excessively fast stirring leads to the undesirable inclusion of air. The ultrasonic homogeniser produces a physically stable emulsion! The applied amplitude is decisive for the yield of the droplet comminution.

5.1.2 Homogenising

If ultrasound is used for homogenising, the particles (solid or liquid) are comminuted in a liquid, resulting in more intensive mixing. There is a wide range of application possibilities. See below for further information on homogenising in sample preparation for analysis.

5.1.3 Extraction

The extraction of ingredients from solid particles in the liquid phase represents yet another extremely interesting field of application. The possible advantages to be achieved for many applications, in comparison with other extraction methods, are:

- higher yield;
- shorter extraction times;
- lower required temperature;
- lower proportion of solvent; and
- complete conversion to aqueous phases.

A combination of ultrasound and other extraction methods is also practical in some cases. The application can be customised to the requirements, and upscaling to production processes is also possible with excellent results. One example of this application is the extraction of mineral components from the soil in the scope of sample preparation for analysis. The extraction is completely finished after 10 seconds, whereas it has to be shaken for 1 hour in the conventional shaker.

5.1.4 Disagglomeration

Agglomerates can be very effectively destroyed with an ultrasonic homogeniser. For example, this is employed in sample preparation for particle size analysis, as preparation for cell count determination in microbiology, for the production of stable protein solutions, etc. The high variability of the power input makes it possible to ensure that precisely the right amount of power that is required for complete disagglomeration without degrading the particles, cells, etc., is applied.

5.1.5 Degassing, defoaming

The removal of air or other gases from liquids is essential for further use in a variety of scenarios, for example for HPLC eluent, for the analysis of sparkling drinks, for the degassing or defoaming of emulsions, varnishes,

etc. Degassing or defoaming with an ultrasonic homogeniser is very fast, simple and effective. Even large sample volumes, including chemical solutions, can be degassed with ultrasound. This is mostly carried out in a flow-through cell that can also be integrated in a production line where, for example, gas is to be expelled from a fluid (a degassing opening must be present).



5.1.6 Sample preparation for analysis – homogenising, extracting, disagglomerating, degassing

These procedures are widely used in the preparation of samples for analysis and are particularly efficient and simple in their use compared with the available alternatives. The sonication takes just a few seconds or minutes. The preparation, use and cleaning are exceptionally simple and uncomplicated. Dismantling of the device for cleaning is not required.

An autosampler can be used. Examples of applications include:

- disagglomeration as sample preparation for particle size analysis
- homogenising of waste, wastewater, food samples for content analysis
- extraction of components, for example minerals from soil, etc.
- degassing of sparkling drinks for undisturbed analysis of the contents

It is possible to sonicate volumes from μl quantities up to 3,000 ml in stationary operation, and up to 30 l/h in a flow-through vessel made of glass or stainless steel.

The solution to be treated can also be routed through the sonication vessel multiple times in a circuit. In the case of samples consisting of large pieces, comminution in advance is often practical. If necessary, simple cooling is also possible (ice bath, flow-through cooling jacket). The pulsation mode (cyclical sonication) avoids rapid warming on the one hand, and achieves good swirling of the sample on the other.

Long probes are especially suited for the sonication of ceramic suspensions or for sample preparation for particle size analysis, for example.

5.1.7 Disruption of cells, microorganisms and tissue

The ultrasonic homogeniser has been established as the standard method for disruption of cells of all types, for decades. It is possible to disrupt bacteria, yeasts, fungi, eukaryotic or plant cells, tissue, algae and even microalgae. The broad range of variation of the power input is particularly relevant in this respect, as it allows control over the degree of disruption. Fragmentation of DNA, for example, is also possible if desired. An excessively high power input may lead to a high degree of disruption or to unnecessary heating of the sample. Cooling is recommended for the majority of cases, in this respect. To some extent, indirect sonication is also given preference. Even very small quantities in the μl range can be sonicated well and with ease.

5.1.7.1 Cell disruption

Sonication with an ultrasonic homogeniser makes it possible to achieve short disruption times, especially for bacteria. 20 ml of a 20% yeast cell solution can be disrupted in 20 min (use of glass beads). In the case of animal cells, which are encased in only one outer membrane, a significantly shorter disruption time is achieved than with alternative methods. The time needed ranges from only a few seconds to 5 min.



In the case of plant cells, up to 15 min are needed since the cells possess one additional shaping membrane. Thermal damage to the cell contents can be prevented by employing pulsation, i.e., periodic interruption of the power supply. In addition, respectively suitable time intervals can be set on the device. Cooling down is possible during the pulse pause.

In addition, cooling vessels made of glass or stainless steel may be used, making temperature control through the use of liquid cooling agents possible during sonication.

The use of rosette cells, in which the sample is repeated and evenly sonicated thanks to the design of the side arms, is also suitable. Cooling is possible with ease, for example by positioning the vessel in an ice bath. Larger quantities can be sonicated in a flow-through vessel which, just like the cooling vessels, is also equipped with a cooling jacket.

Direct sonication with micro tips is helpful for particularly-resistant bacteria, fungi and spores, since this method makes a higher power density possible. It should be mentioned again at this point that the probes are produced from a titanium alloy and are thus both thermally stable and autoclavable.

Direct sonication of μl quantities in 2 ml plastic vials with the SONOPULS mini20 is regularly employed with success in practice. Alternatively, μl quantities can also be sonicated indirectly in the beaker resonator. This can prove the better alternative if too intense splashing occurs in direct sonication. However, the attainable power densities are lower, but cell disruption is still possible in many cases.

5.1.7.2 Tissue disruption

Another interesting application is the use of ultrasound for tissue disruption, particularly for difficult tissues such as the brain, liver, bladder, aorta, kidneys, lungs, skin, muscles, bone, heart muscle and fibrins. If an intact piece of tissue is sonicated, the piece of tissue and the probe must be in contact. Possible rapid heating of the sample may render cooling necessary. The material, shape and size of the sample vessel are also decisive. Sample vessels made of thin glass, such as Pyrex or Vycor, have a tendency to break when the probe is pressed against the walls of the vessel.

The use of stainless steel centrifuge tubes and 'cold shoulder cooling cells' is recommended. These are thin

stainless steel test tubes with a comb shape on the sides and a dimple on the bottom. The comb shape increases the transfer of heat and the dimple provides a "resting place" for the tissue. If the cell is placed in an ice bath, the temperature of the tissue can be kept at 5°C using a magnetic stirrer.

With skin, effective disruption is only possible if the probe is placed on the tissue and pressed against the bottom of the vessel. Even faster results are obtained if glass beads (diameter up to 0.5 mm) are added to the solution, which fall to the bottom of the vessel after sonication and can then be centrifuged or filtered out. A good ratio is 1/3 glass beads to 2/3 solution. With this approach, 4 minutes are required for the disruption of 1 g of skin. If it is not possible to add glass beads, enzymes such as hyaluronidase can be used to dissolve the connected tissue. The sample vessel should be filled with sufficient liquid in order to prevent foaming, although this is only a problem with minute volumes. It is also possible to place a plastic ring or wire on the surface of the liquid, and thus prevent heavy surface or circular movements. Very small tissue pieces can be well disrupted with a micro tip in a narrow vessel.

Cutting the tissue into small pieces is not especially advantageous unless it is to "flow-through freely" beneath the probe. In such a case, the probe may not be positioned directly on the tissue.

If freezing and grinding are possible, the probe must not touch the tissue. It is also possible to sonicate larger quantities. The following is a simple method for sonicating larger quantities, for example 10 g of liver: The tissue is liquefied for 10 s in a high-speed mixer. The probe is then immersed in the liquid and sonicated for 15 s.

If subcellular elements are to remain intact, the operation should use a lower amplitude and perhaps a longer sonication time.

5.1.8 Sonochemistry

The term 'sonochemistry' refers to the use of ultrasound to influence chemical reactions or polymerisation. Effects that are desired and achieved through such use include an increase of the reaction speed and yield overall or of individual reactants / catalysts, or the influencing of the reaction pathway. In some cases, reactions only occur at all if power is applied via an ultrasonic homogeniser. The effects are understandably extremely case-specific and thus the testing and development of methods can prove very beneficial.

5.2 Biology – Microbiology – Life sciences – Human medicine

5.2.1 Branches with ultrasound applications

The disruption of cells or tissue is an established method for obtaining good results with a wide range of cell and tissue types. With respect to the volumes, there are absolutely no restrictions, whether microvials in labs or applications at production level. More detailed information on cell and tissue disruption can be found in Sections 5.1.7.1 and 5.1.7.2.

Fermentation processes can be activated or accelerated and cells disrupted on a grand scale. A special setup optimises the turnover in biogas plants.

5.2.2 Nanomaterials

Nanomaterials are in widespread use today and there is a whole spectrum of products on offer, so it is not surprising that the range of applications for ultrasonic homogenisers in this field is equally broad. Classic applications include the disagglomeration of nanoparticles in solutions for further use, particle size analysis and the suspension of nanoparticles in solutions for further processing, for toxicity tests, etc.

Ultrasonic homogenisers are also used in the production of nanomaterials, where they contribute to acceleration, controlling reactions, preserving defined particle structures, etc. Further tried and tested applications include the positive influencing of the production of surface coatings and functionalisation / phase transfers of nanoparticles. With respect to the volumes, there are absolutely no restrictions, whether of microvials in labs or applications at production level.

5.2.3 Foodstuffs & drinks

Foodstuffs often need to be homogenised in a liquid phase before they can be analysed. This can be achieved very easily, rapidly and efficiently with the ultrasonic homogeniser. The high power input generates smaller particles and thus achieves a more homogeneous distribution. In many cases, the addition of solvents is no longer necessary and smaller sample quantities can be used. The main area of use for ultrasonic homogenisers is the treatment and preparation of samples, homogenising and extracting all types of substances. The variety of samples is extensive. The sonication of hard cheese, cottage cheese, salami and ham, for example, has proven very successful in practice. In the beverage industry, degassing via ultrasonic homogenisers is a particularly

widespread practice both for subsequent analysis and for further processing requirements. 0.5 l beer is degassed, for example, in 1 minute at 100% amplitude and 50% pulsation.



Microbial processes such as fermentation, cell disruption, enzyme activation, etc., can be supported / performed in a myriad of ways. Autosamplers can be employed for larger sample flow-throughs in sample preparation. All processes such as homogenising, dispersing, suspending, emulsifying and degassing can be performed with sonoreactors in individual setups at production level. Different companies and investigating bodies have performed a range of reference investigations in combination with universities. At one university, for example, a process for the rapid and gentle isolation of fat was developed for determination of the intramuscular fat and fatty acid pattern in pork. To this end, 50 pork chop samples were investigated. Puréed meat was compared with ultrasound-homogenised meat.



Using the ultrasonic homogeniser made it possible to save both time and energy, plus a smaller sample quantity was required! Furthermore, for example, 50 g of frozen fish were homogenised in less than 1 min without the addition of a solvent. Cheese, especially cream cheese, is often homogenised in practice for sample preparation for analysis (e.g., nitrate determination) with excellent application advantages, namely simple handling and very rapid cleaning. It has been documented that very reliable analysis results are obtained.



5.2.4 Cosmetics

Emulsions and suspensions are the keystones of products as well as development, analysis and production processes in the cosmetics industry. As already described, the sonication with the ultrasonic homogeniser produces emulsions and suspensions with outstanding characteristics combined with simple handling and optimal flexibility in terms of the setting of the properties (droplet/particle size, stability, etc.). Another field of application is the extraction of contents from plants, which is possible rapidly, efficiently and with high yields. Both the extraction time and the required extraction temperature are more cost-effective for a wide range of applications than with other extraction methods. The combination of classic extraction methods with the ultrasonic homogeniser has also proven successful in some cases. These processes can be employed in a laboratory or at production level with customised technology constellations. The ultrasonic homogeniser has also established itself excellently in the sample preparation for analysis for cosmetics, be it for particle size analysis, the homogenising of hydrophobic substances with high fat contents such as make-up, lipstick and mascara for analysis of the ingredients (e.g., via HPLC), or other analysis techniques.

5.2.5 Chemistry and pharma industry

The broad spectrum of products and processes in these two branches gives rise to the enormous number of possible applications for the procedures described above with the ultrasonic homogeniser, in laboratories and the sonoreactors at production level. On the one hand, there are the physical procedures of suspending and emulsifying for additives such as pigments or other supplementary components for lubricating oils, formula, etc. On the other hand, sonochemistry allows for the direct influencing of chemical reactions or polymerisations with regard to the yield, reaction speed, reaction control, etc. The overlaps between the pharma, chemical, phyto, cosmetics, life sciences and nanomaterials industries are now very high and the transitions are seamless.

As such, applications such as extraction, cell disruption and disagglomeration (for example for particular polymer structures) are also worth mentioning here. In order to avoid unnecessary repetition, these aspects are not all dealt with in detail here. For further information, please refer to the individual parts of Section 4 for the basic application possibilities and other similar topics addressed in this section.

5.2.6 Ink & ink jets

The dispersion of ink pigments is an outstandingly introduced ultrasonic homogeniser application. As particle sizes down to the low nanometre range can be achieved, it is possible to produce particularly finely dispersed inks with resulting products that have correspondingly high-quality characteristics. It is possible to sonicate both water-based and solvent-based inks. An additional advantage is particularly reliable process control. It is also true that both process development at the laboratory level and up-scaling to production processes are possible with good results.

5.2.7 Paints & varnishes, surface coatings

Pigments, fillers and additives of all kinds can be effectively added to varnishes, paints and other surface coatings using ultrasound. Ultrasonic homogenisers are also used very successfully in the field of nanoparticles in laboratories and sonoreactors in production departments. For all tasks involving dispersing, emulsifying, suspending, disagglomeration, defoaming or degassing, ultrasound is a tried and tested means of conducting processes and optimising product features as described above. Ultrasound can also be employed outstandingly in the ever more desired changeover of solvent-based to water-based products and the reduction of VOCs, be it in product development in the laboratory or in the sonoreactor in production following upscaling. Disagglomeration or homogenising for sample preparation via an ultrasonic homogeniser can also be successfully employed in the field of analytics. There are also application possibilities in the field of synthesis, such as mini emulsion polymerisation to name but one example.

5.2.8 Construction industry

Ceramics and cement manufacturers, among others, employ ultrasonic homogenisers for a wide range of applications. The predispersing of slips and the suspension of substances such as aluminium oxide, silicon dioxide, etc., as well as sample preparation for particle size analysis are all examples of practical applications. Here too, the production process, such as the production of cement, can be influenced positively.

6 Detailed applications

A word in advance

The number of applications in a certain application field is not directly related to the suitability of the ultrasonic homogeniser for these applications. It can be largely attributed to the segments in which the use of the ultrasonic homogeniser has been established in practice for many years and those where the viability was only recently “discovered”, often with particular success. The detail provided for each application is another criterion. Whereas individual description of the cell disruption for many different organisms appears practical, in other areas such as that of degassing, a general application is sufficient. Ultimately, we can adopt as many varieties of

the practice examples in this collection as are provided by cooperative users.

The collection of applications is expanding all the time. We are happy to receive any feedback concerning interesting applications,

The overview shows you which applications are already written down in practice reports. We will be delighted to send you the corresponding application notes on request (info@bandelin.com). If the application you are looking for is not there, please contact us. We will surely be able to provide you with some pointers.

6.1. Classification based on process

6.1.1 Dispersing, suspending

| Number | Work area | Branch | Title |
|--------|-----------------------|------------|--|
| C-104 | Dispersing/suspending | Materials | Dispersing of carbon nanoparticles in processing oil |
| C-105 | Dispersing/suspending | Materials | Dispersing of ceramic raw materials and glass powder |
| C-107 | Dispersing/suspending | Pharma | Production of ultrafine pharmaceutical emulsions |
| C-108 | Dispersing/suspending | Polymers | Production of microcapsules with monomers |
| C-109 | Dispersing/suspending | Materials | Dispersing of solids such as aluminium oxide and silicone dioxide |
| C-202 | Dispersing/suspending | Materials | Suspending of multi-walled carbon nanotubes (MWCNTs), GFRPs and other hard-to-dissolve materials |
| C-203 | Dispersing/suspending | Materials | Sample preparation of ceramic suspensions for particle measurement – particle size analysis |
| C-207 | Dispersing/suspending | Polymers | Production of polymer particle suspensions |
| L-102 | Dispersing/suspending | Foodstuffs | Production of hop emulsions |

6.1.2 Disagglomeration

| Number | Working area | Branch | Title |
|--------|---|--------------------|---|
| B-208 | Disagglomeration | Microbiology | Separation of yeasts for determination of the vital cell count |
| C-101 | Disagglomeration / particle size analysis | Materials | Disagglomeration of tungsten powder for subsequent particle size determination |
| C-102 | Disagglomeration / particle size analysis | Materials | Dispersing of fine metal powder (Al) for subsequent particle size determination |
| C-106 | Disagglomeration / particle size analysis | Water /waste-water | Disagglomeration of water sediment samples in preparation for particle size analysis |
| C-111T | Disagglomeration / particle size analysis | Materials | Disagglomeration as sample preparation for particle size analysis – Tabular overview |
| C-204 | Disagglomeration / particle size analysis | Materials | Sample preparation for the particle size measurement of catalyst dispersions |
| C-208 | Disagglomeration / particle size analysis | Foodstuffs | Homogenising of solid food supplements in water for sample preparation for particle size analysis |

6.1.3 Degassing, defoaming

See Section 5.1.5

6.1.4 Extraction

| Number | Working area | Branch | Title |
|--------|--------------|--------------------|---|
| C-201 | Extraction | Soil | Extraction of exchangeable magnesium from soil |
| C-206 | Extraction | Paints / varnishes | Extraction of oily ingredients from dried varnish |

6.1.5 Sample preparation for analysis (except particle size analysis)

| Number | Working area | Branch | Title |
|--------|--------------------|--------------------|--|
| B-114 | Sample preparation | Medicine | Homogenising of sperm for determination of quantity |
| B-212 | Sample preparation | Molecular biology | Dissolving of peptides as sample preparation for analysis |
| C-110 | Sample preparation | Water / wastewater | Sample preparation of wastewater samples |
| C-112T | Sample preparation | Miscellaneous | Sample preparation for analysis for soil and wastewater samples |
| C-205 | Sample preparation | Cosmetics | Homogenising of cosmetics in solvents for sample preparation for analysis |
| C-210 | Sample preparation | Water / wastewater | Sample preparation of wastewater containing particles, for TOC determination as per DIN EN 1484 |
| L-101 | Sample preparation | Foodstuffs | Fast and gentle isolation of fat for fatty acid determination in meat – Method improvement |
| L-103 | Sample preparation | Foodstuffs | Identification of fatty acid distribution in bovine milk |
| L-201 | Sample preparation | Foodstuffs | Sample preparation for determination of nitrate content in cheese (xylenol process) |
| L-202 | Sample preparation | Foodstuffs | Sample preparation for potentiometric determination of chloride content in cheese |
| L-203 | Sample preparation | Foodstuffs | Sample preparation for potentiometric determination of chloride content in cheese |
| L-204 | Sample preparation | Foodstuffs | Sample preparation / homogenising of cheese and other foodstuffs and extraction of relevant analytes |

6.1.6 Sample preparation for particle size analysis

see 6.1.2 Disagglomeration

6.1.7 Cell and tissue disruption

6.1.7.1 Cell disruption

| Number | Working area | Branch | Title |
|--------|-----------------|--------------------------------|---|
| B-101 | Cell disruption | Molecular biology | Cell and tissue disruption, including in µl-batches with indirect sonication in a beaker resonator |
| B-102 | Cell disruption | Molecular biology | Cell disruption of yeast cells |
| B-108T | Cell disruption | Molecular biology | Cell disruption of Escherichia coli bacteria – tests with diverse parameters with the SONOPULS |
| B-109 | Cell disruption | Molecular biology | Cell disruption of Pseudomonas thailandensis |
| B-110 | Cell disruption | Molecular biology | Lysis and fragmentation of cell cultures via indirect sonication in the scope of cancer research |
| B-111 | Cell disruption | Molecular biology | Procurement of proteins for the western blot technique, e.g., for evidence of HIV or other infections |
| B-112 | Cell disruption | Molecular biology | Cell disruption of eukaryotic cells as preliminary step to protein isolation |
| B-113 | Cell disruption | Molecular biology | Cell disruption of insect cells as preliminary step to protein isolation |
| B-115 | Cell disruption | Molecular biology | Cell disruption of mammalian cells |
| B-117 | Cell disruption | Molecular biology | Production of lysates from purchased cell cultures for antibody reactions |
| B-119T | Cell disruption | Molecular biology | Cell disruption of different organisms and cells – Tabular overview |
| B-201 | Cell disruption | Molecular biology | Cell disruption of E. coli in volumes from µl to l |
| B-203 | Cell disruption | Algae | Cell disruption of Haematococcus pluvialis microalgae for carotenoid analysis |
| B-205 | Cell disruption | Molecular biology | Cell disruption of Escherichia coli for protein analysis |
| B-206 | Cell disruption | Molecular biology/ Medicine | Cell disruption of human cells |
| B-207 | Cell disruption | Algae | Cell disruption of microalgae and cyanobacteria |
| B-209 | Cell disruption | Molecular biology | Production of cell lysates of eukaryotic cells in different volumes |
| B-211 | Cell disruption | Molecular biology | Cell disruption for enzyme processing for E. coli or fungi cultures |

6.1.7.2 Tissue disruption

| Number | Working area | Branch | Title |
|--------|-------------------|-------------------|--|
| B-106 | Tissue disruption | Tissue | Tissue disruptions, especially also for difficult tissues |
| B-107 | Tissue disruption | Tissue | Tissue disruption of larger quantities, e.g., liver |
| B-116 | Tissue disruption | Molecular biology | Production of protein lysates from tissue |
| B-118T | Tissue disruption | Tissue | Tissue disruption applications – Tabular overview |
| B-202 | Tissue disruption | Toxicology | Tissue disruption –Homogenising of organs in forensic medicine |

6.1.8 Miscellaneous

| Number | Working area | Branch | Title |
|--------|---------------|-------------------|--|
| B-103 | Miscellaneous | Medicine | Procurement of stroma-free haemolysate from EDTA blood for paternity testing |
| B-104 | Miscellaneous | Molecular biology | Liposome production |
| B-105 | Miscellaneous | Molecular biology | Replication of infectious prions – process acceleration via ultrasound |
| B-204 | Miscellaneous | Molecular biology | Homogenising of peptide with Freund's adjuvant |
| B-210 | DNA isolation | Molecular biology | Disruption of FFPE tissue for DNA isolation |
| C-103 | Miscellaneous | Polymers | Degradation of cellulose using ultrasound |
| C-209 | Miscellaneous | Materials | Phase transfer of iron oxide nanoparticles |

6.2 Classification by branches / working areas

6.2.1 Materials

| Number | Working area | Branch | Title |
|--------|--|-----------|---|
| C-101 | Disagglomeration / particle size analysis | Materials | Disagglomeration of tungsten powder for subsequent particle size determination |
| C-102 | Disagglomeration / particle size analysis | Materials | Dispersing of fine metal powder (Al) for subsequent particle size analysis |
| C-104 | Dispersing/ suspending | Materials | Dispersing of carbon nanoparticles in process oils |
| C-105 | Dispersing/ suspending | Materials | Dispersing of ceramic raw materials and glass powder |
| C-109 | Dispersing/ suspending | Materials | Dispersing of solids such as aluminium oxide and silicone dioxide |
| C-111T | Disagglomeration / particle size analysis | Materials | Disagglomeration as sample preparation for particle size analysis – Tabular overview |
| C-202 | Dispersing/ suspending | Materials | Suspending of multi-walled carbon nanotubes (MWCNTs), GFRPs and other hard-to-dissolve materials |
| C-203 | Dispersing/ suspending | Materials | Sample preparation of ceramic suspensions for particle measurement – particle size analysis |
| C-204 | Disagglomeration / particle size analysis | Materials | Sample preparation for the particle size measurement of catalyst dispersions |
| C-209 | Miscellaneous | Materials | Phase transfer of iron oxide nanoparticles |

6.2.2 Polymers / paints and varnishes

| Number | Working area | Branch | Title |
|--------|---------------------------|----------------------|---|
| C-103 | Miscellaneous | Polymers | Degradation of cellulose using ultrasound |
| C-108 | Dispersing/ suspending | Polymers | Production of microcapsules with monomers |
| C-206 | Extraction | Paints/ varnishes | Extraction of oily ingredients from dried varnish |
| C-207 | Dispersing/ suspending | Polymers | Production of polymer particle suspensions |

6.2.3 Environment

| Number | Working area | Branch | Title |
|--------|---|----------------------|---|
| C-106 | Disagglomeration / particle size analysis | Water/ wastewater | Disagglomeration of water sediment samples in preparation for particle size analysis |
| C-110 | Sample preparation | Water/ wastewater | Sample preparation of wastewater samples |
| C-201 | Extraction | Soil | Extraction of exchangeable magnesium from soil |
| C-210 | Sample preparation | Water/ wastewater | Sample preparation of wastewater containing particles for TOC determination as per DIN EN 1484 |

6.2.4 Life sciences / molecular biology

| Number | Working area | Branch | Title |
|--------|--------------------|--------------------------------|---|
| B-101 | Cell disruption | Molecular biology | Cell and tissue disruption, including in µl-batches with indirect sonication in a beaker resonator |
| B-102 | Cell disruption | Molecular biology | Cell disruption of yeast cells |
| B-103 | Miscellaneous | Medicine | Procurement of stroma-free haemolysate from EDTA blood for paternity testing |
| B-104 | Miscellaneous | Molecular biology | Liposome production |
| B-105 | Miscellaneous | Molecular biology | Replication of infectious prions – process acceleration via ultrasound |
| B-108T | Cell disruption | Molecular biology | Cell disruption of Escherichia coli bacteria – tests with diverse parameters with the SONOPULS |
| B-109 | Cell disruption | Molecular biology | Cell disruption of Pseudomonas thailandensis |
| B-110 | Cell disruption | Molecular biology | Lysis and fragmentation of cell cultures via indirect sonication in the scope of cancer research |
| B-111 | Cell disruption | Molecular biology | Procurement of proteins for the western blot technique, e.g., for evidence of HIV or other infections |
| B-112 | Cell disruption | Molecular biology | Cell disruption of eukaryotic cells as preliminary step to protein isolation |
| B-113 | Cell disruption | Molecular biology | Cell disruption of insect cells as preliminary step to protein isolation |
| B-115 | Cell disruption | Molecular biology | Cell disruption of mammalian cells |
| B-116 | Tissue disruption | Molecular biology | Production of protein lysates from tissue |
| B-117 | Cell disruption | Molecular biology | Production of lysates from purchased cell cultures for antibody reactions |
| B-119T | Cell disruption | Molecular biology | Cell disruption of different organisms and cells – Tabular overview |
| B-201 | Cell disruption | Molecular biology | Cell disruption of E. coli in volumes from µl to l |
| B-204 | Miscellaneous | Molecular biology | Homogenising of peptide with Freund's adjuvant |
| B-205 | Cell disruption | Molecular biology | Cell disruption of Escherichia coli for protein analysis |
| B-206 | Cell disruption | Molecular biology/ Medicine | Cell disruption of human cells |
| B-209 | Cell disruption | Molecular biology | Production of cell lysates of eukaryotic cells in different volumes |
| B-210 | DNA isolation | Molecular biology | Disruption of FFPE tissue for DNA isolation |
| B-211 | Cell disruption | Molecular biology | Cell disruption for enzyme processing for E. coli or fungi cultures |
| B-212 | Sample preparation | Molecular biology | Dissolving of peptides as sample preparation for analysis |

6.2.5 Medicine / toxicology / microbiology / algae

| Number | Working area | Branch | Title |
|--------|--------------------|--------------|---|
| B-103 | Miscellaneous | Medicine | Procurement of stroma-free haemolysate from EDTA blood for paternity testing |
| B-114 | Sample preparation | Medicine | Homogenising of sperm for determination of quantity |
| B-202 | Tissue disruption | Toxicology | Tissue disruption –homogenising of organs in forensic medicine |
| B-203 | Cell disruption | Algae | Cell disruption of Haematococcus pluvialis microalgae for carotenoid analysis |
| B-207 | Cell disruption | Algae | Cell disruption of microalgae and cyanobacteria |
| B-208 | Disagglomeration | Microbiology | Separation of yeasts for determination of the vital cell count |

6.2.6 Foodstuffs

| Number | Working area | Branch | Title |
|--------|--|------------|--|
| C-208 | Disagglomeration/ particle size analysis | Foodstuffs | Homogenising of solid food supplements in water for sample preparation for particle size analysis |
| L-101 | Sample preparation | Foodstuffs | Fast and gentle isolation of fat for fatty acid determination in meat – Method improvement |
| L-102 | Dispersing/ suspending | Foodstuffs | Production of hop emulsions |
| L-103 | Sample preparation | Foodstuffs | Identification of fatty acid distribution in bovine milk |
| L-201 | Sample preparation | Foodstuffs | Sample preparation for determination of nitrate content in cheese (xylene process) |
| L-202 | Sample preparation | Foodstuffs | Sample preparation for potentiometric determination of chloride content in cheese |
| L-203 | Sample preparation | Foodstuffs | Sample preparation for potentiometric determination of chloride content in cheese |
| L-204 | Sample preparation | Foodstuffs | Sample preparation / homogenising of cheese and other foodstuffs and extraction of relevant analytes |

6.2.7 Pharma / cosmetics

| Number | Working area | Branch | Title |
|--------|---------------------------|-----------|---|
| C-107 | Dispersing/ suspending | Pharma | Production of ultrafine pharmaceutical emulsions |
| C-205 | Sample preparation | Cosmetics | Homogenising of cosmetics in solvents for sample preparation for analysis |

6.3. Publications

You can find articles in which a wide variety (thousands) of our SONOPULS ultrasonic homogenisers are explained, in select publications and on the Internet using the keyword **SONOPULS**.

Sample preparation for determination of particle sizes – Disagglomeration with ultrasonic homogenisers

Morten Schonert¹, Richard Winterhalter², Dr. rer. nat. Kirsten Siebertz³

1 Umicore AG & Co. KG, Automotive Catalyst, Hanau, Germany,

2 Bavarian Health and Food Safety Authority, Chemical Security & Toxicology, Bavaria, Germany,

3 TDCLAB Dr. Siebertz GmbH, Nidderau, Germany

Published in GIT Labor-Fachzeitschrift, Issue 01/2018, pp. 24 – 26

Use in laboratory analytics after comparison with conventional method

(use of ultrasonic homogeniser for sample preparation of foodstuffs (cheese))

Susanne Zeller¹, Hagen Nusche², Dr. rer. nat. Kirsten Siebertz³

1 Mecklenburg-Vorpommern State Office for Agriculture, Food safety and Fishery, Neubrandenburg Office, Germany

2 Operating Company for Environment and Agriculture (BfUL), Nossen, Germany

3 TDCLAB Dr. Siebertz GmbH, Nidderau, Germany

Presentation at 2016 VDLUFA congress in Rostock, published in VDLUFA-Schriftenreihe 73 (2016), 598

State-of-the-art sample preparation with ultrasonic homogenisers – applied test for foodstuffs and tissue

Dr. Cora Wunder¹, Susanne Zeller², Dr. rer. nat. Kirsten Siebertz³

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Ultrasound applications in technology and production

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7 FAQs

7.1 FAQs concerning practical application

Sample liquid splashes out of the vessel. What do I need to change? Possible solutions:

- Set a lower amplitude and test whether the result is still satisfactory
- Use conical vessels
- Increase the immersion depth

My sample fluid foams a lot. How can I prevent that?

- Increase the immersion depth
- Add glass beads
- Use a conical vessel
- Place wire on the surface of the sample

How deep should I insert the probe?

- Normally min. 0.5 and max. 2 cm. Immersion that is too deep results in dampening of the probe that is too severe. This results in insufficient application of power to the sample.
- In Eppendorf cups, as far as possible – ensure that the sample does not foam!

Can the probe touch the vessel during sonication?

No. This can result in damage to the probe and the vessel (melting, breakage).

Can I touch the probe with my hands during the sonication process?

No. This can result in bone damage.

I want to separate / disagglomerate cells without destroying them.

What do I need to change?

- Reduce the amplitude or use a probe with a larger diameter.

How is the power for SONOPULS ultrasonic homogenisers measured?

During the measurement of power, the sonication vessel should be used for the standard trials.

This vessel is filled with water. The temperature increase can be measured for a set period of time and the power density calculated from the volume, temperature increase and elapsed time, using the familiar formula. This is done using the following formula ¹:

$$\text{The following applies: } P/V = \frac{c\Delta\theta}{\Delta t}$$

| | |
|-----|---|
| P/V | Power density in water [W/cm ³] |
| c | Specific heat capacity of the water |
| Δt | Length of time between the two temperature measurements |
| Δθ | Temperature difference between the two temperature measurements |
| V | Test water volume |

This method allows for documentation of the input of power in the test series. Further information can be requested from bandelin.com (power determination of SONOPULS ultrasonic homogenisers – 5169).

¹ Note: The formula is only adequate for small volumes.

Can solvents be sonicated?

- Yes, but safe extraction of vapours must be guaranteed!
- Only small amounts!
- Observe the flashpoint; cooling may be required!

7.2 FAQs concerning devices, probes and safety aspects

What should be done if the probe displays mild pitting?

At depths of up to approx. 1 mm, the probes should be carefully polished manually in your facility. For further information, refer to the instructions for use.

Are probes available in different lengths?

No. The probes are always calibrated to the resonance frequency and dictated by the design. They vary in the millimetre range depending on the acoustic properties of the titanium cast used (batch).

Do I need to take anything into consideration when disposing of probes?

- Probes can be disposed of without any problems. They pose no hazards. They do not contain heavy metal and are thus environmentally friendly.
- Scrap dealers offer minor remuneration (titanium weighs very little but is valuable)

Can probes also be produced from another material?

Yes, but with the respective restrictions:

1. Quartz glass – only low amplitudes are possible, as the material cannot withstand high amplitudes.
2. Ceramics – permit higher amplitudes than quartz glass, but is liable to break.
3. Stainless steel – very brittle. Breaks quickly and more likely to heat.
4. Aluminium – too soft. A certain hardness is essential for prolonging cavitation erosion. Limited chemical resistance.

Is hearing protection necessary?

- The ultrasonic homogeniser can be operated in a soundproof box, available for purchase from BANDELIN, please enquire for more information.
- Alternatively, hearing protection should be worn: capsule hearing protection with an HM value of 25 – 30 dB or similar ear plugs or coverings if capsule hearing protection is unsuitable for the respective application.

Safety aspects for use of ultrasonic homogenisers in solvent-based samples

See FAQs – Practical application

7.3 FAQs concerning standards and guidelines

Are ultrasonic homogenisers suitable as medical devices in accordance with Directive 98/79 EC concerning in vitro diagnostics?

Yes, ultrasonic homogenisers are remote controlled and with a CE label they are also classified as medical devices in accordance with Directive 98/79 EC concerning in vitro diagnostics.

Do ultrasonic homogenisers comply with RoHS guidelines?

The devices comply with RoHS guidelines.

8 A final word

We hope to have been able to provide you with a good overview of the options for the practical use of SONOPULS ultrasonic homogenisers. If you have any unanswered questions, please do not hesitate to contact us for a personal consultation. Feel free to send us your ideas for new contents in the application guide. We will also be delighted to adopt your customised methods as an application in our collection for community use.

Our individual applications can be requested in accordance with Section 6 "Detailed applications" from:
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SONOPULS series 4000
ultrasonic homogenisers
HD 4050

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