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BÜCHI Labortechnik AG
Meierseggstrasse 40
Postfach
CH-9230 Flawil 1

E-Mail: quality@buchi.com

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Read this manual carefully before installing and running your system and note the safety precautions in chapter 2 in particular. Store the manual in the immediate vicinity of the instrument, so that it can be consulted at any time.

No technical modifications may be made to the instrument without the prior written agreement of BUCHI. Unauthorized modifications may affect the system safety, the EU conformity or result in accidents.

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*The English manual is the original language version and serves as basis for all translations into other languages.*
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1 About this manual

This manual describes the Encapsulator B-390. It provides all information required for its safe operation and to maintain it in good working order. It is addressed to laboratory personnel in particular. If the instrument is used in a manner not specified in this manual, the protection provided by the instrument may be impaired.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPDM</td>
<td>Ethylene Propylene Dimonomer</td>
</tr>
<tr>
<td>FEP</td>
<td>Fluorelastomer</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
</tbody>
</table>
2 Safety

This chapter points out the safety concept of the instrument and contains general rules of behavior and warnings from direct and indirect hazards concerning the use of the product. For the user's safety all safety instructions and the safety messages in the individual chapters shall strictly be observed and followed. Therefore, the manual must always be available to all persons performing the tasks described herein.

2.1 User qualification

The instrument may only be used by laboratory personnel and other persons, who on account of training and professional experience know the dangers, which can develop when operating the instrument.

Untrained personnel or persons, who are currently being trained, require careful supervision by a qualified person. The present Operation Manual serves as a basis for training.

2.2 Proper use

The Encapsulator B-390 has been designed and built as laboratory instrument.

The Encapsulator B-390 is a semi-automated instrument used for the polymer encapsulation of chemical substances, bio-molecules, drugs, flavor & fragrances, pigments, extracts, cells and micro-organisms under open conditions. Aseptic working conditions are possible, because all parts in contact with the encapsulation mixture are autoclavable.

The bead formation is based on the fact that a controlled, laminar liquid jet is broken into equally sized beads, if vibrated at an optimal frequency.

The Encapsulator B-390 provides just such controlled conditions to generate beads between 0.15 to 2 mm. The instrument is ideally suited to encapsulate particles < 50 µm.

If the instrument is used with potentially toxic or hazardous substances, it has to be installed inside a closed fume hood or glove box. In such case, the complete processing and system handling has to be performed within the ventilated box to avoid toxication and other hazardous situations to the user and the environment.

2.3 Improper use

Applications not mentioned in section 2.2 are considered to be improper. Applications which do not comply with the technical data (see section 3 of this manual) are also considered to be improper.

The operator bears the sole risk for any damages or hazards caused by improper use!

The following uses are expressly forbidden:

- Installation or use of the instrument in rooms, which require ex-protected instruments.
2.4 Safety warnings and safety signals used in this manual

DANGER, WARNING, CAUTION and NOTICE are standardized signal words for identifying risk levels, related to personal injury and property damage. All signal words, which are related to personal injury are accompanied by the general safety sign.

For your safety it is important to read and fully understand the below table with the different signal words and their definitions!

<table>
<thead>
<tr>
<th>Sign</th>
<th>Signal word</th>
<th>Definition</th>
<th>Risk level</th>
</tr>
</thead>
<tbody>
<tr>
<td>!</td>
<td>DANGER</td>
<td>Indicates a hazardous situation which, if not avoided, will result in death or serious injury.</td>
<td>★★★★</td>
</tr>
<tr>
<td>!</td>
<td>WARNING</td>
<td>Indicates a hazardous situation which, if not avoided, could result in death or serious injury.</td>
<td>★★★☆</td>
</tr>
<tr>
<td>!</td>
<td>CAUTION</td>
<td>Indicates a hazardous situation which, if not avoided, may result in minor or moderate injury.</td>
<td>★★☆☆</td>
</tr>
<tr>
<td>☢</td>
<td>NOTICE</td>
<td>Indicates possible property damage, but no practices related to personal injury.</td>
<td>★☆☆☆</td>
</tr>
</tbody>
</table>

Supplementary safety information symbols may be placed in a rectangular panel on the left to the signal word and the supplementary text (see below example).

<table>
<thead>
<tr>
<th>Space for supplementary safety information symbols.</th>
<th>SIGNAL WORD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplementary text, describing the kind and level of hazard/risk seriousness.</td>
<td></td>
</tr>
<tr>
<td>• List of measures to avoid the herein described, hazard or hazardous situation.</td>
<td></td>
</tr>
<tr>
<td>• ...</td>
<td></td>
</tr>
<tr>
<td>• ...</td>
<td></td>
</tr>
</tbody>
</table>

Table of supplementary safety information symbols
The below reference list incorporates all safety information symbols used in this manual and their meaning.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="General warning icon" /></td>
<td>General warning</td>
</tr>
<tr>
<td><img src="image" alt="Electrical hazard icon" /></td>
<td>Electrical hazard</td>
</tr>
<tr>
<td>Hazard Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
</tr>
<tr>
<td>EX</td>
<td>Explosive gases, explosive environment</td>
</tr>
<tr>
<td>×</td>
<td>Harmful to live-forms</td>
</tr>
<tr>
<td>!</td>
<td>Device damage</td>
</tr>
<tr>
<td>⚠️</td>
<td>Pressurized gas/air</td>
</tr>
<tr>
<td>🌡️</td>
<td>Hot Surface</td>
</tr>
<tr>
<td>🩸</td>
<td>Wear laboratory coat</td>
</tr>
<tr>
<td>🕵️</td>
<td>Wear protective goggles</td>
</tr>
<tr>
<td>🖐️</td>
<td>Wear protective gloves</td>
</tr>
</tbody>
</table>

**Additional user information**

Paragraphs starting with NOTE transport helpful information for working with the device/software or its supplementaries. NOTEs are not related to any kind of hazard or damage (see example below).

**NOTE**

*Useful tips for the easy operation of the instrument/software.*
2.5  Product safety

Safety warnings in this manual (as described in section 2.4) serve to make the user alert and to avoid hazardous situations emanating from residual dangers by giving appropriate counter measures. However, risks to users, property and the environment can arise when the instrument is damaged, used carelessly or improperly.

2.5.1  General hazards

The following safety messages show hazards of general kind which may occur when handling the instrument. The user shall observe all listed counter measures in order to achieve and maintain the lowest possible level of hazard.

Additional warning messages can be found whenever actions and situations described in this manual are related to situational hazards.

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or serious injuries by use in explosive environments.</td>
</tr>
<tr>
<td>• Do not operate the instrument in explosive environments.</td>
</tr>
<tr>
<td>• Do not operate the instrument with explosive gas mixtures.</td>
</tr>
<tr>
<td>• Before operation, check all gas connections for correct installation.</td>
</tr>
<tr>
<td>• Directly withdraw released gases and gaseous substances by sufficient ventilation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure increase in the inlet-system due to clogged nozzles.</td>
</tr>
<tr>
<td>Bursting of the inlet system.</td>
</tr>
<tr>
<td>Death or serious poisoning by contact or incorporation of harmful substances at use.</td>
</tr>
<tr>
<td>• Clean nozzle immediately after use, see section 7.4.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or serious injuries by contact with high voltage.</td>
</tr>
<tr>
<td>• Only open the housing of the product when machine is switched off and unplugged.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Notice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of instrument short-circuits and damage by liquids.</td>
</tr>
<tr>
<td>• Do not spill liquids over the instrument or parts of it.</td>
</tr>
<tr>
<td>• Wipe off any liquids instantly.</td>
</tr>
<tr>
<td>• Ensure a safe positioning of the sample vessel.</td>
</tr>
<tr>
<td>• Do not move the instrument when it is loaded with liquid.</td>
</tr>
<tr>
<td>• Keep external vibrations away from the instrument.</td>
</tr>
</tbody>
</table>
2.5.2 Safety measures

Always wear personal protective equipment such as protective eye goggles, protective clothing, and gloves when working with the instrument.

2.5.3 Built-in safety elements and measures

High voltage and electrostatic charges
- Safety current limitation.
- Internal grounding to arrest electrostatic charges.

Air/Gas
- Over pressure safety valve (opens at 1.5 bar)
2.6 General safety rules

Responsibility of the operator
The head of laboratory is responsible for training his personnel. The operator shall inform the manufacturer without delay of any safety-related incidents which might occur during operation of the instrument. Legal regulations, such as local, state and federal laws applying to the instrument must be strictly followed.

Duty of maintenance and care
The operator is responsible for the proper condition of instrument at use and that maintenance, service and repair jobs are performed with care and on schedule by authorized personnel only.

Spare parts to be used
Use only genuine consumables and genuine spare parts for maintenance to assure good system performance and reliability. Any modifications to the spare parts used are only allowed with the prior written permission of the manufacturer.

Modifications
Modifications to the instrument are only permitted after prior consultation with and with the written approval of the manufacturer. Modifications and upgrades shall only be carried out by an authorized BUCHI technical engineer. The manufacturer will decline any claim resulting from unauthorized modifications.

2.7 Disclaimer

Use and marketing of any material produced with the Encapsulator are in the sole responsibility of the operator.
3 Technical data

This chapter introduces the reader to the instrument and its specifications. It contains the scope of delivery, technical data, requirements and performance data.

3.1 Scope of application and delivery

The scope of delivery can only be checked according to the individual delivery note and the listed order numbers.

NOTE
For additional information about the listed products, see www.buchi.com or contact your local dealer.

3.1.1 Standard instrument

<table>
<thead>
<tr>
<th>Table 3-1: Standard instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product</td>
</tr>
<tr>
<td>Encapsulator B-390</td>
</tr>
<tr>
<td>50 – 60 Hz, 100 – 240 V</td>
</tr>
<tr>
<td>Complete Encapsulator B-390 system for microencapsulation under open working conditions with integrated nozzle heating and with liquid pumping by air pressure.</td>
</tr>
</tbody>
</table>
3.1.2 Standard accessories

Table 3-2: Standard accessories

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Order no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set of 8 single nozzles</td>
<td>11057918</td>
</tr>
<tr>
<td>Set of 8 single nozzles with high precision opening of 0.08, 0.12, 0.15, 0.20, 0.30, 0.45, 0.75 and 1.00 mm, made of stainless steel 316L including nozzle rack</td>
<td></td>
</tr>
<tr>
<td>Pressure bottle 500 mL</td>
<td>11058190</td>
</tr>
<tr>
<td>Pressure bottle 1000 mL</td>
<td>11058191</td>
</tr>
<tr>
<td>Glass bottles with fittings, tubes and air filter, working pressure up to 1.5 bar, autoclavable</td>
<td></td>
</tr>
<tr>
<td>Grounding set</td>
<td>11058189</td>
</tr>
<tr>
<td>Operation Manual English</td>
<td>11593477</td>
</tr>
</tbody>
</table>

3.1.3 Optional accessories

Table 3-3: Optional accessories

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Order no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentric nozzle set</td>
<td>11058051</td>
</tr>
<tr>
<td>Set of 7 external nozzles with high precision opening of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.9 mm made of stainless steel, incl. 1000 mL pressure bottle</td>
<td></td>
</tr>
</tbody>
</table>
### 3.1.4 Recommended spare parts

<table>
<thead>
<tr>
<th>Product</th>
<th>Order no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-ring set for single nozzle</td>
<td>11057954</td>
</tr>
<tr>
<td>O-ring set for concentric nozzle</td>
<td>11057955</td>
</tr>
<tr>
<td>Pre-filters for nozzle, diameter 7 mm (10 pcs.)</td>
<td>11057957</td>
</tr>
<tr>
<td>Drain filters for reaction vessel, diameter 35 mm (10 pcs.)</td>
<td>11057958</td>
</tr>
</tbody>
</table>
3.2 Technical data

<table>
<thead>
<tr>
<th>Table 3-5: Technical data Encapsulator B-390</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power consumption</td>
</tr>
<tr>
<td>Connection voltage</td>
</tr>
<tr>
<td>Mains supply voltage fluctuations</td>
</tr>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Fuse</td>
</tr>
<tr>
<td>Dimensions (W × H × D)</td>
</tr>
<tr>
<td>Weight</td>
</tr>
<tr>
<td>Nozzle diameter of single (= core) nozzles</td>
</tr>
<tr>
<td>Nozzle diameter of shell nozzles</td>
</tr>
<tr>
<td>Droplet size range</td>
</tr>
<tr>
<td>Vibration frequency</td>
</tr>
<tr>
<td>Electrode tension</td>
</tr>
<tr>
<td>Heating</td>
</tr>
<tr>
<td>Pump rate by air pressure</td>
</tr>
<tr>
<td>Maximal allowed air pressure in the system</td>
</tr>
<tr>
<td>Reactor gross volume</td>
</tr>
<tr>
<td>Reactor working volume</td>
</tr>
<tr>
<td>Parts in contact with medium</td>
</tr>
<tr>
<td>Sterile working conditions</td>
</tr>
<tr>
<td>Overvoltage category</td>
</tr>
<tr>
<td>Pollution degree</td>
</tr>
<tr>
<td>Environmental conditions:</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>Altitude</td>
</tr>
<tr>
<td>Max. relative humidity (curve parameter)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3-6: Material and Approvals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material in contact with sample</td>
</tr>
<tr>
<td>Approvals</td>
</tr>
</tbody>
</table>

3.3 Materials used

<table>
<thead>
<tr>
<th>Table 3-7: Materials used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
</tr>
<tr>
<td>Bead producing unit</td>
</tr>
<tr>
<td>Nozzles</td>
</tr>
<tr>
<td>Pressure bottle</td>
</tr>
</tbody>
</table>
4 Description of function

This chapter explains the basic working principle of the Encapsulator B-390. It also shows how the instrument is structured and provides a general functional description of its assembly.

4.1 Functional principle

The instrument provides the following key functions:

Reproducible bead size from one production to the next
- Adjustable parameters (nozzle size, liquid flow rate and vibration frequency) determine bead size.

Reproducible bead formation
- In the range of 0.15 mm to 2.0 mm.

High bead size uniformity
- Due to the integrated Electrostatic Dispersion Unit (EDU); approximately 5 % relative standard deviation of bead size using pure alginate.

Immediate process control
- Visual monitoring in the light of a stroboscope lamp.

High cell viability
- Bead formation technique is at low shear stress and under physiological conditions, thus resulting in high cell survival.

Batch size
- The batch size is of 5 mL to 1’000 mL and the dead volume is approximately 2 mL.

Set of 8 single nozzles
- The 8 nozzle sizes of 0.08, 0.12, 0.15, 0.20, 0.30, 0.45, 0.75 and 1.0 mm cover the bead size range of approximately 0.15 mm to 2.0 mm.

Delivery of the polymer mixture
- By air pressure with flow rates from 70 mL/h (0.08 mm nozzle) to 2’500 mL/h (1.0 mm nozzle).

High bead production
- Up to 6000 beads are produced per second depending on encapsulation conditions and polymer mixture.
Figure 4-1: Schematic representation of the Encapsulator B-390

1. Pressure bottle
2. Bead producing unit
3. Vibration unit
4. Single nozzle
5. Electrode
6. Dispersion control
7. Vibration control
8. LED/stroboscope
9. Polymerization bath
10. Magnetic stirrer

(P) Air pressure
The main parts of the Encapsulator B-390 are the control unit, the bead producing unit, and the pressure bottle. All parts of the instrument which are in direct contact with the immobilization mixture can be sterilized by autoclaving.

The product to be encapsulated (active ingredients, enzymes, chemicals and cells) is mixed with an encapsulating polymer (typically alginate) and the mixture put into the pressure bottle. The polymer-product mixture is forced into the bead producing unit by air pressure. The liquid then passes through a precisely drilled nozzle and separates into equal size droplets on exiting the nozzle. These droplets pass through an electrical field between the nozzle and the electrode, resulting in a surface charge. Electrostatic repulsion forces disperse the beads as they drop to the polymerization bath. The polymerization bath must be electrically grounded.

**Bead size**

The bead size is controlled by several parameters including the vibration frequency, nozzle size, flow rate, and physical properties of the encapsulation mixture. In general, the bead size is twice the nozzle diameter. But, by varying the jet velocity and the vibration frequency, the range can be adjusted by about 1 - 15%.

Optimal parameters for bead formation are indicated by visualization of real-time bead formation in the light of a stroboscope lamp. When optimal parameters are reached, a standing chain of droplets is clearly visible. Once established, the optimal parameters can be preset for subsequent bead production runs with the same encapsulating mixture.

Depending on several variables, up to 6000 beads are generated per second and collected in the polymerization bath, which is continuously mixed by a magnetic stir bar. In addition, the reaction solution must be electrically grounded to eliminate the electrostatic charges coming from the charged bead surface. At the conclusion of the production run, the hardening solution is drained off, washing solutions, or other reaction solutions, are added to further process the beads if needed.

### 4.2 Connections at the Encapsulator B-390

**Front connections (See figure 5-2)**

- Main switch
- Air out
- Electrode
- Ground

**Rear connections (See figure 5-1)**

- Electric supply
- Air inlet
- Vibration
- Optional plug(s)
5 Putting into operation

This chapter describes how the instrument has to be installed. It also gives instructions for the initial startup.

**NOTE**
Inspect the instrument for damages during unpacking. If necessary, prepare a status report immediately to inform the postal company, railway company or transportation company. Keep the original packaging for future transportation.

5.1 Installation site

Put the instrument on a stable, horizontal surface. Consider the maximum product dimensions and weight. The instrument must be set up in such a way that the main switch and the mains plug are easily accessible at all times.

Obtain the environmental conditions as described in section 3.2 “Technical data”.

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or serious injuries by use in explosive environments.</td>
</tr>
<tr>
<td>- Do not operate the instrument in explosive environments.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or serious poisoning by contact or incorporation of harmful substances.</td>
</tr>
<tr>
<td>- Wear safety goggles.</td>
</tr>
<tr>
<td>- Wear safety gloves.</td>
</tr>
<tr>
<td>- Wear a laboratory coat.</td>
</tr>
<tr>
<td>- Clean the instrument and all accessories thoroughly to remove possibly dangerous substances.</td>
</tr>
<tr>
<td>- Do not clean dusty parts with compressed air.</td>
</tr>
<tr>
<td>- Store the instrument and its accessories at a dry place.</td>
</tr>
</tbody>
</table>
5.2 Installing the Encapsulator B-390

Place the instrument on the lab bench with convenient access to an AC electrical outlet and to compressed air. Place the instrument in a way that disconnection of the electric supply plug is possible at all times.

Connect the external air/gas supply (=air inlet) and the vibration unit as shown in figure 5-1.

All controlling systems for bead production are incorporated in the control unit. Vibration frequency, light intensity, electrostatic dispersion and heating, are controlled on the touch screen. Air pressure is regulated with the pressure regulating valve. The pressure is electronically indicated on the touch screen. The pumping rate of the polymer mixture is controlled with the liquid flow regulating valve. The integrated stroboscope lamp allows real time jet breakup control. The main switch is on the front panel. The bead producing unit is attached to the carrier plate with two screws (M3×25).
Installation of the air line

A 3 m air tube (2.6×4.0 mm) is included with each Encapsulator to connect it to external compressed air or nitrogen.

1. Stick the air tube into the air inlet plug.
2. Attach the other side of the air tube to the external gas supply.
3. Deliver gas to the Encapsulator at 1.5 to 2 bar (23 to 30 psi) when running the instrument.

**NOTE**
The integrated pneumatic system (valve and fittings) will tolerate up to 7 bar (100 psi) at the inlet. An over pressure safety valve, which opens at 1.5 bar, is incorporated after the pressure regulating valve, so that the maximum air pressure at the air outlet is 1.5 bar. However the working range is from 0 to 1 bar.

### 5.3 Electrical connections

Verify that the electrical requirement of the unit, stated on the type plate of the control unit, corresponds to voltage of your local electrical network. Connect the power plug of the Encapsulator to the mains supply.

<table>
<thead>
<tr>
<th>Caution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of instrument damage by wrong mains supply.</td>
</tr>
<tr>
<td>• External mains supply must meet the voltage given on the type plate.</td>
</tr>
<tr>
<td>• Check for sufficient grounding.</td>
</tr>
<tr>
<td>• Additional electrical safety measures such as residual current breakers may be necessary to meet local laws and regulations! External connections and extension lines must be provided with an earthed conductor lead (3-pole couplings, cord or plug equipment). All used power cords shall be equipped with molded plugs only to avoid risks due to unobservant defective wiring.</td>
</tr>
</tbody>
</table>
5.4 Assembling of the bead producing unit

The bead producing unit is the central part of the Encapsulator B-390. It is fully autoclavable. Figure 5-3 shows the different parts of the bead producing unit. The assembled bead producing unit is attached with the screw \( \overline{\text{screw}} \) on the carrier plate of the control unit. The vibration unit is placed on the magnet holder without the need of further attachment.

Figure 5-3: Parts of the bead producing unit

5.5 Single (inner) nozzles

A high quality nozzle is crucial for homogenous bead production. The holes of the Encapsulator nozzles are precisely drilled using the newest technology. Every Encapsulator B-390 is delivered with a set of 8 nozzles; nozzle aperture sizes are 80, 120, 150, 200, 300, 450, 750 µm and 1.0 mm. They are made completely of stainless steel.

Figure 5-4: Set of 8 nozzles on the nozzle rack

The nozzle rack contains 8 nozzles (80, 120, 150, 200, 300, 450, 750 µm, and 1.0 mm). The size of the O-ring is 4.47x1.78.
5.6 **Electrode**

The electrode is part of the electrostatic dispersion unit. It is attached from below to the heating block. The distance between the electrode and the nozzle tip can be changed as needed. This distance is approximately 3 to 8 mm. It should be set so that the droplets are formed near the upper side of the electrode.

When the beads pass through the electrode they pick up the electrostatic charge. This electrostatic charge is transferred to the polymerization bath and accumulates if the vessel is not grounded. The electrostatic field created by the accumulated charges can be so strong that small beads are repelled over the bath and will no longer enter it. Therefore, the grounding hook attached to the grounding wire is put into the polymerization bath so that the electrostatic charges will flow to ground, see figure 5-8.

![Electrode with two clips](image1)

**Figure 5-5:** Electrode with two clips

![Grounding set](image2)

**Figure 5-6:** Grounding set
Figure 5-7: Position of the electrode below the carrier plate

Figure 5-8: Grounding the polymerization bath
5.7 Pressure bottle

The pressure bottle is an autoclavable container used to push the encapsulation mixture by air pressure into the bead producing unit. Figure 5-9 shows the different parts of the pressure bottle. The glass recipient has a guaranteed pressure resistance of 1.5 bar. The liquid flow rate is controlled at two levels:

1. With the air pressure by the pressure regulation system, and
2. With the liquid flow regulating valve situated on top of the control unit. The reproducibility of the liquid flow rate from one run to the other is usually ±5 %.

![Figure 5-9: Pressure bottle with HEPA filter for sterile pumping of the immobilization mixture with air pressure](image)

- Pressure stable flask of 500 mL or 1,000 mL
- HEPA air filter
- PTFE tube (4×6)
- Silicone tube for liquid (4×7)
- Silicone tube for air (5×8)
- Luer lock male, 4.8 mm ID
- Nipple for quick coupling
- Two port cap
- Cap with PTFE fitting for 6 mm tubes

The air passes through a silicone tube with an inner diameter of 5 mm (5×8 mm). The Hepa-filter prevents contamination of the sterile immobilization mixture and should be replaced according to the manufacturer's instructions or if signs of reduced air passage are noticeable.

The liquid passes from the inside of the bottle through a PTFE tube (3×6 mm) to the silicone tube outside of the bottle. This silicone tube is attached to the bead producing unit with the luer lock male.
5.7.1 Installation of the pressure bottle

1. Assemble and – if needed - autoclave the pressure bottle.
2. Fill the bottle with the immobilization mixture.
3. Attach the silicone tube of the pressure bottle to the luer lock inlet of the bead producing unit.
4. Pass the silicone tube in the liquid regulating flow valve. Squeeze it so that no liquid can pass.
5. Insert the nipple of the air tube into the quick coupling of the air outlet at the control unit.

Figure 5-10: Installed pressure bottle
5.8 **Option: Concentric nozzle system**

The concentric nozzle system (CN system) is an optional kit to the single nozzle unit. It is for the production of capsules in a one-step procedure. The system consists of CN bead producing unit, a set of 7 shell nozzles (0.20, 0.30, 0.40, 0.50, 0.60, 0.70 and 0.90 mm) and one pressure bottle of 1000 mL. The shell liquid is pumped by air pressure using the pressure bottle.

The main parts of the concentric nozzle unit are (refer to figure 5-12):
- the nozzle pair with shell ① and core ② nozzle.
- CN bead producing unit with CN pulsation body ③ and magnet holder ④.

![Figure 5-11: Capsule formation](image)

![Figure 5-12: Schematic description of the concentric nozzle system](image)
Figure 5-13: CN bead producing unit with set of 7 shell nozzles. The following nozzle apertures are standard: 0.20, 0.30, 0.4, 0.50, 0.60, 0.70 and 0.90 mm.

Figure 5-14: Single parts of the CN bead producing unit

1. Screw M3×6
2. Luer-Lock
3. Pre-filter with 50 µm mesh
4. Flat gasket (18/14×1.5)
5. Screw M3×8
6. Screw M3×25
7. O-Ring (3.68×1.78)
8. CN pulsation chamber
9. O-Ring 12.42×1.78
10. CN magnet holder
11. Membrane
12. Magnet
5.8.1 Mounting of CN nozzles

Put the O-ring 12.42×1.78 in the groove of the CN bead producing unit. Put the inner nozzle (with attached O-ring) into the hole of the CN bead producing unit. There is no thread. The inner nozzle is centered and fixed by the shell nozzle.

- Exit of the shell liquid
- O-ring 12.42×1.78

**Figure 5-15:** Mounting of the inner nozzle

Put carefully the shell nozzle over the inner nozzle. Attach the shell nozzle with two screws (M3×6). The shell nozzle centers and fixes the inner nozzle.

**Figure 5-16:** Mounting of the shell nozzle

**Figure 5-17:** Installation of the concentric nozzle system
Attach the complete CN bead producing unit to the carrier plate with two screws (M3×25). Attach the silicone tube of the core liquid to the core inlet port and the silicone tube of the shell liquid to the shell inlet port.

Pass the silicone tubes in the liquid regulating flow valves. Squeeze them so that no liquid can pass.

**Figure 5-18:** Connection of the pressure bottles to the air outlet. A T-piece feeds both pressure bottles.

### 5.9 Final installation check

This check has to be carried out after every installation and prior to the first encapsulation process. All connected supply media (e.g. mains voltage and gas pressure) must match the technical data of the installed system or system set-up.

- Inspect all glass components for damage.
- Check all other electrical connections for proper connection, such as optional or external components, e.g. magnetic stirrer, vibration unit, etc.
6 Operation

This chapter gives examples of typical instrument applications and instructions on how to operate the instrument properly and safely. See also section 2.5 “Product safety” for general warnings.

6.1 Starting up the instrument

- Make sure the Encapsulator B-390 is properly connected to the mains supply.
- Carry out a final installation check (see section 5.9) before every bead production.
- Switch on the Encapsulator B-390. The system runs an internal check.

6.2 Screens and menu functions

All controlling systems for bead production are incorporated in the control unit. Vibration, frequency, heating, light intensity of stroboscope lamp, and electrostatic dispersion (electrode) are controlled on the touch screen. Air pressure is regulated with the pressure regulating valve. The pressure is indicated on the touch screen.

The integrated stroboscope lamp allows real time jet breakup control.

When the Encapsulator is switched on the touch screen runs an initialization program for few seconds. Then the screen shows the start menu with four sub-parts (see figure 6-1 to 6-5) for frequency, electrode, heating and more options concerning frequency. Further are on the screen the indication of the pressure and the button “store” for saving the set values.

NOTE

Icons with a thick bar at the bottom e.g. activate/stop a process or lead to another screen.
The frequency regulation generates the appropriate electric oscillation in the vibration unit. Pressing on the (+) and (-) buttons will change the frequency. Pressing the “on/off” button activates or deactivates frequency. Pressing “Esc” will return you to the start menu and the set value will be kept.

Screen 6-2: Frequency regulation

The electrostatic dispersion unit is used to charge the surface of the beads. The repulsion forces induced by the equally charged surfaces prevent the beads from hitting each other in flight, and from hitting each other as they enter the hardening solution. The applied voltage often lies in the range of 500 to 2000 V, depending primarily on the bead size and the liquid flow velocity. In this way, the Encapsulator B-390 can routinely generate bead batches with homogeneity greater than 95 %.

Pressing on the (+) and (-) buttons changes the electrostatic dispersion parameter. The system needs few moments to reach the set value. Pressing “Esc” will return you to the start menu and the set value will be kept.

Screen 6-3: Electrostatic dispersion unit

Pressing on the (+) and (-) buttons will change the heating parameter. The system will need several minutes to reach the set value. Pressing the “on/off” button activates or deactivates heating. Pressing “Esc” will return you to the start menu and the set value will be kept.

Screen 6-4: Heating regulation

Note
The indicated temperature is the temperature near the heater. The temperature of the bead producing unit and of the nozzle are some degrees lower. When starting the heating; an internal program starts to compensate for this temperature difference as much as possible. Therefore the real heating might be delayed for up to 2 minutes.

<table>
<thead>
<tr>
<th>Caution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot surface while heating is switched on.</td>
</tr>
<tr>
<td>• Do not touch the heating block or the carrier plate while heating is switched on. The surface of both elements becomes hot during heating!</td>
</tr>
<tr>
<td>• Allow both elements to cool down before touching them if heating has been switched on before.</td>
</tr>
</tbody>
</table>

---

6 Operation
The light intensity of the stroboscope lamp and amplitude \(\text{intensity}\) of the vibration can be set from 1 to 9. Above a frequency of 1500 Hz the amplitude can be set from 1 to 12. By increasing the amplitude the vibration becomes stronger. Values above 3 are mainly for solutions with viscosity > 100 mPa s. Pressing on the (+) and (-) buttons will immediately change the parameters. Pressing the “Esc”-button will cause a return to the start menu and the set value will be kept.

**Screen 6-5:** More options concerning amplitude of vibration and light Intensity of the stroboscope lamp.

### 6.3 Menu structure of the control unit

The figure below shows a schematic overview of all menus of the Encapsulator B-390, each with the available functionality.

![Menu structure of the control unit](image_url)

*Figure 6-1: Menu structure of the control unit*
6.4 Manual air pressure control

In the control unit the pressure is manually controlled by the pressure regulating valve, integrated in the front panel of the control unit (see figure 6-2). Set the air pressure at a value which is 0.2 to 0.3 bar higher than the maximal air pressure needed during the encapsulation procedure; but not higher than 1 bar. Turning the knob of the pressure regulating valve clockwise increases the pressure, counter-clockwise decreases the pressure. The knob of the pressure regulating valve has two positions. If it is pushed in, it is locked, if it is pulled out, it is unlocked. Turning the knob counter-clockwise reduces the pressure via the self-venting system in the valve. The pressure is indicated on the touchscreen (see screen 6-1).

Note

- The pressure of air or nitrogen entering the control unit on the rear panel of the Encapsulator should be below 7 bar (100 psi). The preferred range is between 1.5 and 2 bar (20 to 30 psi).
- Be aware that the pressure regulation system reacts relatively slowly, because the displacement of air in or out through the constriction valve is delayed.
- Do not leave the gas supply line on when the Encapsulator is not being used. The self-venting system in the valve would drain the gas tank.
- The maximum pressure at the air outlet is 1.5 bar (20 psi). This value is controlled by an incorporated overpressure safety valve, which opens at 1.5 bar. However the working range is from 0 to 1 bar.

Figure 6-2: Air pressure regulating system for manual air pressure control - turning the pressure regulating valve clockwise increases the pressure.
6.5 Practicing with the Encapsulator, using water

Before working with encapsulation polymers, use water for practicing with the Encapsulator to become familiar with the effects of the controls.

1. Assemble the bead producing unit, screw the 0.30 mm single nozzle to the bead producing unit and attach all on the carrier plate with the screw (M3×25). Fix the electrode. Place the vibration unit on the bead producing unit.

2. Fill the pressure bottle with 200 to 300 mL distilled water and screw on the assembled cap. Pass the silicon tube (4×7 mm) between the blades of the flow regulating valve and attach the male luer lock fitting of the silicon tube to the female luer lock fitting of the bead producing unit. Squeeze the valve by turning the knob clock wise so that the silicon tube is closed.

3. Open the external pressurized air supply. The air inlet pressure is optimally at 1.5 to 2 bar (20 to 30 psi). However the system tolerates air inlet pressures of up to 7 bar (100 psi).

4. Set the air outlet pressure to 0.2 bar with the pressure regulating valve. Check the readout periodically to verify that the air pressure still corresponds to the set value. Activate the vibration control system and set the frequency at 800 Hz.

5. Open the flow regulating valve by turning the knob counter-clock wise until the water flows through the silicone tubing and the bead producing unit to the nozzle where it forms a continuous liquid jet. Adjust the liquid flow and/or the frequency to obtain a good bead chain in the light of the stroboscope lamp. The desired setting is when the beads within the bead chain are clearly separated for several centimeters, starting 3 to 5 mm below the nozzle. Record the position of the flow regulating valve for this desired setting.

6. Increase the vibration frequency until the bead chain becomes unstable. Then increase the liquid flow rate by slowly increasing the air pressure until a uniform bead chain is restored. Repeat this in the opposite direction by decreasing the flow rate and compensating by decreasing the vibration frequency. This may be done until you become familiar with the relationship between these two settings. Record the values in table 6-1.

NOTE
• The liquid flow rate and the vibration frequency influence each other within a given working range. The working range itself is mainly determined by the nozzle diameter and the viscosity of the polymer mixture.
• An air pressure setting between 0.05 to 0.15 bar is sufficient to pump distilled water. Greater working pressures indicate problems such as a clogged nozzle.

General Rules:
• Higher frequencies generate smaller bead sizes.
• Lower liquid flow rates generate smaller bead sizes.
### Table 6-1: Determination of the working field (with pressure bottle)

**Nozzle size:**

<table>
<thead>
<tr>
<th>Air pressure</th>
<th>Clear bead chain without electrostatic tension</th>
<th>Clear bead chain with electrostatic tension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest frequency</td>
<td>Highest frequency</td>
</tr>
<tr>
<td></td>
<td>Electrostatic Voltage</td>
<td>Lowest frequency</td>
</tr>
<tr>
<td></td>
<td>Highest frequency</td>
<td>Highest frequency</td>
</tr>
</tbody>
</table>

**Nozzle size:**

<table>
<thead>
<tr>
<th>Air pressure</th>
<th>Clear bead chain without electrostatic tension</th>
<th>Clear bead chain with electrostatic tension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest frequency</td>
<td>Highest frequency</td>
</tr>
<tr>
<td></td>
<td>Electrostatic Voltage</td>
<td>Lowest frequency</td>
</tr>
<tr>
<td></td>
<td>Highest frequency</td>
<td>Highest frequency</td>
</tr>
</tbody>
</table>
7. Set the liquid flow rate and the vibration frequency to a value where a clear bead chain is obtained. Activate the electrostatic dispersion unit at 300 V and increase the tension stepwise by 100 V until the one-dimensional liquid jet is transformed into a funnel-like multi-line stream. The higher the electrostatic charge the earlier the bead chain is separated. This prevents the beads from hitting each other in flight, and from hitting each other as they enter the hardening solution. Therefore the Encapsulator can routinely generate bead batches with homogeneity greater than 95%. If nothing happens, check that the electrode is connected to the control unit.

8. Change the vibration frequency and the flow rate and observe their influence on the electrostatic tension needed to generate a jet separation. The use of electrostatic tension enlarges the working range.

   It can happen that after some time, the beads no longer enter, or actually jump out of the beaker. This is due to the fact that electrostatic charges have accumulated in the electrically isolated beaker. To avoid this phenomenon, place the supplied stainless steel clip of the grounding wire over the edge of the beaker so it extends into the receiving liquid and connect the green-yellow wire to the grounding plug on the front panel of the control unit. (See figure 6-3).

   **Figure 6-3:** Grounding the open polymerization bath

   **General Rule:**
   *The larger the beads, the higher the electrostatic voltage needed to separate the jet.*

9. Change the amplitude of the vibration. You will observe only slight changes of the bead chain. Very often values between 1 and 3 are optimal for low viscous solutions. If using immobilization mixtures with rather high viscosity (> 150 mPa s), values higher than 3 might be more appropriate.

10. Repeat this procedure with another nozzle size.

   **General Rule:**
   *Smaller nozzles generate smaller bead sizes.*
   *The final bead diameter will be approximately 2 times the nozzle size.*
6.6 Practicing with the Encapsulator, using alginate solution

After getting comfortable with the bead formation controls, perform test runs with non-sterile alginate solutions. Sodium alginate is the most commonly used polymer, but there are others in use with varying properties. We recommend the low viscosity grade alginate. The alginate concentration strongly influences the viscosity and this in turn influences the pressure drop in the nozzle. Therefore, the maximum concentration of the alginate solution is a function of the nozzle diameter (see the following table).

<table>
<thead>
<tr>
<th>Nozzle diameter</th>
<th>Concentration of low viscosity grade alginate</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 to 120 µm</td>
<td>Working range: 0.75 to 1.4 %, Recommended concentration: 1.1 to 1.2 %</td>
</tr>
<tr>
<td>120 to 200 µm</td>
<td>Working range: 1.0 to 1.6 %, Recommended concentration: 1.3 to 1.4 %</td>
</tr>
<tr>
<td>200 to 300 µm</td>
<td>Working range: 1.2 to 1.8 %, Recommended concentration: 1.5 to 1.6 %</td>
</tr>
<tr>
<td>300 to 500 µm</td>
<td>Working range: 1.5 to 2.5 %, Recommended concentration: 1.8 to 2.0 %</td>
</tr>
</tbody>
</table>

**NOTE**
Under normal storage conditions the alginate powder contains 10 - 12 % water. Therefore we refer to the alginate concentration on a dry weight base.

### 6.6.1 Preparation of 1.5 % Na-alginate solution

1. Take a 400 mL beaker and weigh in 3.3 g Na-alginate powder of low viscosity grade.
2. Add 200 mL of deionized water and mix vigorously with a laboratory mixer for 1 to 2 minutes.
3. Alginate has the tendency to get lumpy. Remove the alginate lumps from the beaker and the mixer blades with a spatula and mix again for 1 to 2 minutes. If lumps remain in the liquid, repeat mixing.
4. Then let the mixture stand so that the trapped air bubbles will escape from the liquid.
5. If needed, de-gas the mixture under reduced pressure.
6. Dissolution of alginate with a magnetic stirrer takes much more time and should be done overnight.

**NOTE**
Alginate solutions will support the growth of microorganisms and are stable for about 2 weeks in a refrigerator. An indication of microbial contamination is reduction of the mixture’s viscosity. Alginate solutions can be stored for much longer time, even at room temperature, if sterilized or if preservatives are added, like 0.05 % NaN₃.
6.6.2 Working with alginate solution

1. Attach a 200 µm or 300 µm nozzle to the assembled bead producing unit. Attach all to the carrier plate. Check that the electrode is attached. Put the vibration unit on the bead producing unit. Put a magnetic stirrer below the nozzle and a large beaker on the stirrer. Fill the beaker with 100 mM CaCl₂ so that at least 2 cm (approx. ¾") is filled with the polymerization liquid. Put a magnetic stir bar in the beaker and adjust the stirrer, so that a slight vortex is visible. Also, place the grounded clip over the edge of the beaker and into the liquid. At this time, either cover the beaker with a plate (petri dish) or move it and the stirrer out of the way and position an empty beaker (and the grounding clip) under the nozzle in its place.

2. Fill the pressure bottle with the above described 1.5 % alginate solution and screw on the assembled cap. Pass the silicon tube (4×7 mm) between the blades of the liquid flow regulating valve and attach the male luer lock fitting of the silicon tube to the female luer lock fitting of the bead producing unit. Squeeze the valve by turning the knob clock wise so that the silicon tube is closed.

3. Open the external pressurized air supply. The air inlet pressure is optimally at 1.5 to 2 bar (20 to 30 psi). However the system tolerates air inlet pressures of up to 7 bar (100 psi).

4. Set the air pressure to 0.4 bar at the pressure regulation system. Check the readout periodically to verify that the air pressure still corresponds to the set value. Activate the vibration control system and set the vibration frequency at 1100 Hz for the 200 µm nozzle and at 800 Hz for the 300 µm nozzle.

5. Open the liquid flow regulating valve by turning the knob counter-clock wise until the liquid flows through the silicone tubing and the bead producing unit to the nozzle where it forms a continuous liquid jet. Adjust the liquid flow and/or the frequency to obtain a good bead chain in the light of the stroboscope lamp. The desired setting is when the beads within the bead chain are clearly separated for several centimeters, starting 3 to 5 mm below the nozzle. Record the position of the flow regulating valve for this desired setting.

6. Increase the vibration frequency until the bead chain becomes unstable. Then increase the liquid flow rate by slowly increasing the air pressure or by slowly opening the flow regulating valve until a uniform bead chain is restored. Repeat this in the opposite direction by decreasing the flow rate and compensating by decreasing the vibration frequency. This may be done until you become familiar with the relationship between these two settings.

**NOTE**

An air pressure setting from 0.1 to 0.8 bar is generally sufficient to pump the polymer mixture. Working pressures greater than 1.0 bar should be avoided and are indicative of problems such as:

- Clogged nozzle,
- Overly viscous polymer mixture,
- Under sized nozzle for the polymer mixture in use.

7. Activate the electrostatic dispersion unit at 500 V. Increase the voltage by steps of 100 V to get a circular dispersal of the bead stream 3 to 10 cm (1" to 4") after the electrode. An optimal distance is about 5 cm (approx. 2") below the electrode.
NOTE
The stronger the circular dispersal of the bead stream the better is the bead homogeneity. This does not only depend on the electrostatic tension, but the liquid flow rate and the vibration frequency are also factors. Ideally, the bead should separate from the liquid jet within the electrostatic field between the nozzle and the end of the electrode.

8. As soon as a symmetrical and stable dispersal is obtained, remove the plate from the beaker filled with polymerization solution or replace the beaker of water with the beaker of polymerization solution and the stir plate (and the grounding forceps), and collect the beads for about 1 minute. Record the process parameters in table 6-3 while the beads are accumulating. Cover or switch the beaker and stop the bead production by turning off the electrostatic voltage and vibration control.

NOTE
Clean the nozzle thoroughly immediately after each run with distilled water to avoid nozzle clogging or partial occlusion due to dried out polymer mixture.

9. Check the beads under a microscope with a micrometer scale eyepiece, and record your observations of diameter, uniformity and shape in table 6-3.

10. Repeat this process for each change in process parameters.

<table>
<thead>
<tr>
<th>Table 6-3: Encapsulator trial test work sheet (pressure bottle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nozzle size $[\mu m]$</td>
</tr>
<tr>
<td>Alginate concentr. [%]</td>
</tr>
<tr>
<td>Position of the flow regulating valve</td>
</tr>
<tr>
<td>Vibration frequency [Hz]</td>
</tr>
<tr>
<td>Amplitude</td>
</tr>
<tr>
<td>Approximate bead size $[\mu m]$</td>
</tr>
<tr>
<td>Homogeneity [%]</td>
</tr>
<tr>
<td>Comments</td>
</tr>
</tbody>
</table>

NOTE
When producing small beads with a diameter $<500 \mu m$, it may occur that their shape is not spherical but somewhat oval. This is mainly due to the surface tension of the polymerization solution. A very critical point for the bead is its entrance into the polymerization solution. If the surface tension is high, then the bead is partially held back at the surface and polymerization starts before the bead can regain a round shape. This problem can be eliminated by adding a small quantity of surfactant like Tween 20 to the polymerization mixture.

11. Compare the influence of the electrostatic tension by collecting beads at the same vibration frequency and pumping rate with and without the electrostatic tension function turned on.
6.7 Theoretical background

Equ. 1: \[ f = \frac{v}{\lambda} \text{ [Hz]} \]

When a laminar jet is mechanically disturbed at the frequency \( f \), beads of uniform size are formed\(^1\). The optimal wavelength \( \lambda_{\text{opt}} \) for breakup, according to Weber\(^2\) is given by:

Equ. 2: \[ \lambda_{\text{opt}} = \pi \sqrt{2D} \cdot \sqrt{1 + \frac{3\eta}{\sqrt{\rho \sigma D}}} \text{ [m]} \]

where:
- \( D \) = nozzle diameter
- \( \eta \) = dynamic viscosity [Pa s]
- \( \rho \) = density [kg/m\(^3\)]
  (ca. 1000 kg/m\(^3\) for alginate solutions)
- \( \sigma \) = surface tension [N/m]
  (ca. 55×10\(^{-3}\) N/m for alginate solutions)

\( \lambda_{\text{opt}} \) is the optimal wavelength to get the best bead formation for the given nozzle diameter and the viscosity of the encapsulation mixture. It is possible to change \( \lambda_{\text{opt}} \) by 30 % and still achieve a good bead formation.

The diameter of a bead = \( d \) [m] can be calculated with the flow rate = \( V' \) [m\(^3\)/s] and the frequency of the pulsation \( f \) according to:

Equ. 3: \[ d = \frac{3}{\pi} \sqrt{\frac{6V'}{\pi f}} \text{ [m]} \]

The jet velocity = \( v \) [m/s] and the nozzle diameter = \( D \) [m] are correlated to the flow rate (\( V' \)) according to:

Equ. 4: \[ V' = \frac{\pi v D^2}{4} \text{ [m}^3\text{/s]} \]

Figure 6-4 shows the dependence of the flow rate to the jet velocity and the nozzle diameter as calculated by Equation 4. Because the liquid must flow laminarily the working range of the jet velocity will normally lay between 1.5 and 2.5 m/s, depending on the liquid viscosity and the nozzle diameter.

Figure 6-4: Influence of the liquid jet velocity and the nozzle diameter on flow rate, as calculated by Equation 4.

Figure 6-5 shows the correlation between the vibration frequency and the bead diameter for five different flow rates as calculated by equation 4. Lower flow rates, which corresponds to lower pumping rates, produce smaller beads. Higher vibration frequencies produce smaller beads also.

Figure 6-5: Influence of the vibration frequency and the flow rate on the bead diameter as calculated by Equation 4.
Table 6-4: Optimal working conditions for the Encapsulator determined with alginate solution

<table>
<thead>
<tr>
<th>Nozzle diameter [µm]</th>
<th>Flow rate * [mL/min]</th>
<th>Frequency interval **</th>
<th>Amplitude</th>
<th>Air pressure [bar]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mm</td>
<td>30 to 40</td>
<td>40 to 220 Hz</td>
<td>2 to 6</td>
<td>0.3 to 0.6</td>
</tr>
<tr>
<td>750 µm</td>
<td>19 to 25</td>
<td>40 to 300 Hz</td>
<td>2 to 5</td>
<td>0.3 to 0.5</td>
</tr>
<tr>
<td>450 µm</td>
<td>9 to 14</td>
<td>150 to 450 Hz</td>
<td>2 to 5</td>
<td>0.3 to 0.5</td>
</tr>
<tr>
<td>300 µm</td>
<td>5.5 to 7</td>
<td>400 to 800 Hz</td>
<td>1 to 3</td>
<td>0.3 to 0.5</td>
</tr>
<tr>
<td>200 µm</td>
<td>3.5 to 4.5</td>
<td>600 to 1200 Hz</td>
<td>1 to 3</td>
<td>0.4 to 0.6</td>
</tr>
<tr>
<td>150 µm</td>
<td>2.3 to 2.8</td>
<td>800 to 1800 Hz</td>
<td>1 to 3</td>
<td>0.4 to 0.6</td>
</tr>
<tr>
<td>120 µm</td>
<td>1.5 to 1.8</td>
<td>1000 to 2500 Hz</td>
<td>1 to 4</td>
<td>0.5 to 0.7</td>
</tr>
<tr>
<td>80 µm</td>
<td>1.1 to 1.3</td>
<td>1300 to 3000 Hz</td>
<td>1 to 4</td>
<td>0.5 to 0.7</td>
</tr>
</tbody>
</table>

* Tested with 2 % low viscosity grade alginate solution for 750 µm and 1.0 mm nozzle, with 1.5 % alginate solution for the 150 to 500 µm nozzle and with 1.2 % alginate solution for the 80 and 120 µm nozzles.

**Upper values with application of high voltage.

**NOTE**

For solutions with a viscosity different from the tested one, it can be said that:

- the higher the viscosity the higher the minimal jet velocity
- the higher the viscosity the higher the working flow rate
- the higher the viscosity the lower the optimal frequency
- the higher the viscosity the larger the beads

6.7.1 Bead productivity and cell density

Figures 6-6 and 6-7 indicate the amount of beads formed from 1 mL of liquid. About 30,000 beads with a diameter of 0.4 mm will be formed, but only 2,000 with a diameter of 1 mm.

Figures 6-8 and 6-9 indicate the number of cells which are encapsulated in one bead for a given cell density and bead diameter. These figures may help you select the appropriate cell density in the immobilization mixture. For example, if the immobilization mixture contains $1 \times 10^6$ cells per mL, then about 33 cells are, on average, in each 0.4 mm bead, but, about 520 cells will be in each 1 mm bead.
Figure 6-6: Amount of beads with a diameter of 0.3 to 0.6 mm formed from 1 mL of immobilization mixture.

Figure 6-7: Amount of beads with a diameter of 0.6 to 1.1 mm formed from 1 mL of immobilization mixture.
Figure 6-8: Amount of cells per bead made from different cell concentrations for bead diameters of 0.3 to 0.6 mm.

Figure 6-9: Amount of cells per bead made from different cell concentrations for bead diameters of 0.6 to 1.1 mm.
7 Maintenance and repairs

This chapter gives instructions on maintenance work to be performed in order to keep the instrument in good and safe working condition. All maintenance and repair work requiring the opening or removal of the instrument housing must be carried out by trained personnel and only with the tools provided for this purpose.

Note
Use only genuine consumables and spare parts for any maintenance and repair work in order to assure warranty and continued system performance. Any modifications of the Encapsulator B-390 or parts of it need prior written permission of the manufacturer.

7.1 Customer service

Only authorized service personnel are allowed to perform repair work on the instrument. Authorization requires a comprehensive technical training and knowledge of possible dangers which might arise when working at the instrument. Such training and knowledge can only be provided by BUCHI.

Addresses of official BUCHI customer service offices are given on the BUCHI website under: www.buchi.com. If malfunctions occur on your instrument or you have technical questions or application problems, contact one of these offices.

The customer service offers the following:
- Spare part delivery
- Repairs
- Technical advice

7.2 Housing condition

Check the housing for visible defects (switches, plugs, cracks) and clean it regularly with a damp cloth. The Encapsulator control unit should be handled as with any other piece of electrical equipment. The front panel is covered by a polyamide sheet, so that it may be cleaned with a mild detergent solution or alcohol.

7.3 Sealing conditions

It is recommended to check the integrity of the sealings on a regular base. Gaskets, O-rings and silicone tubing should be replaced periodically (approximately once per year). Check all parts before use and replace if needed.
7.4 Cleaning

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure increase in the inlet-system due to clogged nozzles.</td>
</tr>
<tr>
<td>Bursting of the inlet system.</td>
</tr>
<tr>
<td>Death or serious poisoning by contact or incorporation of harmful substances at use.</td>
</tr>
<tr>
<td>• Clean nozzle immediately after use, see following section.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wear laboratory coat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wear protective goggles</td>
</tr>
<tr>
<td>Wear protective gloves</td>
</tr>
</tbody>
</table>

7.4.1 Cleaning the nozzle after each immobilization run

It is critical to clean the nozzle immediately after use so that the encapsulation medium (alginate, etc.) will not dry and clog the system.

1. Leave the nozzle in place on the bead producing unit.
2. Attach a 20 mL or 60 mL syringe to the bead producing unit and inject 20 to 60 mL of distilled water or of the solvent used for the encapsulation polymer.
3. If needed unscrew the nozzle from the bead producing unit, rinse with deionized water (see figure 7-1) or an appropriate solvent and dry the nozzle with a flush of air.
7.4.2 Cleaning a clogged nozzle

Unscrew the nozzle. Pass air or water through the nozzle as shown in figure 7-1.

If the nozzle tip is not clear, soak the nozzle in water, the appropriate solvent, in 1N NaOH or 1N sulfuric acid (never use HCl) according to the encapsulation mixture for 1 hour at room temperature, with periodic agitation. Sonication of full stainless steel nozzles is also a helpful procedure. Wear appropriate protection equipment. Rinse with distilled water, with air and let dry.

Examine the nozzle tip under a stereoscopic microscope to make sure the passage is clear and clean.

**NOTE**
*If lipophilic immobilization solutions were used, then use appropriate solvents for cleaning. Do not use acid solution for alginate, as this would create a precipitate.*

7.4.3 Cleaning the bead producing unit

Disassemble the bead producing unit. However the magnet holder should not be disassembled! Wash all parts, with a mild detergent solution, 0.01N NaOH or 0.01N sulfuric acid (never use HCl) as appropriate. Rinse thoroughly with hot water, then with distilled water and let dry.
8 Troubleshooting

8.1 Malfunctions and their remedy

The table below lists possible operating errors and their cause. As remedy set the parameter stepwise in the opposite direction or fix the missing part.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstable liquid stream</td>
<td>The liquid flow rate is too low.</td>
</tr>
<tr>
<td></td>
<td>The nozzle is not adequately cleaned (frequent cause).</td>
</tr>
<tr>
<td></td>
<td>The frequency is too high.</td>
</tr>
<tr>
<td></td>
<td>The amplitude is too high.</td>
</tr>
<tr>
<td>Unstable bead chain</td>
<td>The frequency is too high or too low.</td>
</tr>
<tr>
<td></td>
<td>The liquid flow rate is too high or too low.</td>
</tr>
<tr>
<td></td>
<td>The Nozzle is not adequately cleaned.</td>
</tr>
<tr>
<td></td>
<td>The amplitude is too low or too high.</td>
</tr>
<tr>
<td>Non-homogenous bead-size-distribution</td>
<td>The liquid flow rate is too high.</td>
</tr>
<tr>
<td></td>
<td>The frequency is too high.</td>
</tr>
<tr>
<td></td>
<td>The electrostatic tension is too low.</td>
</tr>
<tr>
<td></td>
<td>The immobilization mixture is a non-Newtonian liquid, making it difficult to extrude or to prill.</td>
</tr>
<tr>
<td>The bead chain does not separate</td>
<td>The electrode is not connected to the control unit.</td>
</tr>
<tr>
<td></td>
<td>The electrical tension is too low.</td>
</tr>
<tr>
<td></td>
<td>The electrode is not on.</td>
</tr>
<tr>
<td>Beads are not visible in the strobo light</td>
<td>The vibration unit is not activated.</td>
</tr>
<tr>
<td></td>
<td>The vibration unit is not put on the bead producing unit.</td>
</tr>
<tr>
<td></td>
<td>The vibration frequency is too low or too high.</td>
</tr>
<tr>
<td></td>
<td>The viscosity of the immobilization mixture is too high.</td>
</tr>
</tbody>
</table>
9 Shutdown, storage, transport and disposal

This chapter instructs how to shut down and to pack the instrument for storage or transport. Specifications for storage and shipping conditions can also be found listed here.

9.1 Storage and transport

Switch off the instrument and remove the power cord. Wait until all hot parts (e.g. heating block and carrier plate) have cooled down.

To disassemble the Encapsulator B-390 follow the installation instructions in section 5 in reverse order. Remove all liquids and dusty residues before packaging the instrument.

<table>
<thead>
<tr>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or serious poisoning by contact or incorporation of harmful substances.</td>
</tr>
<tr>
<td>• Wear safety goggles</td>
</tr>
<tr>
<td>• Wear safety gloves</td>
</tr>
<tr>
<td>• Wear a laboratory coat</td>
</tr>
<tr>
<td>• Clean the instrument and all accessories thoroughly to remove possibly dangerous substances</td>
</tr>
<tr>
<td>• Do not clean dusty parts with compressed air</td>
</tr>
<tr>
<td>• Store the instrument and its accessories at a dry place in its original packaging</td>
</tr>
</tbody>
</table>
9.2 Disposal

For instrument disposal in an environmentally friendly manner, a list of materials is given in chapter 3.3. This helps to ensure that the components can be separated and recycled correctly. You have to follow valid regional and local laws concerning disposal. For help, please contact your local authorities!

**NOTE**

When returning the instrument to the manufacturer for repair work, please copy and complete the health and safety clearance form in section 10.2 and enclose it with the instrument.
10 Declarations and requirements

10.1 FCC requirements (for USA and Canada)

**English:**
This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to both Part 15 of the FCC Rules and the radio interference regulations of the Canadian Department of Communications. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.

This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

**Français:**
Cet appareil a été testé et s’est avéré conforme aux limites prévues pour les appareils numériques de classe A et à la partie 15 des réglementations FCC ainsi qu’à la réglementation des interférences radio du Canadian Department of Communications. Ces limites sont destinées à fournir une protection adéquate contre les interférences néfastes lorsque l’appareil est utilisé dans un environnement commercial.

Cet appareil génère, utilise et peut irradier une énergie à fréquence radioélectrique, il est en outre susceptible d’engendrer des interférences avec les communications radio, s’il n’est pas installé et utilisé conformément aux instructions du mode d’emploi. L’utilisation de cet appareil dans les zones résidentielles peut causer des interférences néfastes, auquel cas l’exploitant sera amené à prendre les dispositions utiles pour palier aux interférences à ses propres frais.
Health and Safety Clearance

Declaration concerning safety, potential hazards and safe disposal of waste.
For the safety and health of our staff, laws and regulations regarding the handling of dangerous goods, occupational health and safety regulations, safety at work laws and regulations regarding safe disposal of waste, e.g. chemical waste, chemical residue or solvent, require that this form must be duly completed and signed when equipment or defective parts were delivered to our premises.

Instruments or parts will not be accepted if this declaration is not present.

<table>
<thead>
<tr>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model:</td>
</tr>
</tbody>
</table>

1. A Declaration for non dangerous goods
We assure that the returned equipment
- has not been used in the laboratory and is new
- was not in contact with toxic, corrosive, biologically active, explosive, radioactive or other dangerous matters.
- is free of contamination. The solvents or residues of pumped media have been drained.

1. B Declaration for dangerous goods
List of dangerous substances in contact with the equipment:

<table>
<thead>
<tr>
<th>Chemical, substance</th>
<th>Danger classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We assure for the returned equipment that
- all substances, toxic, corrosive, biologically active, explosive, radioactive or dangerous in any way which have pumped or been in contact with the equipment are listed above.
- the equipment has been cleaned, decontaminated, sterilized inside and outside and all inlet and outlet ports of the equipment have been sealed.

2. Final Declaration
We hereby declare that
- we know all about the substances which have been in contact with the equipment and all questions have been answered correctly
- we have taken all measures to prevent any potential risks with the delivered equipment.

Company name or stamp: ____________________________________________

Place, date: _______________________________________________________

Name (print), job title (print): ______________________________________

Signature: ________________________________________________________
**Quality in your hands**

**BUCHI Affiliates:**

### Europe

<table>
<thead>
<tr>
<th>Country/Sector</th>
<th>Contact Information</th>
</tr>
</thead>
</table>
| Switzerland/Austria | BÜCHI Labortechnik AG  
CH – 9230 Flawil  
T +41 71 394 63 63  
F +41 71 394 65 65  
buchi@buchi.com  
www.buchi.com |
| Benelux | BÜCHI Labortechnik GmbH  
Branch Office Benelux  
NL – 3342 GT Hendrik-Ido-Ambacht  
T +31 78 684 94 29  
F +31 78 684 94 30  
belenux@buchi.com  
www.buchi.be |
| France | BÜCHI Sarl  
FR – 94656 Rungis Cedex  
T +33 1 56 70 62 50  
F +33 1 46 86 00 31  
france@buchi.com  
www.buchi.fr |
| Germany | BÜCHI Labortechnik GmbH  
DE – 45127 Essen  
T +800 414 0 414 0 (Toll Free)  
T +49 201 747 49 0  
F +49 201 747 49 20  
deutschland@buchi.com  
www.buchigmbh.de |
| Italy | BÜCHI Italia s.r.l.  
IT – 20010 Corredado (MI)  
T +39 02 575 12 855  
italia@buchi.com  
www.buchi.it |
| Russia | BÜCHI Russia/CIS  
Russia 122787 Moscow  
T +7 495 36 36 485  
F +7 495 98 10 530  
russia@buchi.com  
www.buchi.ru |
| United Kingdom | BÜCHI UK Ltd.  
GB – Oldham OL9 9QL  
T +44 161 633 1000  
F +44 161 633 1007  
uk@buchi.com  
www.buchi.co.uk |
| China | BUCHI China  
CN – 200052 Shanghai  
T +86 21 6280 3366  
F +86 21 5230 8821  
china@buchi.com  
www.buchi.com.cn |
| South East Asia | BUCHI (Thailand) Ltd.  
TH – Bangkok 10600  
T +66 2 862 08 51  
F +66 2 862 08 54  
bacc@buchi.com  
www.buchi.com |

### America

<table>
<thead>
<tr>
<th>Country/Sector</th>
<th>Contact Information</th>
</tr>
</thead>
</table>
| Brazil | BUCHI Brasil Ltda.  
BR – Valinhos SP 13271-200  
T +55 19 3849 1201  
F +55 19 3849 2907  
brasil@buchi.com  
www.buchi.com |
| USA/Canada | BUCHI Corporation  
US – New Castle, DE 19720  
T +1 877 692 8244 (Toll Free)  
T +1 302 652 3000  
F +1 302 652 8777  
us-sales@buchi.com  
www.mybuchi.com |

### Asia

<table>
<thead>
<tr>
<th>Country/Sector</th>
<th>Contact Information</th>
</tr>
</thead>
</table>
| Korea | BUCHI Korea Inc.  
KR – Seoul 153-782  
T +82 2 6718 7500  
F +82 2 6718 7599  
korea@buchi.com  
www.buchi.kr |
| India | BUCHI India Private Ltd.  
IN – Mumbai-400 055  
T +91 22 667 75400  
F +91 22 667 18986  
india@buchi.com  
www.buchi.in |
| Indonesia | PT. BUCHI Indonesia  
ID – Tangerang 15321  
T +62 21 537 62 16  
F +62 21 537 62 17  
indonesia@buchi.com  
www.buchi.co.id |
| Japan | Nihon BUCHI K.K.  
JP – Tokyo 110-0008  
T +81 3 3821 4777  
F +81 3 3821 4555  
nihon@buchi.com  
www.nihon-buchi.jp |
| Malaysia | BUCHI Malaysia Sdn. Bhd.  
MY – 47301 Petaling Jaya, Selangor  
T +60 3 7832 0310  
F +60 3 7832 0309  
malaysia@buchi.com  
www.buchi.com |
| Singapore | BUCHI Singapore Pte. Ltd.  
SG – Singapore 609919  
T +65 6566 1175  
F +65 6566 7047  
singapore@buchi.com  
www.buchi.com |
| Thailand | BUCHI (Thailand) Ltd.  
TH – Bangkok 10600  
T +66 2 862 08 51  
F +66 2 862 08 54  
thailand@buchi.com  
www.buchi.co.th |

### BUCHI Support Centers:

<table>
<thead>
<tr>
<th>Country/Sector</th>
<th>Contact Information</th>
</tr>
</thead>
</table>
| South East Asia | BUCHI (Thailand) Ltd.  
TH-Bangkok 10600  
T +66 2 862 08 51  
F +66 2 862 08 54  
bacc@buchi.com  
www.buchi.com |
| Middle East | BÜCHI Labortechnik AG  
UAE – Dubai  
T +971 4 313 2860  
F +971 4 313 2861  
middleeast@buchi.com  
www.buchi.com |
| Latin America | BUCHI Latinoamérica Ltda.  
BR – Valinhos SP 13271-200  
T +55 19 3849 1201  
F +55 19 3849 2907  
latinoamerica@buchi.com  
www.buchi.com |

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