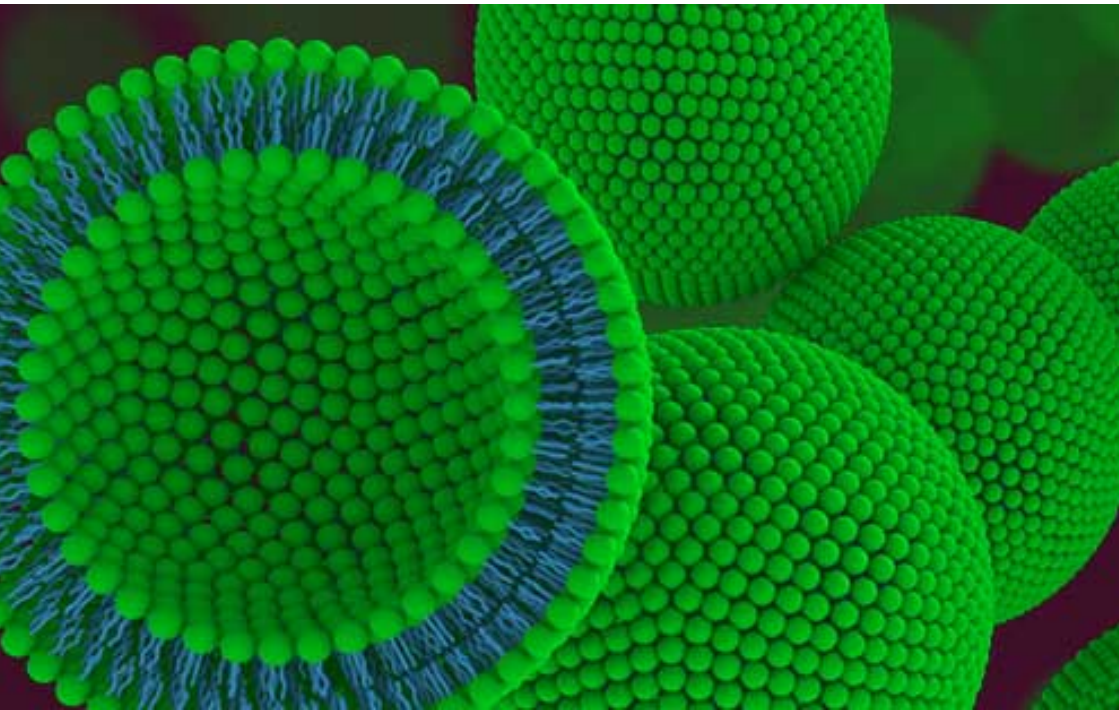
biopharma group

Introduction to
Homogenisation



Introduction to Homogenisation

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Introduction to Homogenisation

Why Homogenise?

High pressure homogenisation is a key technology for particle size reduction, particle or suspension stability, sample homogeneity and nanomolecular disruption.

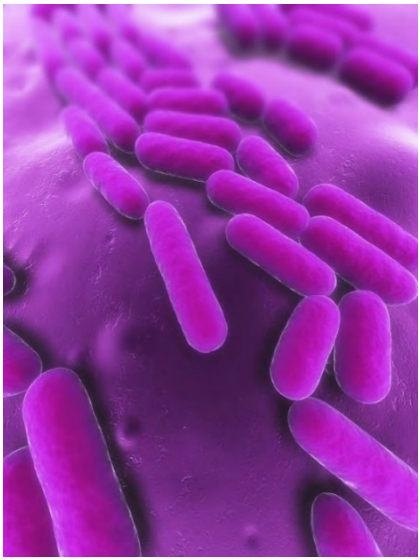
The benefits of this technology include extraction of valuable intracellular products, and improving the shelf life and potency of pharmaceuticals at both small and large scale.

The process is very simple, involving the forced passage of a sample through a narrow orifice/nozzle at high, controlled pressures. Parameter control is vital, particularly when dealing with the extraction of proteins from cells, which can be denatured by a range of factors. These factors must be regulated and controlled for successful processing.

Factors Affecting Homogenisation

Pressure & Temperature

When homogenising, increasing the pressure does not always correlate to an increase in effectiveness. This is due to the fact that different samples can behave differently when subject to higher pressures.



When homogenising biological products, it is vital to apply the correct pressures to avoid denaturing the proteins or completely breaking apart each cellular product of interest.

Temperature and pressure are directly proportional to each other, and so increasing pressures induce temperature increases. As a general rule, a 1000psi (69bar) rise in pressure would result in a 1°C temperature rise.

Number of Passes

Increasing the number of passes through the homogeniser can circumvent the requirement for higher pressures for lysis. In cellular disruption, lower pressures with a higher number of passes tend to give the maximum yields with active and viable products. This is also the case with other applications such as emulsions, liposomes and dispersions, where higher product stability can be achieved with more passes. However, too many passes can risk other forms of damage.

Identifying a solution

As both higher pressures and over-processing can damage sensitive products, it is important to identify the optimal balance of processing pressure, maximum pressures and number of passes.

The Theory of High Pressure Homogenisation

The un-homogenised product is pumped into the homogenising chamber under a small amount of pressure to generate velocity. As the product is pushed through the homogenising valve, the sample is subjected to high pressures as well as forced passage through a narrow orifice. As it exits the orifice, stress forces such as cavitation and shear are generated. The amount of stress on the sample is controlled by controlling the pressure.

Single-Stage or Two-Stage Homogenisation

A single-stage valve assembly is sufficient for processing in many applications such as emulsions, bacteria and yeast. For processing dispersions a single-stage valve assembly usually is preferred.

A two-stage assembly is also available where pressurised extrusion or sterile filtration is undertaken through a membrane. The second stage aims to improve the stability of the most sensitive, size dependent applications, such as multilamellar liposomes.

Multiple-Pass Homogenisation

If a narrow particle-size distribution is required, it may be necessary to homogenise the product more. This can be done by two or more passes by re-circulating the sample.

Examples include IV emulsions, blood substitutes and parenteral emulsions.

Applications

Cell Rupture

Microbial cell disruption is a common application for high pressure homogenisation. Different cell species vary in size, shape and cellular strengths and therefore require different process parameters for effective disruption.

Species	Pressure
Escherichia coli	15-20,000psi (1030-1380bar)
Saccharomyces cerevisiae	25-29,000psi (1700-2000bar)
Pichia pastoris	23-27,000psi (1500-1800bar)

Figure 1: *Commonly processed microbial organisms and their recommended homogenising pressures*

Disruption of sub cellular and intracellular particles with one pass is possible with many types of organisms. Figure 1 illustrates universally used pressures for some common micro-organisms.

Cell rupture is a major application in a variety of biotechnological applications, and with the increasingly high pressures achievable in modern machines, more rapid lysis has become possible.

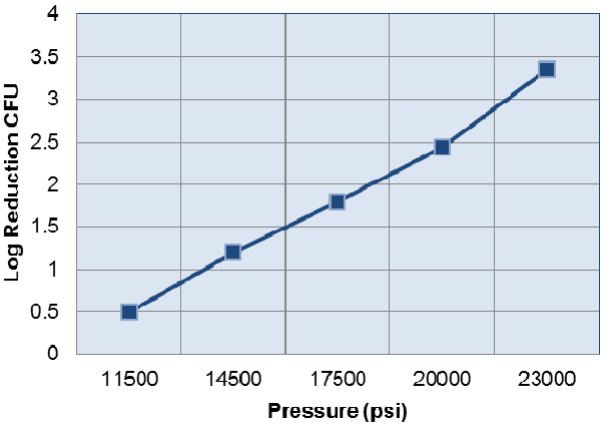


Figure 2: Lysis of E coli MG1655 using EmulsiFlex C5 Source: Laboratory of Food Microbiology, Faculty of Agricultural and Applied Biological Sciences, Catholic University of Leuven, Belgium

However, over-processing or exceedingly high pressures increase the damage done to the intracellular contents and end products of cell lysis. The cells should be subjected to sufficient pressure to break them open without over- processing and causing protein damage. Processing *E. coli* using 7,000psi (480 bar), for example, will require 3 passes to achieve 90% lysis, but viable protein

yield can be as low as 60- 70%. However, at 15,000psi (1,030 bar) 97% lysis can be achieved in a single pass, with 90% yield.

Nano Particles, Emulsions & Liposomes

Many new drug compounds have limited aqueous solubility, which can result in limited and unpredictable bioavailability. One solution gathering popularity is drug particle size reduction. Altering size from the micron to the nm range results in a significant increase in surface area: for example, when the particle size of a drug is condensed from 8µm to 200nm there is 40-fold increase in the surface area to volume ratio. This results in a substantial amplification of the dissolution rate, if the formulation disperses into discrete particles.

For the production of ultrafine stable emulsions pressures from 10,000— 20,000psi (700— 14000 bar) are commonly used in conjunction with membrane filters, resulting in droplet sizes

<50nm.

By passing the combined liquids through the machine at incremental pressures, droplet size is reduced to the point where the immiscible phase is suspended in the substrate to form a stable emulsion, whilst ensuring sterile filtration.

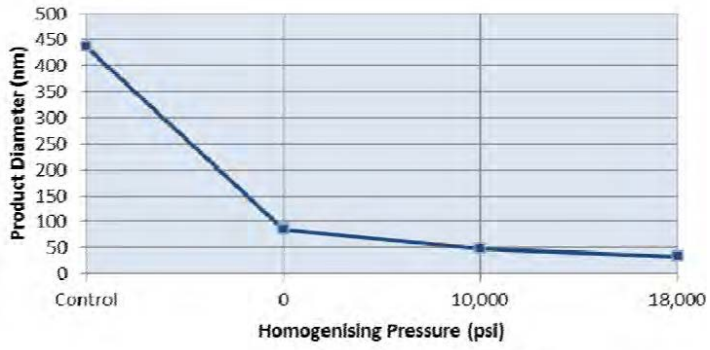


Figure 3: Particle size reduction of pharmaceutical emulsions Diameter before processing 435nm. One pass through the filter extruder with 80nm pore at 0psi following which initiated homogenising.

Multi-Lamellar to Uni-Lamellar Liposomes

Membrane filter/extruder attachments can be used in line with many types of high pressure homogenizer to extrude liposomes to produce unilamellar populations. Combining extrusion with homogenisation can result in a significant reduction in passes to achieve the desired liposomal vesicle size.

Other Applications

Other applications of high pressure homogenisation technology include:

- Pharmaceuticals: nanoparticles, drug delivery, intravenous emulsions/ suspensions, API, liposomes vaccines

- Biotechnology: *E. coli*, yeast, mammalian tissue, other bacteria, algae, fungi and insect cell lysis

- Chemicals: emulsifiers, coatings, lubricants, preservatives, polymers, fuel oil, catalysts, biofuel

- Cosmetics: cream formulations, ointments, emulsions, lotions, fragrances

- Food and beverages: juices, concentrates, beverage emulsions, dressings, flavourings, additives, sauces.

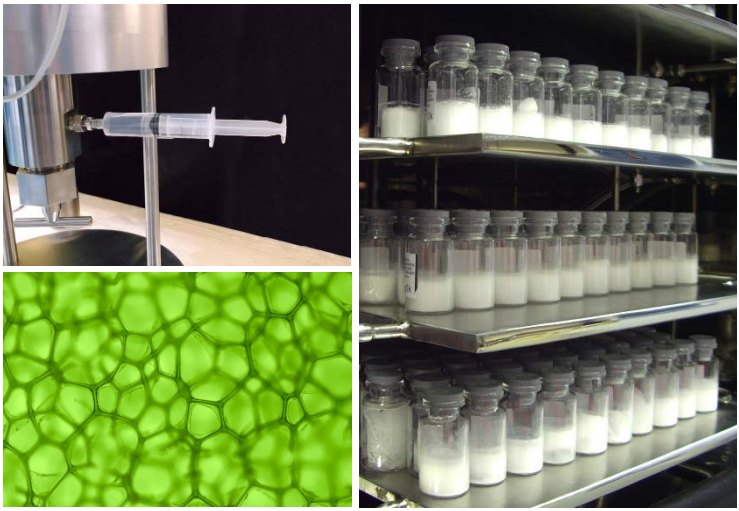


Figure 5: Sterile Application with the Emulsiflex B15 (Top Left), Algal cell for rupture (Bottom left, Vaccine development (Right)

Process Control

Homogenisation under controlled conditions of high pressure and stress with evenly distributed cavitation and shearing force offer a range of advantages including consistent viscosity, product stability and potency, better active ingredient dispersion, particle size reduction in the nanometre range, uniformity, absorbance, shelf life, reaction time, improved flavour and colour.

Avestin Emulsiflex

Avestin's EmulsiFlex homogenisers are designed and manufactured for applications in research, pilot plant as well as scaled up production.

Avestin EmulsiFlex:

- *Ensure particle size reduction in the nm range*
- *Maximise reduction of poly-dispersity*
- *Determine the flow or batch times*
- *Produces higher yield in less time*
- *Near perfect scale up potential*
- *Calculate/define the final product's quality standard*

Keeping You at the Forefront

Cutting edge research in pharmaceutical, biotechnological and chemical laboratories all over the world carries the EmulsiFlex signature. When it comes to developing formulations or small scale products, Avestin high-duty systems are in use on the front line.

Avestin systems provide a smooth transition of formulations and technologies from laboratory to the large-scale production. Findings generated by laboratories world-wide translate from process and recipe development to production scale in a consistently reliable manner.

Unique Dynamic Homogenising Valve

In simple terms, there are two important parts to a high pressure homogeniser: the pressure generator, or pumping system, and the homogenising valve.

The homogenising valve is the heart of any high pressure homogeniser. Broadly speaking, there are two types of valve: a "fixed" valve (a small orifice, or series of orifices) and a "dynamic" valve.

Fixed or "Static" valves are ideal for readily flowing products which are easy to handle. However with more difficult products they are liable to blockage, requiring the user to remove the valve unit and back-flush it before restarting the process. Thus is cost intensive, both in terms of time and product. Dynamic valves allow the user to vary the aperture during processing, which is particularly useful where the product changes its flow characteristics during homogenisation.

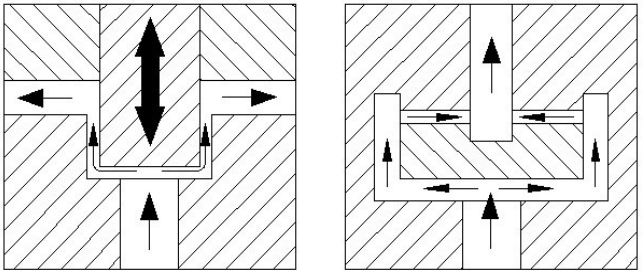


Figure 6: Dynamic Valve (left), Static Valve (Right)

Avestin uses a unique, dynamic self-regulating homogenising valve which maintains itself once the desired pressure is achieved. If the valve should block, the valve opens to allow the blockage to pass with little or no interruption to the process.

Pumping System

The pumping system needs to be easy to be clean and service and be reliable. Avestin builds its own pumps to a high specification to ensure that the high quality and rapid delivery of the machines is not compromised.

Advanced Simplicity

With a user friendly design the EmulsiFlex is quick and easy to disassemble. The homogenising valve parts can be disassembled for inspection in just a few seconds - impossible with most competitive machines!

With minimal moving, parts the EmulsiFlex is easy to maintain, with the user being able to replace seals themselves. There are no O-rings in the product path and nearly all of the contact parts are metal-to-metal or metal-to-ceramic, reducing the risk of leaks. A short product path ensures minimal hold-up volumes, as low as <1ml.

The systems are all suited to cold room operation and some can be fully

Key Features

- *Minimum sample size 10 mL*
- *Capacity up to 1,000 litres per hour*
- *Minimal hold-up volumes, as low as <1ml*
- *Sterile applications*
- *Aseptic applications*
- *High controllable pressures*
- *Clean-In-Place and Sterilise-In-Place*
- *Suitable for cGMP manufacture*
- *Reproducible results*
- *Near perfect scalability*
- *Simple and fast disassembly*
- *Minimal maintenance*
- *Fully autoclavable product path*
- *Leak free design*
- *Continuous or batch operation*

immersed for cold-water temperature control. A range of efficient and sanitary heat exchangers are also available. EmulsiFlex systems are also suitable for Steam-In- Place (SIP) and Clean-In-Place (CIP).

Small Scale to Production Scale

Avestin manufactures cutting edge systems from laboratory scale benchtop systems to large scale production systems for manufacturing.

Bespoke systems can also be designed and are especially useful for pharmaceutical companies, CROs and CMOs.

System	Flow through capacity	Holdback
C5	1-5 L/hr	<1 mL
C3	3 L/hr	<1 mL
C50	15-50 L/hr	<4 mL
D20	20 L/hr	<4 mL
C55	55 L/hr	<8 mL
C160	160 L/hr	<15 mL
C500	500 L/hr	<15 mL
C1000	1000 L/hr	<15 mL

Figure 7: Key specifications of the EmulsiFlex range

Service and Support

Avestin EmulsiFlex homogenisers are engineered for reliability, to minimise service and down-time. Many routine maintenance tasks, such as seal replacements, are straightforward and can easily be carried out by the user. However, Biopharma's experienced after-sales service engineers are also available to provide planned or emergency maintenance.

Testimonials

"*The EmulsiFlex C5 is an ideal system with minimal maintenance, generating quality results and lasting easily for more than a decade"* - *Chief Scientist, Development stage Pharmaceutical Firm, Cambridge*

"Key reasons for choosing the Emulsiflex for emulsification and suspension milling include precise, fine tuning of pressure setting and reproducibility from run to run; robustness of the high pressure pump; allowing for real scaled-down studies" - *Vaccine Scale-Up Senior Manager*


Holistic support from Biopharma UK and Avestin HQ in Germany
Troubleshooting support
Demostrations with client products
Hire opportunities
Robust long life systems with minimal maintenance
Industry leading homogenising efficiency
Personalised, product- specific solution & support
cGMP validation, FAT, IQ/OQ
Aspetic and ATEX solutions
Assistance offered by Biopharma

About Biopharma

Biopharma Group presents expertise in the fields of freeze drying products and technologies, R&D consultancy, analysis, process development, training courses, Genevac Solvent Removal, Sepiatec preparative chromatography and Avestin high pressure homogenisation products.

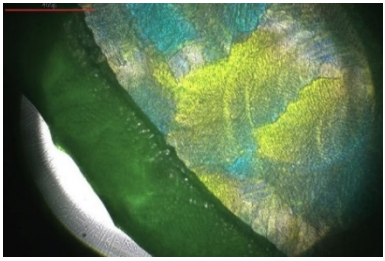
The Biopharma Group is made up of several divisions, including dedicated offices covering the UK, France, Ireland and the USA who offer a variety of our products and services within their geographical regions.

Equipment Sales and Service Division:

Established in 1989, the sales and service division is a leading supplier of equipment to pharmaceutical, biotech and process industries in the UK, Ireland and France, with a specialisation in freeze drying, solvent removal/ evaporation (Genevac), high pressure homogenisation technologies and the related equipment such as Sepiatec preparative chromatography solutions, Kinematics powder fillers, and Penntech vial handling solutions (including full aseptic processing lines).

Biopharma is proud not to be a catalogue company and our aim is to provide our customers with equipment and/ or services that best meet their process requirements whilst remaining on-hand to provide advice and assistance thereafter; it is our expertise and ability to be a 'one-stop shop' when it comes to freeze drying technology and lab processing equipment that keeps Biopharma at the forefront of our field.

The key to our success is the many combined years of experience and expertise in the processing industries and our in-depth knowledge of the equipment we supply. We also have an experienced technical service/ maintenance department and strong links with our suppliers enabling us to support the working life of your equipment. Additionally, our on-site freeze drying laboratory in Winchester, UK, offers a range of services in product and process development and analysis.



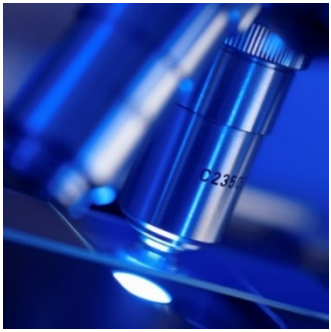
Independent Consultancy Division: R&D consultancy, analysis, process development and training courses (scheduled and/ or customised)

The R&D consultancy and lab analysis division was established in 1997 to provide

independent contract research, analysis, process reviews, product and cycle development services, training and analytical instrumentation to the global biopharmaceutical and related industries. We have decades of experience in the application of all aspects of freeze drying technology and have successfully processed in excess of 1000 substances on behalf of clients.

Together with our knowledge of pilot-scale and industrial freeze-dryers, we offer a uniquely comprehensive service and training courses (scheduled and customized on-site or webinar options are available) covering all aspects of freeze drying technology from pre-formulation through to full-scale production and dried product analysis.

Biopharma is at the forefront of analytical instrument technology developments having, in 2016, launched the most advanced freeze-drying microscope (FDM), the Lyostat5 with its unique tilt-back imaging station for easy sample loading and the Lyotherm3 frozen state solution analyser which combines traditional DTA with electrical impedance technology to provide the most accurate data relating to critical product stages to optimise the efficiencies of freeze drying process development.



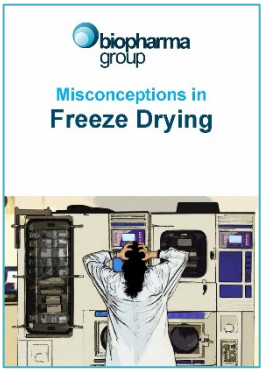
Biopharma's aim is to meet the precise needs of our customers' projects, and will agree a work programme and budget that is appropriate to the size and stage of the project, whether this be a single cycle run, individual analysis or a complete formulation development programme. Our philosophy is to augment our customers in-house expertise and work together to make each project a success.

As with the equipment sales and service division, our R&D Consultancy and lab analysis team is committed to providing a world-class service to our customers. We seek to ensure all our practices are controlled, safe and reliable for staff, customers and the products we handle. Therefore we have adopted a Quality Management System that ensures our laboratory, documentation and health and safety practices are of the highest standard. The Quality Management System ensures compliance with ISO 9001.

Biopharma in France combines elements from equipment sales and service in addition to having access to the expertise of the consultancy division, giving our French-speaking client base a one-stop option, while Biopharma Technology LLC in the USA is focused on providing analytical instruments and independent analytical services for those using freeze drying technologies and BPS Crowthorne in Ireland combines all the expertise offered by Biopharma's UK teams with that of Crowthorne Hi-Tec Services, who are the UK's largest cleanroom validation service providers.

Other Booklets

These introductory booklets are designed to be a helpful reference for anyone new to the fields. To download copies of these other information guides, please use these links:



Misconceptions in Freeze Drying

www.biopharma.co.uk/introduction-to-freeze-drying

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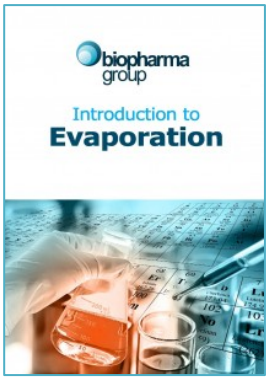

Introduction to Evaporation

http://biopharma.co.uk/bps/about/knowledge-base/guide-to-evaporation/

Alternatively request a copy through the office: bps@biopharma.co.uk, +44 1962 841092.

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