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CHITOVATION

THE COMPANY MAGAZINE BY HEPPE MEDICAL CHITOSAN

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HMC+

HEPPE MEDICAL CHITOSAN GmbH
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Editorial

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» Chitosan is on the upswing «

The outcomes of chitosan research are increasingly detailed - the demand more and more specific. This shows once more that chitin and chitosan are a truly multi-facetted element group.

A minor alteration of a given specification can yield different property characteristics. This versatility and its adjustability through exact specifications make chitosan an ideal material for precise customized applications in medicinal and pharmaceutical products.

We experience these trends and the growing need for special, reproducible chitosans and derivatives with very stringent specifications in our daily business. And - we are proactively taking up any new challenge because it makes our work all the more interesting.

Let me put it this way: Chitosans are on the move and all of us can be curious to learn more about a bunch of new certified pharmaceutical products with this fascinating material to come.

We are looking forward to our cooperation and will be happy to support you. Let your chitosan ideas and developments soar - and we take care of the rest, such as quality and all other essentials.

Sincerely yours

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Thermosensitive chitosan-based *in situ* forming implants for drug delivery

Christoph Porazik, Sabine Kempe, Karsten Mäder

Chitosan and its derivates are attractive for pharmaceutical and biomedical applications. It has been evaluated as a material for wound healing and dressing, dialysis membranes, tissue engineering, contact lenses, liposome stabilization agents and anti tumor uses. There are many chitosan applications in the field of drug delivery. For example, it can be used as a new excipient for tablets and pellets or as a matrix material for micro- and nanoparticles (Illum 1998, Felt et al. 1998). An interesting and both scientifically and industrial attractive chitosan application is its use as stimuli responsive hydrogels former (Chenite et al. 2001). These chitosan-based hydrogels have promising perspectives for applications as carriers or materials in drug delivery systems and scaffold in tissue engineering. The thermogelling properties of chitosan open the avenue for easy injectable *in situ* forming depot formulations.

There was a boost in the research efforts on *in situ* forming implants or gels during the last years. The question arises what are the promises of *in situ* forming implants in the field of drug delivery? And finally what are the advantages and disadvantages of particular systems?

Parenteral depot systems have reached significant research interests in the past years, due to the steadily increasing number of drugs and compounds that cannot be administered via the oral route. Until now, various types of potential formulations are available, e.g. liposomes, mixed micelles, emulsions, microparticles or implants. They generally allow localized or systemic prolonged drug delivery resulting in a decreased application frequency and a drug dosage reduction combined with a lower risk of unwanted side effects. Key roles play implantable drug delivery systems of biocompatible polymers. While the insert of a pre-shaped parenteral depot system requires either surgery (which adds to the costs and the risk of such systems) or the use of a large needle (which causes pain), *in situ* forming implants based on biodegradable polymers avoid this problem and present a non-invasive and more painless alternative. They can be injected as low viscous liquids with small needles. After injection, they solidify and form a subcutaneous depot. *In situ* gelling can be triggered via several stimuli such as changes in temperature, pH, ionic or chemical cross-linking or solvent exchange (*in situ* precipitation). Two poly(lactide-co-glycolide) (PLGA) products are on the market. Atridox® (Steinberg, Friedman 1999) is used for the periodontal delivery of doxycycline. Eligard® (Sartor 2003) forms a subcutaneous depot of the peptide leuproline for the treatment of prostate cancer. For both products, the depot formation is caused by *in situ* solvent exchange (Kempe, Metz, Pereira, Mäder). The poly(lactide-co-glycolide) (PLGA) polymers are dissolved in the organic solvent N-methyl-2-pyrrolidone (NMP). The polymer precipitates after the injection of the solution into the body due to the dissipation of organic solvent into the surrounding body tissue and the compensatory penetration of water as non-solvent. However, the use as drug delivery is limited because the contained organic solvents might cause denaturation of proteins. In addition, the safety profile of NMP is still under debate.

Examples for the development of thermosensitive systems which

show a temperature-dependent reversible so-gel transition are copolymers of poly(ethylene oxide) and poly(propylene oxide) (known as Poloxamers) (Malmsten et al. 1992) and copolymers of N-isopropylacrylamide (Hoffman 1987). But the use of these systems is limited because they are not biodegradable and Poloxamers can cause hyperlipidemia in rats (Wout et al. 1992, Palmer et al. 1997). In addition, the gelation and drug release is highly variable and strongly influenced by the addition of drugs. Block copolymers of poly(ethylene oxide) and poly(lactic acid) were described as alternative biodegradable polymers but the need to heat the solution up to around 45 °C to reach the sol-state limits the use of these systems especially for delivering sensitive proteins or living cells (Jeong et al. 1997, Ruel-Gariépy et al. 2000). A further limitation for thermogelling PEG-PLGA is the instability of the polymers. Thermogelling requires the use of PEG-PLGA with a low molecular weight, which increases polymer degradation.

Therefore, there is the need of a biodegradable and biocompatible stimuli responsive delivery system for *in situ* implants that can be processed under mild conditions. In addition, the use of organic solvents should be avoided and the delivery and degradation performance should be reliable and predictable. A possible solution is the use of aqueous chitosan solutions with added polyol salts like glycerol-, sorbitol-, fructose- or glucose-phosphate salts (Chenite et al. 2000). The combination of chitosan and β-glycerol-phosphate (patent: Chenite, Chaput, Combes, Jalal, Selmani 1999) leads to a unique behavior by allowing the chitosan solutions to remain liquid at room temperature and physiological pH and to turn into gel by heating above the lower critical solution temperature (LCST). By using the right concentrations of chitosan and the polyol it is possible to produce systems, which gel at body temperature. A low viscosity solution can easily be injected into the body. Within the body, a depot is formed *in situ* due to the thermogelling properties of chitosan (Fig. 1)

Drugs, living cells or other therapeutic agents can easily be incorporated within the thermogelling system by simple mixing with the chitosan solution prior to the injection. The injectable hydrogel can homogenously incorporate and suspend cells while allowing the diffusion of hydrophilic nutrients and metabolites of incorporated cells. Gels for tissue engineering have the advantage that the flowable material can fill any shape of a defect. Within our research efforts, we investigated thermogelling chitosan systems with respect to gel formation, thermosensitivity, macro- and microviscosity, and their drug release properties (Kempe et al. 2008, 2010).

In order to characterize the properties of the chitosan gelling system at the molecular scale, we used spin probes and the non-

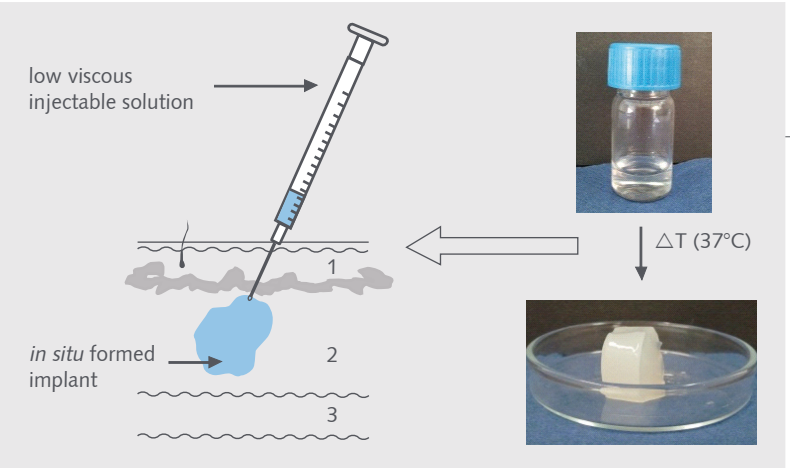


Figure 1

Principle of thermogelling *in situ* forming depots: injection of a low viscous thermogelling chitosan-solution leads to a subcutaneous implant; with schematically 1 dermis, 2 subcutis and 3 muscle.

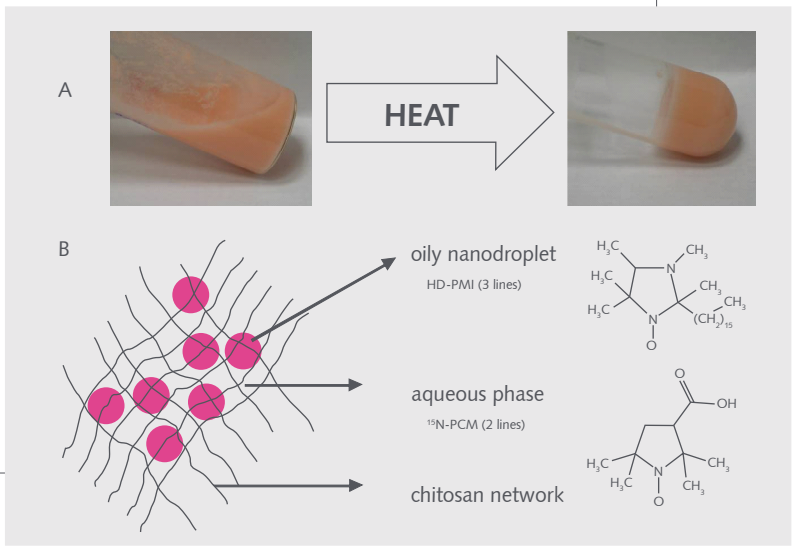
invasive technique of Electron Paramagnetic Resonance (EPR or ESR). EPR permits the direct measurement of microviscosity and micropolarity inside DDS, the detection of microacidity, phase transitions and the characterization of colloidal drug carriers (Kempe et al. 2010). By means of EPR we could monitor microviscosity and the pH inside the gel. The rotation of the spin probe was not affected by the formation of the gel network and huge difference (several orders of magnitude) between macroviscosity (gel) and microviscosity (close to water) was detected. The pore size of chitosan-β-GP gels is large enough to provide small hydrophilic molecules a low viscous environment and to permit rapid release to the buffer or the surrounding tissue sites. In a further study, hydrophilic and lipophilic spin probes were incorporated into *in situ* gelling chitosan-based emulsions as models for low molecular weight drugs (Fig. 2) (Kempe et al. 2010).

Both spectra of the spin probes indicate a high mobility inside the gels during the whole time of release. We were able to follow the release of the water soluble spin probe which was completed within 6 h *in vitro* (Fig. 3) and 3 h *in vivo*. On the other hand, the EPR spectra of the lipophilic spin probe located in the oily nanodroplets remained almost unchanged and were still detectable after 2 months *in vitro* and *in vivo*.

Figure 2

A
Thermally induced gelation of chitosan-based nanoemulsion: oil, here marked with the lipophilic dye Sudan Red, was dispersed in the aqueous chitosan solution.

B
The principle of the simultaneous assessment of multiple sites of an oil/water chitosan-based *in situ* gelling emulsions by EPR. The lipophilic ^{14}N-nitroxide (HD-PMI) is localized in the oily nanodroplets, the hydrophilic ^{15}N-nitroxide (PCM) in the outer aqueous phase.



Another example is the incorporation of spin labeled insulin in thermosensitive chitosan-β-GP gels (Kempe et al. 2008, 2010). The drug is located in the aqueous environment of the gel and a controlled release over several days was achieved. The EPR spectrum also indicates that insulin is released without denaturation and that there is no negative impact on the insulin stability. So, spin labeled drug molecules can give important information about the localization in the drug delivery system and if they undergo changes during their release. In summary chitosan gels are suitable matrices for cell immobilization or enzymes, drugs, growth factors and other potential therapeutic agents.

Chenite et al. showed that thermogelling chitosan-β-GP aqueous formulations can be administered within the body by injection of liquid system and form *in situ* a homogeneous gel-implant in many body compartments, e.g. subcutaneously, intra-muscularly and in bone and cartilage defects (Chenite et al. 2000).

In summary, thermosensitive chitosan-based hydrogels reveal unique features. The gelation at physiological pH and body temperature allows the administration of therapeutic agents and retention of the implant at the site of injection for a predictable residence time with no surgery needed for placement and withdrawal. The mechanism of gelation, which does not involve organic solvents, covalent cross-linkers or other detergents/agents trigger except the temperature change, combined with sustained/controlled release leads to a promising drug delivery system for e.g. sensitive biological substances such as proteins. ■

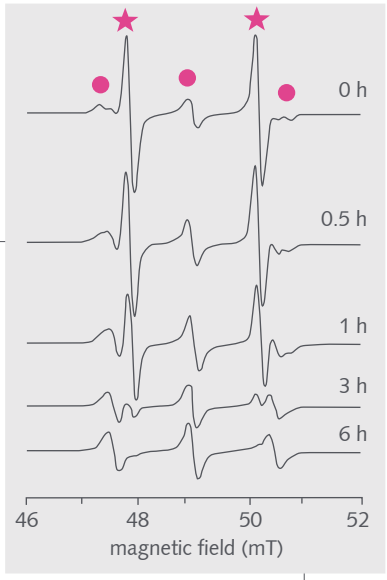


Figure 3

EPR spectra of nanoemulsion loaded *in situ* thermogelling chitosan-gels (see Fig.2). The water soluble and small ^{15}N-nitroxide PCM (2 lines marked with ★) is released within short time. In contrast, the lipophilic nitroxide HD-PMI (3 lines marked with ●) is localized in the oily nanodroplets which are restricted by the chitosan network.

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The scientific investigation of Prof. Mäders group is focused on the development and physicochemical characterization of drug delivery processes and systems.

Multilayered chitosan scaffold for bile duct reconstruction

Roberto Tozzi, Antonello A. Romani, Marina M. Morganti, Anna P. Soliani, Ruggero Bettini, Angelo F. Borghetti

Bile duct injuries have been associated with upper abdominal operations and biliary tract surgical procedures. Their overhelming majority are caused by laparoscopic cholecystectomy and are considered a true health and financial emergency (Savader et al. 1997). Their repair carries a significant mortality rate and can run 4.5 to 26.0 times the cost of the uncomplicated procedure. The standard treatment of neoplastic or degenerative/flogistic diseases that cause stenosis of the main biliary duct involves the resection of the extrahepatic biliary duct and the subsequent tension-free anastomosis between the bile duct stump and the intestine (Roux-en-Y hepaticojeunostomy). Often, this treatment is burden with septic complications (cholangites) and/or anastomotic stenosis. Post operative cholangitis, one of the most common complications in hepatobiliary surgery, accounts for 8-22% of patients following hepaticojeunostomy (Tocchi et al. 2001), and affects more than 50% of pediatric patients following Kasai's operation for biliary atresia (Selvalingam et al. 2002). Considering these common post operative complications (cholangites and stenosis), the present work aimed at developing a novel highly biocompatible chitosan-based polymeric scaffold in form of a tube to be used as a substitute of the human main bile duct. Polymeric tube-shaped scaffolds were manufactured by casting a chitosan solution, prepared, as previously described (Bettini et al. 2008), into cylindrical mold constituted by two coaxial plastic tubes. The solution was, then, frozen and gelified (Bettini et al. 2008). The reproducibility of the method was evaluated by assessing physical parameters of the scaffold such as swelling index, while porosity and microstructural characteristics were assessed by using electron-scanning microscopy. Permeability experiments through the gelled scaffold were carried out in a Resomat II apparatus (Dibbern and Scholz 1969) using a concentrated bovine bile solution in the donor compartment and measuring the concentration of the permeated bile acids in the receptor compartment. Dynamometric measurements were performed to determine the elastic modulus and the elongation to

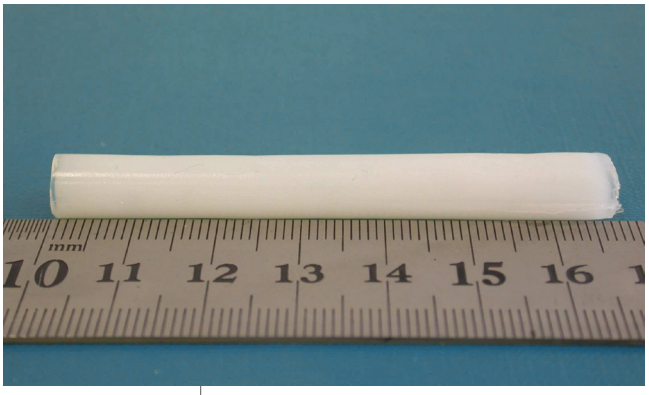

Figure 1 longitudinal view of a chitosan tube

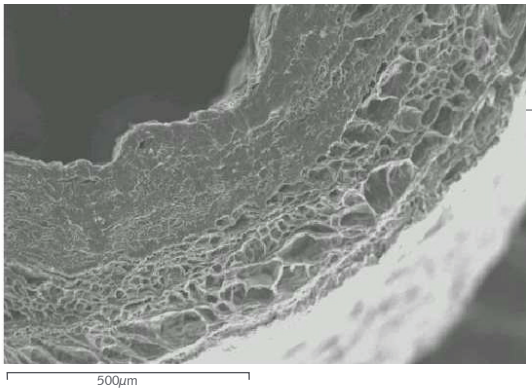

Figure 2 cross section of the tube

break in axial direction, as well as the resistance to surgical suturing. The cytocompatibility of the scaffolds were also evaluated in terms of their ability to support cell colonization. The results so far obtained indicate that the tubular scaffolds, easily created trough a simple freeze-gelation procedure, possess an interconnecting, porous structure. In terms of their ability to organize the cell in 3D environments, the scaffold successfully promotes cell adhesion, viability, proliferation and maintenance of the original differentiation phenotype. ■

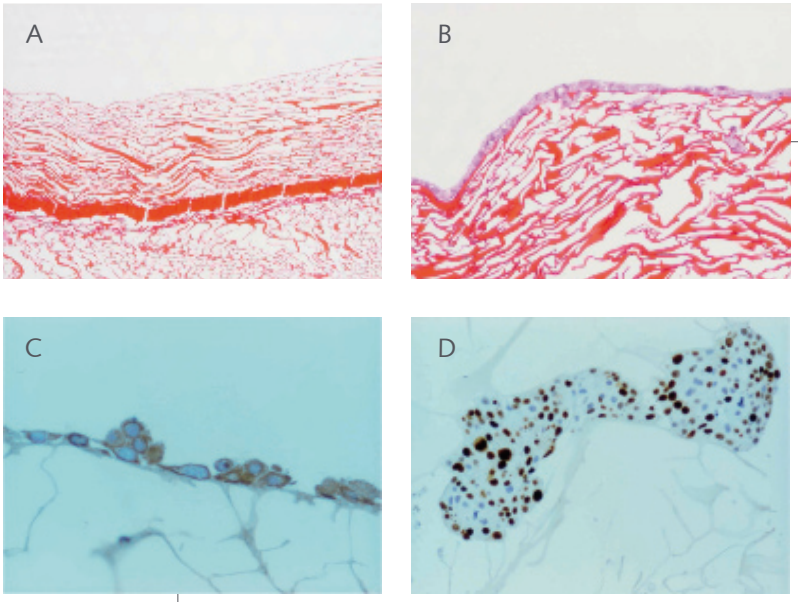

Figure 3

Histology sections of bilayered scaffold 72 h after cell seeding

Panel A shows the bilayered tube without cells. Panel B shows cholangiocarcinoma 72 h after seeding cells and stained with ematoxylin-eosin. The cells lie down on the inner (luminal) tube surface forming a monolayer. Panel C, shows immunohistochemical section tube scaffold stained with cytokeratin 7 (brown color). Panel D shows the Ki-67 staining (proliferation marker, brown color) of cells.

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HMC+ Competences

We have long-standing and in-depth expertise regarding chitin, chitosan and their derivatives and like to share our knowledge with you. Therefore, our offer goes far beyond basic production. The following sections shall give you an idea of the added values and our services.

Production of chitosan specialities

One of our core competences is the development and production of chitosan specialities according to customers' requests and specifications.

These might be chitosans with extraordinary high viscosities of up to 15,000 mPas, especially low degrees of deacetylation down to 60% or non-standard chitosan derivatives.

Furthermore, HMC+ is doing the up-scaling of customer processes (from laboratory to batch scale). And, of course, our customers are always welcome to discuss their requests and the feasibility of their projects with us.

Above all, the production for clients from the pharmaceutical industry can be carried out in compliance with GMP guidelines in clean rooms class C.

Analyses

At HMC+ experienced and well trained personnel perform various highly controlled analyses according to validated methods so that we can assure the high quality of our products.

Thus, our customers can benefit from the well-equipped laboratory, i.e. additional analyses can be carried out or own chitosan samples assayed. The following parameters may be analysed, e.g.:

Identity
Degree of acetylation I Degree of deacetylation
Viscosity
Ash content
Dry matter content
Heavy metals (Pb, Hg, Cd, As)
Microbiology (total viable count, yeast and mould, pathogenic germs)
Endotoxins
Protein content
Molecular weight

Support I Training

Chitosan is a multi-talented raw material for almost every branch of industry. Depending on its specification, chitosan can be used in manifold applications. Nevertheless, this element group is relatively unknown and intensive support is often of greatest importance.

HMC+ is primarily focused on the cosmetics and pharmaceutical sectors. We have a great understanding of chitosan and its specific treatment and usage. And - we know that correct handling has to be understood. We can help and assist you to develop appropriate skills.

To learn more about chitin and chitosan, their structures and properties HMC+ experts offer customized workshops and trainings. Furthermore, brainstorming sessions and consultations present effective tools for identifying the suitable chitosan product to improve or enhance your final applications.

HMC+ Product portfolio

Chitin, chitosans and derivatives

Our passion is chitosan and anything that is derived from it. Being receptive to customers' needs HMC+ offers a unique variety of standardized chitosans and chitosan derivatives in different product lines. Stringent specifications regarding degree of deacetylation and viscosity guarantee reproducible products with consistent properties.

Chitin

Our standard program comprises α- and β-chitin from different species (snow crab, shrimp, squid and krill).

Chitosan

Our standard chitosans are produced from snow crab, other species may be used upon request.

To find the appropriate chitosan customers can combine six degrees of deacetylation (DDA) with twelve viscosity ranges:

DDA / Viscosity	70 %	75 %	80 %	85 %	90 %	95 %
5 mPas	70/5	75/5	80/5	85/5	90/5	95/5
10 mPas	70/10	75/10	80/10	85/10	90/10	95/10
20 mPas	70/20	75/20	80/20	85/20	90/20	95/20
50 mPas	70/50	75/50	80/50	85/50	90/50	95/50
100 mPas	70/100	75/100	80/100	85/100	90/100	95/100
200 mPas	70/200	75/200	80/200	85/200	90/200	95/200
500 mPas	70/500	75/500	80/500	85/500	90/500	95/500
1000 mPas	70/1000	75/1000	80/1000	85/1000	90/1000	95/1000
1500 mPas	70/1500	75/1500	80/1500	85/1500	90/1500	95/1500
2000 mPas	70/2000	75/2000	80/2000	85/2000	90/2000	95/2000
2500 mPas	70/2500	75/2500	80/2500	85/2500	90/2500	95/2500
3000 mPas	70/3000	75/3000	80/3000	85/3000	90/3000	95/3000

Water-soluble chitosans and derivatives

Chitosan oligosaccharides (molecular weight 1-2 kDa)
Chitosan Hydrochloride (Chitosan HCl)
Chitosan Acetate
Chitosan Glutamate
Chitosan Lactate
Carboxymethylchitosan (N,O-Carboxymethylchitosan)
N-Trimethylchitosan

All standard chitosans are available in three and chitosan derivatives in two product lines. Chitoscience® products and standard Chitoceuticals® are available online at: www.medical-chitosan.com.

Chitocare®

Chitocare® products are used in cosmetic applications, e.g. skin and hair care, personal and dental hygiene, deodorants.

Chitoscience®

Chitoscience® chitosans and chitosan derivatives are conform to the specific requirements of academic and industrial research and development units. This product line allows scientists to rely on high quality and consistently stringent specifications. The minimum order quantity is 20 g. Basic analytical results are provided at a reasonable price.

Chitoceuticals®

This high purity line is particularly interesting for research, development and production in medicinal and pharmaceutical environments. According to the official requirements for medical devices or pharmaceutical products, customers may chose among standard Chitoceuticals® and Chitoceuticals® GMP-compliant. High-quality raw materials are being used and the final products are subjected to in-depth analyses to verify their quality. GMP-compliant products are produced in clean rooms according to GMP guidelines and comply with traceability demands. Chitoceuticals® GMP will be available after the official manufacturing license was granted.

Product lines at a glance	Chitocare®	Chitoscience®	Chitoceuticals®		
			Chitoceuticals®	GMP compliant	GMP
Appearance of solid product	+	+	+	+	+
Appearance of solution	+	+	+	+	+
Degree of deacetylation	+	+	+	+	+
Viscosity	+	+	+	+	+
Ash content	+	+	+	+	+
Dry matter content	+	+	+	+	+
Heavy metals: Pb	+	+	+	+	+
Hg		+	+	+	+
Cd		+	+	+	+
As			+	+	+
Microbiology: Total viable count			+	+	+
Yeast & mould			+	+	+
E. Coli				+	+
P. aeruginosa				+	+
St. aureus				+	+
Salmonella				+	+
Endotoxins				+	+
Protein content			+	+	+
Molecular weight: Approx.		Available	+		
Per batch			Available	+	+
Insolubes			+	+	+
Particle size	Flakes	Powder	Powder	Powder	Powder
Production in clean room				+	+
Documentation according GMP				+	+
GMP certificate					+

»» Customers with individual requirements, who need chitosan products with own specifications are kindly invited to discuss production feasibilities together with the highly specialized staff. HMC+ also works according to customer specifications.

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