SPECIFIC APPLICATIONS REQUIRE SPECIALIZED CHITOSANS

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Summary

Chitin and chitosan preparations may differ at the molecular level in their primary amine content, charge distribution, molecular weight and at the macroscopical level in their amorphous or crystalline character, swelling characteristics and solubility and viscosity after dissolution in various solutions. These chemical and physical differences have a profound influence on the applicability of chitosan.

During the last decade, the number of practical applications of chitosan has grown logarithmically. Especially in the medical technological area, many new and specific applications of chitosan have been developed. For any of these developments one should establish the chitosan preparation with optimal operational effectiveness.

An overview will be presented of the most significant differences between various chitosan preparations and how these variations can be controlled. In addition, a number of examples will be presented or referred to showing the importance of making the right choice of the best operational chitosan quality for a specific application.

Introduction

Last decade, many new applications of chitosan have emerged. Practical applications have been described in various fields including water and waste water treatment, food processing and controlled delivery of drugs, whereas a respectable number of new applications are in the pipeline. Especially in the medical biological sector chitosan is a biomaterial of choice in many strategies for bone and would healing, as scaffold in tissue engineering and as protective carrier in DNA transvection. However, in any application one should be aware that chitosan is a complex biomaterial that should be well characterized before use.

The quality of a chitosan product is variable due to many causes. The name chitosan does not stand for one unique product but covers a broad range of amino glycosidic copolymers with a large variety in physico-chemical properties. Chitosan's properties may vary due to variation in the intrinsic properties of the biological source used for its production. Variation in the procedure used to extract the chitosan can lead to considerable differences in the properties of the chitosan. Finally, the chitosan may also be treated after processing to make it more suitable for a specific application. All together, even without manifest chemical modification by addition of side chains to the molecule, there is a nearly unlimited range of chitin and chitosan products. Since unfortunately in daily life many chitosan samples are not produced appropriately, the variety in effectiveness of chitosan preparations is enormous. Although these variations can be of decisive influence on the success of any investigation or application, many chitosan preparations are used as offered by the supplier. Usually chitosan preparations are not subjected to further quality testing or to additional treatments to enhance the chance for success. In this way chitosan is applied sub-optimally and sometimes even in vain, whereas success might lay around the corner.

In this paper consideration will be given to critical factors that might play a role in appropriate chitosan production and that might enhance the success of its subsequent application. The paper is limited to the application of chitosan itself, for chemically modified chitosan reference is made to a review of this topic [1].

The following aspects will be considered: (1) biological resource, (2) physical form, (3) charge and charge distribution, (4) crystallinity, (5) molecular weight (6) swelling (7) dissolution, transparency and precipitation (8) complex formation. The paper will focus mainly but not exclusively on chitosan. A few points regarding chitin are included.

Materials and methods

The chemical procedure to isolate chitosan from shellfish chitin is based on deacetylation by treatment under the harsh conditions of strong alkali (50%, w/v) and elevated temperature (up to 140^{o}C). Although this method usually is cost effective, objections have been raised concerning the quality of the chitosan product and the protection of the environment against the alkaline fluid wastes produced. Research at the Asian Institute of Technology, Bangkok, Thailand has been focused on less aggressive isolation conditions both to protect the product and the environment [1] by improving the conditions of the chemical deacetylation.

The normal industrial protocol is to produce first chitin by treatment of shell fish biowaste by alkali (4 % w/v) and hydrochloric acid (4% w/v). The alkali treatment results in the solubilization of most of the protein and peptides. The acid treatment converts insoluble minerals mainly $CaCO_3$ into soluble material. This treatment has to be carried out with caution due to the sensitivity of the chitin for acid hydrolysis. The resulting chitin is subsequently deacetylated into chitosan in strong alkali and at higher temperature.

For detailed description of specific methods applied, reference is made to the publications cited in this paper.

(1) Considerations related to biological resource

Chitosan is usually produced from crab or shrimp biowaste. In the solid form the chitin obtained is of the so-called alpha type. At this early stage it is important to realize that crab material due to its higher mineral content needs more stringent acid treatment as compared with shrimp shell material. On the other hand the crab chitosan has a higher density, important, as in the case of dietary application, when a maximal amount of material is to be placed in a capsule with a fixed volume. If milder reaction conditions are preferable, sources of beta chitin could be used better. The beta chitin from squid and cuttle fish has a more open structure and is easier to deacetylate. If protein might be a problem due to the allergenic character of shell fish protein, fungal mycelia might be considered as source [2]. In case highly consistent biomaterial is demanded, one should consider to start with biowaste from shrimp produced in a well-programmed way in aquaculture, not with catch from the sea. The quality of the biowaste can be considerably improved by pre-treatment [3]. Particularly washing with acidified water is very efficient [4]. A large part of protein and a significant part of the minerals are removed. The biowaste can also be preconditioned by autofermentation, a storage procedure that allows endogenously present micro-organisms to partially decompose the biowaste, resulting in an easier down stream processing without affecting the quality of the chitin [5]. Although very effective, this is from an esthetical point of view not attractive. Better is to apply a recent finding [6] that treatment with a small amount of benzoic acid can precondition the biowaste in 6-8 hours, resulting in an efficient and more economic isolation of chitin and chitosan.

(2) Considerations related to physical form

In discussion of physical form a few aspects of chitin should be mentioned. Chitin is still underutilized due its lack of solubility in common solvents. Chitin can be dissolved in special solvents like methanol/CaCl2/H2O and dimethylacetamide/LiCl and reprecipitated by excess of water in very fine particles called super fine chitin [7]. Due to the simplicity of the method and the large increase in effective surface, this material will find its way in the market.

Chitin can be made more reactive by peripheral deacetylation to make chitin-os-an, consisting of particles of chitin with the insolubility of chitin but with a thin deacetylated outer layer with the reactivity of chitosan [8]. The material is very suitable for chromatographic purposes.

In several other ways chitin can be given a more open structure. Chitin can be dissolved, mixed with a porogen or exposed to gas bubbles and precipitated again. Dissolved chitin can be mixed with an inactive powdery matrix [9] or nano carbon particles. Upon drying the chitin adheres to the matrix in a distributed form.

Most applications of chitosan start with the solid material that is dissolved (usually in diluted acid). After application it often turns into the solid state if applied as coating, thread, membrane or moulded in a desired form as hollow fibers or as scaffold for tissue engineering.

The considerations related to charge and charge distribution (see below) concern both solid and dissolved state. Considerations on crystallinity and the amorphous state concern primarily the solid state but have consequences for subsequent dissolution. Molecular weight affects primarily liquid state properties including swelling, viscosity and solubility.

(3) Considerations related to charge and charge distribution

The most characteristic property of chitosan is its cationic charge and in case of high degree of deacetylation its high charge density. The charge is generated during the process of deacetylation and most effective at pH values around or below the pK value of the free amine group of chitosan. The consequences of chitosan with a lower or higher degree of deacetylation DD are well known (for a systematic study see [10]. Nevertheless most studies using chitosan are carried with an arbitrary value between 70 and 90 % DD. In many case it

will pay off to vary the DD. In case of chitosan membrane permeability it was shown [11] that the release of the aesthetic lidocaine from a chitosan patch for transdermal application was controlled by the degree of deacetylation of the rate limiting chitosan membrane. The DD is also an important factor that controls the tensile strength of in a chitosan scaffold for tissue engineering [12]In addition it should be mentioned that not only overall charge density of the chitosan is important but also the distribution of the charge along the molecule. Chitosan can be deacetylated in a random fashion but also block wise. In the latter case the remaining N-acetylated moieties in the chitin are present in blocks. The mechanism of this process is well understood [13]. External conditions of temperature and alkali concentration control the ratio block/random deacetylation. Block deacetylation can result in the formation of an insoluble fraction in the chitin, so it is better to avoid it.

(4) Considerations related to crystallinity

Both chitin and chitosan can be highly crystalline. For chitin, the combination of crystallinity and the lack of cationizable side groups are the causes for its insolubility. Application of fully acetylated chitin with high molecular weight is for this reason very limited. Pure chitosan can be highly crystalline so that in the solid state its edges can be knife-sharp and hurt. However, chitosan produced from chitin by gradually removal of acetyl groups is much less crystalline. The deacetylation of chitin will proceed from the outside of the particle into the interior. Due to local differences in the chitin material this will result in micro areas that have been nearly completed deacetylated and micro areas that are still chitinous. The persisting chitinous micro areas will locally inhibit the rearrangement into the chitosan crystalline structure. Thermogravimetric studies have contributed to understand local variations in crystallinity of partial deacetylated chitosans [13]. Amorphous chitosan is highly desirable in case of adsorption of metal or apolar ligands to chitosan. The binding of textile dye in textile factory waste water was shown to be at least 20 times better as compared with standard chitosan with the same degree of deacetylation [14]. There are many cases of applied research in which chitosan acts as adsorbents that would benefit from the use of decrystallized chitosan.

(5) Considerations related to molecular weight

An equally important consideration is the molecular weight and its consequence for viscosity after dissolution. A higher molecular weight chitosan will be applied where upon dissolution a higher viscosity is important. The effect of MW on physico-chemical properties has been studied systematically [15]. High molecular weight chitosan is indicated for application in gelling and for membranes with high tensile strength. The β-glycosidic bond in chitosan is sensitive to acid. To isolate high molecular weight chitosan one should decalcify in less acidic conditions. Low molecular weight chitosan seems to be more effective in applications in agriculture and horticulture [16]. The market for lower molecular oligochitin has expanded very fast. However, most materials are obtained by limited but uncontrolled acid or enzymatic hydrolysis. The products are usually badly characterized and of low quality. Once systems will become available to produce pure and well-characterized oligochitins, the applications of these compounds will fast expand, especially in the medical sector.

(6) Considerations related to swelling

Chitosan in contact with aqueous solutions will swell. The swelling is dependent on the characteristics of the chitosan and on the nature of the solvent. Swelling is important in the process of chemical deacetylation. Swollen chitosan is deacetylated more efficiently. To reach high values of deacetylation in concentrated alkali, the material should be allowed to swell by an intermittent treatment with water. Industrially, swelling can be a problem in ready to use chromatographic chitosan columns for waste water purification. Due to changes in the pH of

the waste water chitosan will swell and shrink, leading to a disruption of the column integrity. Chitosan that does not change its volume in contact in various aqueous solutions might be applied to solve this problem.

(7) Considerations on solubility, transparency, precipitation

Good quality chitosan dissolves completely in a 1% solution in 1% aqueous acetic acid. The higher the degree of deacetylation and the more homogenous the distribution of the remaining acetylated sites, the better the chitosan will dissolve. Similarly, chitosan that is deacetylated block wise and still contains clusters of acetyl-glucosamine moieties will not completely dissolve although that might be expected on basis of the overall degree of deacetylation. Block wise deacetylation occurs if the deacetylation is carried out in a combination of high temperatures (70-90^{o}C) but insufficient alkali concentration (effective concentration lower than 40%) [12]. The amount of insolubles can amount to 10-30% depending on the conditions used. These insolubles cause turbidity of the chitosan solution and are difficult to remove. For high quality chitosan it is essential to meet the appropriate conditions for deacetylation.

The applicability of both solid chitin and solid chitosan can be improved by a treatment by dissolution followed by precipitation. The transformation of chitin flakes into very small superfine chitin particles has been mentioned already above. Chitosan dissolved in acetic acid can be precipitated by alkali. If the alkali is added slowly and under stirring chitosan will precipitate in very small particles. Chitosan's physico-chemical characteristics can change by precipitating from various solvents [14]. Most chitosans precipitate in the crystalline state when precipitated from an acidic aqueous solution. A special case is chitosan dissolved in citric acid. Upon addition of alkali, the citric acid with its three strong carboxylic acid groups forms a complex with the chitosan at a pH were the chitosan still has charge. As a result it precipitates at relative low pH. The precipitated chitosan appeared to be amorphous in contrast to the chitosan that precipitates from other (organic) acids at higher pH and has high crystallinity. The applicability of this chitosan is already mentioned above. Neutralization of acidic chitosan solutions by limited amount of alkali under vigorous stirring results in the formation of a chitosan cream. The consistency of this cream is highly dependent on the ionic surroundings.

(8) Considerations related to complex formation

Chitosan interacting with anionic compounds can result in a complex that makes the chitosan more resistant to acidic conditions. Ionic gelation occurs in combination between chitosan and inorganic poly phosphate or with poly-anionic alginate [17] and other biopolymers. This is the basis for the application of chitosan in material for controlled drug release. In this interplay of multivalent cationic and anionic compounds beneficial effects of both anionic and cation ions should be investigated. The inclusion of Ba^{++} ions in the chitosan alginate resulted in a considerable extension of the period of controlled drug release [18]. Interpenetrating networks of various polymers have been described. A network between chitosan and polyoxanes has been proposed as bandage material for wound healing [19]. A three dimensional scaffold composed of chitosan and gelatine has been investigated as a gene-activated matrix by including plasmid DNA for transforming growth factor (TGF-β1). Chondrocytes homing in the matrix were found to express the TGF protein [20].

The effect of covalent cross linking used for strengthening chitosan against dissolution at lower pH and the use of chemical modification as surveyed by [1] give an even wider other horizon for the application of chitosan but falls outside the scope of this paper. For applications in medicine and food that require registration, the use of unmodified but functionalized chitosan might be of advantage.

Quality control

Quality Control of the chitin/chitosan before before use in research and application is as important as choosing the chitosan in the right physico-chemical form. Quality testing is complicated, laborious and costly and is often neglected by chitosan users. There is no chance to go for the optimal result if the quality of the material to be used does not meet specification. Really unfortunately is that there is no generally accepted framework for quality testing of chitosan. At previous chitosan meetings (Princetown, Bangkok AIT 1996) chitin/chitosan quality testing has been discussed. A practical model has been presented at the Yamaguchi meeting [21]. Further discussion to reach broad consensus is badly needed [22].

Conclusion

In summary, most of the new chitosan applications - including its use in DNA transvection, vaccination, controlled drug release to specific targets in digestive, transdermal and mucosal uptake, in biosensor membrane technology and chemical synthesis - but also its use in more conventional applications - as in cosmetics, in food, in agricultural and in water and waste water treatment - all have to be considered whether the opportunities for chitosan product improvement have been sufficiently explored and exploited. The need for an international quality system is still very actual. Chitosan to be used for advanced applications including those in microelectronics, nanotechnology and in delivery of DNA and drugs to specific sites should be chosen on basis of appropriate charge density, amorphous character, size, ionic counter ions and swelling capacity to serve its purpose optimally. Chitosan is not table salt that can be ordered in the blind from the supplier, efforts to choose the best source and the best processing will pay back in a very short time.

Acknowledgements

The authors are grateful for the encouragement and generous financial support received from the late Prince Leo de Lignac

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(For full reference to general underlying methodology and specific data, see the references quoted in the following papers).

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