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Compression profiles of different molecular weight chitosans

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Abstract

The compression behavior of hydrophilic chitosan has been investigated as a function of molecular weight (MW), ranging from 8-100 kDa. Powder packing, plasticity, elasticity, brittle-fracture nature were evaluated using force displacement curves and Heckel/Kawakita analysis upon compression of different MW chitosan powders. The compacts produced were subjected to ejection work determination and tensile strengths analysis, the later using Leuenberger equation. Molecular modeling was used to correlate the MW-binding energy dependence on the physical strength of the chitosan compacts. The high work of compression of the low MW (≤30 kDa) chitosans is accounted for their brittle nature upon compression. At high compression forces (> 3kN), the low MW chitosan compacts exhibited slightly lower tensile strength due to their increasing extents of elastic recovery and ejection work. Lower particle size of chitosan enhances lower fragmentation, packing, and frictional tendency that can be related to an increase in plastic deformation and compacts tensile strength. In molecular modeling, the calculated higher binding energies of the high MW chitosan (88 kDa) than that of the low MW chitosan (8 kDa) may further explain its rigid, complex nature when compared to low MW chitosan. Upon compression, increasing MW of chitosan is manifested as higher energies for densification which in turn determines the extent of helical packing from intermingled molecular to open structure as the MW is decreased. Such changes in structural configuration are suggested to contribute to the transfer from plastic to brittle-fracture nature with high packing extent of chitosan upon compression when the MW is reduced. The foregoing may justify the slow diclofenac Na drug release upon dissolution testing when low MW chitosan is used in tablet preparation.
Keywords: chitosan, molecular weight, compression force, molecular modeling

1. INTRODUCTION

Chitosan powders have been the subject of various investigations as matrices for immediate/controlled release solid dosage forms [1-9]. Since chitosan is prepared from chitin [10,11], the final molecular weight (MW) of chitosan depends on the source or initial MW of chitin and the deacetylation time and temperature [12]. However, Hwang KT et al [13] demonstrated the complexity of variables, e.g. temperature and time, involved to control the depolymerization and/or the deacetylation of chitosan. Many of these variables were found to manipulate the effective surface charge, electrokinetic mobility, and colloidal stability of chitosan especially upon interfacial interaction inside an aqueous environment [14,15]. Unfortunately, the literature itself lacks sufficient handling of the physicochemical investigations as a function of chitosan MW. Investigations were mostly concerned with applications with few justifications, if present, on the role of chitosan MW at the molecular and morphological level [16-25]. These justifications were confined to structure-MW dependence and supported by the fact that the MW is equivalent to the number of repeating units of D-glucosamine. Onto each of these units, interactive amine groups (NH$_2$) are bonded. The general observation conclusively illustrates that the longer the chain length of chitosan, the more NH$_2$ binding sites are available. Consequently chitosan binding affinity, uptake capacity, rheological properties, particle morphology and subsequently surface area, are all strongly dependent on the number of NH$_2$ groups in a specific polymer mixture, i.e. average MW.
The scientific inputs become even shallower when chitosan is present in the powder state. In this context, the compression behavior of chitosan powder was not understood to be MW related [1,5,6,26-27]. In order to correlate such compression dependency, the particle morphology and density have to be introduced for analysis when the MW of chitosan is varied. These two parameters play a significant role in the packing and arrangement upon powder compression [28]. Fortunately, some attention has been given to try to understand the complex relationship between the MW of chitosan and the structural changes and consequently the powder physical properties, all as a function of chain length (or MW). For example, Goycoolea et al pointed out that chitosan of longer chain lengths tends to form ‘loops’ giving rise to the creation of more expanded structures and to a denser packing [7]. Ofori-Kwakye et al related the change in the intrinsic viscosity of different MW chitosans to the change in particle shape and size [29]. Aggarwal et al highlighted the dependence of chitosan molecular architecture and MW on the mechanical strength of branched chitosans synthesized by grafting low MW chitosan chains [30]. Furthermore, bulk density and flow of powders were found to be dependent on the MW of chitosan when the later is spray-dried with lactose [31]. In this regard, it was noted that the lower the MW of chitosan the higher the bulk density and better flow of their processed mixture. However, the poor powder flow and thereby compressibility have never been investigated as a function of chitosan MW. Consequently, an insight into the behavior of different MW chitosan powders upon compression is of significant interest.

In light of the foregoing remarks which are important parameters in characterizing powder compression properties for direct compression pharmaceutical applications, the
work reported herein addresses the mechanistic densification behavior of depolymerized chitosan powders. The properties of the compressed tablets were assessed in the context of testing tablet mechanical properties as a function of MW.

2. MATERIALS AND METHODS

2.1. Materials

Chitosan (Hong Ju Ginseng Co., Ltd., Dalian Liaoning, China), particle size <150 μm, MW 100 kDa, (93% degree of deacetylation) of 100 kDa MW was supplied as pharmaceutical grade. The 8, 13, 18, and 30 MW chitosan powders were prepared by the Jordanian Pharmaceutical Manufacturing Company (JPM, Naor, Jordan) using the acid hydrolysis of the 100 kDa chitosan. This technique was described in details by Qandil et al [32], who illustrated the de-polymerization of chitosan by acid hydrolysis, the determination of viscosity average molecular weight using the relative and reduced viscosities of the Mark-Houwink technique, and finally the determination of the degree of deacetylation which was approximately 100% for all chitosan samples according to the first derivative UV spectroscopic method [33]. Diclofenac sodium was supplied by CHEM. Co., Ltd (CCS. Xiaoshan Hangzhou, China).

2.2. METHODS

2.2.1. Compression of chitosan samples

Prior to compression the chitosan samples were grinded and sieved using an oscillating granulator with a mesh size 250 μm and collected on a 90 μm mesh. 100 mg of each MW (8, 13, 18, 30, 100 kDa) chitosan sample was compressed using a Gamlen Tablet Press (Gamlen Tableting Ltd. Biocity Nottingham, UK). Initially, the powders were
compressed at a punch size of 6.0 mm diameter, enough to accommodate the chitosan samples. The die was manually filled and the machine operated under an automatic compression force or displacement control. Ejection was carried out in the same direction as compression i.e., the tablets were pushed through the bottom of the die into a tablet holder which was removed from the machine. The test speed was set at 60 mm/min. The applied loads used were; 100, 200, 300, 400, and 500 kg equivalent to 098-4.9 kN compression force or to 34.6-173.2 MPa compression pressure (compression force/punch surface area) applied on a 6 mm diameter circular punch. The Gamlen Tablet Press (GTP) software displayed a full data capture of the work of compression and work elastic recovery on a PC screen. After ejection the thickness of the tablets were immediately measured using a caliber. Other tablets produced in the same manner were directly subjected to tensile strength tests using a hardness tester (Copley, Nottm Ltd., Therwil, Switzerland). The average tensile strength of 10 tablets was recorded at each compression force for each MW chitosan. From the measured tablet hardness, the tensile strength was calculated according to the equation [34]:

\[ \sigma = \frac{2F}{\pi Dh} \]  

(1)

where \( \sigma \) is the tensile strength (Pa), \( F \) is the tablet hardness (N), \( D \) the tablet diameter (m), and \( h \) the tablet thickness (m).

2.2.2. Work of compression, elastic recovery and tablet ejection, and extent of powder packing

A typical illustration of the force displacement curve is presented in Fig. 1 for the 100 kDa chitosan (100 mg) whereby the input load was set at 100 kg (4.91 kN). The upper
punch, at a distance of 27.379 cm from the base, displays an automatic displacement starting from the zero position (point A), followed by point B where the upper punch makes the first contact with the powder. The powder then undergoes compression whereby the recorded curve represents an increase in the amount of force as a function of the upper punch displacement. The maximum recorded force terminates the punch movement in the downward direction and is presented by point C of the displacement value D. However, the powder’s elasticity exerts an upward force to the punch in the form of a decreasing force profile until it reaches zero at point E.

From Fig. 1, the area under the curve BCD is the work of compression in J (N.m). On the other hand, the area under the curve ECD represents the work of elastic recovery. In both cases, the data are tabulated by the software of the GTP into two columns, one for the position of the upper punch (mm) and the other for the load applied (kg). The total area was numerically calculated by approximation using areas of rectangles. The width and height of such rectangles were taken as the difference between two successive displacement points and their corresponding ‘load value’ length respectively. The summation of all rectangles areas represents the area under the curve (AUC).

The packing extent can be estimated from the final volume of the powder after being compressed compared to its initial bed volume before compression. Empirically, the packing extent can be estimated from Fig. 1 using the following equation;

\[
Packing \ extent = \left( \frac{D - B}{27.379 - B} \right) \times 100\%
\]  

(2)
where \((D - B)\) and \((27.329 - B)\) represent the final (after compression) and initial (before compression) heights of the powder bed respectively, mm.

Generally, the die-wall friction can be usually estimated by either of the following methods; by calculating the difference in the work of compression of unlubricated and lubricated granules [45], or by calculating the difference in the work of compression for the upper punch forces and that for the lower punch [46]. However, both methods were not adopted in this work as lubricants, in the first case, may disrupt the arrangement of particles through agglomeration [47]. The extent of such disruption may vary between different MW of chitosans. In the second case, the GTP does not provide a lower punch for compression, it can, however, measure the work for tablet ejection by the upper punch from within the die. A typical force-displacement profile for the ejection of the 100 kDa chitosan tablets compressed at the input load of 500 kg (4.9 kN) is presented in Fig. 2. The AUC, which represents the work for tablet ejection, was calculated for all force-displacement curves for each MW of chitosan and then plotted at each compression force. Accordingly, after the powder was being compressed into a tablet, the GTP setting was turned into the ejection mode using the same upper punch and inverting the closed die base position into an open position.

2.2.3. Analysis of compression data

Heckel, Kawakita, and Leuenberger analysis were used to characterize the compression properties of different MW chitosan samples. The Heckel equation (Eq. 3) describes the change of powder porosity as a function of the applied pressure upon powder densification [38,39].
\[ \ln \frac{1}{(1 - D)} = KP + A \]  

(3)

where \( D \) (compact density/true density) is the relative density of a powder compact at pressure \( P \) (MPa), whereby compact density is the tablet weight (100 mg)/volume of 6 mm diameter tablet. The true densities, measured using a helium pycnometer (Ultrapyenometer 1000 v. 2.2, Quatachrome Co., Odelzhausen, Germany) of the 100, 30, 18, 13, and 8 kDa chitosans were 1.351, 1.549, 1.592, 1.758, and 1.853 g/cm\(^3\) respectively. \( K \) (MPa\(^{-1}\)) is the slope and it is a measure of the plasticity of a material. The inverse of the slope is the yield pressure \( (P_y) \) of the materials (MPa) [40], which describes the tendency of the material to deform either by plastic flow or fragmentation. \( P_y \) is inversely related to the ability of the material to deform plastically under pressure. The constant \( A \) is related to the die filling and particle rearrangement before deformation and bonding of the discrete particles.

For comparison purpose, the compression behavior of the samples can be also evaluated using Kawakita analysis of powder compression data. Such a model is usually used to linearize the non-linear pressure-porosity relationship provided by the Heckel model into a linear pressure-volume reduction relationship. Therefore such a model is dependent upon the initial powder bulk density instead of the true density of the powder, as in the case of the Heckel model. The Kawakita equation (Eq. 4) is used to study powder compression via the degree of volume reduction, \( C \) (Eq. 5) under an applied pressure, \( P \) (MPa).

\[ \frac{P}{C} = \frac{P}{a} + \frac{1}{ab} \]  

(4)
\[ C = \frac{V_0 - V}{V_0} = \frac{abP}{1 + bP} \]  \hspace{1cm} (5)

where \( V_0 \) is the initial bulk volume and \( V \) is the volume at pressure of \( P \). \( a \) and \( b \) are constants which are obtained from the slope and intercept of the \( P/C \) versus \( P \) plots.

The constant \( a \) is the initial porosity of the material before compression, while the constant \( b \) relates to the degree of plasticity of the material. The reciprocal of \( b \) or \( P_k \) defines the pressure required to reduce the powder bed by 50% \([41, 41]\).

In addition to the previous particle size of chitosan adopted for compression analysis, i.e. between 250 \( \mu \)m and 90 \( \mu \)m, all of the aforementioned compression tests were repeated for the 100 kDa and the 8 kDa chitosans at two additional particle sizes. The first particle size represents the coarse grade whereby the chitosan powder was passed over a mesh size 500 \( \mu \)m and collected on a 250 \( \mu \)m mesh. The second particle size represents the fine particles that pass through the 90 \( \mu \)m mesh.

The last compression analysis carried out in this work is the Leuenberger analysis. In this model, the correlation of the tablets tensile strength with the compression pressure is found to be further dependant on the tablets density. Blattner and Leuenberger analysis describe such correlation whereby there is a non linear relationship between the tensile strength (\( \sigma \)) and the product of applied pressure (\( P \)) with the tablets relative density (\( \rho_R \)) as Eq. 6 implies \([43]\).

\[ \sigma = \sigma_{\text{max}} \left( 1 - e^{-\gamma \times P \times \rho_R} \right) \]  \hspace{1cm} (6)
where $\sigma_{\text{max}}$ is the maximum tensile strength when $P$ is infinite and $\rho_R$ (the relative density of the tablet) = 1, $\gamma$ is the compression susceptibility which represents the decrease in porosity under pressure. $\gamma$ is related to the how fast (high $\gamma$) or how slow (low $\gamma$) is the transition state between a powder and tablet when compacts are formed under compression pressure [44]. The obtained data were analyzed by using GraphPad Prism (GraphPad Software Inc., La Jolla, CA).

2.2.4. Molecular modeling

Computations were performed with Hyperchem® (6.03 professional, Hyperchem Inc., Waterloo, Canada), using the Amber Force field implemented in Hyperchem. All molecular calculations were carried out using MM+ force fields (bond dipoles). The partial atomic charges were calculated using AM1 semi-empirical methods. Energy minimizations were performed using the Polak-Ribiere algorithm (0.01 and 0.1 kcal/mol.Å for D-glucosamine and chitosan, respectively) in order to obtain the stable conformations of chitosan. For simplicity, chitosan polymers were built up from amine groups (100% deacetylated). Chitosan monomer (D-glucosamine) was also built up from natural bond angles, as defined in the Hyperchem software and then optimized at the HF- \textit{ab initio} level using the 3-21G* basis set (structures I). A set of named selections has been made to define the HEAD and TAIL for building the polymer. An additional set of named selections was made to define the atoms involved in the torsion angles used to assemble the monomers. The HEAD is assumed to be connected to x which in turn is connected to x’. The TAIL is assumed to be connected to y which in turn is connected to y’. The torsional angles connecting the monomers are therefore x’–x–y–y’, with retention of the internal structure of the monomer. The torsion angle used to bring the monomers
together may be specified to have a random value rather than the constant value described earlier. The chitosan polymer was constructed with a suitable length from the monomer unit [45].

2.2.5. Dissolution of diclofenac sodium

Three preparations of diclofenac sodium tablets were formulated so that each tablet (150 mg) contained 50 mg diclofenac sodium and 100 mg of the 100, or 8, or the 30 kDa chitosan powders. The foregoing MWs represent the insoluble, the lowest MW soluble and the highest MW soluble chitosans respectively. All components were physically mixed for each preparation and then directly compressed at pressures of 30-35 kN using a single punch tabletting machine (Manesty, Merseyside, UK) in which a 10-mm flat circular punch was fitted. Compression was carried out at different forces (30-35 kN) to maintain similar tablet tensile strength at 3.0 (±0.1) MPa for the purpose of having a fair comparison in drug release data. The tablets had an average tablet density of 6.25 (± 0.01) g/cm³. For drug dissolution studies, eight tablets were used according to USP specifications (USP30-NF25, 2007) for the dissolution media and apparatus.

3. RESULTS

3.1. Extent of powder packing and compression, elastic recovery, and tablet ejection works

For each MW, the extent of powder packing, the work of compression, elastic recovery, and tablet ejection were calculated for each chitosan powder from the force-displacement curves measured using the Gamlen tablet press. Figures 3, 4, 5, and 6 illustrate the plots of powder packing extent and the works for powder compression, elastic recovery, and
tablet ejection, respectively as a function of the five applied forces; 1-5 kN which are equivalent to the GTP compression loads from 100-500 kg on 100 mg of the chitosan samples.

In Figures 3 and 4 the increase in powder packing extent and compression work as the compression force increases is clearly evident for all the different MW chitosan samples (8 kDa-100 kDa). Moreover, decreasing the MW of chitosan from 100 kDa to 8 kDa shows an increase in both the powder packing extent (at compression forces > 2.94 kN) and the work for compression.

The work for elastic recovery calculated from the force-displacement curves is illustrated in Fig. 5 for the same chitosan samples. It is clear from the data in Fig. 5 that increasing the compression force for all samples results in an increase in the elastic recovery work. Such behavior manifests the high amount of elastic energy stored at higher compression forces which is then recovered upon termination of the applied force. In addition, the data in Fig. 5 shows that all of the low MW chitosans (8, 13, 30 kDa) show lower elastic recovery work than the 100 kDa chitosan for all compression forces below 2.94 kN. Higher than 2.94 kN, the elastic work of all the low MW chitosans approach that of the 100 kDa. The foregoing suggests that although higher compression work is displayed for chitosan samples of lower MW at all compression forces (Fig. 4), there appear to be force-related factors that contribute to the observed elastic recovery trend when compared with 100 kDa chitosan.

Since tablet ejection depends on the frictional forces between the tablet and the die, the work of tablet ejection measured using the GTP was used to indicate variations with
compression forces and chitosan MW. Fig. 6 illustrates the foregoing criteria. In this regard, with the exception of the 100 kDa chitosan, the work increases with increasing the compression force and decreasing the MW. In contrast, for the 100 kDa chitosan, ejection work decreases with increasing the compression force.

3.2. Heckel, Kawakita, and Leuenberger compression analysis

The properties and behavior, e.g. plastic/elastic deformation, brittle fracture, and extent of particles arrangement, of powder compacts upon compression may justify the changes obtained in the work for compression and elastic recovery when increasing the applied force on one hand and decreasing the MW of chitosan on the other. Heckel and Kawakita analysis of the compression data are considered herein in an attempt to evaluate the behavior of chitosan powders when subjected to different compression forces.

Fig. 7 illustrates the Heckel plots of the samples whereby non-linear trends are obtained over all the compression pressure data range (i.e. 34.6-173.2 MPa). It is clear from the Figure that powder porosity decreases when the compression pressure increases and when the MW of chitosan deceases. The lowest porosity is thus obtained for chitosan of 8 kDa MW. From the same Figure, linear portions can be drawn through three successive points (porosity-pressure points). For example, for all the samples, except for chitosan of 8 kDa, the first three points were considered whereas the 3rd, 4th, and the 5th points were considered for chitosan of 8 kDa. The slopes and the intercepts of the straight line portions are tabulated in Table 1.

Kawakita analysis was carried out using the same compression data obtained by the GTP. In this model, bulk densities were considered for calculations of the powder volume
reduction upon compression instead of the true densities used in Heckel analysis when calculating porosity changes upon compression. Kawakita parameters; $a$ and $1/b$ ($P_K$) were calculated from the slopes and intercepts of Kawakita equation (Eq. 4). Results are tabulated in Table 2.

From the compression analysis using Leuenberger equation, the radial tensile strength at each compression force, calculated using Eq.1, was plotted against the product of the pressure of compression and the relative density of the different MW chitosans as Figure 8 illustrates. By choosing a logarithmic regression for curve fitting of the data, the unknown parameters representing the compression susceptibility and the maximum tensile strength can be displayed. Values of these parameters are presented in Table 3. The first two high (100 and 30 kDa) and the lowest (8 kDa) MW chitosans had low compression susceptibility, whilst values of the 18 and the 13 kDa chitosans were the highest. Thus the maximum tensile strengths (Table 3) of the 18 and the 13 kDa are reached faster than all the other MW chitosans. However, the $\sigma_{\text{max}}$ of the 18 and 13 kDa chitosans were the lowest indicating poor bonding at higher compression pressures.

The tensile strength of chitosan compacts are rearranged in Table 4 at each compression force for the whole MW range of the samples. Generally, increasing the compression force, allows closer packing of the powder bed, thus minimizing the porosity and forming a more coherent compact. The tighter less porous structure of the compacts, the higher their tensile strengths when compressed at increasing force, as the data in Table 4 illustrates. In addition, the highest tensile strengths were obtained for the highest MW chitosan, i.e. 100 kDa at the 4.9 kN compression force. In contrast, the same MW manifested the lowest compacts tensile strengths when compressed at 0.98 and 1.96 kN.
compression forces. It can be further noticed that, the tensile strengths of the 18 and the 13 kDa chitosans started to form stronger compacts than the other MW chitosans at the compression pressure of 2.94 MPa. Further increase in the compression force (>2.94 MPa), however, resulted in weaker compacts for the same foregoing MWs of chitosan. On the other hand, all the other MWs encountered a tensile strength increase with pressures higher than 2.94 MPa.

3.3. Particle size effect on compression properties

All compression properties were found to be affected by chitosan particle size (Table 5) using the highest (100 kDa) and lowest (8 kDa) MWs. Although the packing extent decreases as the particle size is decreased, the work of compression is higher for smaller particle size chitosans. Such behavior is contradictory to Figures 3 and 4 which indicate that the recorded high compression work belongs to high chitosan packing when low MW chitosans are compressed at 4.9 kN. On the other hand, the work of elastic recovery and tablet ejection are reduced when lower chitosan particle size is used. Heckel analysis of the three particle size chitosans indicates the decrease in the yield pressure ($P_Y$) and rearrangement values when the particle size is reduced. Finally, high tensile strength of the compacts is achieved for the compression of low particle size chitosans.

3.4. Molecular modeling

In molecular modeling using Hyperchem, the chemical structure of D-glucosamine (chitosan monomer) built up from natural default bond angles is shown in Fig. 9(A). Building chitosan polymers (high MW chitosan and low MW chitosan) were constructed with a suitable length from the monomer unit. The high MW chitosan (Fig. 9(B)) was
constructed using 365 monomer units (MW is 66 kDa), while the low MW (Fig. 9(C)) chitosan from 45 D-glucosamine units (MW is 8 kDa). Accordingly, both high and low MW chitosans exist in two different orientations owing to the chain length. Compared to the low MW chitosan, the high MW chitosan, Fig. 9(B), shows a longer and more extended structure (more extensive H-bonding). The interior space interactions within polymeric species were studied manually by allowing two chitosan polymers of the same MW to approach each other, and then system optimization was carried out under the conditions described in the experimental section.

It is noteworthy that the high MW chitosan polymer units approach more closely to each other than the low MW chitosan, allowing more extensive intermolecular H-bonds when compared to the less extended low MW chitosan polymers (Figures 10(A) and (B)). The calculated binding energy per monomer unit, Ecal, is determined from the following equation:

$$E_{cal} = \frac{E_{system} - (E_{p1} + E_{p2})}{n}$$

(7)

Where $E_{system}$ is the energy of the optimized system, $E_{p1}$ and $E_{p2}$ are the energies of the isolated gas phase polymers in similar conformations, with $N$ being the number of D-glucosamine units in the polymer.

Dissolution data for diclofenac sodium tablets containing different MW chitosan powders is presented in Fig. 11. The 100 kDa chitosan tablets showed fast drug release whereas slow drug release was the case for the 30 kDa and the 13 kDa chitosan tablets. The extent of powder packing was tested for the three chitosan MWs used in the diclofenac product.
preparation (Fig. 12). There is clearly an increasing extent of packing the higher the MW of chitosan.

4. DISCUSSION

The increase in compression work as the compression force increases is clearly evident for all the chitosan samples. Generally, upon powder compression, increasing the applied force by the GTP allows either more folding for plastically deforming materials or high extent of particle fragmentation for brittle materials. Thus, in the course of compression by the upper punch there will either be a change in the fracture behavior of the materials, or extra displacement to be undertaken when achieving a complete input load, or the establishment of both of the foregoing changes. In the current investigation, the extent of powder packing using force-displacement curve has been used to give measurable estimates of the amount of these changes taking place as a function of compression force and chitosan MW. In this regard, the effect of compression force on the powder packing was found to be more significant than chitosan MW whereby, as expected, higher compression forces induce extensive packing (Fig. 3). Examining the effect of chitosan MW, the extent of powder packing was found to increase with increasing the MW and decrease with increasing the MW for the lowest and highest tested compression forces respectively. In other words, decreasing the chitosan MW implies more packing extent attained with increasing the compression force. Such finding explains the obtained data in Fig. 4 whereby there is more displayed work of compression when lower MW chitosans are used. Since the work required for compression is the sum of work necessary to rearrange the particles, deform, and finally to fragment them [46], it is suggested that decreasing the MW of chitosan implies that the compacts undergo excessive folding.
and/or fragmentation at the micro level upon compression. Furthermore, such particles behavior will have an impact on the resulted profiles of elastic recovery work for each MW (Fig. 5). In this regard, the profiles of the elastic recovery work of all MW chitosans except for the 100 kDa are similar, with values lower than the 100 kDa chitosan for compression forces less than 2.94 kN. Above 2.94 kN, the aforementioned profiles start to approach that of the 100 kDa chitosan. Such observation is in harmony with the packing extent as a function of MW (Fig. 3) whereby above 2.94 kN the packing extent is clearly in increasing order with chitosan MW. This indicates that higher powder packing induced by higher compression forces manifests higher elastic recovery which increases with MW. Since elastic recovery is related to the amount of stored energy during compression [47] and is released during decompression, then the apparent high packing/elastic recovery extents are thought to be correlated to higher ability of the lower MW to store more energy than the higher MW chitosans. The foregoing will be given a further attention in the molecular modeling work. In the meanwhile, compression data analysis using Heckel and Kawakita equations may assist to elucidate the impact of the MW of chitosan on powder properties upon compression.

Heckel and Kawakita analysis determine optimum tableting properties usually obtained with plastically deforming materials. Generally, plastic materials exhibit greater degree of folding resulting at higher compression pressures thus allowing the appearance of new surfaces for binding to take place. With regard to Heckel analysis, the lower the yield pressure of a material is an indication of higher extent of plastic deformation. Thus the data in Table 1 shows that the lower the MW of chitosan (e.g. 8 kDa), the lower the extent of plastic deformation. On the contrary, the 100 kDa chitosan displays the highest
plastically deforming sample. In fact, there is a tendency for the lower MW chitosan samples to undergo brittle fracture upon compression. Such a conclusion is supported by the fact that lower MW chitosan samples showed higher intercept values (A) of the Heckel analysis implying that there is greater tendency of the lower MW chitosan to undergo fragmentation and to fill the die.

The results for the Kawakita analysis of the experimental data are shown in Table 2. It is clear from the data in Table 2 that the highest porosity (highest ‘a’ Kawakita parameter) was obtained for the highest MW chitosan, i.e. 100 kDa, whereas the lowest porosity (lowest ‘a’ Kawakita parameter) was obtained for the 8 kDa chitosan. The foregoing is agreement with the results of Fig. 3 whereby the extent of packing increases, and subsequently porosity is decreased, when lower MW chitosans are used. On the other hand, the $P_K$ values are lower for the higher MW chitosan samples indicating that they readily deform plastically under pressure. This MW-plasticity relationship is in agreement with the yield pressure obtained by Heckel analysis of compression data.

Optimal tablettng properties are generally attained for powders with maximum amount of plastic deformation and/or with minimum amount of elastic recovery [48,49]. The highest MW chitosan (100 kDa) showed the highest compacts tensile strength (Table 4) as its total amount of plastic deformation was the highest revealed by its lowest yield pressure (Table 1). However, despite their higher work of compression, the low MW chitosan samples exhibited less plastic behavior and therefore slightly lower tensile strength of the compacts than the 100 kDa chitosan. This result contradicts the findings by Picker-Freyer et al who investigated the slope of the Heckel plot as a function of
different MW chitosans [50]. They found that chitosan with a MW of 87.2 kDa behaves in a similar way to microcrystalline cellulose as a highly plastically deforming excipient. The other higher MW chitosans ((173.3 and 210.5 kDa) showed a lower Heckel slope and thus higher resistance against deformation. They suggested that the higher Heckel slope - thus a higher and easier deformation- of the 87.2 kDa chitosan are correlated to its low MW. In re-evaluating such behavior, the aforementioned judgment did not consider differences in the physical properties of the chitosans used. For example all their investigated MW chitosans differ in the degree of N-deacetylation, bulk density, and particle size. All these parameters affect the packing and extent of deformation upon compression. In addition, the determination of the Heckel slopes includes plastic and elastic deformation and therefore the contribution of the undetermined elastic recovery might have added up to the Heckel slope estimation [51-55]. The foregoing is justified in the current work as the high plastically deforming character of the 100 kDa chitosan presented the highest work of elastic recovery (Fig. 5). All the other lower MW chitosans (8, 13, 18, 30 kDa) had lower elastic recovery due to their lower extent of plastic deforming or to their brittle-fracture character. Such behavior is, however, valid up to a compression force of 2.94 kN (Fig. 5) which is almost equivalent to the linear portion range considered for the Heckel plots (Fig. 7). Higher than that, the elastic recovery work seems to be almost the same for all MW chitosans. It is suggested that, under high compression forces (>2.94 kN), the low MW chitosans deviate away from brittle-fracture deformation and start to exhibit plastically deforming character as their elastic recovery work becomes equivalent to the 100 kDa chitosan. Patel et al highlighted such behavior for dicalcium phosphate where they noted that as the pressure is increased, more and
more primary particles fragment to smaller particles, which have higher resistance against deformation due to the appearance of elastic character [56].

In summary, the recorded increase in the work for compression is due to appearance of brittle-fracture characteristics. The foregoing implies the appearance of new smaller granules that require extra work to compress and exhibit less elastic recovery work. The increased (A) values of the Heckel analysis for chitosan MW<100 kDa implies more dense packing due to high fragmentation which again justifies the brittle-fracture behavior upon compression.

Changes in the compacts tensile strength with increasing the compression pressure (Table 4) are interestingly in agreement with the deduced parameters using Leuenberger analysis of the compression data (Fig. 8/Table 3). For example, the high compression susceptibility parameters (γ) for the 18 and 13 kDa chitosans (Table 3) indicate that high tensile strengths are attained at lower pressure of compression compared to the other MW chitosans. The foregoing is in agreement with Table 4 which indicates that the 18 and the 13 kDa reach a higher tensile strength values at 2.94 MPa than the other MW chitosans. The 100, 30, and 8 kDa chitosans showed low γ values, thus higher compression pressures are required to produce stronger tablets. However, their σmax values (Table 3) are higher than that for the 13 and 8 kDa chitosans indicating stronger compacts at higher pressures. Table 4 confirms the foregoing finding as the highest tensile strengths are achieved by the 100, 30, and 8 kDa chitosans.

In order to rationalize differences in tablets strength with pressure for different MW chitosans, the powder elastic recovery and compacts ejection work have to be considered
hereafter. Initially, when the tensile strength of the compacts in the whole applied force range (Table 4) is examined for chitosan lower than 100 kDa, up to an applied force of 2.94 kN, almost all samples exhibited higher tensile strength than the 100 kDa chitosan. Increasing the applied force above 2.94 kN the tensile strengths for the same samples were lower than that for the 100 kDa. Examining the profiles of the elastic recovery work in Fig. 5 for forces lower and higher than 2.94 kN, shows that for chitosan < 100 kDa, the elastic recovery was much lower than the 100 kDa chitosan up to 2.94 kN. Above 2.94 kN, the elastic recovery work of the low MW chitosans started to approach the elastic recovery work value of the 100 kDa. This trend correlates well with the tensile strength trend obtained below and above 2.94 kN. In other words, compacts with lower tendency for elastic recovery work have higher tensile strength values. On the other hand, when the tablets ejection work is considered (Fig. 6), at the lowest compression force (0.98 kN), the compact of the 100 kDa chitosan displayed the highest ejection work indicating the highest frictional forces with the die wall. Such high forces may have further contributed to the lowest tensile strength of the 100 kDa at 0.98 kN. Higher compression forces render smoother ejection of the 100 kDa compacts and thus higher tensile strengths compared to the other MW chitosans. In contrast, lower MW chitosan manifest higher ejection work when the compression pressure is increased thus lower compacts tensile strength.

The high mechanical strength of the high MW chitosan has been previously reported and attributed to the entanglement network of the high MW chitosan [57]. The 100 kDa sample shows a β conformation whereby the individual chains are arranged in a parallel manner. Hence giving rise to the appearance of ordered structure and thereby to a semi-
Acharya et al. reported that lowering the MW of chitosan results in a decrease in crystallinity due to a larger $d$-spacing as a result of an increase in the dimensions of the unit cell. Such a change results in a weaker network of hydrogen-bonds between chitosan chains as evidenced by FT-IR experiments. The later technique indicated a change in the environment of primary -OH groups and thus in the overall hydrogen bonding network [59].

When lower chitosan particle size is used (Table 5), the reduced packing extent indicates that the powder bed is of less porous structure resulting in lower volume reduction upon compression of both MWs at 4.9 kN. Such reduced powder porous structure takes place concurrently with enhanced powder deformation resulting in higher work of compression for lower particle size of chitosan. The powders are found to undergo increased plastic deformation (Lower $P_Y$) when smaller particle size chitosan is used. Such increased plasticity, and thereby extent of particle deformation, results in the appearance of fresh surfaces for binding. Therefore more coherent and stronger compacts are produced when lower chitosan particle size is compressed. The lower $A$ Heckel parameter for lower particle size indicates the lower particle rearrangement and thereby explains the lower volume reduction obtained. Consequently, having less porous powders, more coherent compacts are produced with less frictional forces with the die wall as the work of tablet ejection is lower for lower particle size.

The molecular structure of the high MW chitosan has been previously shown to be of open helical orientation whereas the low MW chitosan has been shown to be of closed and highly packed helical complex [45]. Such finding was established when chitosan molecular modeling was carried out in water. In contrast, when chitosan molecular
modeling was carried out in vacuum, as in the current work, the high MW chitosan showed high extended and closely packed molecular structure. This result is reasonable with regard to the fact that high MW chitosan has more hydrogen bonds across parallel polymeric chains, and consequently, more stable as compared to low MW chitosan. Therefore, it can be postulated that low MW chitosan requires higher energy to yield compacted helical structures where molecules can be intermingled. For high MW chitosan of highly packed structure and, accordingly low d-spacing, requires lower energy to bind. This was evident in the current work (Fig. 9) using molecular modeling when calculating the binding energies between two polymeric species of two different MW chitosans (88 and 8 kDa). The binding energy of high MW chitosan (-2.12 kCal/mol per unit) is higher than that for the low MW chitosan (-1.56 kCal/mol per unit). Such high binding energy leads to a more rigid structure of the high MW chitosan. Consequently high MW chitosan requires less applied compression forces to bind and allows less energy (as measured herein from the force displacement curve) to get the two polymeric species closer. On the other hand, a greater compression force and energy are required to get the small helical strands closer for the low MW chitosan.

The powder compression and tablet properties dependence on chitosan MW will clearly impart variations in drug dissolution profiles when the MW is changed. For diclofenac sodium (Fig. 11) the relatively fast drug release for the 100 kDa chitosan tablets reflects appropriate application of such chitosan immediate release tablet preparations. On the contrary, the slow drug release for the 30 kDa and the 13 kDa chitosan tablets reflects suitability to be used in controlled release drug applications.
The high MW chitosan in its own is not generally used for sustained release applications; in contrast, it is used as an immediate disintegrating additive [60]. The reason behind that is due to the fact that high MW chitosan, if not modified, does not undergo gelling which represents a perquisite for sustained action to take place [61]. On the contrary, the high water uptake of chitosan makes it a good candidate for immediate release applications [60]. Thus, in order to prolong the release, chitosan is generally combined with anionic polymers which form swellable non-erodible gels that are capable of prolonging the drug release [62-65]. On the other hand, low MW chitosan, in its own, has not been investigated thoroughly and compared with higher MW chitosans in regards to drug release behavior. However, the low MW chitosan was noticed to cause prolonged release action on some drugs [66]. In the current investigation, tablet swelling, as a factor in hindering the drug release, is excluded in having a contribution since the drug dissolution media was not acidic (phosphate buffer pH 6.8 according to USP specifications) [67]. Thus the apparent slow drug release using low MW chitosans is suggested to be attributed to other factors such as the slow erosion rate. On the other hand, both modes of drug release, i.e. immediate and sustained, are preliminary attributed to the behavior of packing extent in response to increasing the MW. Such behavior is valid irrespective of the absence (Fig. 3) or the presence (Fig. 12) of diclofenac Na with chitosan whereby lower MW chitosans induce higher packing extents. The net result, therefore, conclusively demonstrates the suitability of chitosan to be used in immediate and sustained release applications for the high and low MW chitosan powders respectively.
5. CONCLUSIONS

The change from high to low MW chitosan brings about changes in the mechanical properties. Above specific compression force limits, low MW (≤30 kDa) chitosans manifest higher levels of: fragmentation and brittle-fracture tendency, compression work, packing, and, to some extent, compacts ejection work, relative to the 100 kDa chitosan. All of the aforementioned changes depend on two main factors; i.e. the extent of compression force applied and the chitosan particle size. In the first factor, Leuenberger analysis revealed that stronger compacts of high MW chitosans (≥30 kDa) are reached slower, upon increasing the compression forces, than the other lower MW chitosans. The foregoing has been attributed to the increasing elastic recovery and ejection work of the low MW chitosans upon increasing the compression pressure thus contributing to weaker compacts. In the second factor, lowering the chitosan particle size, for the highest (100 kDa) and the lowest (8 kDa) MWs, reduces the particle rearrangement and volume reduction upon compression resulting in less porous powders and more coherent compacts. Modeling high MW chitosan interacting polymeric pairs appears more extended and shows more extensive hydrogen bonding parallel chains leading to more compact and stable structure than the low MW chitosan. The aforementioned results explain the low energy required to compress the high MW chitosan as indicated by the force displacement curves. Differences in compression properties may further correlate to the immediate release profile of diclofenac with the 100 kDa chitosan and the sustained release profile with the 30 and 8 kDa chitosans upon dissolution of the drug.
CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

REFERENCES


Captions:

Tables

Table 1: Heckel parameters; yield pressure ($P_Y$) and the intercept ($A$), of different MW chitosan powders upon compression using the GTP.

Table 2: Kawakita parameters; compressibility ($a$) and $P_K$, of different MW of chitosan powder upon compression using the GTP.

Table 3: Compression analysis using Leuenberger equation of different MW chitosans compressed using the GTP. The tensile strength and density of the 6 mm tablets were calculated from their hardness and tablet thickness values.

Table 4: Tensile strengths (MPa) of the different MW chitosan samples compressed at a force range 0.98-4.91 kN (equivalent to 100-500 kg load applied by the GTP).

Table 5: Compression and tablet properties of different particle size chitosans for the 100 kDa and the 8 kDa MWs.

Figures

Fig. 1.: A typical force-displacement curve displayed by the GTP for the compression of 100 kDa chitosan (100 mg sample) at 100 kg (4.91 kN) compression force.

Fig. 2: A typical force-displacement curve displayed by the GTP for the tablet ejection compressed at 100 kg (4.91 kN) compression force for the 100 kDa chitosan (100 mg sample).

Fig. 3.: Extent of powder packing after compression relative to the initial bed volume for each chitosan MW at different compression forces.
Fig. 4.: Compression work of different MW chitosan powders as a function of compression force.

Fig. 5.: Elastic recovery work of different MW chitosan powders as a function of compression force.

Fig. 6.: Tablet ejection work of different MW chitosan powders as a function of compression force.

Fig. 7.: Heckel plots of different MW chitosan samples compressed at a pressure range 34.6-173.2 MPa.

Fig. 8.: The tensile strength versus the product of the pressure of compression and the relative density of different MW chitosan powders. The powders were compressed using the GTP.

Fig. 9.: Chemical structure of D-Glucosamine (A), high MW (B) and low MW (C) chitosans.

Fig. 10.: A section of the high MW/more extended (A) and low MW/less extended (B) chitosans showing extensive and less extensive intermolecular hydrogen bonds.

Fig. 11.: Dissolution of diclofenac sodium tablets containing different MW chitosan powders; 100 kDa, 30 kDa and 13 kDa chitosans.

Fig. 12.: Extent of powder packing of diclofenac/chitosan preparations after compression relative to the initial bed volume for each chitosan MW at different compression forces.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9
Figure 10
Figure 11
Figure 12
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Table 5

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*AUC: area under the curve which is equal to work (x 10^{-2} J)
Graphical abstract

A

B

C

Chemical structure of D-Glucosamine (A), high MW (B) and low MW (C) chitosans.
Highlights

- Tensile strength of chitosan compacts is associated with chitosan molecular weight.
- Low chitosan compacts tensile strength is attributed to high elastic recovery.
- High MW chitosan has a more stable structure than the low MW chitosan.
- High MW chitosan provides immediate release properties in solid form preparations.
- Low MW chitosan provides sustained release properties.