Contents lists available at ScienceDirect





Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Dissolution of chitosan nanocrystals in aqueous media of different acidity. Molecular dynamic study



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ARTICLE INFO	A B S T R A C T
Keywords:	The process of dissolution of chitosan nanocrystals with molecular mass of polymer up to 12.8 kDa in aqueous
Chitosan dissolution	media of various pH was studied by molecular dynamic simulations with the use of the improved force field
Molecular dynamics	GROMOS 56A _{CARBO, CHT} specially developed for the chitosan polymers description. The effect of the media
Polymer structure	acidity and polymer molecular weight on the dissolution process kinetics has been studied and the regression
	expressions for kinetic parameters were established. The calculated solution viscosity, Mark-Houwink-Sakurada
	equation parameters, and pH values of the dissolution beginning are in good agreement with the available
	experimental data. The uniform/non-uniform distribution of protonated amino groups and hydrogen bonds
	along the polymeric chains is found to be of key importance parameter for the dissolution process which can be
	considered as a criterion of dissolution ability.

1. Introduction

The derivatives of amine-substituted polyglycans, in particular chitosan (poly-1,4-(N-acetyl)-β-D-glucopyranosamin-2), are currently considered promising agents for encapsulating drugs and transportating them inside the human body (Aydın & Pulat, 2012; Ghaz-jahanian, Abbaspour-Aghdam, Anarjan, Berenjian, & Jafarizadeh, 2014; Li, Ma, Dang, Liang, & Chen, 2014; Patel, Patel, & Patel, 2010; Riva et al., 2011). These are convenient, non-toxic and easily removable agents that interact with biological structures (cell membranes, walls, receptors, etc.). The last property is especially important for the development of modern medicinal systems with active targeting (Ghaz-jahanian et al., 2014; Li et al., 2014). However, the practical application of these compounds is complicated by the lack of data on the structure and the possibility of the association of their chains or globules in aqueous solutions, as well as on the thermodynamic properties and kinetic parameters of complexation in aqueous media. In particular, the interpretation of the structure of the polymer chain in solution on the basis of electron microscopy data was the subject of discussion (Li, Song, Yang, & Fan, 2006; Pedroni Viviana, Gschaider María, & Schulz Pablo, 2003; Pedroni, Schulz, Gschaider, & Andreucetti, 2003). Data on the stability constants of chitosan derivatives interacting with various protein agents are practically absent, and the dissolution process and its kinetics were mainly studied using experimental methods based on the formal kinetic approach.

Chitosan is a mixture of polymers with varying degrees of acetylation, molecular weight and the arrangement of acetyl groups in the polymer chain, and its aqueous solutions are characterized by the additional protonation of free amino groups. Therefore, experimental research can often provide only apparent or averaged physicochemical characteristics. In this respect, the method of molecular dynamics (MD) applied to the processes of chitosan dissolution and its complex formation ensures the determination of true microscopic physicochemical constants. Furthermore, molecular dynamics modeling allows us to investigate in details the patterns of changes in these constants with a wide variation of the structural characteristics of the polymer including the number, type and charge of substituents.

Previously, several studies on the structure and association of chitosan in aqueous solutions have been conducted. Skovstup et al. (Skovstrup, Hansen, Skrydstrup, & Schiøtt, 2010) investigated flexibility of glycosidic linkage in chitin/chitosan oligomers using molecular mechanics and Monte Carlo methods with implicit water. It was found that the flexibility of the glycosidic linkage is determined by the nature of the sugar ring at the reducing end, and the presence of an Nacetylated ring on it provides increased flexibility of the molecule. Franca et al. (Franca, Lins, Freitas, & Straatsma, 2008) studied structure of single chains of chitosan and nanoparticle of chitin in water using molecular dynamics with explicit water. It was shown that solubility in chitosan is controlled mainly by electrostatic interactions. Authors found a relationship between acidity of media, intrachain H-bonds and

https://doi.org/10.1016/j.carbpol.2018.12.019

Received 5 October 2018; Received in revised form 18 November 2018; Accepted 9 December 2018 Available online 11 December 2018 0144-8617/ © 2018 Elsevier Ltd. All rights reserved.

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solubility of polymers. Another research (Franca Eduardo, Freitas Luiz, & Lins Roberto, 2011) was focused on flexibility of chitosan of different degrees of acetylation (*DA*) and influence of *DA* on the structure and solubility of polymers in water. The relationship between flexibility of alpha-chitosan crystals, *DA* and alignment of acetyl groups was also demonstrated in (Yu & Lau, 2017). According to molecular dynamics modeling, flexibility increases as the number of acetylated monomers becomes fewer, with the exception of some cases of the distribution of acetyl groups.

One of the most recent research (Esteban et al., 2018) is devoted to conformational properties of chitosan and chitlac (1-deoxy-lactit-1-yl chitosan derivative). Authors observed the influence of various factors (temperature, ionic strength, ionization, degree of substitution) to glycosidic angles, H-bonding and helicity of chtilac 10-monomeric oligomers. The chitosan association has been studied with the coarse graining (CG) models in (Pigaleva et al., 2014). It was established that chitosan macromolecules self-organize into elongated rod-like structures. However, the CG models behavior is frequently dependent on the system parameters varying in the process (i.e. density or concentrations). Thus, the results of such a modeling should be proved with independent quantum chemical, MD, or experimental data. The above studies were focused on establishing the structure of the polymer in aqueous media, but not directly on the dissolution process. In particular, the numerical estimates of dissolution kinetic constants and influence of the acidity of the medium on them were not reported and the effect of polymer characteristics including molecular weight and the distribution of protonated groups in the polymer chain were not considered previously. At the same time, such estimates and patterns of their changes are extremely important for biomedical, pharmaceutical and cosmetological industries, where the key processes for obtaining or using the final product are the association, dissociation and co-association of chitosan molecules with peptides, nucleic acids or nanoparticles. From this point of view, the dissolution process of pure chitosan nanocrystals is one of the simplest and fundamental cases of such processes.

In this article, we study the process of dissolution of chitosan nanocrystals using molecular dynamic simulation of oligomeric chitosan nanocrystals in an aqueous medium of various acidity under conditions close to standard ones. For this purpose, we use a modified version of new force field 56A_{CARBO} which was specially developed for description of aminoglycane polymers. Earlier, we demonstrated that the modified force field GROMOS 56A_{CARBO CHT} (Naumov & Ignatov, 2017) gives very reliable results on chitosan conformations in the aqueous solutions of various acidity and allows proper description of the thermodynamic properties of these polymers in a solution. Using this improved force field, we investigate the effect of molecular weight, protonation degree, and the charge distributions in the polymer chains on the dissolution kinetics and make the direct comparison with the observed characteristics as well as with the dissolution kinetic equations derived earlier both from the experimental and theoretical studies. The main goal of the study is determination of the kinetic constants of dissolution depending on the structural characteristics of polymers and environmental conditions. Additionally, we formulate the criteria of nanocrystal dissolution useful for the prediction of association/dissociation properties of chitosan of various molecular weights under different physicochemical conditions.

2. Calculation details

The nanocrystal consisted of 18 polymer chains surrounded with water in rectangular box was used as a model system. The polymer chains of different length were considered to estimate the influence of molecular weight (M_w) on dissolution kinetics. The molecular weights of single polymeric chains were of 1.6, 3.2, 6.4, 9.6 μ 12.8 kDa (10, 20, 40, 60 and 80 monomeric units). All polymeric chains of the crystal had the same length. Due to the difference in the kinetics of dissolution, we

will distinguish systems consisting of polymers with a molecular weight of 1.6 and 3.2 kDa (hereinafter referred to as models A) and systems consisting of polymers with a molecular weight of 6.4, 9.6, and 12.8 kDa (referred to as models B).

Chitosan has several crystal modifications (Ogawa, Yui, & Okuyama, 2004; Okuyama, Noguchi, Miyazawa, Yui, & Ogawa, 1997; Yui et al., 1994). In our calculations we used crystal structure of anhydrous chitosan determined by combined X-ray diffraction analysis and stereochemical model refinement (Yui et al., 1994). The snapshots of initial structures are presented in Figures S1 and S2 of Supplementary information. The choice is based on assumption that the crystal has a developed system of H-bonds which keeps polymeric chains together. H-bonds in amorphous chitosan are not so ordered and aligned, that leads to less resistance to dissolution. The crystal model proposes avoiding strong influence of reptation to dissolution process to make an analysis simpler. All chitosan amino-groups were considered as completely deacetylated (DA = 0%).

Crystals were put in rectangular periodic box filled with water molecules (SPC-model). Box sizes were from 20.8x12.4x11.7 to 41.7x12.4x11.7 nm depending on polymer length. The GROMACS 5.1 (Abraham et al., 2015) with $56A_{CARBO_{CHT}}$ force field (Naumov & Ignatov, 2017) were used for the calculations. PLUMED plugin (Bonomi et al., 2009) was used in analysis of output trajectories. Energy minimization and 100 ps equilibration dynamics were made before the production runs. NPT-ensemble with reference pressure 1 bar maintained by Berendsen barostat (characteristic time of 2.0 ps) was used in equilibration calculations. Parameters of production MD calculations were as follows: NVT-ensemble with the temperature of 300K controlled by the Berendsen thermostat with characteristic time of 0.1 ps, time of modeling from 40 to 240 ns, integration time step of 1 fs. The PME-type method was used for accounting the electrostatic interactions, and LINCS algorithm was used for geometry constraints.

The acidity of the aqueous media was modeled using the different protonation degree (*PD*) of chitosan chain, i.e. the ratio of monomeric units with linked NH_3^+ groups in a polymeric chain:

$$PD = \frac{N_p}{N}$$

Here, N_p is the number of monomeric units with protonated aminogroups; N – total number of monomeric units in a polymeric chain. Protonation degree and media acidity are connected by the Henderson–Hasselbalch equation:

$$pH = pK_{1/2} - n\log\left(\frac{PD}{1 - PD}\right)$$

where $pK_{1/2}$ and *n* are constants dependent on polymer properties and obtained from experimental data (Q. Z. Wang et al., 2006). Due to the direct connection between pH and PD, both these characteristics can be used for the description of medium acidity. However, PD value is directly connected to the structure of polymer (controlled by us) whereas pH value, although more convenient for chemists, requires additional constant $pK_{1/2}$ for its determination. The values of this constant are different for various experimental conditions and chitosan samples. Thus, in the following, we use PD as a main characteristic of protonation. pH values calculated for some $pK_{1/2}$ and *n* are given in Table S1 of Supplementary information.

Protonation degrees of 0, 30, 50, 70 and 90% were modelled for crystals consisted of polymers with molecular weights of 3.2, 6.4 and 9.6 kDa. Simulations at PD = 90% are impossible for chitosan with molecular weight 1.6 kDa (10 monomeric units) because terminal units cannot be protonated due to the force field restrictions. Therefore, maximum *PD* of this system was 80%. The modeling of the polymers with $M_w = 12.8$ kDa implies the large simulation box size with about 340,000 atoms. Due to the long simulation time in such a large system, the calculations for this system were carried out only with PD = 0, 50, and 90%. In all the cases, the protonated monomeric units were

randomly distributed in a whole crystal. The corresponding number of water molecules was replaced with chlorine anions to maintain electric neutrality.

3. Results and discussion

Visual inspection of modeled systems after 40–150 ns of MD simulation shows that dissolution does not take place in the case of absence of protonation (PD = 0%) for all molecular weights and for low protonation (PD = 30%) of models B. The spiral lengthwise twisting of both whole crystals and each separate chain occurs immediately after the start (typically within 1–5 ns) of calculation. This kind of deformations is typical for cellulose crystals in water (Paavilainen, Róg, & Vattulainen, 2011) and recently were reported for chitosans of different *DA* (Yu & Lau, 2017). Twisting behavior of unprotonated chitosan crystal is presented in Figure S3 of Supplementary information.

If the protonation degree is large enough ($PD \ge 50\%$), dissolution proceeds with different speed in dependence on molecular weight as well as on the number and distribution of protonated groups. The dissolution process starts from the swelling of nanocrystals when solvent molecules and chlorine ions penetrate to crystal structure. Then, during 10-50 ns of modeling, the separation of polymer chains or small groups of chains from the outer layer of crystal occurs. Sometimes (see below), the splitting of nanocrystals to smaller fragments takes place. Separated molecules (or aggregates of molecules) diffuse into solvent. In dissolved systems (except the cases of high protonation degree), the aggregates consisted of several polymer molecules are present. The general picture of dissolution is illustrated by Fig. 1 for the 40-monomer polymer with PD = 50%. Details of system evolution during MD simulations are presented in Figures S4-S7 of Supplementary information. As a whole, this picture is in agreement with the data reported by (Thevarajah, Bulanadi, Wagner, Gaborieau, & Castignolles, 2016).

In models A at high protonation degrees (80 and 90% respectively for 10- and 20-monomer chains), crystal body splitting occurs. Crystal breaks into few small particles in first hundreds of picoseconds and these particles further separate into individual molecules. In case of "big models" and high degrees of protonation the first stage of dissolution looks much more like swelling neither than splitting.

It is obvious that the main physical effects determining the dissolution kinetics rate are the balance between the number of H-bonds and tight van der Waals contacts, the number of protonated groups as well as their linear density, and the typical diffusion coefficient of a system connected with the polymer length. Steric hindrances induced by the interlacement of polymeric chains in an amorphous polymer are a frequently discussed effect reducing the dissolution rate. However, for the dissolution of ordered nanocrystals, this effect cannot be determining, and the dissolution of amorphous and crystalline polymers should be described by the different kinetic mechanisms. It is also wellknown that the crystalline fragments of chitosan are frequently incorporated into the amorphous matrix and the dissolution of these fragments occurs slower than the dissolution of the remaining matrix (Lu, Song, Cao, Chen, & Yao, 2004; Sogias Ioannis, Khutoryanskiy Vitaliy, & Williams Adrian, 2009). Below, we consider all the mentioned physical effects separately.

3.1. Viscosity of solution

Viscosity of liquid systems are extremely sensitive to interatomic potentials and inter/intramolecular interaction models used for the description of a liquid dynamics. Thus, this gives very sensitive tools for the verification of the proper description of chitosan solutions. It can also be a criterion for the description of solubility of large systems at higher concentrations because the direct MD modeling of such systems requires extremely long evolution times. Instead, the viscosity can describe the system behavior under the gel-like state conditions using relatively short-time simulations.

Viscosities of pure water as a reference system and the chitosan solutions were calculated using the transverse-current autocorrelation-functions method (Palmer, 1994). This gives shear (dynamic) viscosity (η) which can be easily converted to relative (η_{rel}), specific (η_{sp}) and intrinsic viscosity ([η]) by equations:

$$\eta_{rel} = \frac{\eta}{\eta_0}$$
$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \eta_{rel} - 1$$
$$[\eta] = \frac{2 \cdot (\eta_{sp} - \ln(\eta_{sp}))^0}{C}$$

where η_0 is dynamic viscosity of pure solvent (water) and *C* is the massconcentration of polymer in a solution. The last formula was obtained by Solomon and Ciută (Solomon & Ciută, 1962) for low-concentrated polymer solutions. In the case of our calculations, this has only restricted applicability because of high concentrations of chitosan in modelling cell.

The intrinsic viscosity dependence on the polymer molecular weight was described according to the empirical Mark–Houwink–Sakurada (MHS) equation:

$$[\eta] = KM_w^\alpha$$

where *K* and α are viscosimetric constants. Earlier, several experimental studies were carried out to estimate the viscosimetric constants of chitosan (Anthonsen, Vårum, & Smidsrød, 1993; Berkovich, Timofeyeva, Tsyurupa, & Davankov, 1980; Gamzazade et al., 1985;



Fig. 1. Process of chitosan dissolution. Crystal of 18 chitosan chains (each chain consisted of 40 monomeric units), PD = 50%. Red – protonated monomeric units; Cyan, Gray and Tan – unprotonated monomeric units; Gray and Tan – chain-side monomeric units; Blue Bubbles – chloride anions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1

The estimated viscosity and the viscosimetric constants in comparison with the available experimental data.

PD, %	C, g/dL	M_w ·10 ³ , Da	η, cP	[η], dL/g	<i>K</i> ·10 ⁵ , dL/g	α
water	_	_	0.444	_	_	_
0	5.63	1.6	0.514	2.664	-	-
	7.75	3.2	0.596	3.984		
	9.60	6.4	0.719	5.450		
	10.47	9.6	0.777	5.881		
	10.87	12.8	0.800	6.004		
30	5.63	1.6	0.550	3.921	-	-
	7.75	3.2	0.658	5.421		
	9.60	6.4	0.769	6.298		
	10.47	9.6	0.838	6.774		
50	5.63	1.6	0.562	4.342	613	0.27
	7.75	3.2	0.667	5.631		
	9.60	6.4	0.811	6.972		
	10.47	9.6	0.884	7.426		
	10.87	12.8	0.911	7.506		
70	5.63	1.6	0.569	4.601	626	0.27
	7.75	3.2	0.679	5.887		
	9.60	6.4	0.822	7.148		
	10.47	9.6	0.884	7.425		
80	5.63	1.6	0.573	4.726	1080	0.21
90	7.75	3.2	0.691	6.145		
	9.60	6.4	0.826	7.203		
	10.47	9.6	0.858	7.053		
	10.87	12.8	0.905	7.419		
Experiment	al data	2			-	
Ref		<i>M_w</i> ·10 ³ , Da	DA, %	[η], dL/g	<i>K</i> ·10 ⁵ , dL/g	α
(Berkovich	et al., 1980)	1.6 – 15	≤ 10	3.4 – 7.0	1115	0.147
(Anthonsen	et al.,	15 – 310	0	1.46 –	559.0	0.58
1993)				9.31		
(Anthonsen 1993)	et al.,	35 – 245	15	1.46 – 9.31	58.5	0.78
(Gamzazado	e et al.,	61 – 150	15 ± 3	1.07 – 3.50	13.8	0.85
(Rinaudo et	tal 1993)	100 - 600	2	7.50	82.0	0.76
(Rinaudo et	t al. 1993)	100 - 600	11.5	9.80	76.0	0.76
(Wang et al		194 - 937	0	4.60 -	16.8	0.81
()	,,			15.30		
(Wang et al	l., 1991)	211 - 1260	9	2.90 -	6.59	0.88
				14.60		
(Wang et al	l., 1991)	536 - 1850	16	3.30 – 12.00	1.42	0.96

Rinaudo, Milas, & Dung, 1993; Wang, Bo, Li, & Qin, 1991) resulting in the values varying in rather broad ranges of 1.42-1115 for *K* and 0.147 - 0.96 for α . It was shown that these constants are dependent on acidity of media, ionic strength and deacetylation degree (Kasaai, 2007).

We have calculated *K* and α for the systems where dissolution occurred during the calculation time (70–240 ns). The obtained estimates of viscosity and viscosimetric constants are presented in Table 1 in comparison with the most relevant experimental estimates.

As was reported in ref. (Palmer, 1994), the method of shear viscosity calculation used here typically underestimates the viscosity values of water by about 2 times relatively the experimental value 0.8903 cP (Korson, Drost-Hansen, & Millero, 1969). We assume that the same underestimation takes place for chitosan solution. The calculation of intrinsic viscosity by Solomon-Ciută formula uses relative viscosity. Therefore, underestimation of shear viscosity doesn't influence to values of intrinsic viscosity and MHS parameters. Values of MHS equation parameters are in good agreement with the experimental data of (Anthonsen et al., 1993; Berkovich et al., 1980) but deviate significantly from the results of (Anthonsen et al., 1993; Gamzazade et al., 1985; Rinaudo et al., 1993; Wang et al., 1991). To our opinion, this deviation is due to the difference in the molecular mass and degree of acetylation of chitosan. The results obtained for the low mass chitosan samples with DA lower than 10% are in good agreement with our results. Whereas the data for M_w greater than 30 kDa and DA greater than 10% leads to significant deviations. Thus, the differences in the

viscosity parameters observed experimentally are first of all due to the differences in polymer mass and degree of acetylation.

3.2. Diffusion constant, acidity of media and molecular weight

Mean square displacement of polymer molecule centers of masses (MSD_{CM}) averaged over all polymer chains with the subtracted center of mass displacement for the whole system was used to evaluate the rate and kinetic parameters of dissolution. It was found in a series of test calculations that the mean square displacement of selected atoms of monomers or monomer centers of masses is not applicable for the description of dissolution process, because it is influenced by changing of polymer shape. Its time dependence demonstrates unpredictable oscillations in the case of elongated polymers. At the same time, the time dependence MSD_{CM} value is much more monotonous. Therefore, this quantity will be used in the following for evaluation of kinetic parameters.

Time dependences of MSD_{CM} for various protonation degrees values and molecular weights of polymers are shown in Fig. 2.

Small rising of MSD_{CM} in the cases when dissolution does not occur are the result of spiral twisting of molecules which leads to displacement of centers of masses from the initial position. The small slope of the graph shows that the crystal does not swell in the media and only small twisting and bending take place slightly increasing the MSD_{CM} value.

The centers of masses diffusion coefficients of polymer molecules (D_{CM}) obtained as the first derivative of MSD_{CM} by time are presented in Table 2. The full parameters of linear fitting MSD_{CM} vs time are in Table S2 of Supplementary materials.

Because the diffusion coefficient increases with PD and decreases with M_w , we fitted the data from Table 2 using the power-law regression model:

$$D_{CM} = a \cdot PD^k + b \cdot \frac{1}{M_w^l} + c$$

where *a*, *b*, *c*, *k*, *l* are the regression coefficients. In the case of PD = 0%, the dissolution does not occur. In the case of PD = 30%, dissolution occurs only for small models. Therefore, we used three different data sets to obtain the values of *a*, *b* and *c*: (1) full data, (2) data with excluded D_{CM} values of PD = 0% cases, (3) data only for the systems where dissolution occurs. However, for all the data sets, *k* and *l* values are quite close to 1 whereas some data fits are not completely robust due to the lack of data values. Therefore, the diffusion coefficient D_{CM} were fitted on the basis of essentially linear model relatively to *PD* and $1/M_w$ of polymers:

$$D_{CM} = a \cdot PD + b \cdot \frac{1}{M_w} + c \tag{1}$$

In the limit of very long polymer chains, this equation is getting to be purely linear:

$$D_{CM} \approx a \cdot PD + c; \ (M_w \to \infty).$$

From the Eq. (1) the protonation degree of the dissolution beginning (PD_{start}) could be found and the acidity of dissolution beginning (pH_{start}) also could be obtained using the Henderson–Hasselbalch equation:

$$D_{CM} = 0$$

$$PD_{start} = \frac{-c}{a}$$

$$pH_{start} = pK_{1/2} - n \log\left(\frac{PD_{start}}{1 - PD_{start}}\right)$$

The value of molecular weight of polymer ($M_{w,start}$) under which any protonation will initialize the dissolution process is

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Fig. 2. *MSD_{CM}* vs simulation time up to 90 ns. The full traces are presented in Figure S8 of Supplementary information. Lines are named according to the number of monomeric units (m) and protonation degree (p).

Table 2

Average diffusion coefficients of chitosan molecules of estimated from the time dependencies of *MSD_{CM}*.

M _W , kDa	D_{CM} , $10^{10} \mathrm{m}^2/\mathrm{s}$					
	PD = 0 %	PD = 30 %	PD = 50 %	PD = 70 %	PD = 90 %	
1.6	0.0224 ^a	1.2346	1.8333	1.9833	2.6833 ^b	
3.2	0.0767 ^a	0.3455	1.0124	0.8643	1.2675	
6.4	0.0141 ^a	0.0188^{a}	0.4006	0.8822	1.1217	
9.6	0.0159 ^a	0.0320^{a}	0.1635	0.3458	0.8671	
12.8	0.0171^{a}	-	0.1226	-	0.6227	

^a dissolution doesn't happen.

^b PD = 80%.

$$M_{w, start} = \frac{b}{-c}; \ (D_{CM} = 0; PD = 0)$$

The fitted values of coefficients *a*, *b* and *c*, adjusted determination coefficients, the values of PD_{start} pH_{start} and $M_{w,start}$ are given in Table 3. pH_{start} values were calculated using two different experimental values of $pK_{1/2}$ and *n* in the Henderson–Hasselbalch equation: (a) for chitosan with high deacetylation degree 94%, (b) for chitosan with relatively low molecular weight 60 kDa (Wang et al., 2006).

Fig. 3 shows the dependence D_{CM} from *PD* and M_w for full data set from Table 3. The obtained regression model allows direct comparison of diffusion coefficients obtained by MD simulations with the experimental data of pH_{start} . Namely, the experimental values of pH_{start} reported in (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Pillai, Paul, & Sharma, 2009; Qin et al., 2006) (*pH* 6.0–6.5) are in good

Table 3

Regression coefficients of Eq. (1) a, b, c, the adjusted determination coefficient of regression, and the starting values of dissolution process.

data set	а	b	с	Adj.RSq	PD _{start} ,%	pH_{start}^{a}	pH_{start}^{b}	<i>M_{w,start}</i> , kDa
Full PD = 0% excluded Dissolution only Experiment ^c	0.0145 0.0171 0.0183	2.3734 3.0466 3.1331	- 0.6308 - 0.9838 - 1.1068	0.807 0.925 0.914	43.5 57.5 60.5	6.3 6.0 5.9 6.0 - 6.5	6.5 6.2 6.2	3.8 3.1 2.8

^a $pK_{1/2} = 6.17$, n = 1.20 (highly deacetylated chitosan).

 $p_{1/2} = 6.39$, n = 1.09 (chitosan with relatively low molecular weight).

^c (Kumar et al., 2004; Pillai et al., 2009; Qin et al., 2006).



Fig. 3. Surface of dependence D_{CM} from *PD* and M_w for full data set from Table 3.

agreement with the values obtained with Eq. (1).

3.3. Number of H-bonds and charge density

The changes in the number of H-bonds between chitosan chains are determined on the basis of geometry criteria as implemented in the GROMACS program are shown in Fig. 4. Cut-off radius and cut-off angle for detecting H-bond were 0.35 nm and 30°, respectively. The H-bonds are relatively short-ranged interactions and the number of H-bonds should decrease during the dissolution. However, as is shown in Figure, the number of H-bonds does not decrease in the systems without protonation. Moreover, the number of H-bonds between chitosan molecules in such systems is getting even higher. This is due to the fact of twisting of the nanocrystals in an aqueous media described above. Thus, the number and/or the energy of H-bonds per unit length of a polymer can be used as a numerical characteristic of chitosan dissolution.

As is evident from Fig. 4, values about 0.3 H-bonds per monomeric unit correspond to completely dissolved systems, H-bonds are mainly intramolecular (excepted short-time occasional intermolecular bonds between unprotonated monomeric units). 0.7 H-bonds per monomeric unit is the edge of dissolution. The area between 0.3 and 0.7 H-bonds per monomeric unit represents chitosan solutions with different degrees of polymer aggregation. The area above 0.7 corresponds to different structures of undissolved chitosan.

3.4. Influence of length of unprotonated areas

The data in Table 2 and 3 shows that models A dissolve even at low protonation which is also in agreement with experimental observations (Kubota, Tatsumoto, Sano, & Toya, 2000; Qin et al., 2006). The visual analysis of trajectories also reveals that the polymer chains remain

connected for a long time if they have significantly long regions which are not protonated. These regions play a role of links providing very long resistance to dissolution even after the remaining parts of polymers are dissociated. Thus, we conclude that the criterion of dissolution is not only the relations between the number of H-bonds (van der Waals contacts) binding the polymer chains but also the distribution of the protonated group along the polymer chain. In order to quantify this effect, we introduce the value of length fraction of unprotonated regions:

$$P_L = 1 - \frac{1}{N_{np}} \sum_{i=1}^L n_i$$

Here, L – number of monomeric units in an unprotonated region, N_{np} – total number of unprotonated regions in a whole polymer, n_i – number of regions with a length of *i* monomeric units.

Distribution of lengths of unprotonated regions for PD = 30% (dissolution happens only in "small models") and PD = 50% (all crystals dissolve) are shown in Fig. 5.

Fig. 6 demonstrates the dependence of P_L (in percent) of unprotonated regions longer than the length L for polymers with PD = 30% and longest polymers with PD = 50% (these two kinds of systems are close to the "dissolution boundary", the remaining ones are either readily soluble or strongly insoluble). The soluble systems in Fig. 6 are designated with dashed lines and the systems which do not dissolve during 100 ns of modeling are designated with solid lines. As is evident from the Figure, the most notable feature of insoluble systems is the "tail" corresponding to the long unprotonated regions with the L values up to 18 and 19. Thus, the easily soluble systems have only short unprotonated regions (L < 10) whereas the polymers with essentially longer unprotonated regions (L > 10) dissolve hardly. It is also interesting to note that the left part of graphs in Fig. 6, although is different for the soluble and insoluble polymers, does not allow distinguish between them. Namely, the soluble system ($M_w = 20$, PD = 30) is closer to the bunch of curves characterizing the insoluble systems than to the soluble ones.

Thus, the distribution of unprotonated regions of polymers can give a criterion of their solubility and, probably, the ability for an association. This fact can give a hint for the chemical modifications of chitosan to make it soluble or insoluble at the prescribed acidity which is necessary for the technological applications.

4. Conclusions

In the current study, we carried out the molecular dynamic simulation of the chitosan nanocrystal dissolution using the new improved modification of the $56A_{CARBO}$ force field specially designed for the description of the polyglicane chain dynamics and its behavior in the liquid media. We analyzed the influence of the molecular weight and the protonation degree of the chitosan chains on the kinetics and molecular mechanisms of the dissolution process as well as the viscosity of the gel state solution. The last parameter is directly related to the pH value of a aqueous solution. We found that the dependence of viscosity of the chitosan solution is in good agreement with the experimental data



Fig. 4. The number of H-bonds between chitosan chains vs simulation time. Lines are named according to number of monomeric units (m) and protonation degree (p).



Fig. 5. Distribution of lengths of unprotonated regions. Bar charts are named according to number of monomeric units (m) and protonation degree (p). Bar charts are shaded for insoluble models.



Fig. 6. Dependence of P_L of unprotonated regions longer than the length *L* for polymers. Lines are named according to number of monomeric units (m) and protonation degree (p). Solid lines are for insoluble models, dashed lines are for soluble models.

obtained for the oligomeric chitosan ($M_w < 30$ kDa) whereas the experimental viscosity parameters for the heavy polymeric fractions are significantly deviate from the calculated results. This demonstrates that the experimental differences in the viscosity parameters which is frequently discussed (Kasaai, 2007) is dependent mainly on the molecular-mass distribution, not on the experimental details or features.

During the dissolution, we established the clear linear dependence of the dissolution rate on the protonation degree of the chitosan chains which is directly connected to the media pH and on reverse molecular mass of the chitosan. These dependencies are in good agreement with the observed value of the pH value of the dissolution beginning which is reported in many experimental studies. This also demonstrates the validity of the force field modification proposed earlier (Naumov & Ignatov, 2017).

The detailed analysis of the chitosan dissolution process demonstrates that the main barrier impeding the crystal dissociation to the separate chains is the formation of continuous deprotonated regions of polymeric chains. These regions provide strong binding between the chains due to the formation of several hydrogen bonds and van der Waals contacts which are stronger than the Coulombic repulsion of the charged groups of dissociated polymer regions. For our models, the limiting number of the monomeric units providing the strong binding between chains and preventing dissociation for a long time was 10. The presence of such regions in polymers can be a criterion of bad solubility of the crystals.

In our study, we do not discuss the question of reptation of chitosan chains and, thus, the results are related mainly to the polymer crystals or to the crystallite regions of the partially crystalized polymers. It is, however, well-known, that the crystallite regions of partially crystalized polymers are the regions providing the slowest rate of dissolution. Thus, the results obtained here can be an upper boundary of the dissolution parameters if applied to the amorphous or glassy chitosans with the comparable molecular mass and deacetylation degree.

Acknowledgments

Study was supported by RFBR (projects № 18-33-00721, №-17-03-00912). Molecular dynamic calculations were performed on the supercomputer "Lobachevsky" of the University of Nizhny Novgorod.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

online version, at doi:https://doi.org/10.1016/j.carbpol.2018.12.019.

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