

CHITOSAN DICHLOROACETIC ACID SALTS

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Abstract

The salts of dichloroacetic acid were obtained from chitosans of different deacetylation degrees (69–97%) and viscosity-average molecular weights (61–327 kDa). The mole ratio of dichloroacetic acid to amine groups was 1. The chemical and physical properties of the salts were evaluated by determining the dry weight, the content of ash, acid, insoluble substances, kinematic viscosity, and pH. The studied salts had a low contents of water (<10%), inorganic pollutants (<0.2%), and a low insoluble substances fraction (<0.15%). The determined acid content was similar to the theoretical content. The physical properties of the chitosan influenced the kinematic viscosity of the salt solutions. The kinematic viscosity decreased when the viscosity-average molecular weights of the chitosan decreased. The properties of chitosan did not significantly impact the pH of the salt solutions. This depended on the pKa of the acid. The chemical and physical properties of the salts can be altered by neutralizing the dichloroacetate solutions to a pH at which no distinct change in clarity occurs.

Key words: *chitosan, dichloroacetic acid.*

1. Introduction

Chitosan dichloroacetic acid salts can be characterized by a specific biological activity based on the modification of the metabolism of cancer cells. This is indicated by the results of research conducted to date on the anti-carcinogenic properties of chitosan and sodium dichloroacetate. Among other things, sodium dichloroacetate inhibits the occurrence of mitochondrial pyruvate dehydrogenase kinase, which reduces the mitochondrial membrane potential, and shifts the metabolism from glycolysis to glucose oxidation [1]. Chitosan limits the glycolytic activity of tumor cells by inhibiting the production of lactate and lowering levels of ATP [2 - 4]. An influence of the degrees of deacetylation and polymerization upon the anticarcinogenic activity of chitosan was observed [2, 3, 5, 6].

There is little information in the literature regarding chitosan dichloroacetates. This refers primarily to how salts are obtained, but only from polymers of a known deacetylation degree [7, 8].

The aim of the present study was to determine the impact of the properties of the substrates used to produce chitosan dichloroacetic acid salts on the physicochemical characteristics of these salts.

2. Materials and Methods

2.1. Substrates

The study materials were dichloroacetic acid (ReagentPlus®, Sigma-Aldrich) and chitosan from krill chitin. The polymers were obtained and characterized according to the methods described previously [9]. The physicochemical properties of chitosan are presented in Table 1.

2.2. Chitosan salts

Chitosan dichloroacetic acid salts were obtained according to Wojtasz-Pająk [9]. The dry weight of the salts was determined by freeze drying to a constant weight, while the ash and acid contents were determined according to the methods described previously [9].

Table 1. Chemical and physical properties of chitosan; ^a deacetylation degree, ^b viscosity-average molecular weight.

Sample symbol	DD ^a , %	\bar{M}_{Vb} , kDa	Deacetylation reaction parameters	
			Temperature, °C	Time, min
CHN 1	69	279	115	20
CHN 2	78	277	100	120
CHN 3	84	327	95	2×20
CHN 4	93	279	115	3×20
CHN 5	73	68	115	30
CHN 6	84	74	90	2×20
CHN 7	90	76	140	90
CHN 8	97	61	140	2×90

10]. Tests of pH and kinematic viscosity were performed on the salts obtained. A solution was prepared by dissolving the salt in cold, distilled water for an hour while continually mixing vigorously. The samples were left at room temperature for 24 hours, then they were filtered through Schott 3 funnels, which were then rinsed with distilled water (3×50 ml) and dried to a constant weight.

Kinematic viscosity (η_k) was determined by measuring the flow rate of salts at concentration ranges of 0.0002 to 0.0015 g/ml at a temperature of 25 ± 0.01 °C with an AVS-350 (Schott Geräte) using a Ubbelohde 53210/I viscometer with a constant of $K = 0.009852$ mm²/s².

Measurements of the pH of solutions of the chitosan dichloroacetic acid salts ($c = 1$ g/100 ml) were conducted using a 702 SM Titrimo apparatus (Metrohm).

3. Results and discussion

3.1. Chemical properties of salts

Table 2 presents the chemical properties of the tested salts.

The products obtained by freeze-drying the salt solutions had a low water content that did not exceed 10%, as is demonstrated by the salt dry weights presented in **Table 2**. The only way to determine the water content of the studied salts is to freeze-dry them to a constant weight. Attempts to use thermal methods to determine the dry weight demonstrated that chitosan dichloroacetic acid salts are highly susceptible to higher temperatures. While being heated for a longer period at a temperature of 105 °C, the salts changed color from white to brown. Such a distinct color change indicated that thermal treatment caused a fundamental change in the chemical structure of the salts. The dry weight determined with this method was about 7% lower than the samples that had been lyophilized (data not presented). Pettersen et al. [11] observed similar changes in color in chitosan chloride and chitosan glutamate that were subjected to high temperature (105 °C and 120 °C). These results indicate that these salts are more susceptible to the effects of temperature than are the

Table 2. Chemical properties of the chitosan dichloroacetate; ^aS_{69/279} – salt from chitosan of DD 69 %, *M_v*, 279 kDa.

Sample symbol	Dry weight, %	Ash content, %	Water-insoluble fraction content, %	Acid content determined, %	Theoretical acid content, %
S _{69/279}	97.0	0.06	0.07	34.3	33.9
S _{78/277}	97.1	0.04	0.07	37.2	37.0
S _{84/327}	95.6	0.02	0.12	39.9	39.3
S _{93/274}	93.2	0.06	0.07	42.9	42.4
S _{73/67}	97.4	0.02	0.10	35.8	35.2
S _{84/74}	98.0	0.10	0.07	39.9	39.4
S _{90/76}	97.9	0.06	0.03	41.5	41.2
S _{97/61}	97.4	0.16	0.11	43.4	43.4

chitosan that they are obtained from. The browning of polymer was noted by de Britto and Campana-Filho [12] when it had been degraded at a temperature of 280 °C. According to these authors, chitosan undergoes dehydration, deacetylation, and depolymerization during thermal processing.

Factors other than temperature and time that effect changes in the structure of the salts probably include the type of acid used to obtain them. This is indicated by the results of studies of the solid residues of chitosan salts formed with adipic, succinic and fumaric acids after heating at a temperature of 105°C [9]. Under these conditions neither the color nor the chemical structure of the samples changed significantly. The contents of inorganic pollutants and the water-insoluble fraction in the salts tested were both low (**Table 2**). The determined acid content was similar to theoretical values. The differences did not exceed 0.6% (**Table 2**). These results confirm that freeze-drying the tested salts prevented the disadvantageous changes in chemical structure that are associated with thermal processing.

3.2. Physical properties of the salts

The salts tested dissolved well in water at a temperature of about 25 °C. The content of insoluble substances in dichloroacetate chitosan did not exceed 0.15% (**Table 2**).

The dependency of the kinematic viscosity of chitosan dichloroacetates on the salt concentration in solution is presented in **Figures 1** and **2**.

Salts obtained from chitosans of similar viscosity-average molecular weights had similar kinematic viscosity (**Figures 1** and **2**). The molecular weight of the chitosan used to obtain the salts had the greatest influence on the kinematic viscosity measured. The lower the viscosity-average molecular weight of the polymer was, then the lower the kinematic viscosity was.

The pH of the salt solutions ($c = 1\text{g}/100\text{ ml}$) varied within the relatively narrow range of 3.3 to 3.8 (**Table 3**). This indicates that the properties of the chitosan from which the salts were obtained did not have a significant impact on pH values.

Table 3. pH of solutions chitosan dichloroacetic acid salts; ^aS_{69/279} – salt from chitosan of DD 69 %, \bar{M}_v 279 kDa.

Sample symbol	pH
S _{69/279}	3.6
S _{78/277}	3.3
S _{84/327}	3.6
S _{93/274}	3.8
S _{73/67}	3.3
S _{84/74}	3.3
S _{90/76}	3.6
S _{97/61}	3.7

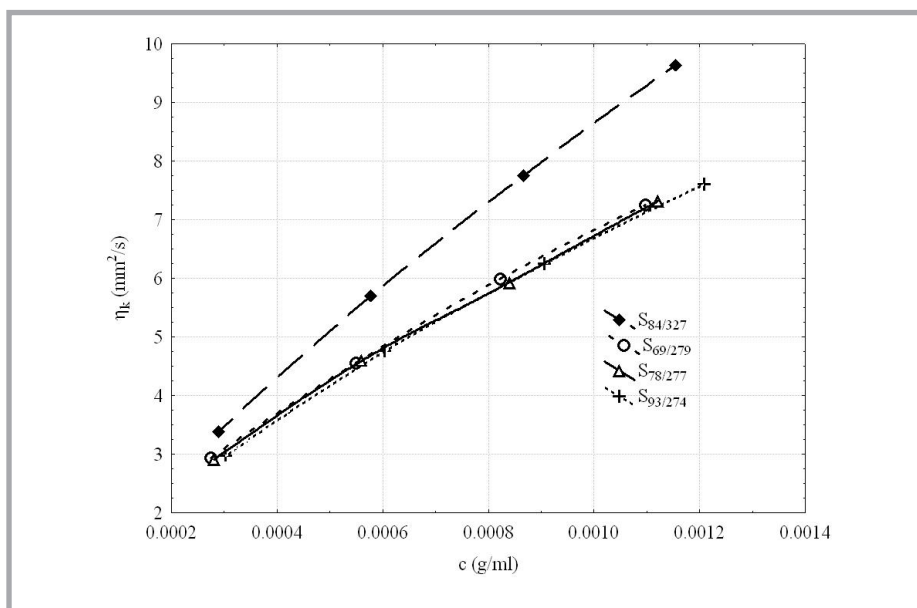


Figure 1. Kinematic viscosity of chitosan dichloroacetic acid salts obtained from polymers with high viscosity-average molecular weights.

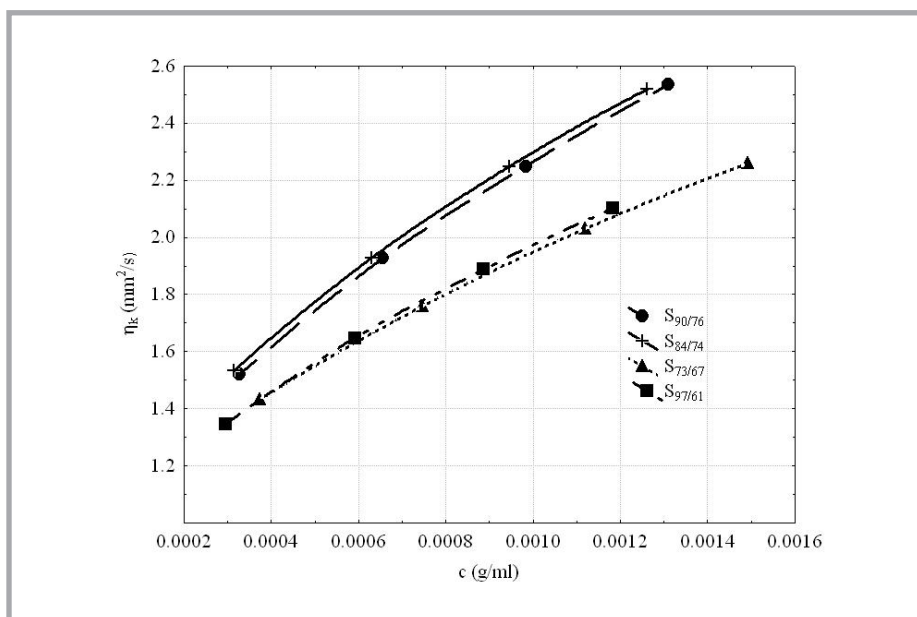


Figure 2. Kinematic viscosity of chitosan dichloroacetic acid salts obtained from polymers with low viscosity-average molecular weights.

The solutions of the chitosan dichloroacetic acid salts that were tested had a lower pH than did dicarboxylic acid salts obtained at the same mole ratio of acid to amine group [9]. In the case of the salts of dicarboxylic acids, this changed from 4.9 to 5.5. These results indicate that the type of acid used to obtain the salts has an impact on the pH, and the higher the strength of the acid then the lower is the pH of the chitosan carboxylic salt solution. Of all the acids discussed above, dichloroacetic acid exhibited the highest dissociation constant. These results concur with data published by Hamdine et al. [13].

The relatively low pH of the solutions of the tested salts might limit their use, especially for medical or veterinary preparations. For this reason, the range within which pH can be changed without precipitation of chitosan from solutions was tested. Solutions of the salts at a concentration of 1 g/100 ml were titrated with 0.1 N NaOH until distinct changes in clarity were noted.

The pH of the tested solutions of chitosan dichloroacetic acid salts can be changed to about 6.3. The degree of deacetylation of the chitosan used to prepare a given chitosan dichloroacetic acid salt determines the pH at which the chitosan begins to precipitate from the solution. Chitosan that was 97% deacetylated precipitated at a lower pH (5,6) than did chitosan that was 73% deacetylated (6,3). These results are consistent with data reported previously [14, 15].

The sodium dichloroacetate formed on neutralisation can be removed from the solution through dialysis. This is indicated by an attempt to dialyse a S_{97/61} solution that had been neutralised to pH 5.6. The salt dissolved almost totally in water.

4. Conclusions

Salts with specific properties can be obtained from dichloroacetic acid and chitosans of varied degrees of deacetylation and viscosity-average molecular weights.

The physical properties of the chitosan have an impact on the kinematic viscosity of the salts.

The pH of a chitosan salt solutions depends on the pK_a of the acid used in its preparation.

The chemical and physical properties of the salts can be modified by neutralizing solutions of the chitosan dichloroacetic acid salts to a pH at which no distinct change occurs in their clarity.

5. References

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