



Article Strontium-Induced Gelation of Sodium Alginate in the Presence of Carbon Nanotubes: Elemental Analysis and Gel Structure

Olga S. Zueva ^{1,*}, Tahar Khair ¹, Svetlana R. Derkach ², Mariia A. Kazantseva ^{3,4} and Yuriy F. Zuev ^{3,*}

- Institute of Electric Power Engineering and Electronics, Kazan State Power Engineering University, 51 Krasnoselskaya Str., 420066 Kazan, Russia; taharch937@gmail.com
- ² Department of Chemistry, Murmansk State Technical University, Sportivnaya Str. 13, 183010 Murmansk, Russia; derkachsr@mstu.edu.ru
- ³ Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS, 2/31 Lobachevskogo Str., 420111 Kazan, Russia; masha353kazan@gmail.com
- ⁴ School of Applied Mathematics HSE University, Tallinskaya Str., 34, 123458 Moscow, Russia
- * Correspondence: ostefzueva@mail.ru (O.S.Z.); yufzuev@mail.ru (Y.F.Z.)

Abstract: The elemental composition and structural features of the junction zones of a strontiumalginate hydrogel and their alteration under the intercalation of multi-walled carbon nanotubes into the hydrogel structure were studied. It was shown that the crosslinking with Sr^{2+} cations due to electrostatic interactions leads to the association of polymer chains into junction zones with incompletely filled cells. It was found that in strontium alginate, the average cell occupation number of Sr^{2+} cations is less than 1 and approximately equal to 0.64. In nanocomposite hydrogels including multi-walled carbon nanotubes, its increase to 0.81 indicates the appearance of a more ordered structure of alginate chains in junction zones. The information about the most preferred types of eggbox cells for binding with Sr^{2+} cations was analyzed. The existence of Sr^{2+} cations in nonequivalent positions was established. The possibility of separating the contributions of chemical adsorption due to ionic bonds with alginate chains and physical adsorption due to the appearance of local energy minima near alginate chains, leading to the appearance of ordered secondary structures, was demonstrated. It has been shown that the addition of carbon nanotubes to a hydrogel changes their sorption capability, leading, first of all, to an increase in the possible sites of physical adsorption.

Keywords: strontium-alginate hydrogel; multi-walled carbon nanotubes; elemental analysis; structure

1. Introduction

In food industry, biocompatible natural carbohydrates find extensive applications, where they are commonly used as thickeners, moisture-retaining, emulsifying, structuring, gelling or foaming agents [1–4]. Recently, the technologies for their usage in medical, pharmaceutical and cosmetic industries, for tissue engineering, creation of films and coatings, development of bio-inspired materials and in many other fields have been widely developed [5–13].

It is known that natural polysaccharides can also be used for the adsorption and binding of various contaminants from effluent and wastewater. The solution to similar problems is also important for biomedical applications, for example, for the introduction and subsequent controlled release of drugs. Biopolymer chains of polysaccharides contain carboxyl, hydroxyl and other active functional groups that can react with heavy metals via ion exchange [14–17]. Metal–polysaccharide microspheres prepared via ion-induced gelation [18] and micro fibrils of ionotropic gels are even more effective sorbents of heavy metals and toxic organic compounds. Obtained during ion-induced gelation using divalent metal salts, these materials can be used both as immobilizing carriers to maintain the



Citation: Zueva, O.S.; Khair, T.; Derkach, S.R.; Kazantseva, M.A.; Zuev, Y.F. Strontium-Induced Gelation of Sodium Alginate in the Presence of Carbon Nanotubes: Elemental Analysis and Gel Structure. *J. Compos. Sci.* **2023**, *7*, 286. https:// doi.org/10.3390/jcs7070286

Academic Editor: Francesco Tornabene

Received: 3 June 2023 Revised: 3 July 2023 Accepted: 10 July 2023 Published: 12 July 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). biological activity of microorganisms and enzymes [19,20] and as adsorbents capable of bio sorption of heavy metal ions [21,22] and hazardous organic compounds [23–27].

Alginate gels, prepared with divalent alkaline earth metals, primarily calcium and strontium, are among the most promising materials for environmental and biomedical applications. Natural strontium appears as a composition of four stable isotopes. It is included in growers and organisms and is a low-toxic element, which in its properties is an analogue of calcium. Strontium alginate is less studied. Apparently, this is due to the fact that calcium is a much more prevalent element of the earth's crust and the environment than strontium. However, the low toxicity of strontium and its properties similar to some extent to those of calcium, but still different, are used to create composite materials with unique properties. In particular, strontium is the key chemical element in the technologically important perovskites [28]. For biomedical applications, it is essential that Sr^{2+} is an osteogenic divalent ion, which is beneficial to bone growth and regeneration, available for the creation of the bone regeneration biomaterials [29-32]. Osteogenic ions of strontium and zinc in trace amounts are known to accelerate bone healing due to their role in the regulation of osteoblasts and osteoclasts in addition to their antibacterial properties. The formation of strontium-alginate hydrogel films may be used as a method to introduce these ions through alginate composites for bone tissue engineering [33].

When drops of sodium alginate enter a concentrated solution of strontium chloride salt, the neighboring polymer chains are connected due to the formation of ionic bonds, coordination and complexation between strontium ions and the carboxyl groups of alginate chains, leading to the transformation of solution droplets into gel microspheres [34,35]. Modification of solutions used by the addition of nanoparticles and the creation of composite hydrogels can significantly transform the properties of developed materials and expand the scope of their possible application [20,36–40].

The purpose of this research was to study the structural features of polysaccharide alginate chains' association in gels obtained by the crosslinking of alginates with alkaline earth metal (Sr²⁺) cations, to determine the most probable structure of their connection zones and to evaluate the sorption capability of strontium–alginate hydrogels, including ones incorporated with multi-walled carbon nanotubes (MWCNTs) Taunit added to enhance their mechanical strength [41–44]. This study was carried out on the basis of elemental analysis and electron microscopy data using combinatorial methods. The effect of nanoparticles on the elemental composition of metal–alginate hydrogels has not been previously considered.

2. Materials and Methods

2.1. Materials

For hydrogel preparation, we took sodium alginate of medium viscosity (A-2033 Sigma, Burbank, CA, USA). This naturally occurring anionic polysaccharide is found in brown (*Phaeophyta*), red (*Phodophyta*) and some green algae and is produced by some bacterial species (e.g., *Azotobacter vinelandii*). Linear alginic acid molecules are built from residues of β -D-mannuronic (M units) and α -L-guluronic acids (G units) [45–47], jointed by 1, 4 linkages.

In fact, alginate is a natural copolymer having an irregular block structure consisting of M and G units, where the number of M units usually prevails over the number of G ones. Typically, the ratio (M/G) is about 1.5–2. Alginate used by us was reported to have the ratio of M/G equal to 1.56 [48,49]. The alginic acid has chemical formula $(C_6H_8O_6)_n$, which reflects the composition of both M units and G units. The spatial structures formed by the MM, MG, GG blocks differ quite strongly.

Strontium chloride SrCl₂ (Tatkhimprodukt, Russia) was used to prepare metal–alginate microspheres. For solutions, the purified water prepared in the "Arium mini" ultrapure water system (Sartorius, Gottingen, Germany) was employed, as well as medical ethanol (95%, Rosbio, Russia). To modify the alginate hydrogel, we used MWCNTs of carbon

nanomaterial Taunit produced by NanoTechCenter (Tambov, http://www.nanotc.ru) with an average diameter of 20–40 nm and a length of several hundred nanometers.

2.2. Preparation of Supernatant (MWCNTs Solution in Ethanol)

For preparation of MWCNT ethanol solution, 100 μ L of 95% ethanol was added to 1.5 mg of MWCNTs, and the resulting mixture was kept for 20 min in an ultrasonic bath (35 kHz, 100 W) at 35 °C. The resulting solution was centrifuged for 10 min (10,000 × *g*); in further work, the supernatant with an MWCNT content of 0.3 mg·mL⁻¹ was used.

2.3. Preparation of Strontium–Alginate Microspheres

The sodium alginate aqueous solution with concentration 2 wt.% was prepared in accordance with stereotype procedure [17,18]. Sodium alginate was dissolved at temperature of 70 °C for 3 h, followed by swelling until the next day in a switched off, gradually cooling thermostat (15 h). The next day, 10 μ L of supernatant of MWCNT ethanol solution was added to 500 μ L of aqueous sodium alginate solution (2 wt.%). The weight ratio of alginate and MWCNTs were 3300:1. Next, the samples with and without MWCNTs were warmed and processed to ultrasound (35 kHz, 100 W) at a temperature of 70 °C during 60 min in a water bath of the Bandelin SONOREX TK52 ultrasonic disperser (Germany). To obtain hydrogel microcapsules, a hot (2 wt.%) sodium alginate solution was dropped into a quenching bath with strontium chloride solution (1 M) at a temperature of 20–23 °C using a syringe. When drops of sodium alginate solution entered the salt solution, microcapsules of crosslinked hydrogels with a diameter of about 2 mm were instantly generated, in which monovalent sodium ions were interchanged by divalent metal ions. After dripping, the microcapsules were kept in solution for 20 min, then rinsed twice and frozen in liquid nitrogen for freeze-drying.

2.4. Scanning Electron Microscopy

Freeze-dried microspheres were examined using scanning electron microscope Auriga Crossbeam Workstation (Carl Zeiss AG, Oberkochen, Germany) in the Shared Research Center of Kazan National Research Technical University "Applied Nanotechnology" (Kazan National Research Technical University).

2.5. Energy Dispersive X-ray Spectroscopy

The good analytical method for elemental analysis is energy-dispersive X-ray spectroscopy. It was performed using field emission scanning electron microscope Auriga Crossbeam Workstation (Carl Zeiss AG, Oberkochen, Germany), equipped with INCA X-Max silicon drift detector for energy dispersive X-ray microanalysis (Oxford Instruments, Abingdon, OX, UK). SEM images of the surface and internal cells of freeze-dried microcapsules were investigated, their elemental composition was determined and the chemical formula satisfied to present composition was found.

3. Results

3.1. Theoretical Background

Sodium alginate biopolymer chains are polyelectrolytes because both M and G units contain carboxyl groups capable of dissociation. In solution, monomeric structural units form a bent polymer chain that forms negatively charged cavities of various depths (Figure 1).

When alkaline earth cations Me²⁺ penetrate into the bulk of polysaccharide solution, their complexes with biopolymer chains are formed, leading to cooperative bindings of different macromolecules, resulting in ionotropic gel formation [47]. First, the integration of two linear alginate chains into a dimer occurs (Figure 2a,b). Then, dimers are combined in a flat sheet of a junction zone (Figure 2d) called the "egg-box" in [50]. Proposed by Grant et al. [50], the egg-box model characterizes the crosslinking of alginate chains with divalent ions. Moreover, this term is often used to describe an individual cell formed by four monomer units and including cation Me²⁺.



Figure 1. β -D-mannuronic (M) and α -L-guluronic (G) acid monomeric units in alginate chain.



Figure 2. Combination of biopolymer chains into a dimer (**a**); simplified form of a dimer with completely (**b**) and partially connected cells (**c**); types (**d**–**f**) of junction zones under the integration of dimers. Blue spheres correspond to Me^{2+} cations.

Subsequently, in [51–63], the egg-box model was modified. Particularly, it turned out that the crosslinking of biopolymer chains by divalent alkaline earth metal ions realizes in some stages [57]. At the first phase, the most energetically favorable process is the cation incorporation into the cavity formed by the adjacent guluronic blocks of two neighboring chains. Then, integration of these chains due to the occurrence of ionic bonds and complex formation is carried out. Such integration leads to the construction of an egg-box cell formed by the GG block of one chain and the GG block of neighboring chain, connected by cation built into this cavity. MM and MG blocks constitute shallower cavities. Nevertheless, the formation of even one reliable bond stimulates further "zipping" of chains. This leads to complete or partial filling of cells that arise when the chains approach each other with ions and, thereby, to integration of two neighboring chains into a dimer. It should be noted that the interaction of various alkaline earth cations with different blocks of alginate chains has its own characteristics. Nevertheless, in all cases the places in the cells from GG blocks are the most optimal for cations, and therefore they are always filled.

The combination of two polymer chains into a dimer (Figure 2a) can be conveniently represented as two nearly spaced guluronic chains consisting of successively connected GG blocks with Me²⁺ alkaline earth metal ions embedded in the formed cavities, as described in [15]. Binding of all GG blocks leads to complete dimer construction (Figure 2b). The existence of blocks with M units, which are not always optimum for alkaline earth cations incorporation, leads to dimers with partially empty cells (Figure 2c). As in Grant's work [50], the alginate chains in Figure 2b,c are conventionally depicted as zigzag lines. Thus, we do not take into account the fact of different depths of cells formed by GG, MM and MG blocks. The lack of binding due to a suboptimal cell configuration is represented as the lack of divalent cation (blue sphere) in the cell. The resulting surplus charge of carboxyl groups is neutralized by sodium or hydrogen ions, not shown in the figure.

Farther lateral association of dimers, characteristic for alginates, can lead to appearance of junction zones in the form of flat egg-box sheets. Fully crosslinked packing (Figure 2d),

as shown in our work [64], corresponds to the alginates of some transition metals, and coupled dimers linked by van der Waals interactions and hydrogen bonds are detected for calcium alginates (Figure 2e) [57]. In the case of calcium alginate, half of the carboxyl groups are not involved in the construction of bonds. Nevertheless, the possibility of additional binding of chains by calcium ions in the zones of interdimer association exists, but the probability of this process does not exceed a few percent [64]. Such situation can be realized if a GG–GG cell is formed in the interdimer space. Despite the low probability of the appearance of GG–GG structures in the whole sample, their appearance is important for gelation, because contributes to the launch of the zipping mechanism for combining chains into dimers. Barium and strontium alginates are characterized by cross-linked packing of dimers with partially connected cells that have lost their individuality (Figure 2f) [64].

The investigation of the elemental composition of the junction zones makes it possible not only to obtain information on the degree of the fill of the egg-box cells but also to draw derivations about the value of cations' interaction with polymer chains, to suggest the nature of the binding of alginate dimers into junction zones and to clarify the knowledge about the types of the egg-box cells which are the most preferable for binding with these cations [64]. Since divalent metal cations bind two-by-two monomer units of polymer chains, the chemical formula of Na-alginate is best considered for a block of two units, writing it as $(C_{12}H_{14}O_{12}Na_2)_n$. Interaction with metal cations leads to the crosslinking of chains and construction of junction zones. However, various kinds of divalent cations give the difference in the composition and microstructure of gels [65–67].

The chemical formula of complex of divalent metal Me^{2+} with alginate is converted to the form $(C_{12}H_{14}O_{12}Me_X)_n$. The symbol X designates the medium amount of divalent ions per C_{12} block, i.e., the average cell occupation number. The limit meaning of this number is 1. The case X = 1 corresponds to the entirely filled egg-box cells of flat zones from connected alginate chains (Figure 2d). This case is realized for the complexation of transition metals with alginate, when the long-range electrostatic interactions are added with the prepotent coordinate-covalent bonding of ions with alginate units [67]. This is partly due to the small size of transition metal cations, which facilitates incorporation into the cells of various types.

The formation of complexes between alkaline earth ions and alginate units takes place only due to ionic bonds, i.e., owing to the electrostatic interaction. For alkaline earth calcium, when alginate dimers are linked only by van der Waals interactions and hydrogen bonds without losing their individuality (Figure 2e), there cannot be more than one metal cation per cell formed by four monomeric units. Therefore, in the absence of metal cations between dimers, the average number of Me^{2+} ions per C₁₂ block should not exceed 0.5. If the dimer cells are not completely filled, the average block occupation number will be even less. Figure 2e shows a case approximately corresponding to X = 0.35. A similar experimental result was obtained by us for calcium alginate [64]. If metal ions bond in the interdimer space, but the cells are not completely filled, this average cell occupation number can increase to the value X = $0.6 \div 0.7$ (Figure 2f), corresponding to alkaline earth barium and strontium [64]. With completely filled cells, the occupation number increases to the limiting value X = 1 (Figure 2d), which we observed for transition metal alginates (copper, nickel and manganese). It should be noted that there is a correlation between the average cell occupation number and the magnitude of cation interaction with alginate blocks, which is associated with the appearance of additional contribution of coordinatecovalent interactions in transition metals. Thereby, the medium amount of metal cations per cell received by elemental analysis with applying the egg-box model produces significant information on the formation of alginates structure, the kind of their junction zones, and even on the value of interaction of divalent metal cations with biopolymer matrix.

3.2. Experimental Results for Strontium Alginate

The method of energy-dispersive X-ray spectroscopy does not allow us to determine the quantitative composition of hydrogen in structural formula. Therefore, all further formulas are given without hydrogen, the presence of which is simply implied in the above proportions as $(C_{12}H_{14})$.

The image of the elements of a Sr–alginate microsphere and the information on the elemental analysis of a surface region of this microsphere, received by dropping an alginate solution into a SrCl₂ solution, are demonstrated in Figure 3. The received elemental composition, contrary to double washing, corresponded to chemical formula $(C_{12}O_{11.6}Cl_{2.3}Sr_{1.8}Na_{0.1})_n$. The investigation of the elemental composition of the eight more internal regions of the microcapsules (Table 1) led to similar results, namely, to the ratio $(C_{12}O_{9.1}Sr_{2.06}Cl_{2.84}Na_{0.07})_n$ averaged over eight results. A distinctive feature of these formulas from the theoretical supposition is the attendance of chlorine and a significantly bigger quantity of strontium atoms per block. This composition displays the presence of structures inside the sample which are not intrinsic to Sr–alginate.



Figure 3. SEM image of elements of Sr–alginate and its formula corresponding to average result for eight internal regions after standard wash.

Table 1. Elemental composition of outer and eight inner regions of walls of strontium–alginate microspheres (weight %) and chemical formula corresponding to this composition.

Spectrum	С	0	Na	C1	Sr	Chemical Formula
Spectrum out	25.25	32.50	0.40	14.27	27.58	$C_{12}O_{11.6}Sr_{0.65}Na_{0.1} + 1.15(SrCl_2)$
Spectrum 1	22.76	22.32	0.48	19.81	34.63	$C_{12}O_{8.83}Sr_{2.50}Cl_{3.54}Na_{0.13}$
Spectrum 2	26.39	27.07	0.00	16.63	29.91	C ₁₂ O _{9.24} Sr _{1.86} Cl _{2.56} Na ₀
Spectrum 3	25.17	26.37	0.28	17.59	30.60	C ₁₂ O _{9.44} Sr _{2.0} Cl _{2.84} Na _{0.07}
Spectrum 4	24.44	26.54	0.27	17.08	31.67	C ₁₂ O _{9.78} Sr _{2.13} Cl _{2.84} Na _{0.07}
Spectrum 5	26.45	26.94	0.32	16.33	29.96	C ₁₂ O _{9.17} Sr _{1.86} Cl _{2.51} Na _{0.08}
Spectrum 6	25.39	24.63	0.28	18.33	31.36	C ₁₂ O _{8.74} Sr _{2.03} Cl _{2.94} Na _{0.07}
Spectrum 7	24.31	24.62	0.00	18.04	33.04	C ₁₂ O _{9,12} Sr _{2,24} Cl _{3,02} Na ₀
Spectrum 8	27.26	25.67	0.48	16.20	30.40	$C_{12}O_{8.48}Sr_{1.83}Cl_{2.42}Na_{0.11}$
Average						$C_{12}O_{9.1}Sr_{0.64}Na_{0.07} + 1.42(SrCl_2)$

The obtained formula leads to derivation about the availability of strontium atoms in at least two fundamentally various nonequivalent positions: (a) in the binding of the neighboring Sr–alginate chains, i.e., functioning as crosslinking factors; (b) in the constitution of SrCl₂ associates adsorbed by alginate chains. It should be pointed out that SrCl₂ structures subsist namely in the format of associates; their probable dissociation in water should lead to the emergence of chloride ions, which are neutral to the alginate structure and can be readily deleted by washing [64]. The separation of the contributions of nonequivalent atoms shows that approximately 0.64–0.65 Sr^{2+} crosslinking ions per each C₁₂ block perform the connection of chains, leading to the formation of the egg-box cells.

Moreover, the data obtained indicate that for each C_{12} block of Sr–alginate there are mean 1.15 associates of strontium chloride SrCl₂ in the near-surface region and 1.42 associates corresponding to the internal regions of microspheres. Taking into account that the presence of adsorption abilities is a specific peculiarity of natural carbohydrates, we supposed that the connecting of SrCl₂ by hydrogel structures watched in this case is most likely owing to the existence of local energetically auspicious locations for these associates beside alginate chains, which accords with physical adsorption. In spite of the thing that SrCl₂ associates do not constitute either covalent or ionic chemical bonds with alginate chains, they can be hold around them due to weaker (mainly van der Waals) interactions. To detect the elemental composition of the main structures of strontium alginate corresponding to the near-surface region, we took away 1.15 Sr atoms connected with 2.3 Cl atoms, obtaining the formula ($C_{12}O_{11.6}Sr_{0.65}Na_{0.1}$)_n. For internal regions, a similar operation led to the separation of composition into the main volume corresponding to strontium alginate ($C_{12}O_{9.1}Sr_{0.64}Na_{0.07}$)_n and additional inclusions of 1.42 SrCl₂ associates per each C_{12} alginate block.

To examine the hypothesis of the availability of different kinds of strontium ions connections with alginates, we supposed that the energy of interaction of SrCl₂ associate with biopolymer chains accords to the physical adsorption interaction. In this event, it will not be much more than its thermal motion energy in water. This is indicated by the reduced concentration of associates in the near-surface regions compared to the inner regions of the microspheres. Accordingly, it should be expected that a rise in the wash time should lead to the deletion of weakly connected SrCl₂ associates. In the event of complex formation based on ionic electrostatic interactions, the wash will not alter the results.

We conducted an investigation of anew produced and thoroughly rinsed out microcapsules of Sr–alginate. The rinsing aqua was varied five times, with an interval of 2 h. Salt concentration in the rinsing aqua was monitored using an InoLab Cond 7310 SET1 conductometer (Hungary). The wash occurred at room temperature until the electrical conductivity of the rinsing aqua reached the value of the electrical conductivity of distilled water (which happened after 6 h of wash).

The SEM images of the Sr–alginate microsphere and elemental analysis data of one region of this microsphere after additional washings are shown in Figure 4. After an intensive wash, the strontium–alginate microspheres proved to be much more uniform in construction and cell dimensions (Figure 4). The approximate cell size was found as $60 \times 40 \ \mu\text{m}$. The elemental analysis performed for two strontium–alginate microsphere areas gave renewed results, (a) $(C_{12}O_{11.1}\text{Sr}_{0.70})_n$ and (b) $(C_{12}O_{11.3}\text{Sr}_{0.65})_n$, which do not fundamentally vary from the formulas obtained earlier. This points out that the used calculation of the elemental composition was rather true. The result obtained can be considered as an oblique confirmation of the physical adsorption of SrCl₂.

Early, we suggested [64] that the availability and number of such connecting sites with hydrogel structures for associates (physical adsorption), can be interpreted by the sorption capacitance of alginate hydrogels and, hence, can be correlated with it. We showed that the summary number of feasible connecting sites (of both physical and chemical nature) in the surface regions of strontium and calcium alginates is approximately idem being 1.8 per block of two monomeric polymer units. The decrease in the number of sites at which calcium ions, compared with strontium ions, can enter into the complex formation reaction, leading to chain integration, increases the number of sites valid for physical adsorption. This circumstance gives us to include that nontoxic calcium and strontium alginates can be employed to obtain effective materials for use in environmental and biomedical technologies. It should so be noticed that when these hydrogels are used as adsorption materials, heavy metal ions absorbed by them from the environment lead to the strengthening of their structure [15].



Figure 4. SEM image of Sr–alginate elements, its formula and elemental analysis data after additional washings with strontium–alginate formula corresponding to average result.

3.3. Experimental Results for Strontium-Alginate Eith Carbon Nanotubes

We also studied the structure and elemental composition of strontium–alginate hydrogel in the presence of MWCNTs added to hydrogels to enhance their mechanical strength. The SEM image of the cells of Sr–alginate microsphere prepared with the addition of MWC-NTs and the formula of strontium alginate corresponding to average over eight internal regions result for strontium–alginate microsphere after standard washing are shown in Figure 5.



Figure 5. SEM image of Sr–alginate cells in availability of MWCNTs and formula of strontium alginate averaged over eight internal regions after standard wash.

Our investigation of the elemental composition of the eight internal regions of the microcapsules (Table 2), obtained by dropping sodium alginate into a concentrated solution of strontium chloride, followed by double washing and freeze-drying, corresponded to the average ratio ($C_{12}O_{8.51}Sr_{2.78}Cl_{3.93}Na_{0.06}$)_n. The separation of contributions of nonequivalent strontium ions led to the formula ($C_{12}O_{8.51}Sr_{0.81}Na_{0.06}$)_n, which shows that for each C_{12} block, on average, 0.81 Sr²⁺ crosslinking ions perform spatial integration of chains.

Spectrum	С	0	Na	Cl	Sr	Chemical Formula
Spectrum 1	18.58	16.92	0.00	24.04	40.46	C ₁₂ O _{8,2} Sr _{3,58} Cl _{5,26} Na ₀
Spectrum 2	20.84	17.86	0.00	22.59	38.71	C ₁₂ O _{7.72} Sr _{3.06} Cl _{4.41} Na ₀
Spectrum 3	27.14	26.16	0.46	17.01	29.23	$C_{12}O_{8.68}Sr_{1.77}Cl_{2.55}Na_{0.11}$
Spectrum 4	18.90	17.61	0.00	22.94	40.55	C ₁₂ O _{8.39} Sr _{3.53} Cl _{4.93} Na ₀
Spectrum 5	24.95	26.38	0.41	18.05	30.21	C ₁₂ O _{9.52} Sr _{1.99} Cl _{2.94} Na _{0.10}
Spectrum 6	24.80	22.79	0.38	19.86	32.16	C ₁₂ O _{8.28} Sr _{2.13} Cl _{3.26} Na _{0.10}
Spectrum 7	23.73	23.80	0.53	17.64	34.30	C ₁₂ O _{9.03} Sr _{2.38} Cl _{3.02} Na _{0.14}
Spectrum 8	18.44	16.89	0.00	22.98	41.68	$C_{12}O_{8.25}Sr_{3.72}Cl_{5.06Na0}$
Average						$C_{12}O_{8.51}Sr_{0.81}Na_{0.06} + 1.96(SrCl_2)$

Table 2. Elemental composition of eight inner regions of strontium–alginate microspheres (weight %) prepared with MWCNTs' addition and chemical formula corresponding to this composition.

Moreover, the data obtained indicate that for each C_{12} block of Sr–alginate, there are also on average 1.96 associates, revealing the presence of additional sites for physical adsorption near the alginate chains. The shape and size of the cells of strontium–alginate microspheres, which include carbon nanotubes, do not fundamentally differ from the system without MWCNTs.

4. Discussion

We considered the fundamental aspects of cross-linking by Sr^{2+} ions and their effect on the morphology and elemental composition of Sr–alginate hydrogels, including those reinforced with carbon nanotubes. In the case of alginate, the result of crosslinking with alkaline earth cations was the pairwise integration of alginate chains and following lateral integration of crosslinked dimers. These processes led to the formation of flat junction zones. In the case of strontium alginate, we showed that junction zones corresponding to Figure 2f appeared in this system. Moreover, the value X = 0.64 of the average cell occupation number of Sr^{2+} ions for the inner regions of junction zones was obtained for MWCNT-free hydrogel, but for MWCNT-reinforced hydrogels, this number increased to X = 0.81. In both cases, the experimental value of X turned out to be greater than 0.5 but less than 1, which indicates that alkaline earth strontium does not fill all the cells of the egg-box sheet due to a relatively weak electrostatic interaction [64]. Some of the cells remained vacant and can be occupied by adsorbed heavy metal ions [15].

To explain the results obtained, we used the available structural data on alginates. We assumed that the medium number of the cell fillings in the junction zones in the cases where X < 1 should be determined not only by the type of crosslinking cations but also by the initial composition of studied alginate. It should depend on the sequential structure of alginate, primarily on M/G in the alginate chain. We developed an approach [64] based on combinatorial methods to assess the possible structures of junction zones. Alginate consists of the units of two uronic acids, M (mannuronic) and G (guluronic), with an irregular alternation sequence. The spatial structures formed by the MM, MG and GG blocks of each chain and by pairwise-connected blocks when they are connected into a dimer differ quite strongly despite the fact that the chemical formula of alginic acid for M and G units is the same. It was taken into account that the M/G ratio in the used alginate sample is 1.56 [48,49]. From this, for the convenience of calculations, we assumed that, on average, there are three M units for every two G units, i.e., G:M = 2:3. Based on the variant tabulation method, the approximate probabilities of block formation from two monomeric units were calculated. The probabilities of the appearance of blocks GG, GM, MG and MM turned out to be equal to 16%, 24%, 24% and 36%.

The structure of the junction zones of strontium–alginate hydrogel, according to the eggbox model and the obtained experimental data, consists of cells of various types (Figure 2f), and some cells do not contain metal cations. Using the calculated probabilities for the appearance of certain blocks in each chain, we also found the approximate probabilities for combining alginate chains, containing GG, GM, MG and MM blocks, into various egg-box structures. The results are shown in Table 3.

Table 3. Approximate probabilities of integration of cells GG, GM, MG and MM into different egg-box structures for alginate with $M/G\sim1.5$.

Ν	Type of Structure	Probability	Ν	Type of Structure	Probability
1	GG-GG	16/625 = 2.56%	4	GM-GM	72/625 = 11.52%
2	CC CM	06/625 - 15 26%	5	GM-MG	72/625 = 11.52%
2	GG-GM	90/023 = 15.50 %	6	GM-MM	216/625 = 34.56%
3	GG-MM	72/625 = 11.52%	7	MM-MM	81/625 = 12.96%

Next, using the literature data, we tried to guess which cells are filled first. The existing experimental data indicate that strontium prefers to bind to GG and MG blocks [65,66,68–77]. Some of the blocks, apparently MM, remain unbound by these ions. If, as the most probable structures containing Sr²⁺, we select cells that do not include MM blocks, specifically GG-GG, GG–GM, GM–GM and GM–MG, we receive X = 0.41. The discrepancy between this quantity and the experimental data indicates that some cells comprising MM blocks can also include Sr²⁺ ions. Particularly, the calculations showed that the filling of the GG–GG, GG–GM, GG–MM, GM–MM structures gives a total cell filling likelihood equal to 64%. This allows us to determine the average cells occupation number as X = 0.64, which is close to the values $X = 0.64 \div 0.65$ obtained in our experiments (Figure 3). This means that our assumption about the filling of these cells may be correct. Thus, despite the fact that the experimental technique used by us does not allow us to conduct conclusions about specific crosslinking sites, some considerations about the role of GG, MGand MM blocks in the binding of Sr^{2+} cations by alginate chains can even so be expressed. In addition, this result means that 36% of free cells can adsorb heavy transition metal ions, which have a high degree of binding to alginate chains due to the appearance of additional contribution of the coordinate-covalent bond [67].

A study of gel microspheres reinforced by carbon nanotubes showed a significant difference in results. In particular, the average cells occupation number in junction zones increased to X = 0.81, which indicates the emergence of a more ordered structure from alginate chains when they are connected in flat zones. This result may be related to the enhancement of electrostatic interactions (including strong Coulomb and weak van der Waals interactions) of the elements of biopolymer chains, as well as cations, in the presence of nanoparticles (MWCNTs) polarized in an aqueous medium [44]. The polarization effect of nanoparticles consists in the formation of double electric layer at MWCNT/water interface and appearance of bound negative charge on the MWCNTs' surface [78]. The polarization of the water boundary layer is expressed in the ordering of arrangement of water molecules with respect to the surface of nanoparticle, i.e., in the occurrence of a preferential orientation of dipole moments of water molecules perpendicular to interface. The combination of emerging effects leads to an increase in the interaction of strontium cations with biopolymer chains and the emergence of opportunities for incorporation into cells that were previously not optimal for these cations, for example, GM-GM and GM–MG. The results obtained (X = 0.81) make it possible to assume that in the presence of MWCNTs, only the MM–MM cells remain mostly unoccupied. The complete filling of all other cells gives a total probability of cells filling equal to 87%, i.e., X = 0.87. The remaining unoccupied 6% of the cells, corresponding to the difference between the theory and experiment, are most likely due to some uneven distribution of CNTs in the solution or their insufficient concentration.

Changes in the structure of the inner walls of strontium–alginate microspheres are illustrated by Figure 6. In the absence of carbon nanotubes (Figure 6, left), the inner walls have a very developed microstructure, represented by cells 7–10 μ m in size. Their walls, in turn, have an even finer structure with characteristic size about 1 μ m. In the presence of CNTs (Figure 6, right), the microstructure of inner walls demonstrates somewhat larger

size, 10–12 μ m with a much less developed internal microstructure. It should be noted that early we observed similar changes in the structure of protein–polysaccharide hydrogels, when in the presence of CNTs the network became clearer and more regular and the cell characteristic sizes slightly increased [42]. The changes in the structure of strontium– alginate hydrogels do not contradict the assumption that electrostatic interactions between biopolymer chains are enhanced in the presence of nanoparticles. The changes in the size, shape and structure of the cell walls should undoubtedly affect the functional properties of nanocomposite materials.



Figure 6. SEM images of Sr–alginate microcapsule walls in the absence (**left**) and presence (**right**) of MWCNTs.

According to our experimental data, in addition to chemical bonds with alginates, there is the opportunity for physical adsorption of certain associates, which can be retained near the alginate chains through weaker (generally van der Waals) interactions. The existence of many optimum but still various connecting sites for metal ions with alginate chains has also been established by molecular dynamics methods [60,67,75]. The presence of such sites, some of which remain unoccupied, can lead to the entry of various molecules there and their binding by Sr–alginates on account of physical adsorption, the connecting energy of which is less than the energy of the ionic bond of ions with alginate units and is comparable to their thermal motion energy. Interesting features of hydrogels were revealed when studying the quantitative composition of excess salts used to prepare microspheres and remaining in the gel structure after a standard 20 min double washing. It should be noted that washing leads to the elimination of "extra" ions and associates from the places of their physical adsorption, but not from all the places of their possible binding, which determines the sorption capacity of the alginates. In particular, for the internal regions of strontium–alginate microcapsules the additional physical adsorption of SrCl₂ associates was observed, corresponding to a value of 1.42 per each C_{12} block, indicating good sorption capabilities of strontium alginate. In the presence of MWCNTs, the number of sites for possible additional physical adsorption of SrCl₂ increased and began to correspond to a value of 1.96 for per each C_{12} block. Thus, the addition of carbon nanotubes to the hydrogel structure alters their sorption capabilities, leading first of all to an increase in physically adsorbed molecules.

5. Conclusions

In this work, we studied the elemental composition and structural features of freezedried hydrogel microspheres obtained by the Sr^{2+} crosslinking of sodium alginate chains in water solutions and MWCNT water dispersions. It was demonstrated that in metal alginate hydrogels, the average cells occupation number of Me^{2+} cations of different metals, which theoretically should be equal to 1, in the case of alkaline earth strontium is approximately equal to 0.64 for strontium–alginate hydrogels without MWCNTs and equal to 0.81 in nanocomposite hydrogels containing MWCNTs. It was shown how combinatorial methods and information on the types of the egg-box cell most preferred for binding to Sr^{2+} cations can explain the experimentally obtained occupation numbers and relate them to the structural features of the junction zones.

The study of carbon nanotubes intercalation into a gel showed the availability of a more ordered and more completely filled structure in comparison with MWCNT-free systems. This result is associated with the enhancement of electrostatic interactions (involving strong Coulomb and weak van der Waals interactions) of the elements of alginate chains, as well as cations, with incorporated nanoparticles (MWCNTs), polarized in an aqueous medium. The combination of effects arising during the polarization of nanoparticles leads to an increase in the interaction of strontium cations with biopolymer chains and the emergence of opportunities for incorporation into the cells that previously were not optimal for these cations, for example, GM–GM and GM–MG. The results obtained made it possible to assume that in the presence of MWCNTs, only the most non-optimal MM–MM cells remain mainly unoccupied. Thus, the use of combinatorial methods allows us to express some considerations about the role of various blocks in the crosslinking of alginate chains with Sr²⁺ ions. It was shown that the introduction of MWCNTs into the hydrogel composition leads to the changes in its internal structure, in particular, to the change in characteristic cell size.

Using Sr–alginates as an example, it was determined that the availability of unequal connecting sites for metal cations with biopolymer chains can lead to the emergence of ordered secondary structures through the physical adsorption of ions and molecules from the environment. For the internal regions of strontium–alginate microcapsules, the additional physical adsorption of SrCl₂ associates was observed, corresponding to a value of 1.42 per each C_{12} block, indicating good sorption capabilities of strontium alginate. With the addition of MWCNTs, the number of sites for additional physical adsorption of SrCl₂ increased by almost 40% and corresponded to a value of 1.96 per each C_{12} block. Thus, the addition of carbon nanotubes to the hydrogel changes their sorption capability first of all owing to an increase in physically adsorbed molecules. An additional advantage of using nanocomposite hydrogels is their significantly improved mechanical properties [41–43]. The obtained information on the structure and properties of the resulting nanocomposite hydrogel can promote the development of new materials with improved properties for targeted applications in environmental protection, biotechnology, as well as in any other relevant areas.

Author Contributions: Conceptualization, O.S.Z., Y.F.Z. and S.R.D.; methodology, O.S.Z.; investigation, O.S.Z. and M.A.K.; formal analysis, O.S.Z. and T.K.; writing—original draft preparation, O.S.Z., Y.F.Z. and S.R.D.; writing—review and editing, Y.F.Z., O.S.Z. and S.R.D.; visualization, T.K. and M.A.K.; supervision and project administration, Y.F.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, project No. 23-64-10020 (ion-induced alginate gelation, M.A.K., Y.F.Z.).

Data Availability Statement: The data in this study are available on reasonable request from the corresponding author.

Acknowledgments: O.S.Z. gives thanks for the support of Strategic Academic Leadership Program "PRIORITY-2030" of KSPEU. Electron microscopy investigation was performed using the equipment of the Shared Research Center of Kazan National Research Technical University "Applied Nanotechnology".

Conflicts of Interest: The authors declare no conflict of interest.

References

- Pascuta, M.S.; Varvara, R.-A.; Teleky, B.-E.; Szabo, K.; Plamada, D.; Neme, S. S.-A.; Mitrea, L.; Martau, G.A.; Ciont, C.; Calinoiu, L.F.; et al. Polysaccharide-Based Edible Gels as Functional Ingredients: Characterization, Applicability, and Human Health Benefits. *Gels* 2022, *8*, 524. [CrossRef] [PubMed]
- Lafarga, T.; Acién-Fernández, F.G.; Garcia-Vaquero, M. Bioactive Peptides and Carbohydrates from Seaweed for Food Applications: Natural Occurrence, Isolation, Purification, and Identification. *Algal Res.* 2020, 48, 101909. [CrossRef]
- 3. Shao, P.; Feng, J.; Sun, P.; Xiang, N.; Lu, B.; Qiu, D. Recent Advances in Improving Stability of Food Emulsion by Plant Polysaccharides. *Food Res. Int.* **2020**, *137*, 109376. [CrossRef] [PubMed]
- 4. Muthukumar, J.; Chidambaram, R.; Sukumaran, S. Sulfated Polysaccharides and Its Commercial Applications in Food Industries— A Review. J. Food Sci. Technol. 2021, 58, 2453–2466. [CrossRef]
- Mohammed, A.S.A.; Naveed, M.; Jost, N. Polysaccharides; Classification, Chemical Properties, and Future Perspective Applications in Fields of Pharmacology and Biological Medicine (A Review of Current Applications and Upcoming Potentialities). J. Polym. Environ. 2021, 29, 2359–2371. [CrossRef]
- Rosellini, E.; Zhang, Y.S.; Migliori, B.; Barbani, N.; Lazzeri, L.; Shin, S.R.; Dokmeci, M.R.; Cascone, M.G. Protein/Polysaccharide-Based Scaffolds Mimicking Native Extracellular Matrix for Cardiac Tissue Engineering Applications. J. Biomed. Mater. Res. Part A 2018, 106, 769–781. [CrossRef]
- Makshakova, O.N.; Bogdanova, L.R.; Makarova, A.O.; Kusova, A.M.; Ermakova, E.A.; Kazantseva, M.A.; Zuev, Y.F. κ-Carrageenan Hydrogel as a Matrix for Therapeutic Enzyme Immobilization. *Polymers* 2022, 14, 4071. [CrossRef]
- Wang, M.; Chen, L.; Zhang, Z. Potential applications of alginate oligosaccharides for biomedicine—A mini review. *Carbohydr. Polym.* 2021, 271, 118408. [CrossRef]
- 9. Sahoo, D.R.; Biswal, T. Alginate and its application to tissue engineering. SN Appl. Sci. 2021, 3, 30. [CrossRef]
- 10. Zhong, H.; Gao, X.; Cheng, C.; Liu, C.; Wang, Q.; Han, X. The structural characteristics of seaweed polysaccharides and their application in gel drug delivery systems. *Mar. Drugs* **2020**, *18*, 658. [CrossRef] [PubMed]
- Mahmood, A.; Patel, D.; Hickson, B.; DesRochers, J.; Hu, X. Recent Progress in Biopolymer-Based Hydrogel Materials for Biomedical Applications. *Int. J. Mol. Sci.* 2022, 23, 1415. [CrossRef]
- 12. Shabadrov, P.A.; Safronov, A.P.; Kurilova, N.M.; Blyakhman, F.A. Design of Spherical Gel-Based Magnetic Composites: Synthesis and Characterization. J. Compos. Sci. 2023, 7, 177. [CrossRef]
- 13. Liu, J.; Zhorabek, F.; Chau, Y. Biomaterial design inspired by membraneless organelles. Matter 2022, 5, 2787–2812. [CrossRef]
- 14. Papageorgiou, S.K.; Katsaros, F.K.; Kouvelos, E.P.; Nolan, J.W.; Le Deit, H.; Kanellopoulos, N.K. Heavy metal sorption by calcium alginate beads from Laminaria digitata. *J. Hazard. Mater.* **2006**, *137*, 1765–1772. [CrossRef]
- 15. Kong, C.; Zhao, X.; Li, Y.; Yang, S.; Chen, Y.M.; Yang, Z. Ion-Induced Synthesis of Alginate Fibroid Hydrogel for Heavy Metal Ions Removal. *Front. Chem.* **2020**, *7*, 905. [CrossRef]
- 16. Arıca, M.Y.; Kaçara, Y.; Genç, Ö. Entrapment of white-rot fungus Trametes versicolor in Ca-alginate beads: Preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. *Bioresour. Technol.* **2001**, *80*, 121–129. [CrossRef]
- 17. Bayramoglu, G.; Tuzun, I.; Celik, G.; Yilmaz, M.; Arica, M.Y. Biosorption of mercury(II), cadmium(II) and lead(II) ions from aqueous system by microalgae Chlamydomonas reinhardtii immobilized in alginate beads. *Int. J. Miner. Process.* 2006, *81*, 35–43. [CrossRef]
- 18. Poncelet, D.; Neufeld, R.J.; Goosen, M.F.A.; Burgarski, B.; Babak, V. Formation of microgel beads by electric dispersion of polymer solutions. *AIChE J.* **1999**, *45*, 2018–2023. [CrossRef]
- 19. Bogdanova, L.R.; Rogov, A.M.; Zueva, O.S.; Zuev, Y.F. Lipase Enzymatic Microreactor in Polysaccharide Hydrogel: Structure and Properties. *Russ. Chem. Bull.* **2019**, *68*, 400–404. [CrossRef]
- 20. Bogdanova, L.R.; Zelenikhin, P.V.; Makarova, A.O.; Zueva, O.S.; Salnikov, V.V.; Zuev, Y.F.; Ilinskaya, O.N. Alginate-Based Hydrogel as Delivery System for Therapeutic Bacterial RNase. *Polymers* **2022**, *14*, 2461. [CrossRef] [PubMed]
- 21. Guo, B.; Kamura, Y.; Koilraj, P.; Sasaki, K. Co-sorption of Sr^{2+} and SeO_4^{2-} as the surrogate of radionuclide by alginate-encapsulated graphene oxide-layered double hydroxide beads. *Environ Res.* **2020**, *187*, 109712. [CrossRef]
- 22. Davis, T.A.; Volesky, B.; Mucci, A. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.* 2003, 37, 4311–4330. [CrossRef] [PubMed]
- 23. Ching, S.H.; Bansal, N.; Bhandari, B. Alginate gel particles–A review of production techniques and physical properties. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 1133–1152. [CrossRef]
- 24. Rocher, V.; Siaugue, J.-M.; Cabuil, V.; Bee, A. Removal of organic dyes by magnetic alginate beads. Water Res. 2008, 42, 1290–1298. [CrossRef]
- 25. Li, Y.; Sui, K.; Liu, R.; Zhao, X.; Zhang, Y.; Liang, H.; Xia, Y. Removal of methyl orange from aqueous solution by calcium alginate/multi-walled carbon nanotubes composite fibers. *Energy Procedia* **2012**, *16*, 863–868. [CrossRef]
- Sui, K.; Li, Y.; Liu, R.; Zhang, Y.; Zhao, X.; Liang, H.; Xia, Y. Biocomposite fiber of calcium alginate/multi-walled carbon nanotubes with enhanced adsorption properties for ionic dyes. *Carbohydr. Polym.* 2012, 90, 399–406. [CrossRef] [PubMed]
- 27. Bogdanova, L.R.; Makarova, A.O.; Zueva, O.S.; Zakharova, L.Y.; Zuev, Y.F. Encapsulation of diagnostic dyes in the polysaccharide matrix modified by carbon nanotubes. *Russ. Chem. Bull.* **2020**, *69*, 590–595. [CrossRef]
- Eglitis, R.I.; Bocharov, D.; Piskunov, S.; Jia, R. Review of First Principles Simulations of STO/BTO, STO/PTO, and SZO/PZO (001) Heterostructures. *Crystals* 2023, 13, 799. [CrossRef]

- Li, S.; Li, L.; Guo, C.; Qin, H.; Yu, X. A promising wound dressing material with excellent cytocompatibility and proangiogenesis action for wound healing: Strontium loaded Silk fibroin/Sodium alginate (SF/SA) blend films. *Int. J. Biol. Macromol.* 2017, 104, 969. [CrossRef]
- 30. Gu, Z.; Xie, H.; Li, L.; Zhang, X.; Liu, F.; Yu, X. Application of strontium-doped calcium polyphosphate scaffold on angiogenesis for bone tissue engineering. *J. Mater. Sci.: Mater. Med.* **2013**, *24*, 1251–1260. [CrossRef]
- 31. Zhao, D.; Wang, X.; Tie, C.; Cheng, B.; Yang, S.; Sun, Z.; Yin, M.; Li, X.; Yin, M. Bio-functional strontium-containing photocrosslinked alginate hydrogels for promoting the osteogenic behaviors. *Mater. Sci. Eng. C* 2021, *126*, 112130. [CrossRef] [PubMed]
- Schmidt, R.; Gebert, A.; Schumacher, M.; Hoffmann, V.; Voss, A.; Pilz, S.; Uhlemann, M.; Lode, A.; Gelinsky, M. Electrodeposition of Sr-substituted hydroxyapatite on low modulus beta-type Ti-45Nb and effect on in vitro Sr release and cell response. *Mater. Sci. Eng. C Mater. Biol. Appl.* 2020, 108, 110425. [CrossRef] [PubMed]
- Iskandar, L.; Rojo, L.; Di Silvio, L.; Deb, S. The effect of chelation of sodium alginate with osteogenic ions, calcium, zinc, and strontium. J. Biomater. Appl. 2019, 34, 573–584. [CrossRef]
- Zueva, O.S.; Makarova, A.O.; Zuev, Y.F. Carbon Nanotubes in Composite Hydrogels Based on Plant Carbohydrates. *Mater. Sci. Forum* 2019, 945, 522–527. [CrossRef]
- 35. Makarova, A.O.; Bogdanova, L.R.; Zueva, O.S. Use of Natural Biopolymers to Create Nanocomposite Materials. *Solid State Phenom.* **2020**, 299–304. [CrossRef]
- 36. Zdiri, K.; Cayla, A.; Elamri, A.; Erard, A.; Salaun, F. Alginate-Based Bio-Composites and Their Potential Applications. J. Funct. Biomater. 2022, 13, 117. [CrossRef]
- 37. Sa, V.; Kornev, K.G. A method for wet spinning of alginate fibers with a high concentration of single-walled carbon nanotubes. *Carbon* **2011**, *49*, 1859–1868. [CrossRef]
- 38. Qin, Y. Alginate fibres: An overview of the production processes and applications in wound management. *Polym. Int.* **2008**, 57, 171–180. [CrossRef]
- Lima, A.M.F.; de Lima, M.F.; Assis, O.B.G.; Raabe, A.; Amoroso, H.C.; de Oliveira Tiera, V.A.; de Andrade, M.B.; Tiera, M.J. Synthesis and Physicochemical Characterization of Multiwalled Carbon Nanotubes/Hydroxamic Alginate Nanocomposite Scaffolds. J. Nanomater. 2018, 2018, 4218270. [CrossRef]
- 40. Raus, R.A.; Nawawi, W.M.F.W.; Nasaruddin, R.R. Alginate and alginate composites for biomedical applications. *Asian J. Pharm. Sci.* 2021, *16*, 280–306. [CrossRef]
- Alshehri, R.; Ilyas, A.M.; Hasan, A.; Arnaout, A.; Ahmed, F.; Memic, A. Carbon Nanotubes in Biomedical Applications: Factors, Mechanisms, and Remedies of Toxicity: Miniperspective. J. Med. Chem. 2016, 59, 8149–8167. [CrossRef] [PubMed]
- 42. Zueva, O.S.; Gubaidullin, A.T.; Makarova, A.O.; Bogdanova, L.R.; Zakharova, L.Y.; Zuev, Y.F. Structural Features of Composite Protein-Polysaccharide Hydrogel in the Presence of a Carbon Nanomaterial. *Russ. Chem. Bull.* **2020**, *69*, 581–589. [CrossRef]
- Gubaidullin, A.T.; Makarova, A.O.; Derkach, S.R.; Voron'ko, N.G.; Kadyirov, A.I.; Ziganshina, S.A.; Salnikov, V.V.; Zueva, O.S.; Zuev, Y.F. Modulation of Molecular Structure and Mechanical Properties of κ-Carrageenan-Gelatin Hydrogel with Multi-Walled Carbon Nanotubes. *Polymers* 2022, 14, 2346. [CrossRef] [PubMed]
- 44. Makarova, A.O.; Derkach, S.R.; Kadyirov, A.I.; Ziganshina, S.A.; Kazantseva, M.A.; Zueva, O.S.; Gubaidullin, A.T.; Zuev, Y.F. Supramolecular Structure and Mechanical Performance of κ-Carrageenan–Gelatin Gel. *Polymers* **2022**, *14*, 4347. [CrossRef] [PubMed]
- Atkins, E.; Nieduszynski, I.; Mackie, W.; Parker, K.; Smolko, E. Structural components of alginic acid. I. The crystalline structure of poly-beta-D-mannuronic acid. Results of x-ray diffraction and polarized infrared studies. *Biopolymers* 1973, 12, 1865–1868. [CrossRef]
- Atkins, E.; Nieduszynski, I.; Mackie, W.; Parker, K.; Smolko, E. Structural components of alginic acid. II. The crystalline structure of poly-α-L-guluronic acid. Results of X-ray diffraction and polarized infrared studies. *Biopolymers* 1973, 12, 1879–1887. [CrossRef] [PubMed]
- 47. Usov, A.I. Alginic acids and alginates: Analytical methods used for their estimation and characterisation of composition and primary structure. *Russ. Chem. Rev.* **1999**, *68*, 957–966. [CrossRef]
- Gómez-Ordóñez, E.; Rupérez, P. FTIR-ATR Spectroscopy as a Tool for Polysaccharide Identification in Edible Brown and Red Seaweeds. *Food Hydrocoll.* 2011, 25, 1514–1520. [CrossRef]
- Park, J.; Lee, S.J.; Lee, H.; Park, S.A.; Lee, J.Y. Three Dimensional Cell Printing with Sulfated Alginate for Improved Bone Morphogenetic Protein-2 Delivery and Osteogenesis in Bone Tissue Engineering. *Carbohydr. Polym.* 2018, 196, 217–224. [CrossRef]
- 50. Grant, G.T.; Morris, E.R.; Rees, D.A.; Smith, P.J.C.; Thom, D. Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Lett.* **1973**, *32*, 195–198. [CrossRef]
- Rees, D.A.; Welsh, E.J. Secondary and tertiary structure of polysaccharides in solutions and gels. *Angew. Chem., Int. Ed. Engl.* 1977, 16, 214. [CrossRef]
- 52. Morris, E.R.; Rees, D.A.; Thom, D.; Boyd, J. Chiroptical and stoichiometric evidence of a specific, primary dimerisation process in alginate gelation. *Carbohydr. Res.* **1978**, *66*, 145–154. [CrossRef]
- 53. Mackie, W.; Perez, S.; Rizzo, R.; Taravel, F.; Vignon, M. Aspects of the conformation of polyguluronate in the solid state and in solution. *Int. J. Biol. Macromol.* **1983**, *5*, 329–341. [CrossRef]
- Braccini, I.; Pérez, S. Molecular Basis of Ca²⁺ -Induced Gelation in Alginates and Pectins: The Egg-Box Model Revisited. *Biomacromolecules* 2001, 2, 1089–1096. [CrossRef] [PubMed]
- 55. Donati, I.; Holtan, S.; Mørch, Y.A.; Borgogna, M.; Dentini, M.; Gudmund, S. New hypothesis on the role of alternating sequences in calcium–alginate gels. *Biomacromolecules* **2005**, *6*, 1031–1040. [CrossRef]

- 56. Fang, Y.; Al-Assaf, S.; Phillips, G.O.; Nishinari, K.; Funami, T.; Williams, P.A.; Li, L. Multiple Steps and Critical Behaviors of the Binding of Calcium to Alginate. *J. Phys. Chem. B* 2007, *111*, 2456–2462. [CrossRef] [PubMed]
- 57. Sikorski, P.; Mo, F.; Skjåk-Bræk, G.; Stokke, B.T. Evidence for Egg-Box-Compatible Interactions in Calcium–Alginate Gels from Fiber X-Ray Diffraction. *Biomacromolecules* **2007**, *8*, 2098–2103. [CrossRef]
- Li, L.; Fang, Y.; Vreeker, R.; Appelqvist, I.; Mendes, E. Reexamining the egg-box model in calcium-alginate gels with X-ray diffraction. *Biomacromolecules* 2007, *8*, 464–468. [CrossRef]
- 59. Borgogna, M.; Skjåk-Bræk, G.; Paoletti, S.; Donati, I. On the initial binding of alginate by calcium ions. The tilted egg-box hypothesis. *J. Phys. Chem. B* 2013, 117, 7277–7282. [CrossRef]
- 60. Hecht, H.; Srebnik, S. Structural Characterization of Sodium Alginate and Calcium Alginate. Biomacromolecules 2016, 17, 2160–2167. [CrossRef]
- 61. Hecht, H.; Srebnik, S. Sequence-dependent association of alginate with sodium and calcium counterions. *Carbohydr. Polym.* **2017**, 157, 1144–1152. [CrossRef] [PubMed]
- 62. Wang, H.; Wan, Y.; Wang, W.; Li, W.; Zhu, J. Effect of Calcium Ions on the III Steps of Self-Assembly of SA Investigated with Atomic Force Microscopy. *Int. J. Food Prop.* **2018**, *21*, 1995–2006. [CrossRef]
- Cao, L.; Lu, W.; Mata, A.; Nishinari, K.; Fang, Y. Egg-box model-based gelation of alginate and pectin: A review. *Carbohydr. Polym.* 2020, 242, 116389. [CrossRef] [PubMed]
- 64. Makarova, A.O.; Derkach, S.R.; Khair, T.; Kazantseva, M.A.; Zuev, Y.F.; Zueva, O.S. Ion-Induced Polysaccharide Gelation: Peculiarities of Alginate Egg-Box Association with Different Divalent Cations. *Polymers* **2023**, *15*, 1243. [CrossRef] [PubMed]
- Hu, C.; Lu, W.; Mata, A.; Nishinari, K.; Fang, Y. Ions-induced gelation of alginate: Mechanisms and applications. *Int J Biol Macromol.* 2021, 177, 578–588. [CrossRef]
- Brus, J.; Urbanova, M.; Czernek, J.; Pavelkova, M.; Kubova, K.; Vyslouzil, J.; Abbrent, S.; Konefal, R.; Horský, J.; Vetchy, D.; et al. Structure and Dynamics of Alginate Gels Cross-Linked by Polyvalent Ions Probed via Solid State NMR Spectroscopy. *Biomacromolecules* 2017, *18*, 2478–2488. [CrossRef] [PubMed]
- 67. Agulhon, P.; Markova, V.; Robitzer, M.; Quignard, F.; Mineva, T. Structure of Alginate Gels: Interaction of Diuronate Units with Divalent Cations from Density Functional Calculations. *Biomacromolecules* **2012**, *13*, 1899–1907. [CrossRef]
- Hassan, R.M. Prospective and comparative Novel technique for evaluation the affinity of alginate for binding the alkaline-earth metal ions during formation the coordination biopolymer hydrogel complexes. *Int. J. Biol. Macromol.* 2020, 165, 1022–1028. [CrossRef] [PubMed]
- Santhanes, D.; Teng, L.Y.; Sheng, F.S.; Coombes, A.G.A. Exploiting the versatility of oral capsule formulations based on high M-alginate for targeted delivery of poorly water soluble drugs to the upper and lower GI tract. *J. Drug Deliv. Sci. Technol.* 2018, 46, 384–391. [CrossRef]
- 70. Zhang, X.; Wang, L.; Weng, L.; Deng, B. Strontium ion substituted alginate-based hydrogel fibers and its coordination binding model. *J. Appl. Polym. Sci.* 2020, 137, 48571. [CrossRef]
- Smidsrød, O.; Whittington, S.G. Monte Carlo Investigation of Chemical Inhomogeneity in Polymers. *Macromolecules* 1969, 2, 42–44. [CrossRef]
- 72. Larsen, B.; Painter, T.J. The periodate-oxidation limit of alginate. Carbohydr. Res. 1969, 10, 186–187. [CrossRef]
- 73. Montanucci, P.; Terenzi, S.; Santi, C.; Pennoni, I.; Bini, V.; Pescara, T.; Basta, G.; Calafiore, R. Insights in Behavior of Variably Formulated Alginate-Based Microcapsules for Cell Transplantation. *BioMed Res. Int.* **2015**, 2015, 965804. [CrossRef] [PubMed]
- Mørch, Ý.A.; Donati, I.; Strand, B.L.; Skjåk-Bræk, G. Effect of Ca²⁺, Ba²⁺ and Sr²⁺ on Alginate Microbeads. *Biomacromolecules* 2006, 7, 1471–1480. [CrossRef] [PubMed]
- Bajpai, S.K.; Sharma, S. Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca²⁺ and Ba²⁺ ions. *React. Funct. Polym.* 2004, 59, 129–140. [CrossRef]
- 76. Zimmermann, U.; Mimietz, S.; Zimmermann, H.; Hillgärtner, M.; Schneider, H.; Ludwig, J.; Hasse, C.; Haase, A.; Rothmund, M.; Fuhr, G. Hydrogel-Based Non-Autologous Cell and Tissue Therapy. *BioTechniques* 2000, 29, 564–581. [CrossRef]
- 77. Huang, S.-L.; Lin, Y.-S. The Size Stability of Alginate Beads by Different Ionic Crosslinkers. *Adv. Mater. Sci. Eng.* 2017, 2017, 9304592. [CrossRef]
- 78. De la Cruz, E.F.; Zheng, Y.; Torres, E.; Li, W.; Song, W.; Burugapalli, K. Zeta Potential of Modified Multi-Walled Carbon Nanotubes in Presence of Poly (Vinyl Alcohol) Hydrogel. *Int. J. Electrochem. Sci.* **2012**, *7*, 3577–3590. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.