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# Preparation and Preliminary Characterization of Hybrid Alginate – Carrageenan Aerogel: Effect of Gelation Methods

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**Abstract:** Aerogels are a class of nanoporous structured material with a high specific surface area, large porosity and open pore structure. Usually, they are produced by substituting the solvent of a stable gel with air without affecting the 3-D network of the gel. It is possible to engineer the produced material by controlling the precursors, gelling mechanism and drying process. In this work, hybrid aerogel based on alginate and three different types of carrageenan was produced using supercritical fluid technology. $CO_2$-induced gelation, as well as GdL-induced gelation, were evaluated for their effect on final textural properties of the produced aerogel. $CO_2$-induced gelation method shows enhanced aerogel properties and can be further investigated for the scale-up application. Nevertheless, GdL-induced gelation is easier to perform and produced a smaller specific surface area aerogel if compared with $CO_2$-induced gelation method. Hybrid alginate-carrageenan aerogels were produced with high surface area (390-566) $m^2 \cdot g^{-1}$ and large pore volume (4.2-6.8) $m^3 \cdot g^{-1}$ and with a mesoporous structure (3.2 – 26.8) nm. The produced aerogels have great potential for future biotechnological and pharmaceutical applications.

**Keywords:** Hybrid Aerogel, Alginate, Carrageenan, Supercritical Fluid Extraction, Drug Carrier.

## Introduction

Aerogels are a class of nanoporous materials that are prepared by removing the swelling solvent of a gel without substantially affecting the 3-D network of its structure [1]. The hierarchical structure of aerogels produces many unique properties, such as open pore structure with extremely high porosities (greater than 90%), low thermal conductivity (10-30 W/m·K), very low density (as low as 0.003 $g \cdot cm^{-3}$) and high surface areas (200-1000 $m^2 \cdot g^{-1}$). As a result, aerogels have gained great attention for a various range of applications, such as aeronautics, energy conservation, environmental applications, food, biomedicines, drug delivery, among many other applications [2–7].

Due to non-toxicity, stability, availability and renewability, natural polysaccharides and their derivatives-based aerogels are gaining more attention for biomedical and pharmaceutical applications. Furthermore, they allow coupling aerogel properties with their biodegradability, biocompatibility and surface functional properties [9, 10].

In addition, the broad portfolio of bio-based polysaccharides allows their use in

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pharmaceutical products with different routes of delivery, target organs and/or drug release profiles [5, 6, 11, 12]. Therefore, the drug loading capacity is largely influenced by the chemical structure of the matrix, porosity and surface area of the aerogels. Surface alteration of the gel can hugely play an important role in the release and bioavailability of the entrapped drug, whereas the absorption and release of drugs can be adjusted by matrix synthesis conditions [13].

Polysaccharides are known also as glycans, belonging to the carbohydrates. They are built from mono-saccharides or sugars that are covalently linked together by the glycosidic bond, forming a linear or branched polymeric chain [14–16]. Alginate is a well-known biopolymer known for its biocompatibility, low toxicity, relatively low cost, stability, availability, renewability and simple gelation methods. It is considered a prominent component for food, textile and paper industries, as well as for pharmaceutical and medical products [17]. Alginate is a negatively charged linear polysaccharide composed of 1,4-linked ß-D-mannuronate (M) and 1,4-linked α-L-guluronate (G) residues. G-blocks of alginate can generate an "egg-box"-like structure hydrogel in contact with divalent cations, such as $Ca^{2+}$, $Ba^{2+}$ and $Sr^{2+}$ (Fig. 1) [18].

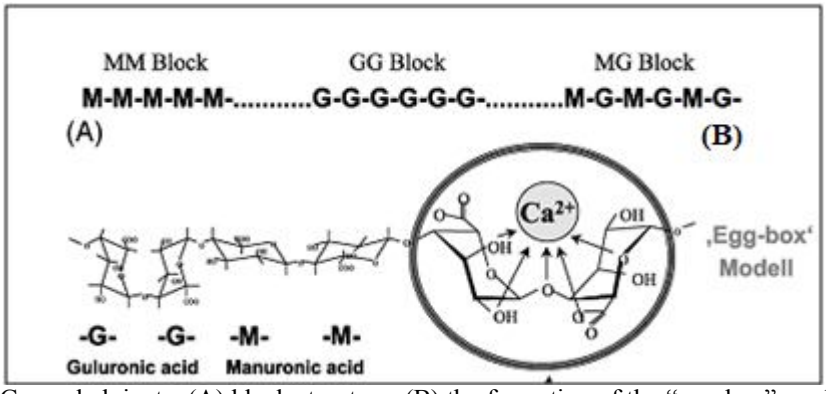


FIG. 1. General alginate, (A) block structure; (B) the formation of the "egg-box" model [14].

On the other hand, carrageenans are a family of seaweed derived polysaccharides composed of alternating α-(1,3) and ß-(1,4) glycosidic links of D-galactose and 3,6-anhydro-L-galactose repeat units. Carrageenan contains high (15 – 40%) sulfate ester contents. There are three major types of carrageenans designated by the Greek letters kappa, iota and lambda, which differ in the degree of galactose unit sulforation and dehydration. The sulfate groups impart an anionic character and accordingly carrageenans can undergo either thermotropic or ionotropic gelation using cations, such as potassium and calcium ions [19].

Hybrid aerogel has been investigated by different researchers; it is composed of two or more chemically or physically bound components [20–22]. The main advantage of such hybrid components is that they inherit the intrinsic properties of aerogel with enhanced and adjustable mechanical properties, wettability and chemical functionality [23].

The use of $CO_2$ as a gelation-inducing method for alginate has been reported previously [21, 24]. The technique has shown some advantages over traditionally used methods [25–27], such as the process allowing to avoid ambient pressure solvent exchange with the possibility of being directly combined with subsequent supercritical drying, in addition, fast depressurization leads to foam like hydrogel [28].

The aim of this study is to develop a hybrid aerogel composed of alginate and different types of carrageenans as the second biopolymer for potential future work in drug delivery. Moreover, this work demonstrates the use of high-pressure $CO_2$ as well as the addition of glucono delta-lactone (GdL) to induce cross-linking of the hybrid biopolymers.

This preliminary characterization of hybrid aerogel will form the basis for future work to be carried on and for extending the use of such aerogels into pharmaceutical applications as drug loadings.

## Materials and Methods

### Materials

Sodium alginate, κ-carrageenan, ί-carrageenan, λ-carrageenan and calcium carbonate were purchased from Sigma Aldrich. D-(+)-Glucono-delta-Lactone (GdL) was obtained from Guangzhou Fischer Chemical Co. Absolute ethanol was supplied by Solvochem, Holland. Carbon dioxide ($CO_2$ 99.99%) was provided by the Jordanian Gas Co., Jordan.

All chemicals were used as supplied without any further modification.

### Methods

Fig. 2 shows the general procedure of preparation of aerogel from polysaccharide precursors followed in this work. Firstly, the precursor should be hydrated with deionized water to prepare the needed concentration of the biopolymer. Then, the sol is cross-linked using a chemical or physical cross-linking method to obtain a 3-D network hydrogel structure. After the gel is aged, a stepwise solvent exchange is used to convert the hydrogel into alcogel. Finally, the solvent of the alcogel is removed using supercritical fluid extraction leaving the 3-D nanoporous structure intact.

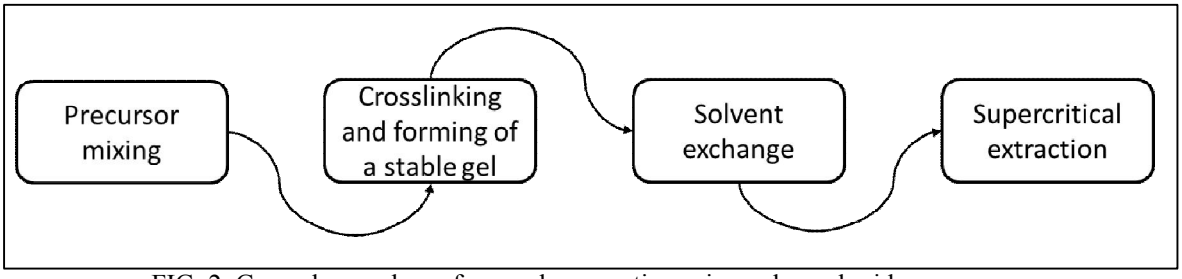


FIG. 2. General procedure of aerogel preparation using polysaccharide precursors.

### Preparation of the Hybrid Aerogel

TABLE *1* shows the prepared samples in this work. Two main process parameters were investigated; namely: (1) carrageenan type and (2) cross-linking method. Other parameters like $CO_2$-induced gelation conditions (temperature, pressure and time), solvent exchange process, biopolymer ratios and biopolymer concentration are still under investigation.

TABLE 1. Sample preparation conditions used in this work.

| Sample ID | First polymer | Second polymer | Mixing ratio | Cross-linking method |
|---|---|---|---|---|
| A1 | Alginate 1wt% | Alginate 1 wt % | 1:1 | $CO_2$-induced gelation |
| L1 | Alginate 1wt% | λ-carrageenan 1 wt% | 1:1 | $CO_2$-induced gelation |
| I1 | Alginate 1wt% | ί-carrageenan 1 wt% | 1:1 | $CO_2$-induced gelation |
| K1 | Alginate 1wt% | κ-carrageenan 1 wt% | 1:1 | $CO_2$-induced gelation |
| A2 | Alginate 1wt% | Alginate 1 wt % | 1:1 | GdL |
| L2 | Alginate 1wt% | λ-carrageenan 1 wt% | 1:1 | GdL |
| I2 | Alginate 1wt% | ί-carrageenan 1 wt% | 1:1 | GdL |
| K2 | Alginate 1wt% | κ-carrageenan 1 wt% | 1:1 | GdL |

The detailed procedure of the hybrid aerogel preparation process is described below.

### Preparation of the Biopolymer Solution

Sodium alginate and three different types of carrageenan solutions (kappa κ, iota ί and lambda λ carrageenans) were prepared by dissolving a certain amount of dry biopolymer powder in deionized water to form the needed concentration of the biopolymer solution. The solutions were left under mixing for 24 hours to ensure complete hydration of the biopolymer. An amount of 0.1825 g $CaCO_3$ was mixed vigorously with alginate solution for each gram of sodium alginate used in the preparation. The carrageenan solution was then added to the alginate-$CaCO_3$ suspension to prepare the desired ratio.

### Gelation

Two different procedures were used to induce the release of $Ca^{+2}$ and make it available for

cross-linking. The first approach used was $CO_2$-induced gelation. The second approach was the addition of GdL to slowly reduce the pH of the hybrid solution to induce cross-linking. In the first one, the mixed suspension of the previous step was transferred to cylindrical moulds and placed in a 1L high-pressure stainless vessel. $CO_2$ was pumped to the cylinder slowly to obtain a pressure of 50 bar. This condition was kept overnight at room temperature. The second approach is based on the addition of GdL to the alginate/carrageenan mixture to make a molar ratio of 0.5 $CaCO_3$:GdL. The resultant suspension was transferred to cylindrical moulds and left overnight to gel.

**Hydrogel to Alcogel**

The water phase of the gel was replaced with ethanol following a stepwise solvent exchange process. The solvent exchange steps were: 10, 30, 50, 70, 90, 100, 100 wt%. In each step, equilibrium was allowed over 24 hours.

**Alcogel to Aerogel**

The resulting alcogel produced in the previous step was dried using a continuous flow supercritical extraction unit described elsewhere [29]. Briefly, the wet gel was placed in a temperature-controlled 500-ml stainless steel vessel. $CO_2$ was pumped continuously from the bottom of the vessel at a constant flow of 100 g/min using a high-pressure piston pump. The vessel pressure was controlled using a pneumatic backpressure valve and kept constant at 100 bar. The $CO_2$-ethanol phase was driven to a separation vessel. The condition of the separation vessel was kept at about 60 bar and 40 °C to ensure phase separation. The solvent was collected from the bottom, while the ethanol lean $CO_2$ was recycled to the extraction vessel. The extraction process was performed for over six hours. Fresh $CO_2$ was introduced several times to the extraction cycle.

**Scanning Electron Microscopy (SEM)**

The surface morphology of the samples was obtained using Quanta FEG 450, SEM (FEI, US). Before performing SEM analysis, the samples were placed on stubs and coated with platinum under vacuum atmosphere using Q150R Rotary-Pumped Sputter Coater/ Carbon Coater (Quorum Technologies, UK).

**Surface Area and Porosity Analysis**

The specific surface area and porosity of the prepared particles were determined by Autosorb-1 Series surface area and pore size analyzer, Quantachrome, USA.

## Results and Discussion

Alginate is a natural ionic polysaccharide composed of repeated β-(1–4) linked D-mannuronic acid and α-L-guluronic acid units. In the presence of divalent cations like $Ca^{2+}$ alginate form stable gel [30]. Carrageenan is a natural polysaccharide composed of alternated 1,3-linked β-D galactose and 1,4-linked α-D-galactose with various degrees of sulfatation. Because of the half ester sulfate, carrageenans are strong anionic and can form gel in the presence of mono-or divalent cations [31]. Hybrid aerogel based on alginate and three different types of carrageenan was prepared following two different procedures. Both procedures are categorized under the internal setting method for the preparation of alginate gel [8, 10, 24, 32, 33]. In this technique, the well-dispersed $CaCO_3$ in the biopolymer solution will release the $Ca^{+2}$ ions upon lowering the pH of the sol phase. After that, the cations will be available for cross-linking and a homogeneous cross-linking will take place.

$CO_2$ induced gelation method is relatively new and was firstly proposed by Raman et al. as a promising step toward continuous production of alginate-based aerogels [24]. The pH of the sol phase is reduced by forming carbonic acid upon dissolving of $CO_2$ in the water phase at 50 bar pressure. the extent of carbonic acid formation depends mainly on the system temperature and pressure [34, 35]. The second approach uses GdL (glucono-delta-Lactone), which is the ester of gluconic acid. Upon contact with water, the ester hydrolyzes forming gluconic acid which in turn reduces the pH of the sol phase [36–38].

TABLE *2* shows the textural properties of the prepared samples, where a high specific surface area from all preparations was achieved. The maximum surface area was obtained from alginate sample without any hybridization with another biopolymer (566 $m^2/g$). Nevertheless, hybrid aerogels have also a relatively high specific surface area compared to alginate alone (390 – 525 $m^2/g$). Moreover, all preparation results give a mesoporous structure with a pore size range of 17.2 – 26.8 nm. The pore volume

of the prepared samples was relatively large (4.2 - 6.8) $cm^{-3}$ $g^{-1}$.

It is possible to state that both approaches produced comparable aerogel properties with a slight improvement for the $CO_2$-induced gelation preparations in comparison with GdL preparations. The slight difference can be due to the nature of pH reduction. In $CO_2$-induced gelation, carbon dioxide will continue to dissolve in the water phase and pH will be reduced slowly until equilibrium is reached. This allows a slow release of $Ca^{2+}$ ions and the formation of a homogeneous network. Using GdL reduces the pH instantaneously upon addition to the polymer solution and makes the $Ca^{2+}$ ions available for cross-linking at once. L1 and L2 preparations contain λ-carrageenan-produced aerogel with the highest specific surface area of 525 and 511 $m^2/g$, respectively. This can be due to the presence of three sulfated groups available for cross-linking with $Ca^{2+}$ ions, whereas ί-carrageenans contain two sulfated group and κ-carrageenans contain one sulfated group. Nevertheless, κ-carrageenan aerogel preparation shows better specific surface area if compared with ί-carrageenan. However, all preparations result in a comparable pore volume and pore size distribution. Raman et al. reported the production of hybrid alginate-based aerogel and other biopolymers, like starch, pectin and carrageenan. The reported specific surface area for λ-carrageenan was 446 $m^2/g$ with 2.23 $cm^3/g$ as a pore volume. With alginate alone, these values were 586 $m^2/g$ and 5.97 $cm^3/g$ for specific surface area and pore volume, respectively [24]. Gonçalves et al. proposed alginate-based aerogel microparticles for mucosal drug delivery. In their publication, pH was reduced using acetic acid. Alginate aerogel microparticles were produced with 330 $m^2/g$ and 1.7 $cm^3/g$ surface area and pore volume, respectively. Further, they produced alginate-κ-carrageenan aerogel with 415 $m^2/g$ and 3.6 $cm^3/g$ for surface area and pore volume, respectively [21]. Robitzer et al. on the other hand reported the preparation of alginate aerogel following the diffusion method, where alginate solution is dropped into $CaCl_2$ solution. They reported 495 $m^2/g$ and 3.9 $cm^3/g$ for surface area and pore volume, respectively. It is possible to say that the method used in this work produces better textural properties of hybrid aerogel in comparison to what is available in the literature. Nevertheless, a complete parametric study of the presented process is needed to understand the significance of each parameter and to develop an optimized process for the production of hybrid aerogel materials. Further, the effect of available functional groups on the hybrid aerogel surface will be further evaluated by testing the adsorption capacity of each preparation for different types of drugs. These two major points will be the subject of our next publication.

TABLE 2. Surface properties of the prepared samples in this study.

| Sample ID | Surface area ($m^2$ $cm^{-3}$) | Pore volume ($cm^3$ $g^{-1}$) | Pore size (nm) |
|---|---|---|---|
| A1 | 566 ± 28 | 6.8 ± 0.3 | 26.8 ± 1.3 |
| L1 | 525 ± 26 | 5.6 ± 0.2 | 17.2 ± 2.3 |
| K1 | 503 ± 25 | 5.5 ± 0.3 | 21.4 ± 1.1 |
| I1 | 390 ± 19 | 5.6 ± 0.3 | 21.8 ± 2.1 |
| A2 | 482 ± 24 | 4.2 ± 0.2 | 22.5 ± 1.1 |
| L2 | 511 ± 25 | 6.5 ± 0.2 | 17.3 ± 0.7 |
| K2 | 482 ± 24 | 5.7 ± 0.1 | 22.3 ± 1.1 |
| I2 | 419 ± 21 | 4.2 ± 0.1 | 17.1 ± 0.9 |

Fig. 3 shows the SEM images of the samples prepared by the $CO_2$-induced gelation method. Although the textural properties of all prepared samples were comparable, it is possible to differentiate between the samples in terms of the surface structure. Sample A1 shows a more dense and uniform structure in comparison with other samples. Samples L1, K1 and I1 show a more intense fibrous airy structure with relatively more loose interconnectivity. These textural properties can be explained in light of the preparation procedure; the gelation of all hybrid combinations was based on cross-linking of alginate and the co-gelation of carrageenan. Such a process allows the presence of more voids and less intensity of cross-linking. In addition, $Ca^{2+}$ can be used as well for the cross-linking of carrageenans [29, 39, 40].

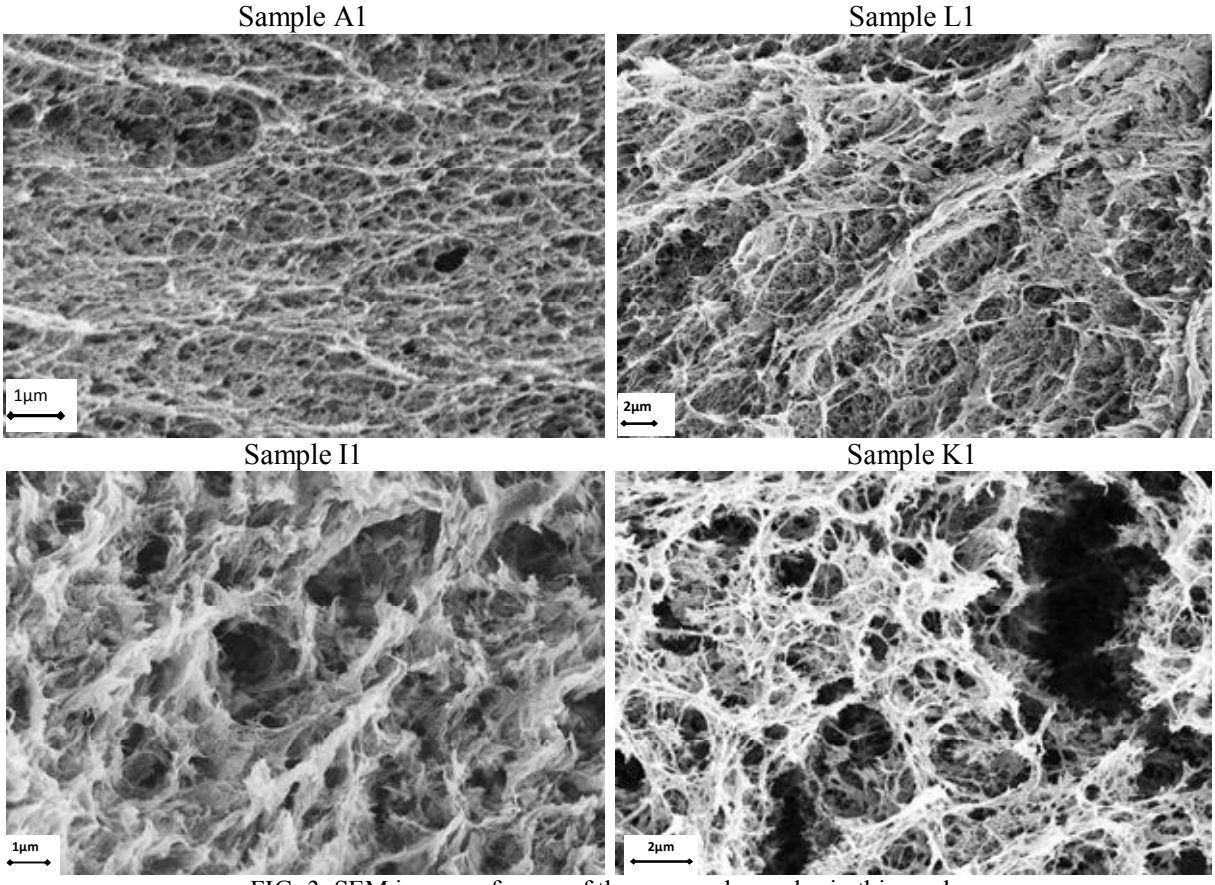


FIG. 3. SEM images of some of the prepared samples in this work.

As can be seen from the results in TABLE *2* and Fig. 3, nanoporous structured materials based on polysaccharides with a high surface area and accessible pore volume were produced. The produced material can serve as a potential drug carrier [21, 41]. The drug can be loaded into the aerogel structure using supercritical $CO_2$ or prior to the extraction process during the solvent exchange process [42]. The loading of the drug can be controlled by varying the surface functional group through hybridization with different polysaccharides or different mixing ratios [43].

The drug loading of different active materials on the aerogel structure will be investigated in a future study. The effect of different backbone structures of the prepared aerogels on the release properties of the loaded drugs will be evaluated *in vitro* and *in vivo*.

## Conclusion

Hybrid aerogels based on alginate and three different types of carrageenan were successfully prepared. $CO_2$-induced gelation and GdL-induced gelation methods were compared in terms of the final textural properties of the produced aerogels. Both procedures result in an aerogel with high surface area (390-566) $m^2\ g^{-1}$ and large pore volume (4.2-6.8) $m^3\ g^{-1}$ with a mesoporous structure (3.2 – 26.8) nm. $CO_2$-induced gelation method shows a slight improvement of aerogel properties in comparison with GdL-induced gelation method. $CO_2$-induced gelation is a promising technique that can be scaled up to industrial scale and promote the use of aerogels in industrial applications. The produced hybrid aerogel will be further investigated and proposed as a possible drug carrier for drug delivery applications.

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