Studies on aging mechanism of agarose gels

Summary

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論文題目 Title	STUDIES ON THE AGING MECHANISM OF AGAROSE GELS		

Gel is a state of matter that consists of the polymer network and huge amount of solvent which are formed from colloidal polysaccharides, proteins, and synthesized polymers. Because of the structure, gels are widely used in food, bio-medical, pharmaceutical, cosmetic, paint, and chemical industries. One of the most widely used food gel is agarose. Agarose is a linear and sulfate-free polysaccharide extracted from red seaweeds. It is widely used in food and separation technologies due to its gelling properties. At high temperature, agarose chains appear to be in random coil conformation and reaching gelling temperature; coils reorder to form helices and subsequently aggregate to form a gel network. Thick bundles of aggregates form in the network of this polysaccharide thereby forming rigid and turbid gels. The formation of thick bundles leads to the thinning of polymer chains among the bundles because of which the local viscosity is reduced, which in turn markedly affects the molecular diffusion in gels. Food gels, like agarose, are commonly used as food additives as thickener and stabilizers and are stored at different storing conditions as based on the convention of how it is processed in food hence it is a common practice that food hydrocolloids are stored and aged. However, there is a poor knowledge on how aging effect would affect the structural changes in gels. It is therefore of considerable interest to investigate the aging mechanism of food gels and agarose is known as the model of food hydrocolloids. In this present work, aging effect in agarose gels was studied using different techniques, NMR and electrophoresis.

Nuclear Magnetic Resonance (NMR) Spectroscopy is a powerful tool to study the physical, chemical, and biological properties of matter. It is used in different applications in several areas of science and routinely used to study the chemical structure and properties of polymers. In food hydrocolloid systems, NMR techniques play an important role in characterizing the structures and dynamics of food polymers. Self-diffusion is the random translational motion of molecules driven by internal kinetic energy. This translational diffusion is the basic mechanism by which molecules are distributed in space and is considered to play a central role in any chemical reaction since the reacting species have to collide before the reaction can occur. The classical description of diffusion is via Fick’s laws. Regarding the molecular mobility of polymers, the tumbling and translational motions of chains change markedly during aggregation and gelation, which affect the *D* and NMR relaxation times. The *D* measured by field gradient NMR reflects the displacement of a molecule by self-diffusion which in turn describes

the interspaces in the gel. Synthetic gels mostly have chemically cross-linked network structures in which the network polymer chains in the gel are more immobile than the polymer chains in dilute solutions. As a result, the polymer concentration is usually homogeneous above a length scale of several nm. On the other hand, physically cross-linked gels such as agarose gel, have concentration fluctuations over larger length scales. This is due to the aggregation of the agarose polymer chains that are responsible in the gelation process. Analysis on the probe polymer diffusion in gel systems will give information on the structure and dynamics of the hydrogels. In some cases, the network polymer may exist as a spatial obstruction in gel systems that consist of probe polymer, network polymer without any functional groups interacting strongly with probe polymer.

Changes of the network structure in agarose gels by aging during storage were studied using PGSTE ^{1}H NMR and gel electrophoresis. Electrophoresis technique is widely used in separation of DNA into different molecular sizes. 20bp DNA ladder is used in the experiment and the changes in the electrophoretic distance with increasing storage time is measured. Results showed a considerable change of the physical properties during storage which is considered to come from the change of the network structure. The gel electrophoresis using DNA ladder in the aged agarose gels were carried out and the *D* of DNA were calculated from the friction on the DNA movement under the electric field to show an increase of *D* with storage to allow a faster movement suggesting structural network changes in the agarose gel during storage. These changes of network structure in agarose gels by aging during storage were also studied using the pulsed-field-gradient stimulated echo (PFGSTE) ^{1}H NMR. PGSTE NMR method is used to determine the network structure and aging behavior of agarose solutions in different storage conditions. The decay of the echo signal intensity with increased gradient strength reflects the displacement of a molecule due to self diffusion which is used as the molecule's diffusion coefficient relating to the viscosity and the local interspatial environment of the medium. Pullulan which is a maltotriose trimer made up of α-(1 → 6)-linked (1 → 4)-α-d-triglucosides with a chemical formula of $C_6H_{10}O_5$ is used as a probe molecule in the agarose gel. The aim of this chapter is to indicate the advantages of NMR diffusion measurements in clarifying the mobility and dynamics of molecules in gel. Measurements of the diffusion coefficient (*D*) by NMR revealed that *D* of the pullulan (D_{pull}) added as a probe polymer increased with storage time suggesting that the formation of thicker bundles of agarose aggregates made larger spaces to allow the faster diffusion. The hydrodynamic mesh size of network was estimated from the degree of restriction in the diffusion to shed light on the change in the microscopic environment of the agarose gel during aging. The PGSTE NMR method was used to investigate the change in the diffusivity of pullulan as a probe polymer which provided information of the change in the network structure of aged agarose gels. The restriction on diffusion of pullulan in the agarose gel was treated within the frame of the hydrodynamic approach. The increase of D_{pull} / $D_{pull,0}$

observed during storage was deduced that the very loose agarose aggregates subsequently involved in the formation of aggregates and the significant change of the agarose network structure happened after 3 days of storage. The change in the hydrodynamic mesh size, ξ_H, yielded a physical picture of the change in the microscopic environment by the aging during storage.

In food industry, it is a concern that water movement is likely due to the water separation during storage and is seen as one of the challenges in the food products as it will determine food shelf-life. In this study, the effect of the addition of iota carrageenan on the aging mechanism of agarose gels. The addition of KCl was also investigated to introduce the formation of gel network of IC gels. PGSTE NMR measurements revealed the microscopic change of the agarose and agarose and IC networks by aging during storage. Results showed an increase of D_{pull} in pure agarose which supports the change in the network structure of agarose gels by aging during storage. With the addition of IC proton peak remained with increased storage suggest that solute IC chains remained in the interspaces of the network. Addition of KCl promote formation of loose aggregates of IC with increased storage time. This was also evident from the decrease of the IC peak. No significant changes were observed from the D_{pull} for sample with addition of IC due to the hydrodynamic restriction of the iota short chains on the pullulan. Consequently, no D_{pull} significant changes were also observed in the sample with the addition of IC and KCl even though KCl promotes aggregation of IC chains due to the assumption of formation of interpenetrating agarose and IC gel network that restricts the hydrodynamic behavior of pullulan.

The aging mechanism of agarose gel from the NMR and electrophoresis measurements and the effect of additional of iota carrageenan and potassium chloride on the behaviors of agarose gels. Furthermore, this study is essential to separation technologies to get accurate results in fractionating biopolymers by their molecular sizes. Alternatively, this is also industrially important to products that use agarose as additives to help understand the challenges on the food aging which relates to the palatability and safety of consumers, flavor release and textural attributes.