Article

Gallic Acid/2-Hydroxypropyl-β-cyclodextrin Inclusion Complexes Electrospun Nanofibrous Webs: Fast Dissolution, Improved Aqueous Solubility and Antioxidant Property of Gallic Acid

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Gallic acid(GA) is a kind of natural polyphenolic compound, but its low aqueous solubility restricts its application in the fields of food and medicine. Cyclodextrin can form inclusion complexes with guest molecules(*e.g.*, essential oils, food supplements) through cavities with special properties to improve aqueous solubility, thermal stability, and bioavailability of guest molecules. In this research, gallic acid/2-hydroxypropyl-β-cyclodextrin inclusion complexes(GA/2-HP-β-CD/ICs) were formed in a highly concentrated solution of 2-HP-β-CD. Bead-free and uniform nanofibrous webs(GA/2-HP-β-CD/IC-NWs) were produced successfully by electrospun GA/HP-β-CD/IC aqueous solution. The initial molar ratio(GA: 2-HP-β-CD=1:1) of GA/2-HP-β-CD/IC in the solutions was largely maintained in GA/2-HP-β-CD/IC-NW. The aqueous solubility of GA was enhanced and GA/2-HP-β-CD/IC-NW has displayed fast dissolution property. Furthermore, in comparison with GA powder, GA/2-HP-β-CD/IC-NW demonstrated improved antioxidant capacity. The results suggested that GA/2-HP-β-CD/IC-NW have broad application prospects as orally fast dissolution systems for food supplements.

Keywords Gallic acid; 2-Hydroxypropyl-β-cyclodextrin; Inclusion complex; Electrospun; Fast dissolution

1 Introduction

Gallic acid(GA) is a kind of natural phenolic compound, commonly presents in plants, such as rhubarb, eucalyptus grandis and dogwood, and usually exists in the form of monohydrate. It has anti-oxidation, anti-diabetes, anti-inflammation, anti-cancer, antibacterial and other effects[1]. However, gallic acid has a poor aqueous solubility, which restricts its application in the food and medicine industry[2,3]. It is of great significance to take appropriate measures to increase its solubility and bioavailability and improve its antioxidant activity of gallic acid.

Cyclodextrin(CD) is a circular oligomeric maltose with special hydrophilic and hydrophobic cavities formed from *D*-pyranoid glucose units connected with α-(1→4) glycoside bonds[4]. The cavity with special properties can form inclusion complex with guest molecules(*e.g.*, essential oils and food supplements) to enhance the aqueous solubility, thermal stability and bioavailability of the guest molecules[5—8]. Natural cyclodextrin has lower aqueous solubility at room temperature. The hydroxypropylated cyclodextrin, methylated cyclodextrin and sulfobutylated cyclodextrin obtained by chemical modification have high aqueous solubility, and curcumin, menthol, eugenol and other biologically active substances are encapsulated by modified cyclodextrin, which improves their solubility in water[9—11].

In recent years, with the rapid rise of nanotechnology, electrospun has become an effective method for direct and continuous preparation of nanofibers due to its simple operation and mild conditions[12,13]. It has been commonly used in food industry as an emerging nano-encapsulation technique, such as encapsulating antibacterial agents, antioxidants, vitamins, probiotics and so on[14—17]. Compared with other encapsulating techniques for bioactive compounds, electrospun has unique advantages, such as nanoscale fibers and adjustable nanofiber diameter, large specific surface area, extremely high porosity, and very high encapsulation efficiency[18]. Due to its unique structural advantages, the nanofibrous webs(NWs) obtained by electrospun hydrophilic compounds have the property of fast dissolution in water, which is greatly beneficial for the fast-dissolving delivery systems. In recent study, Celebioglu *et al.*[7] achieved fast-dissolving of alpha-lipoic acid through electrospun cyclodextrin inclusion complexes to obtain nanofibrous webs, as a potential orally fast dissolution food supplement. Yildiz *et al.*[19] reported electrospun cinnamaldehyde/cyclodexin inclusion complexes nanofibers(CD-IC NFs) have potential applications in food, oral-care, healthcare, and pharmaceutics due to their fast dissolution, improved aqueous solubility and

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high temperature stability. However, as far as we know, no research has reported the gallic acid/cyclodextrin inclusion complexes of electrospun nanofibers to develop orally fast-dissolving food supplements.

In this research, the electrospun of highly concentrated aqueous solutions of cyclodextrin inclusion complexes of 2-hydroxypropyl-betacyclodextrin(2-HP-β-CD) was successfully prepared in order to obtain nanofibrous webs(GA/2-HP-β-CD/IC-NW)(Fig.1). The nanofibrous webs should improve the aqueous solubility of gallic acid significantly. At the same time, since no additional polymer matrix and no organic solvent have been used, the safety of the obtained nanofibrous webs is improved obviously, which is crucial for food applications. The morphology and structure of GA/2-HP-β-CD-IC nanofibers were characterized in detail. By comparing with gallic acid powder, the properties of solubility, dissolution and disintegration, thermal stability and antioxidant activity of nanofibrous webs were studied.

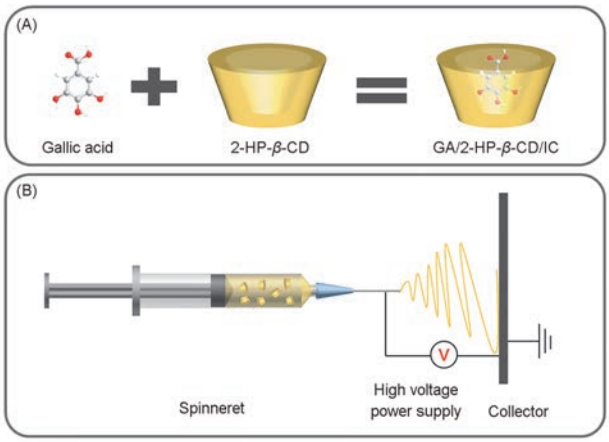

Fig.1 Schematic diagrams of IC formation between GA and 2-HP-β-CD(A) and the electrospun of GA/2-HP-β-CD/IC-NW(B)

2 Experimental

The information of this part can be seen in the Electronic Supplementary Material of this paper.

3 Results and Discussion

3.1 Phase Solubility

The phase solubility study was demonstrated that the apparent solubility of gallic acid improved with increasing 2-HP-β-CD concentration. The GA/2-HP-β-CD solutions of different concentrations were stirred for 12 h to achieve dynamic complexation equilibrium. Then the undissolved gallic acid in the solution was removed from each sample and the absorbance of the solution was analyzed using UV-Vis spectroscopy.

Fig.2 shows the phase solubility graph, which indicated the solubility trend of gallic acid when the concentration of 2-HP-β-CD was improved from 0 to 240 mmol/L. Gallic acid solubility(*ca.* 67 mmmol/L) was increased *ca.* 4 times in the 240 mmmol/L concentrated solutions of 2-HP-β-CD due to the formation of GA/2-HP-β-CD inclusion complexes. According to the report of Celebioglu *et al.*[9], the stoichiometric changes of cyclodextrin and guest molecules during inclusion complexation determine the different types of phase solubility diagrams. A-type phase solubility diagram is mainly found in the modified cyclodextrin system. There are three subtypes, A_P, A_N and A_L, which respectively represent the positive deviation, negative deviation and linear increase of cyclodextrin concentration on guest solubility[9]. As is illustrated in Fig.2, the phase solubility graph of GA/2-HP-β-CD inclusion complexes was A_L type. With the concentration of 2-HP-β-CD increasing, the solubility of gallic acid increased linearly, which also indicated the 1:1 molar ratio inclusion complexes formation of gallic acid and 2-HP-β-CD. The stability constant(K_s) of GA/2-HP-β-CD/IC can be calculated by the phase solubility graph. The K_s value represents the binding strength between gallic acid and 2-HP-β-CD cavity. The larger the K_s, the greater the binding strength between gallic acid and 2-HP-β-CD cavity. In our research, the K_s value of 203.4 L/mol was determined for GA/2-HP-β-CD system, which is larger than the reported one in another research[20]. Our finding indicated that more stable inclusion complexes were formed between gallic acid and 2-HP-β-CD.

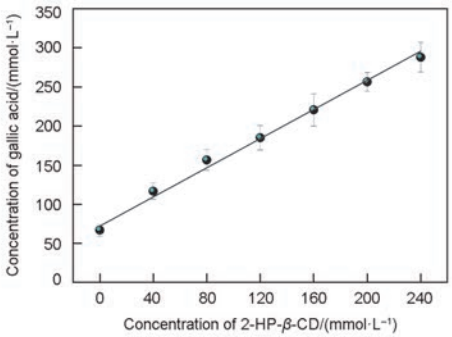

Fig.2 Phase solubility graph of GA/2-HP-β-CD systems

3.2 Morphology Characterization

Fig.3(A) shows the morphological characteristics of 2-HP-β-CD-NW. It may be that the molecular weight of 2-HP-β-CD is low, which is not conducive to electrospun. Although it seemed that the 2-HP-β-CD-NW had a good morphological characteristic, a lot of fracture phenomena of nanofibers were observed under SEM. The average diameter of 2-HP-β-CD nanofibers was (303.5±68.9) nm. It can be observed in Fig.3(B) that the diameter of GA/2-HP-β-CD nanofibers increases to (805.6±223.5) nm due to the presence of gallic acid, but the

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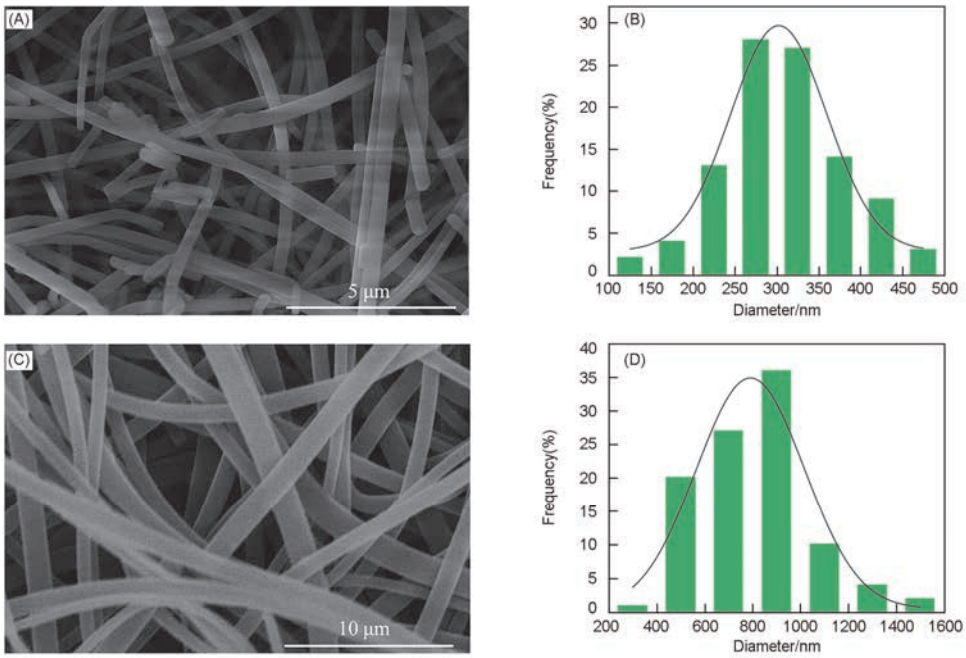

Fig.3 Morphology(A, C) and diameter analysis(B, D) of electrospun nanofibers
(A) and (B) 2-HP-*β*-CD nanofibers, mean diameter: (303.5±68.9) nm; (C) and (D) GA/2-HP-*β*-CD-IC nanofibers, mean diameter: (805.6±223.5) nm.

condition of nanofiber fracture is improved. Wutticharoenmengkol *et al*.[21] also observed that the kinetic viscosity values increased with the enhancement of gallic acid content and the diameter of the GA-loaded electrospun cellulose acetate nanofibers increased. In addition, it was observed that GA/2-HP-*β*-CD-NW has good porous structure, which provides favorable conditions for fast dissolution.

3.3 Structural Characterization

FTIR is one of the most common methods to confirm the presence of guest molecules in inclusion complex structure[22]. Generally, the fingerprint region was defined as 800—1600 cm^{-1}, the region of C—H stretch was defined as 2800—3000 cm^{-1}, and finally the region of O—H stretch was defined as 3000—3600 cm^{-1}[23]. The FTIR spectra of gallic acid powder, 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW are given in Fig.4(A). The prominent peaks at around 3000—3600, 2930, 1650 and 1370 cm^{-1} refer to the primary/secondary —OH stretching, C—H stretching, O—H bending and —CH_3 bending vibrations of 2-HP-*β*-CD, separately[9]. The other prominent absorption bands can be observed at 1033, 1082 and 1153 cm^{-1} owing to the vibrations of coupled C—C/C—O stretching and antisymmetric C—O—C glycosidic bridge stretching of 2-HP-*β*-CD[24]. The characteristic absorption bands of gallic acid powder in the region of 3200—3500 cm^{-1} can be referred to O—H stretching and hydroxyl group and/or hydrogen bond[25]. The region of 1500—1800 cm^{-1} corresponded as C═O stretching of conjugated acids[26]. The bending vibrations of C—H in the ring and O—H of the phenol alcohol are mainly characterized by bands in the region of 1320—1022 cm^{-1}[27]. The absorption peaks of gallic acid and 2-HP-*β*-CD overlap, which complicates the identification of each compound of gallic acid/2-HP-*β*-CD/IC-NW. Nonetheless, there are obvious absorption peaks of GA/2-HP-*β*-CD/IC-NW at 1250 and 1585 cm^{-1} . This result demonstrated the presence of gallic acid in GA/2-HP-*β*-CD /IC-NW.

Gallic acid is a crystalline molecule. Whether gallic acid molecules are distributed in the fiber matrix in crystalline or amorphous form, XRD can provide useful information[9]. When the inclusion complexes are formed, gallic acid molecules are located in the 2-HP-*β*-CD cavities and therefore the crystallites cannot be formed because they are separated from each other by the 2-HP-*β*-CD molecules. In the uncomplexed state, gallic acid molecules may be separated from the 2-HP-*β*-CD molecules and crystalline aggregates can be formed. The state of inclusion complexation between gallic acid and 2-HP-*β*-CD in GA/2-HP-*β*-CD/IC-NW was confirmed by XRD. Fig.4(B) shows the XRD patterns of gallic acid standard card(PDF No.: 30-1731), 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW. Gallic acid had distinctive XRD peaks at 2θ=11.869°, 16.131°, and 19.712°. 2-HP-*β*-CD is amorphous cyclodextrin type, thus, the XRD pattern of 2-HP-*β*-CD-NW had a large halo with no obvious diffraction peaks. The XRD pattern of GA/2-HP-*β*-CD/IC-NW did not have any unique diffraction peaks for crystalline gallic acid, indicating that gallic acid molecules were in inclusion state.

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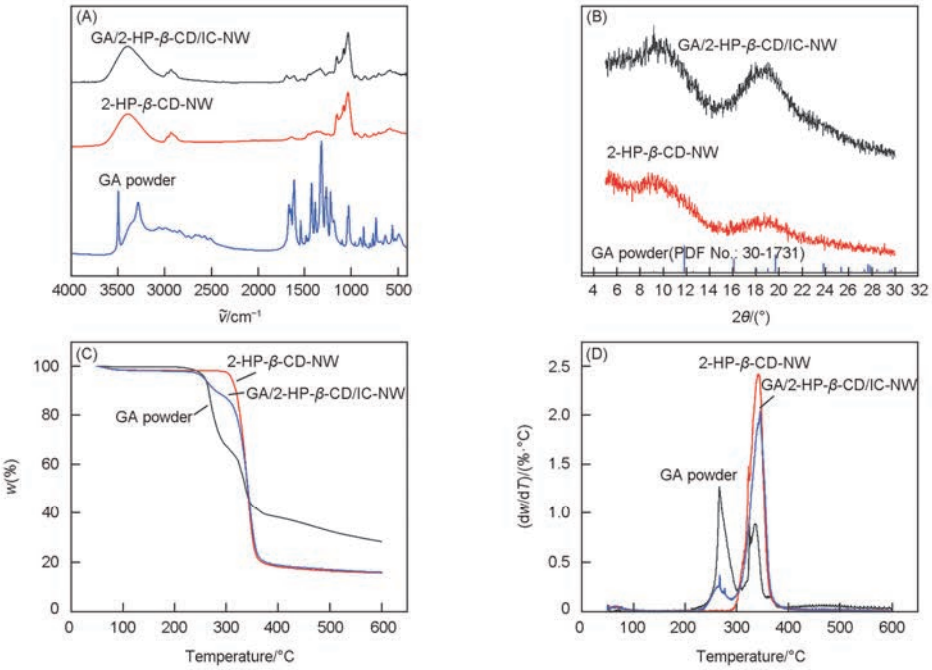

Fig.4 Full range FTIR spectra(A), XRD patterns(B), thermogravimetric analysis(TGA, C) and derivative thermogravimetric analysis(DTG) of GA powder, 2-HP-*β*-CD-NW, and GA/2-HP-*β*-CD/IC-NW(D)

The mass change of the electrospun fibers was studied by thermogravimetric analysis(TGA). The mass loss curves of gallic acid powder, 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW are presented in Fig.4(C). The mass loss of 2-HP-*β*-CD-NW can be divided into two stages with 295 °C as the critical point. Aytac *et al.*[20] believed that the former was the evaporation of water molecules in 2-HP-β-CD cavity, and the latter was the thermal degradation of 2-HP-β-CD. Gallic acid powder exhibited two stages degradation, which started at onset temperatures of 210 and 320 °C(T_{onset}) owing to the decomposition of gallic acid. This was similar to the previous research results[25]. In the thermogram of derivative thermogravimetric analysis(DTG), the temperature with the highest mass loss rate can be explained as the degradation temperature(T_d) of the components. Fig.4(D) reveals that the T_d of gallic acid powder was about 265 °C, which corresponds to the melting temperature of crystallization region. The mass loss of GA/2-HP-*β*-CD/IC-NW can be separated into three stages, corresponding to the evaporation of water molecules, and the thermal degradation of gallic acid and 2-HP-*β*-CD. The findings suggested the formation of GA/2-HP-*β*-CD inclusion complexes without increasing the thermal degradation temperature of gallic acid. The result obtained from TGA demonstrated that gallic acid was loaded in 2-HP-*β*-CD nanofibers successfully.

3.4 Encapsulation Efficiency

In order to calculate the encapsulation efficiency of gallic acid, UV-Vis spectrometry was used to record the absorbance of GA/2-HP-*β*-CD/IC-NW at 259 nm, and the encapsulation efficiency(EE, %) was calculated(see the Electronic Supplementary Material of this paper). Three replicates showed similar encapsulation efficiency(*ca.* 94.6%). These values are quite high and similar to the previously reported results of using modified cyclodextrin as the encapsulation matrix to encapsulate other natural compounds by electrospun[7,9].

3.5 Solubility and Antioxidant Activity Test

The aqueous solubility enhancement of gallic acid in GA/2-HP-*β*-CD/IC-NW was confirmed by the UV-Vis spectroscopy. For solubility test, gallic acid powder and GA/2-HP-*β*-CD/IC-NW containing the same mass of gallic acid were dissolved in water and stirred for 12 h at room temperature. Then, each sample was filtered to remove undissolved gallic acid and the absorbance of the final solution was measured on a UV-Vis spectrometer. The UV-Vis spectra of the final solutions of gallic acid powder and GA/2-HP-*β*-CD/IC-NW samples are given in Fig.5(A). As can be seen, the absorbance peak of gallic acid solution at the specific wavelength of gallic acid(259 nm) was much weaker than that of GA/2-HP-*β*-CD/IC-NW solution. The consequences obviously demonstrated that the aqueous solubility of gallic acid was improved due to the inclusion complexation.

In biological systems and biological molecules, reactive oxygen species(ROS) and free radicals could trigger food aging

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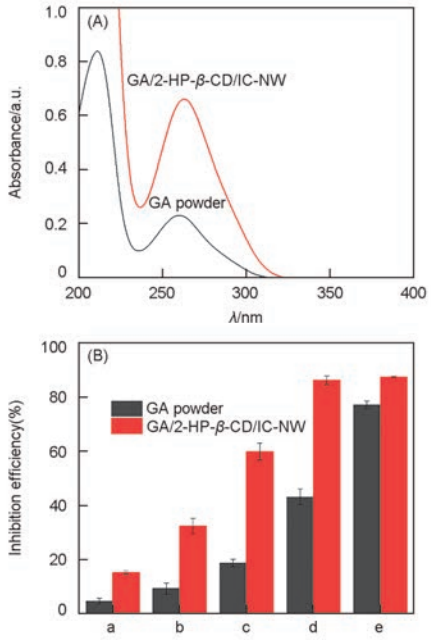

Fig.5 UV-Vis spectra of aqueous solutions of GA powder and GA/2-HP-*β*-CD/IC-NW(A) and concentration dependent antioxidants performance graphs of GA powder and GA/2-HP-*β*-CD/IC-NW(B)

(B) Concentration of nanofibrous web/(μg·mL^{-1}): a. 25; b. 50; c. 100; d. 250; e. 300.

or deterioration due to lipid peroxidation, and even can oxidize a variety of biological molecules(lipids, proteins, DNA, etc.), which induces a variety of human diseases, such as stroke, cancer, etc.[28]. Gallic acid is an effective antioxidant, which shows significant antioxidant activity by supplying H atoms in phenol groups to free radicals[29]. In our study, the antioxidant capacity of gallic acid and GA/2-HP-*β*-CD/IC-NW were examined using the diphenyl picryl hydrazinyl(DPPH) radical scavenging assay. In the experiment, the color of the solution changed from violet to yellow, and the absorbance intensity at 517 nm was reduced obviously, which was attributed to the reduction of DPPH. Fig.5(B) presents the radical scavenging test results of gallic acid powder and GA/2-HP-*β*-CD/IC-NW. As it is expected, DPPH radical scavenging rate increased with the increase of gallic acid concentration. Although the initial mass of gallic acid contained in GA/2-HP-*β*-CD/IC-NW was the same as that of gallic acid powder at each concentration, the inclusion complexes formed by gallic acid and cyclodextrin increased the solubility of gallic acid in water, therefore the DPPH radical scavenging rate was higher, especially at low concentrations. This is consistent with the conclusion in Fig.5(A) that the aqueous solubility of gallic acid is improved.

3.6 Dissolution and Disintegration Studies

Gallic acid has lower solubility, however, it can be seen from the study of phase solubility that the formation of inclusion complexes improves the aqueous solubility of gallic acid obviously. In the experiment, two different approaches were used to visualize the fast dissolution behavior of GA/2-HP-*β*-CD/IC-NW(Videos S1 and S2, see the Electronic Supplementary Material of this paper). For comparison, gallic acid powder and 2-HP-*β*-CD-NW were also placed into vials, as well. After 10 mL of purified water was added to the vials, the 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW dissolved immediately. However, there was still a large amount of undissolved gallic acid powder at the bottom of the vial[Fig.6(A)]. Due to the high specific surface area and porosity of nanofibrous webs and high aqueous solubility of 2-HP-*β*-CD, GA/2-HP-*β*-CD/IC-NW dissolved in water immediately, which proved the fast dissolution property of GA/2-HP-CD/IC-NW. The disintegration behavior of 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW was further explored using wet filter paper to simulate oral cavity environment[30]. Fig.6(B) and Video S2 show the disintegration behavior of 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW. 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW dissolved rapidly in artificial saliva. The results of these two experiments indicated that GA/2-HP-*β*-CD-IC/NW can provide rapid release of gallic acid by fast dissolution.

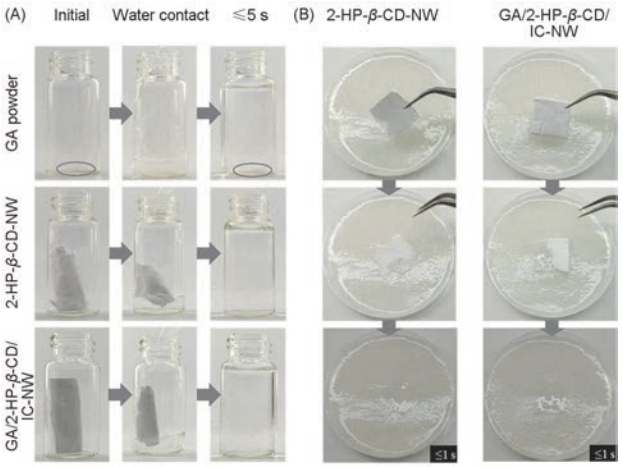

Fig.6 Dissolution behavior of GA powder, 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW in purified water(A) and disintegration behavior of 2-HP-*β*-CD-NW and GA/2-HP-*β*-CD/IC-NW at the artificial saliva environment(B)

The images were obtained from Video S1 and Video S2.

4 Conclusions

In this research, GA/2-HP-*β*-CD/IC-NW was produced *via* electrospun, which achieved fast dissolution of gallic acid. 2-HP-*β*-CD with high aqueous solubility was selected as the fiber matrix to encapsulate gallic acid, and 2-HP-*β*-CD formed inclusion complexes with gallic acid improved aqueous solubility of gallic acid. The XRD analysis confirmed the

formation of inclusion complexes between gallic acid and 2-HP-β-CD with the disappearance of gallic acid crystalline peaks in the XRD patterns, and FTIR analysis confirmed the presence of gallic acid in the nanofibrous webs. GA/2-HP-β-CD/IC-NW showed fast dissolution characteristic in water and disintegrated immediately after being moistened with artificial saliva. In addition, the GA/2-HP-β-CD/IC-NW enhanced the solubility of gallic acid in water greatly and thus the antioxidant capacity was enhanced obviously. In brief, GA/2-HP-β-CD/IC-NWs have broad application prospects as orally fast dissolution systems for food supplements.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s40242-021-0014-0.

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Conflicts of Interest

The authors declare no conflicts of interest.

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