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Preparation and stability investigation of the inclusion complex of sulforaphane with hydroxypropyl-β-cyclodextrin

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ABSTRACT

Sulforaphane (SF) is an anticancer agent present naturally in widely consumed cruciferous vegetables, and it can easily be decomposed by heat, oxygen and alkaline conditions. In order to enhance stability of SF, the inclusion complex of SF with hydroxypropyl-β-cyclodextrin (HP-β-CD) was prepared successfully using co-precipitation method and the inclusion ratio was found to be 1:1. The results showed that the stability of the inclusion complex against heat, oxygen and alkaline conditions was greatly enhanced compared with that of SF. Furthermore, FTIR, ^{1}H NMR and UV/visible spectroscopy were performed to prove the formation of the inclusion complex SF/HP-β-CD. Therefore, it is a very effective method to maintain the anticancer and antioxidant function of SF by preparing the inclusion complex SF/HP-β-CD.

1. Introduction

Sulforaphane (SF), one of the most powerful food-derived anticarcinogens, is an isothiocyanate derived from cruciferous vegetables such as broccoli, cauliflower, kale and cabbage. As an inducer of phase II enzymes (Zhang, Talalay, Cho, & Posner, 1992), SF enhances detoxification of carcinogens based on a mechanism contributing to anticarcinogenic activity (Verhoeven, Verhagen, Goldbohm, Van den Brandt, & Van Poppel, 1997; Zhang, Tang, & Gonzalez, 2003). A large number of studies have shown that SF can prevent, delay, or reverse preneoplastic lesions and has a promising effect on many kinds of cancers involving breast, hepatic, bladder, osteosarcoma, pancreatic, melanoma, etc. (Cho, Li, Hu, Jiang, & Lu, 2005; Jackson & Singletary, 2004; Kuroiwa et al., 2006; Shen, Xu, Chen, Hebbar, & Kong, 2006; Singh et al., 2004). Also, SF is able to offer cellular protection in several models of brain injury (Dash, Zhao, Orsi, Zhang, & Moore, 2009). However, as a kind of unstable oil, SF is susceptible to degradation by oxygen, heat and alkaline conditions (Jin, Wang, Rosen, & Ho, 1999; Qian, Hao, & Qipeng, 2007). Thus, it is difficult to manufacture and distribute SF in food and pharmaceutical industries due to its instability.

Cyclodextrins (CDs) are non-toxic cyclic oligosaccharides constituted by six (α-cyclodextrin), seven (β-cyclodextrin) and eight (γ-cyclodextrin) glucopyranose units linked by α-(1,4) bonds. Cyclodextrin structure provides a hydrophilic outer surface and hydrophobic interior hollow. The inner part of cyclodextrin molecules is made apolar by glycosidic oxygens and methine protons, while the external surface is polar by presence of secondary and primary hydroxyls at the edge of the ring (Del Valle, 2004). As a result, CDs are able to form inclusion complexes with a large number of hydrophobic compounds and these complexes have been successfully used to improve chemical stability, solubility, and bioavailability of many compounds (Dodziuk, 2002; Peggy, Maria, & Beatriz, 2010). Hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative, is prepared by reacting β-CD with propylene oxide in alkaline aqueous solutions. Due to the fact that hydrogen bonds are replaced by hydroxyl groups, HP-β-CD can increase the aqueous solubility compared with α-, β- and γ-CD (Chao, Zhengyu, Xueming, Haining, & Wangyang, 2008; Sarah & Robert, 2005; Seoung et al., 2007). Besides, there are toxicological studies pointing out that HP-β-CD is well tolerated in humans by either oral or intravenous administration (Sarah & Robert, 2005).

So far, several studies have been performed on the reaction between HP-β-CD and some poorly water-soluble or unstable organic compounds, but the inclusion complex of SF has not yet been reported. The aim of our research is to increase stability of SF by preparing the inclusion complex of SF with HP-β-CD. The stoichiometry of the inclusion complex was confirmed by the continuous variation Job's method (Job, 1928). Additionally, the stability of the resultant complex against oxygen, heat and alkaline conditions was also investigated.

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2. Materials and methods

2.1. Materials

SF samples (95%) were prepared in our laboratory by separation and purification from broccoli seeds which were kindly provided by Vegetables and Flowers Institute of China Academy of Agriculture Science. SF standard was purchased from Sigma Chemical Co. (St. Louis, MO.). Acetonitrile was HPLC grade. HP-β-CD (>98%, DS = 5.0) was purchased from Zibo Qianhui Fine Chemical Co., Ltd. (Zibo, Shandong, China). All other reagents were of analytical grade. The water used was double distilled and deionised.

2.2. Methods

2.2.1. Preparation of the inclusion complex of SF with HP-β-CD

The inclusion complex of SF with HP-β-CD at 1:1 molar ratio was prepared using the co-precipitation method. The accurately weighed HP-β-CD was dissolved in distilled water to get a saturated solution. Then SF solution in ethanol was slowly added and a suspension was formed. The suspension was reacted completely by ultrasonic for 20 min and maintained stirring at room temperature for 24 h. The obtained mass was filtered through 0.45 μm membrane filter then dried in a vacuum rotavapor. The dried complex was ground to fine powder and screened through an 80-mesh sieve before use.

2.2.2. Determining SF content by reverse phase HPLC

SF was analyzed using an Hitachi HPLC apparatus equipped with Hitachi model L-7100 pumps, an L-7420 variable wavelength detector, and a reversed-phase C_{18} column (250 mm × 4.6 mm, 5 μm, Diamodsil™). The solvent system consisted of 20% acetonitrile in water, and changing linearly over 10 min to 60% acetonitrile, then raising to 100% immediately and running isocratically for 2 min to purge the column. The column oven temperature was set at 30 °C. The flow rate was 1.0 ml/min, and 10 μL aliquots were injected into the column. Sulforaphane was detected at UV 254 nm (Hao, Chunfang, Qipeng, & Fank, 2007).

2.2.3. The stoichiometry of the SF/HP-β-CD inclusion complex

The stoichiometry of inclusion complex SF/HP-β-CD was determined by the continuous variation Job's method (Job, 1928). To implement Job's method experimentally, equimolar 2.8×10^{-3} mol l^{-1} solutions of SF and HP-β-CD were mixed to a standard volume (1 ml:9 ml; 2 ml:8 ml; 3 ml:7 ml and so on) containing a fixed total concentration of the species. In the solutions the R (R = [SF]/{[SF] + [HP-β-CD]}) is systematically varied from large to small, and the amount of SF in the inclusion complex was measured by HPLC after stirring. The maximum amount of the complex SF should occur at the stoichiometric ratio.

2.2.4. The stability of the SF/HP-β-CD inclusion complex

Comparative tests involving the stability of free and complex SF were tested in several conditions: (a) at a temperature of 50 °C (during 24 h); (b) in the pH range from 2 to 8 (during 10 h); (c) increasing the amount of oxidant H_2O_2. After a fixed period of time, the retention amount of free or complex SF was measured respectively to evaluate the embedding effect of the inclusion complex SF/HP-β-CD.

2.2.5. Characterization

UV spectra of the inclusion complex, pure SF and HP-β-CD were obtained by UV/vis Cecil CE 8020 spectrophotometer. The scans were registered from 200 to 800 nm.

Infrared spectrum of the inclusion complex was conducted using a Nexus 8700 FTIR spectrometer according to potassium bromide disk method. The FTIR spectra of pure SF and HP-β-CD were also obtained by the same procedure for comparison. The samples were placed in the light path and the spectra were obtained (from 4000 to 500 cm^{-1}).

^{1}H NMR measurement of the inclusion complex was obtained using a Bruker Av600 spectrometer NMR using D_2O as solvent. The ^{1}H NMR spectra of pure SF and HP-β-CD were also performed by the same procedure for comparison to get further evidence.

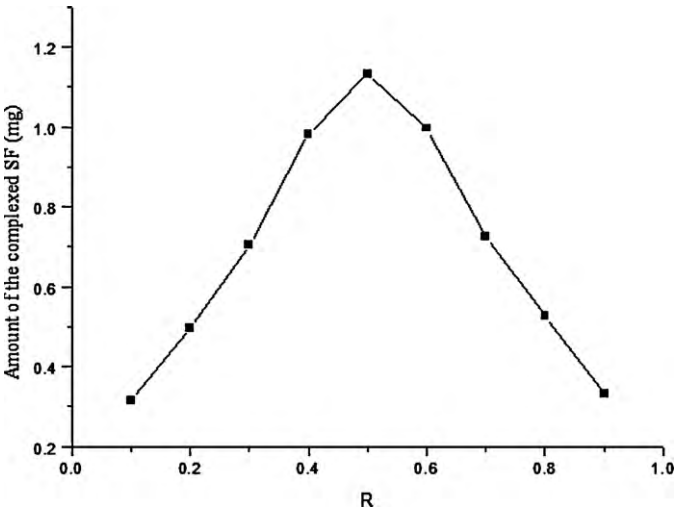

Fig. 1. Job's plot of SF/HP-β-CD inclusion complex.

3. Results and discussion

3.1. Stoichiometry of inclusion complex

According to the continuous variation Job's method, the concentration of the complex of a set of samples with continuously variable molar fraction of components can be measured, and the maximum concentration of complex will be present in the sample where the molar ratio R corresponds to the stoichiometry of the complexation (Misiuk & Zalewska, 2009). The maximum amount of the complex SF was observed for $R = 0.5$ (Fig. 1), which indicated that the main stoichiometry is 1:1.

3.2. Stability of inclusion complex

As shown in Fig. 2(a), effect of heat on the degradation of free or complex SF was verified at 50 °C, respectively. Within 24 h free SF had a severe degradation of 15.2%, whereas there was no obvious change of the complex SF with only a decrease of 3.9%. The results showed that the effect of heat on the inclusion complex was insignificant, but the effect of heat on free SF was apparent. The reason may be that the inclusion complex afforded a powerful protection for SF. Hence, the stability of the inclusion complex against heat was greatly enhanced when the SF and HP-β-CD formed inclusion complex.

Fig. 2(b) illustrates the effect of pH on the retention rate of free or complex SF. After 10 h, SF and the inclusion complex reduced slowly in the acidic environment while free SF had a more severe decrease in neutral or alkaline conditions. It was noted that in pH 8.0 free SF had a 41.9% loss, but the complex with HP-β-CD reduced this figure to only 11.3%. Probably, HP-β-CD inhibited the hydrolyzation of SF after the isothiocyanic group was included in the cavity of HP-β-CD. It was indicated that the chemical stability of SF was also greatly improved when SF and HP-β-CD formed inclusion complex.

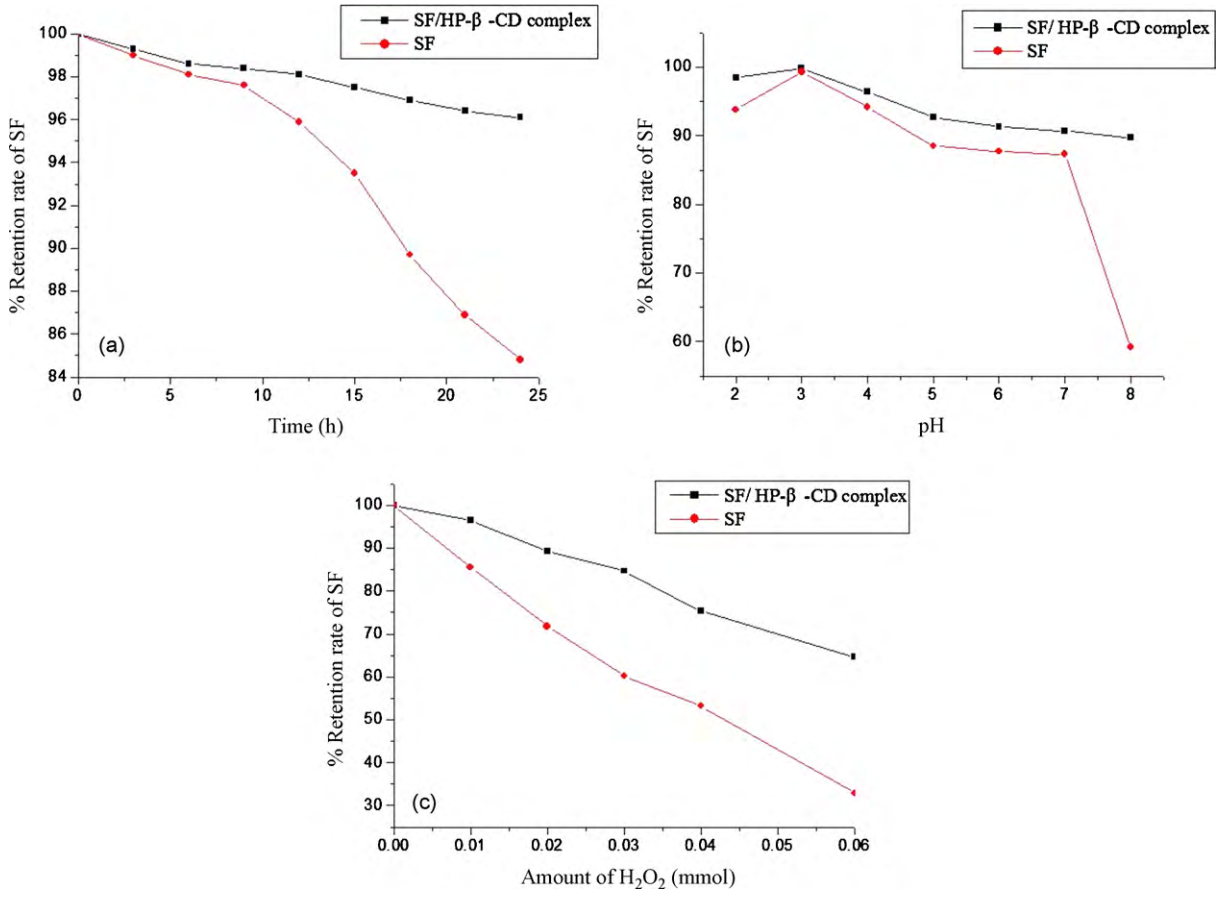

Fig. 2. The stability of SF and SF/HP-β-CD inclusion complex (a) at a temperature of 50 °C; (b) in the pH range from 2 to 8; (c) with the change of amount of H_2O_2.

In Fig. 2(c), the retention rates of the free and complex SF with the change of amount of H_2O_2 were shown respectively. The results presented that free SF reduced more rapidly than the complex SF with the increasing of the amount of H_2O_2. The effect of oxidant on the inclusion complex was less substantial than that on free SF. It is likely that HP-β-CD inclusion could afford some protection from the oxidant H_2O_2 which is harmful to the stability of free SF. Therefore, the stability of SF against oxidant was also greatly increased for the formation of SF/HP-β-CD inclusion complex.

3.3. Characterization of inclusion complex

3.3.1. Absorption spectrum of inclusion complex

The absorption spectra of SF/HP-β-CD complex, SF and HP-β-CD were recorded respectively, according to the procedure of UV spectra. Fig. 3 shows that HP-β-CD had no absorption in the range 225–400 nm. The absorption peaks of SF were near 250 nm. The SF/HP-β-CD inclusion complex in absorption spectrum shape was quite similar to SF, but the absorbance wavelength of inclusion complex appeared slightly blue shifts. It was speculated that blue-shifted hydrogen bond should be formed between SF and HP-β-CD molecular, indicating the formation of the inclusion complex between SF and HP-β-CD.

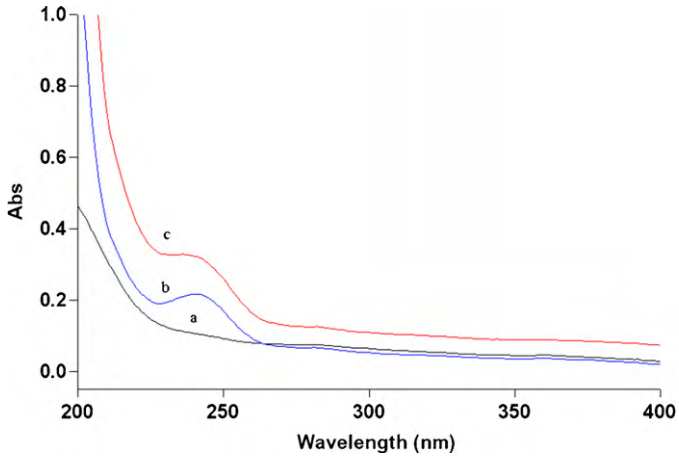

Fig. 3. UV absorption spectra of HP-β-CD (a), SF (b) and SF/HP-β-CD inclusion complex (c).

3.3.2. FTIR analysis

The FTIR spectra of pure SF, HP-β-CD and the inclusion complex SF/HP-β-CD were performed and the complete band assignments are shown in Table 1 and Fig. 4.

In the FTIR spectrum of SF, strong absorbance at 2182 and 2109 cm^{-1} were for the –N=C=S stretching vibration. The absorption peak at 1021,1274 and 692 cm^{-1} was respectively assigned to the S=O, C–N and C–S bond. 1452 and 1350 cm^{-1} were noted for deformation vibration of C–H from CH_3. Compared with that of HP-β-CD, characteristic absorbance at 2183 and 2108 cm^{-1} of the IR spectrum of the inclusion complex should be for the –N=C=S bond in the molecule of SF and the signal was greatly weakened. However, the absorption bands at 1452 and 1350 cm^{-1} almost disappeared in the SF/HP-β-CD inclusion complex, indicating that the group $–CH_2–$ were entrapped into the host cavities. Meanwhile, the characteristic absorption of the bonds S=O, C–N and C–S also disappeared in the inclusion complex since their stretching vibration

Table 1
Wavenumbers (cm^{-1}) and assignments for the bands observed in the FTIR spectra of SF, HP-β-CD and the inclusion complex SF/HP-β-CD.

SF	HP-β-CD	Inclusion complex
3424: ν (O–H) from H_2O adsorbed	3413: ν (O–H)	3413: ν (O–H)
2942 and 2868: ν (C–H) from CH_3 and CH_2	2970 and 2928: ν (C–H)	2968 and 2928: ν (C–H)
2182 and 2109: ν (–N=C=S)	1459 and 1373: δ (C–H) from CH_2 and CH3	2183 and 2107: ν (–N=C=S)
1452 and 1350: δ (C–H) from CH_3 and CH_2	1334: coupled δ (C–C–H), δ (C–O–H), δ (H–C–H)	1456 and 1356: δ (C–H) from CH_3 and CH_2
1274: ν (C–N)	1154 and 1084: ν (C–O), ν (C–C), δ (C–O–C)	1155 and 1084: ν (C–O), ν (C–C), δ (C–O–C),
1021: ν (S=O)	1035: δ (O–C–H), δ (C–C–H), δ (C–C–O)	1034: δ (O–C–H), δ (C–C–H), δ (C–C–O)
740: ω (C–H) from CH_2	948: skeletal vibration involving α-1,4 linkage	947: skeletal vibration involving α-1,4 linkage
692 and 634: ν (C–S)	854: δ (C–C–H), ν (C–O), ν (C–C) from anomeric vibration	855: δ (C–C–H), ν (C–O), ν (C–C) from anomeric vibration

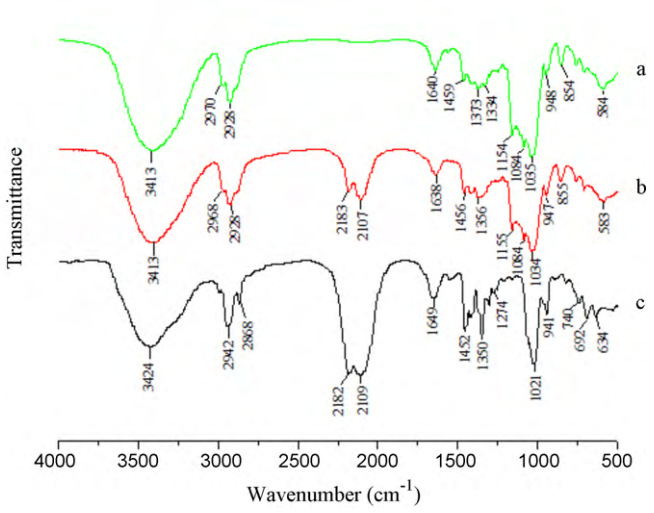

Fig. 4. The FTIR spectra of HP-β-CD (a), SF (c) and SF/HP-β-CD inclusion complex (b).

was restricted after the formation of the inclusion complex. In addition, the absorption bands at 1459 and 1373 cm^{-1} for C–H bond in the molecule of HP-β-CD shifted to low wavenumbers in the SF/HP-β-CD inclusion complex that might be caused by the formation of hydrogen bonds between the molecule of SF and the molecule of HP-β-CD. It was concluded that the inclusion complex of SF with HP-β-CD was obtained according to the IR spectra.

3.3.3. ^1H NMR spectroscopy

The formation of the SF/HP-β-CD inclusion complexes can be proved from the changes of chemical shifts in ^1H NMR spectra because the physical or chemical environment affected may be felt by hydrogens of SF or the HP-β-CD cavity if the inclusion occurs. SF has four types of hydrogen: H-1, H-2, H-3 and H-4 (Fig. 5). The ^1H chemical shift values of SF before and after forming the inclusion complex are shown in Table 2. As can be seen from Table 2, the complexation caused a downfield shift of SF protons and the shift values of H-1, H-2, H-3 and H-4 protons exhibit relatively weak but significant changes. However, the shifts of H-2 (0.034 ppm), H-3 (0.027 ppm) and H-4 (0.041 ppm) protons were larger than those of H-1 protons (0.011 ppm) because these hydrogen atoms

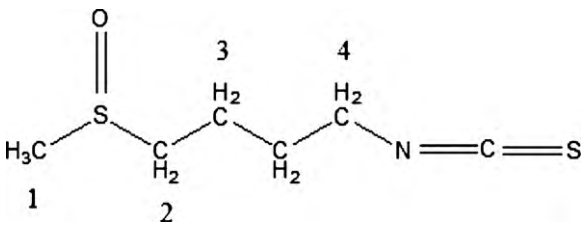

Fig. 5. Structure of SF with the corresponding proton numbering.

Table 2
Changed chemical shifts of SF before and after forming inclusion complex from ^1H NMR spectra.

^1H assignment	δ SF free (ppm)	δ SF complexed (ppm)	Δδ (SF complexed – SF free) (ppm)
H-1	2.677	2.666	−0.011
H-2	2.916	2.882	−0.034
H-3	1.863	1.836	−0.027
H-4	3.652	3.611	−0.041

belongs to the $-CH_2-$ group adjacent to the bond –N=C=S which is highly hydrophobic. It is possible that the hydrogen atom of the $-CH_2-$ group and the oxygen of the glucoside in cyclodextrin interior formed hydrogen bond. Based on these chemical shifts values, we can deduce that the molecule of SF should penetrate deeply into the cavity of HP-β-CD.

4. Conclusion

As an anticancer compound, the isothiocyanate SF has a promising control effect on various kinds of cancers. However, SF is sensitive to heat, alkaline conditions and oxygen, so it is necessary to improve the stability of SF.

In the present work, the inclusion complex of SF with HP-β-CD was prepared successfully with a 1:1 stoichiometry. The formation of the inclusion complex SF/HP-β-CD has been verified by IR, ^1H NMR and UV/visible spectroscopy. The studies have indicated that the thermal stability and the chemical stability of SF were greatly enhanced when the inclusion complex formed. Moreover, by preparing the inclusion complex SF/HP-β-CD, we can change the liquid state of SF into microcrystalline powders with better stability and better market prospects.

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