sciendo

*Polish Journal of Chemical Technology, 25, 1, 20—27, 10.2478/pjct-2023-0004*

# Encapsulation of hydroxycitronellal in β-cyclodextrin and the characteristics of the inclusion complex

**Guangyong Zhu[1, 4], Zuobing Xiao[1, 2, *], Rujun Zhou[1], Junhua Liu[1], Guangxu Zhu[3]**

[1]*No. 100 Haiquan Road, Shanghai Institute of Technology, Shanghai 201418, PR China*
[2]*No. 800 Dongchuan Road, Shanghai Jiao Tong University, Shanghai, 200240, PR China*
[3]*3035 Sable Ridge Dr. Ottawa. ON K1T 3R9, Canada*
[4]*Yancheng City Chunzhu Aroma Co., Ltd., Jiangsu, 22400, PR China*
[*]*Corresponding author: e-mail: zbingxiao@sina.com*

Hydroxycitronellal has been widely used in foods, beverages, perfumery and cosmetics. It can also be used to treat anxiety. The major drawbacks regarding the use of hydroxycitronellal are related to water insolubility, volatility, instability, and sensitization. To overcome these concerns, β-cyclodextrin was adopted as wall material to encapsulate hydroxycitronellal in this work. Hydroxycitronellal-β-cyclodextrin inclusion complex was prepared and the product was characterized. The interaction of hydroxycitronellal and β-cyclodextrin, and the assembly of hydroxycitronellal-β-cyclodextrin inclusion complex were investigated by molecular simulation (MM). The results showed that hydroxycitronellal loading capacity was 8.5%. The thermal stability and lastingness of hydroxycitronellal were improved by the formation of the inclusion complex. The minimum binding energy was –151.2 kJ/mol. Among the perpendicular, staggered parallel and ideally parallel orientation of the inclusion complexes, the minimum energy value was found for the staggered parallel arrangement. These basic data are useful to understand the interaction between hydroxycitronellal and β-cyclodextrin.

**Keywords:** food additives, encapsulation, aroma, controlled release, flavor.

## INTRODUCTION

Hydroxycitronellal (with a synonym: 3,7-dimethyl-7-hydroxyoctanal), which has been reported found in pepper, is usually synthesized by hydration of citronellal[1]. Its chemical structural formula is shown in Figure 1.

Hydroxycitronellal has a sweet, flowery odor reminiscent of lily of valley and linden blossom. As a common flavor and fragrance material, hydroxycitronellal has been widely used in foods, beverages, perfumery and cosmetics. At 50 ppm, hydroxycitronellal has a taste characteristic of green, waxy, sweet, melon and floral notes[2]. Due to its fine aroma, hydroxycitronellal is used in large quantities in the flavor and fragrance industry. As a composition of flavors, a small quantity of hydroxycitronellal can be adopted in blending flavors such as cucumber, melon, apple, and mango to impart a fresh feeling. A large quantity of hydroxycitronellal usually is used in many perfume compositions for creating lily of the valley and linden blossom notes due to its fine flowery odor. Hydroxycitronellal can also be used in imitating blossom fragrances such as cyclamen, honeysuckle, and lily[1, 2]. Most importantly, hydroxycitronellal can present an anxiolytic-like effect and can be used to treat anxiety[3]. Furthermore, its anxiolytic-like activity does not present adverse effects such as neuromuscular impairment and sedation commonly caused by benzodiazepines[3].

Hydroxycitronellal is a slightly viscous, colorless liquid at room temperature. It is soluble in ethyl alcohol, propylene glycol and most fixed oils. But hydroxycitronellal is insoluble in glycerin and water[2]. In some cases, good water solubility is required. For example, some beverages require flavors to have good water solubility. Hydroxycitronellal is a volatile liquid and it cannot last long. However, lastingness is one of the most important properties of flavor and fragrance. The performance of fragrance and flavor is often measured by its lastingness and pleasantness[4].

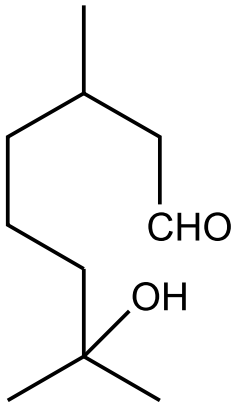


**Figure 1.** The chemical structural formula of hydroxycitronellal

Although the application scope of hydroxycitronellal is wide, it is somewhat unstable. Toward alkali and acid, hydroxycitronellal is relatively unstable. In ethyl alcohol, it tends to form acetal[5]. Furthermore, hydroxycitronellal is prone to polymerization reaction and is easily oxidized when exposed to air[6]. Therefore, there is a need to improve the stability of hydroxycitronellal.

Except for its drawbacks such as water-insolubility, instability and volatility, hydroxycitronellal is often considered as an allergen. It showed a positive patch test[7]. Calnan[8] investigated cases of allergic contact dermatitis to perfumes. Using a patch test, Calnan found that hydroxycitronellal could elicit an allergic contact dermatitis of the face. The potential of hydroxycitronellal to elicit human sensitization reaction or induce hypersensitivity was also investigated by Steltenkamp et al.[9]. They found that, at exposure concentrations greater than 5%, sensitization to hydroxycitronellal appeared to be induced. For its sensitizing potential in human skin, hydroxycitronellal was studied by Ford et al. They found that induction at 12% produced sensitization in 8 of 11 tests. When 100 of the non-reacting panelists were re-exposed in the same way, allergic sensitization reactions appeared during the induction period with concentrations as low as 2.5%[10]. In

recent years, the safety of the use of hydroxycitronella in consumer products has attracted widespread attention. How to reduce the irritation and improve the safety of the hydroxycitronellal is of great interest.

β-Cyclodextrin can be used in the reduction of skin irritation[11]. β-cyclodextrin is nonallergenic and nontoxic. It is not regarded as irritating to the skin. In some cases, the complexation of a guest molecule with β-cyclodextrin can reduce a possible irritating effect of a guest molecule[12]. It was reported that the skin irritation of retinol could be relieved and the service life of retinol products could be extended[13]. Cyclodextrin molecules have hollow hydrophobic cavities and hydrophilic outer surfaces. Encapsulation of hydrophobic organic molecules in the cavities of cyclodxtrin molecules can protect these hydrophobic organic molecules, improve their water solubility and stability, and prolong their shelf-life activity[14–16]. Kayaci et al.[17] found that high thermal stability and enhanced surability of geraniol were achieved by encapsulation of geraniol in cyclodextrins. Siva et al. reported that significant enhancement of isoeugenol water solubility was confirmed after the formation of the inclusion complex with 2-hydroxypropyl-β-cyclodextrin[18]. Real et al. reported that a significant improvement in the aqueous solubility of triclabendazole by complexation with cyclodextrins was achieved[19].

The major drawbacks regarding the use of hydroxycitronellal are related to water insolubility, volatility, instability, and sensitization. In this work, β-cyclodextrin was adopted as wall material to encapsulate hydroxycitronellal to overcome these concerns. Hydroxycitronellal-β-cyclodextrin inclusion complex was prepared and the product was characterized by optical microscope, Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). The interaction of hydroxycitronellal and β-cyclodextrin was investigated by molecular simulation (MM). The optimal structure of the inclusion complex and binding energy (BE) were obtained by MM2 calculation.

## MATERIALS AND METHODS

### Materials

Hydroxycitronellal (slightly viscous colorless liquid, molecular weight: 172, chemical pure) was provided by Pu-Jie Aroma Chemicals (Shanghai) Co., Ltd. β-cyclodextrin (molecular weight: 1135, white powder, chemical pure) and ethyl alcohol (analytically pure) were provided by Sinopharm Chemical Reagent Co. Ltd. Without further purification, all these materials were directly used as received. Deionized water was used throughout the experiment.

### Hydroxycitronellal-β-cyclodextrin inclusion complex preparation

The precipitation method, with some modifications, as elucidated in literature[20] was adopted to prepare hydroxycitronellal-β-cyclodextrin inclusion complex. A digital display electric stirrer (JB200-S, Shanghai Suoying Instrument Equipment Co., Ltd.) was turned on. Then, 11 g of β-cyclodextrin was weighed and added slowly to the deionized water (600 ml) to form β-cyclodextrin aqueous solution. 2 g of hydroxycitronellal (dissolved in 10 ml ethyl alcohol) was added slowly to the β-cyclodextrin aqueous solution. The mixture was kept at 35 °C in a thermostatic water bath and was stirred for another 3 h. After the complexation reaction finished, the mixture was stored in a refrigerator and the temperature of the refrigerator remained constant at 5 °C. After 24 h, the formed precipitate was recovered by vacuum filtration, which was then washed with ethyl alcohol 3 times to remove hydroxycitronellal on the surface of β-cyclodextrin. A freezer dryer (FD-1A-50, Shanghai Bilon Instrument Manufacturing Co., Ltd.) was adopted to dry the products. The dry sample was stored in a desiccator for further analysis.

### Characterization of the surface shape of hydroxycitronellal-β-cyclodextrin inclusion complex

The surface shape of hydroxycitronellal-β-cyclodextrin inclusion complex was characterized using optical microscopy (LW600LJT, Shanghai Cewei Photoelectric Technology Co., LTD). The images of the samples were obtained by a built-in charge-coupled device (CCD) camera.

### Characterization of hydroxycitronellal, β-cyclodextrin, physical mixture and hydroxycitronellal-β-cyclodextrin inclusion complex by FTIR

The FTIR determination method was adopted as described in reference[21]. Hydroxycitronellal, β-cyclodextrin, physical mixture, and the inclusion complex were determined by a Vertex 70 Fourier Transform Spectrometer (Bruker, German). The FTIR spectra were recorded in the frequency range of 400 to 4000 $cm^{-1}$.

### Characterization of hydroxycitronellal, β-cyclodextrin, physical mixture and hydroxycitronellal-β-cyclodextrin inclusion complex by TGA

The weight loss and the rate of weight loss of hydroxycitronellal, β-cyclodextrin, physical mixture and the inclusion complex were performed with a thermogravimetric analyzer (TGA-Q5000IR, TA Instruments, USA). Approximately 5 mg of the sample was loaded in a ceramic crucible. During the pyrolysis process, $N_2$ was used and its flow was 20 ml/min to avoid oxidation. The heating rate used in the experiment was 10 °C/min and the pyrolysis temperature was in the range of 30 °C to 600 °C.

### Determination of the loading capacity of hydroxycitronellal

The loading capacity of hydroxycitronellal is defined as the mass ratio of hydroxycitronellal to hydroxycitronellal-β-cyclodextrin inclusion complex. TGA can provide the mass loss of the samples with the increase of temperature. During the heating process of β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complex, mass loss occurs because of volatiles. As a volatile, hydroxycitronellal can release from its inclusion complex when the temperature reaches a certain point. The loading capacity of hydroxycitronellal can be obtained from the mass loss difference between hydroxycitronellal-β-cyclodextrin inclusion complex and β-cyclodextrin.

### Investigation of the interaction of hydroxycitronellal and β-cyclodextrin by molecular simulation calculations

With some modifications, the molecular simulation method described in references[22, 23] was used to study the interaction of hydroxycitronellal and β-cyclodextrin. Hydroxycitronellal-β-cyclodextrin inclusion complex structure optimization and binding energy calculation, defined as Eq. (1), were performed by Chem 3D Ultra (CambridgeSoft Corporation, MA, USA).

$$BE = E_{complex} - (E_{guest} + E_{host}) \quad (1) \qquad (1)$$

where $BE$ is binding energy, $E_{guest}$ is the minimum total energy of hydroxycitronellal, $E_{host}$ is the minimum total energy of β-cyclodextrin, and $E_{complex}$ is the minimum total energy of hydroxycitronellal-β-cyclodextrin inclusion complex.

The total energy is formed from stretch energy, bend energy, stretch-bend energy, torsion energy, non-1,4-Van der Walls energy, 1,4-Van der Walls energy, and dipole/dipole energy.

The docking strategy was to push hydroxycitronellal molecule stepwise through the β-cyclodextrin orifice along the Z axis as shown in Figure 2. The minimum total energy of the complex was calculated at each step and MM2 calculation was used. The Z coordinate of C6 atom in hydroxycitronellal molecule was used to mark the relative position between hydroxycitronellal and β-cyclodextrin.

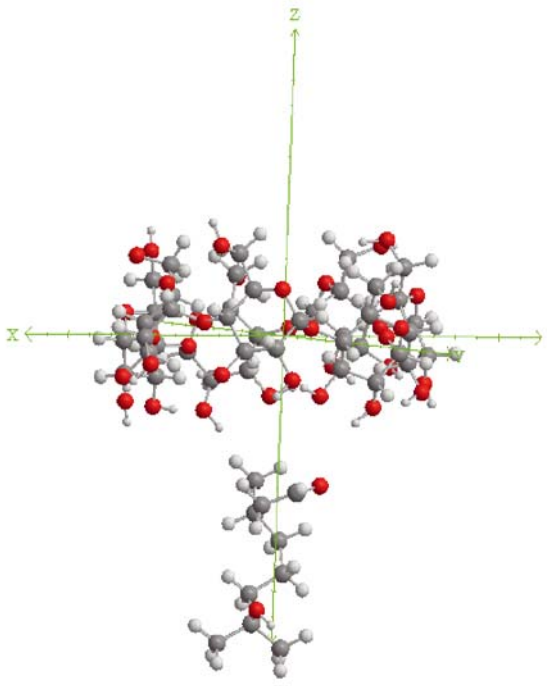


**Figure 2.** The movement direction of hydroxycitronellal to the cavity of β-cyclodextrin along the Z axis

## RESULTS AND DISCUSSION

### The surface shapes of β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complex

Hydroxycitronellal-β-cyclodextrin inclusion complex, and cyclodextrin alone, processed for the preparation of the inclusion complexes with hydroxycitronellal, were investigated with an optical microscope. The surface shapes of β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complex were shown in Figure 3.

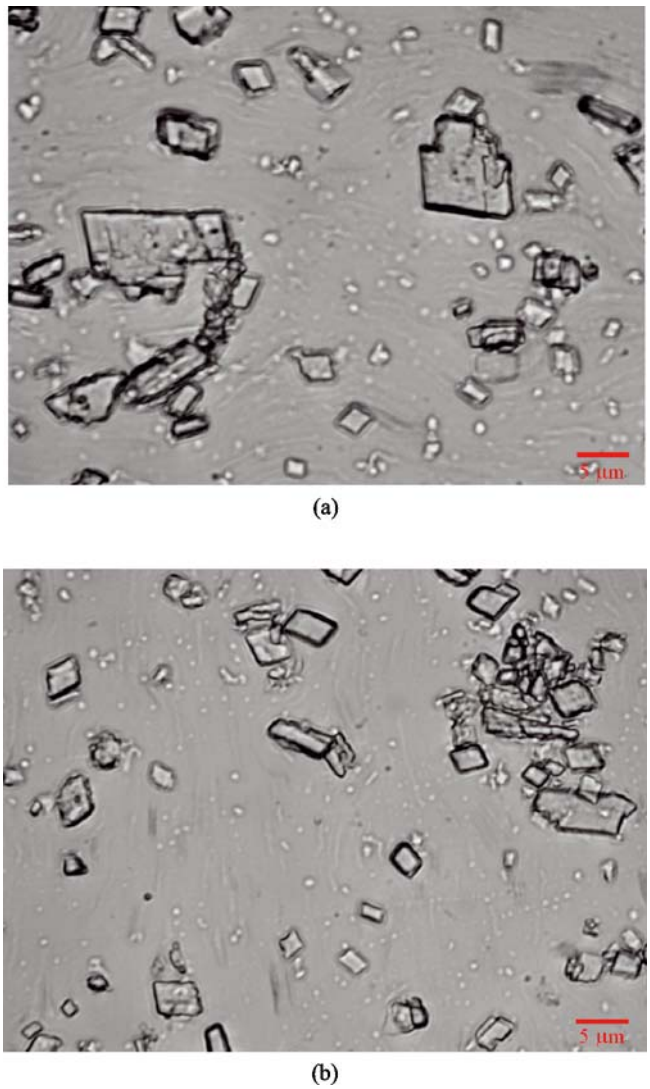


**Figure 3.** The surface shapes of β-cyclodextrin (a) and hydroxycitronellal-β-cyclodextrin inclusion complex (b)

As shown in Figure 3, the size of most β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complexes is in the range of 2 to 10 μm. Although relatively large aggregates can be observed from Figure 3(a), the shape β-cyclodextrin is similar to that of hydroxycitronellal-β-cyclodextrin inclusion complex. During the process of preparation of hydroxycitronellal-β-cyclodextrin inclusion complexes, or cyclodextrin alone processed as for the preparation of the inclusion complexes with hydroxycitronellal, the products formed aggregates which took on different shapes. Some products have irregular shapes, while some of the products are quadrangular or rhombic in appearance as shown in Figure 3. Cyclodextrin is not a cylindrical molecule but rather a slightly conical circular molecule. β-cyclodextrin has a truncated cone-shaped molecular structure. In aqueous suspension, hydroxycitronellal-β-cyclodextrin inclusion complex molecules may line up in staggered parallel arrangement. The schematic diagram of hydroxycitronellal-β-cyclodextrin inclusion complex molecules in staggered parallel arrangement is shown in Figure 4.

In this way, some products with the shapes of parallelogram and rhombus can be formed. This structure has been reported in the literature[24]. It was reported

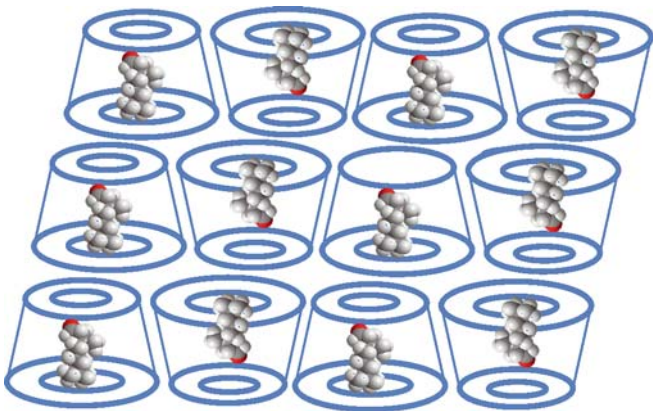

**Figure 4.** The schematic diagram of hydroxycitronellal-β-cyclodextrin inclusion complex molecules in staggered parallel arrangement

that β-cyclodextrin inclusion complexes to line up in staggered parallel arrangement were energetically favorable[25]. Therefore, the appearance of some aggregates of hydroxycitronellal-β-cyclodextrin inclusion complex is parallelogram or rhombus as shown in Figure 3.

**The FTIR spectra of hydroxycitronellal, physical mixture, β-cyclodextrin, and hydroxycitronellal-β-cyclodextrin inclusion complex**

The FTIR spectra of hydroxycitronellal, β-cyclodextrin, physical mixture and hydroxycitronellal-β-cyclodextrin inclusion complex were shown in Figure 5.

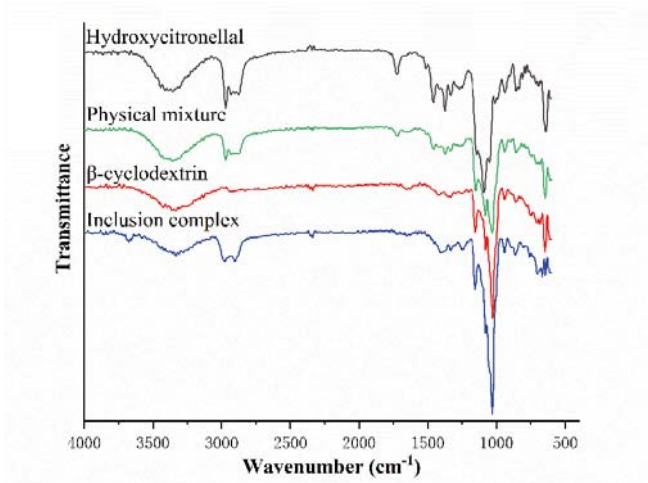


**Figure 5.** The FTIR spectra of hydroxycitronellal, β-cyclodextrin, physical mixture, and hydroxycitronellal-β-cyclodextrin inclusion complex

Hydroxycitronellal contains the C-OH group. In the FTIR spectrum of hydroxycitronellal, the broad peak appearing at 3355 $cm^{-1}$ is due to the O-H stretching vibration of hydroxyl group absorption. The peak appearing at 1147 $cm^{-1}$ is due to the C-O stretching vibration of C-OH group. Hydroxycitronellal contains C=O carbonyl group. The peak appearing at 1724 $cm^{-1}$ can be assigned to aldehyde C=O stretching vibration. The asymmetrical and symmetrical C-H stretching absorption of $CH_3$ occurred at 2970 $cm^{-1}$ and 2876 $cm^{-1}$ respectively. The peak appearing at 1374 $cm^{-1}$ can be assigned to the symmetrical C-H deformation vibration of $CH_3$. The frequency due to C-H deformation vibration of $CH_2$ appears at 1458 $cm^{-1}$ [26].

In the FTIR spectrum of β-cyclodextrin, the broad peak due to the O-H stretching vibration of hydroxyl group absorption appears at 3334 $cm^{-1}$. The peaks appearing at 1154 $cm^{-1}$ and 1028 $cm^{-1}$ can be assigned to the C-O stretching vibration. The FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex shows resemblance to that of β-cyclodextrin. In the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex, the broad peak due to the O-H stretching vibration of hydroxyl group absorption also appears at 3334 $cm^{-1}$, and the peaks due to the C-O stretching vibration appear at 1155 $cm^{-1}$ and 1130 $cm^{-1}$. However, compared with the FTIR spectrum of β-cyclodextrin, two new peaks appear at 2978 $cm^{-1}$ and 2905 $cm^{-1}$ respectively in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex. It may be caused by the asymmetrical and symmetrical C-H stretching absorption of $CH_3$ in hydroxycitronellal molecule, which resulted in the peaks at 2970 $cm^{-1}$ and 2876 $cm^{-1}$ respectively in the FTIR spectrum of hydroxycitronellal. In the FTIR spectrum of physical mixture of hydroxycitronellal and β-cyclodextrin, two small peaks still occur at 2970 $cm^{-1}$ and 2876 $cm^{-1}$ respectively. Furthermore, the peak at 1724 $cm^{-1}$ due to aldehyde C=O stretching vibration in the FTIR spectrum of hydroxycitronellal disappears in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex[27]. In the FTIR spectrum of physical mixture, the peak at 1724 $cm^{-1}$ still appeared. The peaks occurring at 1458 $cm^{-1}$ and 1374 $cm^{-1}$ in the FTIR spectrum of hydroxycitronellal still appear in the FTIR spectrum of the physical mixture, but disappear in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex. These changes in FTIR spectrum suggest the successful encapsulation of hydroxycitronellal in β-cyclodextrin.

**The TG and DTG curves of hydroxycitronellal, β-cyclodextrin, physical mixture, and hydroxycitronellal-β-cyclodextrin inclusion complex**

TGA can be used to investigate the thermal stability and lastingness of the guest molecule encapsulated in β-cyclodextrin[28]. The TG and DTG curves of hydroxycitronellal, β-cyclodextrin, physical mixture, and hydroxycitronellal-β-cyclodextrin inclusion complex obtained were shown in Figure 6.

As shown in Figure 6, from 32 °C to 68 °C, a slight mass loss of hydroxycitronellal can be observed in the TG curve hydroxycitronellal. In this stage, hydroxycitronellal vaporized slowly. With the increase of temperature, hydroxycitronellal vaporized quickly, and the rate of mass loss attained its maximum value at 133.6 °C. A strong peak appeared at this temperature in the DTG curve of hydroxycitronellal. Although the atmospheric boiling point of hydroxycitronellal is 241 °C, hydroxycitronellal almost vaporized completely at the temperature of 180 °C.

Compared with the TG curve of hydroxycitronellal, from 32 °C to 68 °C relatively high mass losses can be observed in the TG curves of β-cyclodextrin, physical mixture and hydroxycitronellal-β-cyclodextrin inclusion complex. These mass losses were mainly due to the desorption of bound water on the surface of β-cyclodextrin. From 68 °C to 150 °C, relatively high mass loss can be observed again in the TG curve of the physical mixture.

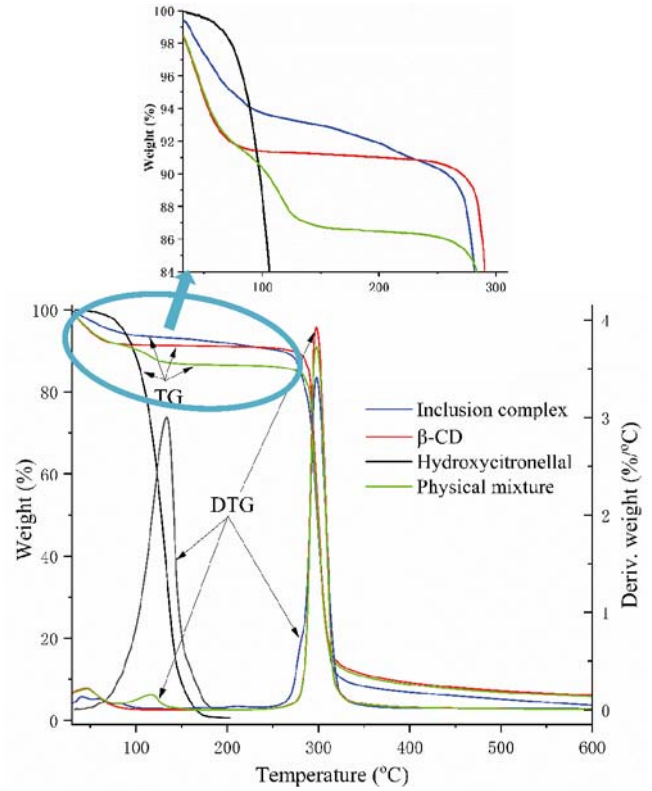


**Figure 6.** The TG and DTG curves of hydroxycitronellal, β-cyclodextrin, physical mixture, and hydroxycitronellal-β-cyclodextrin inclusion complex

This mass loss was mainly due to the volatilization of hydroxycitronellal in the physical mixture. From 180 °C to 269.5 °C, the TG curve of physical mixture was almost parallel with that of β-cyclodextrin. The TG curve of physical mixture was similar to that of β-cyclodextrin in the temperature range of 180 °C to 269.5 °C, and very low mass loss occurred in this stage. From 68 °C to 269.5 °C, a very slight mass loss of β-cyclodextrin occurred, and the TG curve of β-cyclodextrin in this stage was almost parallel with the temperature axis. While on the contrary, a relatively high mass loss of hydroxycitronellal-β-cyclodextrin inclusion complex occurred from 68 °C to 269.5 °C, and a downward tilt can be observed in the TG curve of hydroxycitronellal-β-cyclodextrin inclusion complex. The mass losses of β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complex were 1.0% and 3.4% respectively in the temperature range of 180 °C to 269.5 °C. Free hydroxycitronellal almost vaporized completely before 180 °C. It can be inferred that hydroxycitronellal encapsulated in β-cyclodextrin results in this difference in mass loss. From 180 °C to 269.5 °C, the mass loss due to hydroxycitronellal was 2.4%.

At 269.5 °C, β-cyclodextrin started to decompose. With the increase of temperature, β-cyclodextrin decomposed quickly and dramatic mass loss occurred. At 297.7 °C, 297.8 °C, and 297.9 °C, three strong peaks can be observed in the DTG curves of β-cyclodextrin, physical mixture and hydroxycitronellal-β-cyclodextrin inclusion complex respectively. At the peak temperature, the rate of mass loss of β-cyclodextrin attained its maximum value. From the temperature of about 327 °C to 600 °C (the final temperature of the experiment), slight continued mass losses can be observed in the DTG curves of β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complex again. In this stage, the residuals continuously decomposed at a very slow rate.

The temperature of the initial decomposition of β-cyclodextrin is about 269.5 °C. A more interesting thing was that the mass loss of hydroxycitronellal-β-cyclodextrin inclusion complex was higher than that of β-cyclodextrin during the initial period of decomposition of β-cyclodextrin. From 269.5 °C to 286.6 °C, the mass losses of hydroxycitronellal-β-cyclodextrin inclusion complex and β-cyclodextrin were 9.2% and 3.1% respectively. The difference, 6.1%, can also be attributed to the release of hydroxycitronellal. When β-cyclodextrin decomposed, the release of hydroxycitronellal from its inclusion complex still occurred. Free hydroxycitronellal almost vaporized completely when the temperature reached 180 °C under the experimental conditions. Hydroxycitronellal-β-cyclodextrin inclusion complex still gave off hydroxycitronellal at 286.6 °C. It indicated that hydroxycitronellal was successfully encapsulated in β-cyclodextrin. Compared with free hydroxycitronellal, the release of hydroxycitronellal from its inclusion complex at a relatively higher temperature supported the formation of an inclusion complex. It also revealed that the thermal stability and lastingness of hydroxycitronellal were improved by the formation of the inclusion complex. From 180 °C to 286.6 °C, the mass loss difference between hydroxycitronellal-β-cyclodextrin inclusion complex and β-cyclodextrin was about 8.5%. The loading capacity of hydroxycitronellal, which is defined as the mass ratio of hydroxycitronellal to hydroxycitronellal-β-cyclodextrin inclusion complex, was 8.5%.

### The binding energy and the optimized structure of hydroxycitronellal-β-cyclodextrin inclusion complex obtained by molecular simulation

Hydroxycitronellal molecule entered the cavity of β-cyclodextrin molecule along the Z-axis. When hydroxycitronellal molecule was at different position in the cavity of β-cyclodextrin molecule, the obtained binding energies by MM2 calculation were different. The Z coordinate of C6 atom in hydroxycitronellal molecule was used to mark the relative position between hydroxycitronellal and β-cyclodextrin. The binding energy change with the Z coordinate of C6 atom in hydroxycitronellal molecule was shown in Figure 7.

As shown in Figure 7, the values of binding energies are negative. It means that energy was released during the

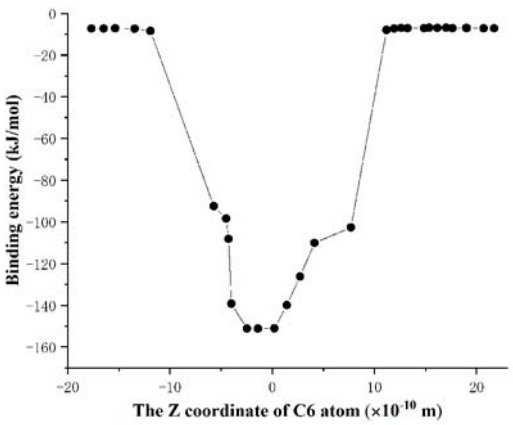


**Figure 7.** The binding energy change with the Z coordinate of C6 atom in hydroxycitronellal molecule

formation of hydroxycitronellal-β-cyclodextrin inclusion complex. With the increase of the Z coordinate of C6 atom in hydroxycitronellal molecule from $-17.7 \times 10^{-10}$ m to $-11.9 \times 10^{-10}$ m, a slight change in binding energy can be observed as shown in Figure 7. With further increase of the Z coordinate of C6 atom from $-11.9 \times 10^{-10}$ m, a dramatic decrease in binding energy occurred. The binding energy is minimum when the Z coordinate of C6 is $-2.5 \times 10^{-10}$ m. The minimum binding energy is –151.2 kJ/mol. The binding energy is formed from Stretch energy change (–1.5 kJ/mol), bend energy change (4.8 kJ/mol), stretch-bend energy change (–0.5 kJ/mol), torsion energy change (–3.8 kJ/mol), non-1,4-Van der Walls energy change (–125.9 kJ/mol), 1,4-Van der Walls energy change (3.1 kJ/mol), and dipole/dipole energy change (–27.4 kJ/mol). The biggest contribution to the decrease of the binding energy comes from non-1,4-Van der Walls energy change. Dipole/dipole energy change comes second. Torsion energy change, stretch energy and stretch-bend energy change also contribute to the decrease of the binding energy. Bend energy change and 1,4-Van der Walls energy change do not contribute to the decrease of binding energy. From $-2.5 \times 10^{-10}$ m to $0.2 \times 10^{-10}$ m, slightly change in binding energy occurred. With the increase of the Z coordinate of C6 atom from $0.2 \times 10^{-10}$ m to $11.2 \times 10^{-10}$ m, a dramatic increase in binding energy can be observed as shown in Figure 5. With further increase of the Z coordinate of C6 atom from $11.2 \times 10^{-10}$ m to $21.8 \times 10^{-10}$ m, the change in binding energy was slight again.

When hydroxycitronellal molecule entered the cavity of β-cyclodextrin molecule, energy was released during this process. The maximum value of energy release was 151.2 kJ/mol when the Z coordinate of C6 is $-2.5 \times 10^{-10}$ m. The more energy was released, the more stable the inclusion complex was formed. Therefore, the structure of the hydroxycitronellal-β-cyclodextrin inclusion complex with the minimum energy is the most stable one. The optimal structure with the minimum energy obtained by MM2 calculation is shown in Figure 8.

As shown in Figure 8, hydroxycitronellal molecule was encapsulated in the cavity of β-cyclodextrin molecule. Two hydrogen bonds can be observed in the hydroxycitronellal-β-cyclodextrin inclusion complex molecule. One hydrogen bond was formed between the oxygen atom of aldehyde group in hydroxycitronellal molecule and the hydrogen atom of hydroxy group in β-cyclodextrin molecule, and the other hydrogen bond was formed between the hydrogen atom of hydroxy group in hydroxycitronellal molecule and the oxygen atom of hydroxy group in β-cyclodextrin

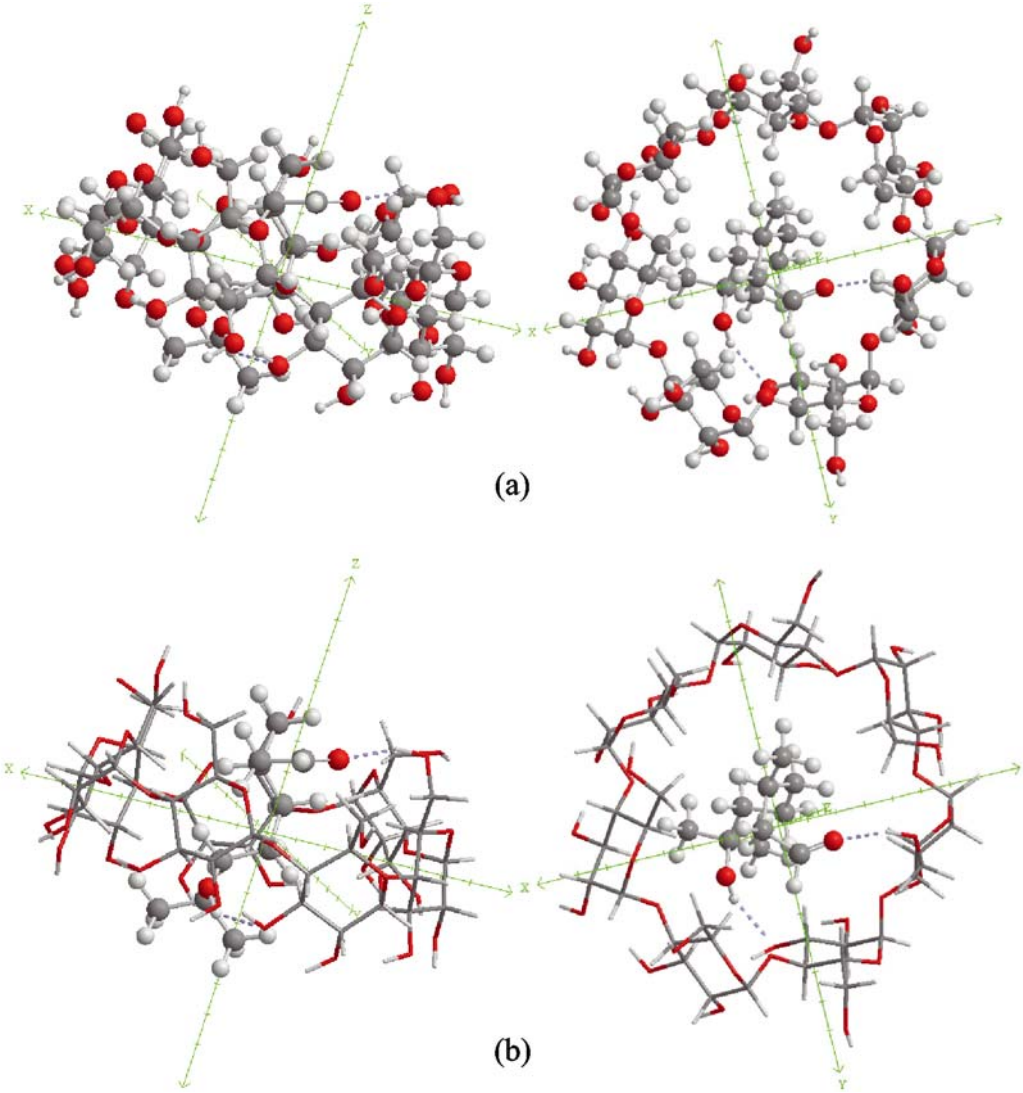


**Figure 8.** The optimal structure with the minimum energy obtained by MM2 calculation ((a) β-cyclodextrin and hydroxycitronellal display mode: ball and stick (b) β-cyclodextrin display mode: stick, hydroxycitronellal display mode: ball and stick)

molecule. The formation of hydrogen bond is one reason for the stability of hydroxycitronellal-β-cyclodextrin inclusion complex. It can be seen from Figure 6 that aldehyde group in hydroxycitronellal molecule is encapsulated in the cavity of β-cyclodextrin molecule after the formation of hydroxycitronellal-β-cyclodextrin inclusion complex. Therefore, the FTIR peak at 1724 $cm^{-1}$ due to aldehyde C=O stretching vibration in the FTIR spectrum of hydroxycitronellal disappeared in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex. It can also be seen from Figure 8 that the methyl group in hydroxycitronellal molecule is not completely encapsulated in the cavity of β-cyclodextrin molecules. The methyl group in hydroxycitronellal molecule is sticking out. Therefore, compared with the FTIR spectrum of β-cyclodextrin, two new peaks at 2978 $cm^{-1}$ and 2905 $cm^{-1}$ appeared in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex because of the asymmetrical and symmetrical C-H stretching absorption of the uncovered $CH_3$ in hydroxycitronellal molecule. The results of molecule simulation are helpful to understand the interaction between hydroxycitronellal and β-cyclodextrin.

**The assembly of hydroxycitronellal-β-cyclodextrin inclusion complex molecules obtained by molecular simulation**

β-Cyclodextrin can form aggregates in water. For a better understanding of the assembly of hydroxycitronellal-β-cyclodextrin inclusion complex molecules, molecular simulation was also performed. The structure of hydroxycitronellal-β-cyclodextrin inclusion complex molecule was optimized previously by MM2. This optimal structure was used as starting geometry to investigate the assembly of two hydroxycitronellal-β-cyclodextrin inclusion complex molecules. Three types of structures for hydroxycitronellal-β-cyclodextrin inclusion complex were evaluated: perpendicular, staggered parallel and ideally parallel orientation of the inclusion complexes. The energy changes ($\Delta E$) with the distances of one hydroxycitronellal-β-cyclodextrin inclusion complex molecule from another were shown in Figure 9.

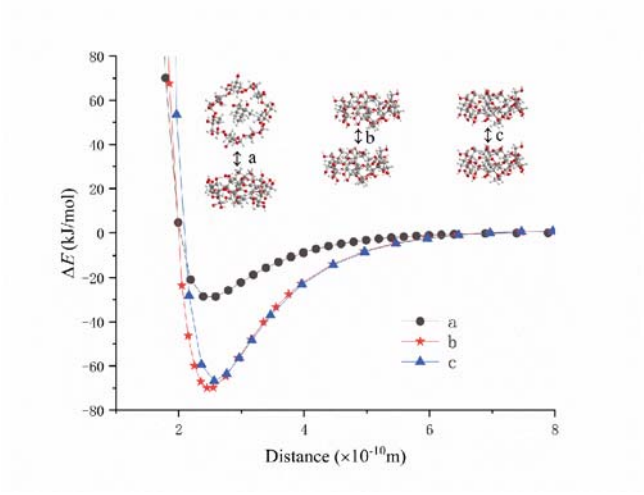


**Figure 9.** The energy changes with the distances of one hydroxycitronellal-β-cyclodextrin inclusion complex molecule from another. Orientation of the inclusion complexes: perpendicular (a), staggered parallel (b), and ideally parallel (c)

As shown in Figure 9, when the distance of the two hydroxycitronellal-β-cyclodextrin inclusion complex molecules is in the range of $7 \times 10^{-10}$ to $8 \times 10^{-10}$ m, the energy changes are approximately equal to 0 kJ/mol. The interaction of the two hydroxycitronellal-β-cyclodextrin inclusion complex molecules is very weak. For the three orientations, as the two hydroxycitronellal-β-cyclodextrin inclusion complex molecules approach each other from $6 \times 10^{-10}$ to $2.6 \times 10^{-10}$ m, the energy of the system tends to go down. The values of $\Delta E$ are negative. The interaction of hydroxycitronellal-β-cyclodextrin inclusion complex molecules to each other is attractive. When the distances of the two molecules are at $2.6 \times 10^{-10}$, $2.5 \times 10^{-10}$, $2.6 \times 10^{-10}$ m for perpendicular, staggered parallel and ideally parallel orientation respectively, the energy changes reach their bottoms. These values are –28.6, –69.9, –66.8 kJ/mol respectively. Among them, the minimum value is found for the staggered parallel arrangement. With the decrease of the distances of the two molecules, the values of $\Delta E$ increase. When the distances of the two molecules are less than $2.0 \times 10^{-10}$ m, the energy changes increase rapidly and the values of $\Delta E$ are positive. Highly positive value of $\Delta E$ means that the interaction of hydroxycitronellal-β-cyclodextrin inclusion complex molecules to each other is strongly repulsive.

Based on Figure 9, it can be inferred that it is energetically favorable for hydroxycitronellal-β-cyclodextrin inclusion complex molecules to line up in staggered parallel arrangement. Thus, it resulted in the structure shown in Figure 4. Similar structures were also reported for (-)-menthol-β-cyclodextrin inclusion complex and dimethyl sulfide-β-cyclodextrin inclusion complex[29, 30]. Some of these products were in the shape of parallelograms.

## CONCLUSIONS

Hydroxycitronellal was successfully encapsulated in the cavity of β-cyclodextrin and hydroxycitronellal-β-cyclodextrin inclusion complex was prepared. The loading capacity of hydroxycitronellal was about 8.5%. The products take on different shapes (parallelogram, rhombus, and irregular shapes). The size of most of the products is in the range of 2 to 10 μm. The peak at 1724 $cm^{-1}$ due to aldehyde C=O stretching vibration in the FTIR spectrum of hydroxycitronellal disappears in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex. Two new peaks at 2978 $cm^{-1}$ and 2905 $cm^{-1}$ appeared in the FTIR spectrum of hydroxycitronellal-β-cyclodextrin inclusion complex because of the asymmetrical and symmetrical C-H stretching absorption of the uncovered $CH_3$ in hydroxycitronellal molecule. These changes in FTIR spectrum suggest the successful encapsulation of hydroxycitronellal in β-cyclodextrin. Thermal stability and hydroxycitronellal release characteristics were obtained by thermal analysis. Free hydroxycitronellal almost vaporized completely when the temperature reached 180 °C. Hydroxycitronellal-β-cyclodextrin inclusion complex still gave off hydroxycitronellal at 286.6 °C. It also revealed that the thermal stability and lastingness of hydroxycitronellal were improved by the formation of the inclusion complex. The optimal structure of the inclusion complex and binding energy were obtained by molecule simulation. When the Z coordinate of C6 is $-2.5 \times 10^{-10}$ m,

the binding energy is minimum (–151.2 kJ/mol). The assembly of hydroxycitronellal-β-cyclodextrin inclusion complex molecules was also investigated by molecule simulation. It revealed that β-cyclodextrin inclusion complexes to line up in staggered parallel arrangement were energetically favorable. These basic data are useful to understand the interaction between hydroxycitronellal and β-cyclodextrin.

## ACKNOWLEDGMENTS

The authors thank financial supports from National Natural Science Found of China (31972196) and Shanghai Alliance Program (LM201844).

## LITERATURE CITED

1. Surburg, H. & Panten, J. (2006). Common Fragrance and Flavor Materials. Weinheim, Wiley-Vch Verlag GmbH & Co. KGaA.

2. Burdock, G.A. (2010). *Fenaroli's handbook of flavor ingredients*. Boca Raton, CRC Press.

3. Andrade, J.C., Monteiro, Á.B., Andrade, H.H.N., Gonzaga, T.K.S.N., Silva, P.R., Alves, D.N., Castro, R.D., Maia, M.S., Scotti, M.T., Sousa, D.P. & Almeida, Reinaldo N. (2021). Involvement of GABAA receptors in the anxiolytic-like effect of hydroxycitronellal. *BioMed Res. Int.* Article ID 9929805. DOI: 10.1155/2021/9929805

4. Teixeira, M.A., Rodríguez, O., Gomes, P., Mata, V. & Rodrigues, A.E. (2013). Perfume Engineering: Design, Performance & Classification, New York, Elsevier Ltd.

5. Zhou, M. & Liu, J. (2000). Study on the permeability of cinnamic aldehyde and hydroxycitronellal in vitro. Chinese J. Health Labor. Technol., 10(2), 135–137. DOI: CNKI:SUN:ZWJZ.0.2000-02-003.

6. Liu S. (2000). Technological Manuals of Synthetic Aroma Chemical, Beijing, China Light Industry Press Ltd.

7. Heydorn, S., Andersen, K.E., Johansen, J.D. & Menné, T. (2003). A stronger patch test elicitation reaction to the allergen hydroxycitronellal plus the irritant sodium lauryl sulfate. *Contact Dermatitis* 49, 133–139. DOI: 10.1111/j.0105-1873.2003.00175.x.

8. Calnan, C.D. (1979). Unusual hydroxycitronellal perfume dermatitis. *Contact Dermatitis* 5(2), 123–123. DOI: 10.1111/j.1600-0536.1979.tb04816.x.

9. Steltenkamp, R.J., Booman, K.A., Dorsky, J., King, T.O., Rothenstein, A.S., Schwoeppe, E.A., Sedlak, R.I., Smith T.H.F. & Thompson, G.R. (1980). Hydroxycitronellal: a survey of consumer patch-test sensitization. *Food Cosm. Toxicol.* 18, 407–412. DOI: 10.1016/0015-6264(80)90198-4.

10. Ford, R.A., Api, A.M. & Suskind, R.R. (1988). Allergic contact sensitization potential of hydroxycitronellal in humans. *Food Chem. Toxicol.* 26, 921–926. DOI: 10.1016/0278-6915(88)90090-7.

11. Liu, X., Wu, J., Wang, M. & Zhang, M. (2021). Research progress of cometic delivery system: application of pharmaceutical new dosage forms and new techniques in cosmetics. *Flavour Frag. Cosmetics*, 6, 99–105. (in Chnises).

12. Amann, M. & Dressnandt, G. (1993). Solving problems with cyclodextrins in cosmetics. *Cosmetics and Toiletries*, 108, 90–95.

13. Chen, N., Zhang, H. & Li, L. (2022). The carrier structure of retinol and retinol derivatives and its application in cosmetics. *Chem. World* 63(1), 51–56. (in Chinese). DOI: 10.19500/j.cnki.0367-6358.20200814.

14. Celebioglu, A., Yildiz, Z.I. & Uyar, T. (2018). Thymol/cyclodextrin inclusion complex nanofibrous webs: Enhanced water solubility, high thermal stability and antioxidant property of thymol. *Food Res. Int.* 106*,* 280–290. DOI: 10.1016/j.foodres.2017.12.062.

15. Kibici, D. & Kahveci, D. (2019). Effect of emulsifier type, maltodextrin, and β-cyclodextrin on physical and oxidative stability of oil-in-water emulsions. *J. Food Sci.* 84(6), 1273–1280. DOI: 10.1111/1750-3841.14619.

16. Perinelli, D.R., Palmieri, G.F., Cespi, M. & Bonacucina, G. (2020). Encapsulation of Flavours and Fragrances into Polymeric Capsules and Cyclodextrins Inclusion Complexes: An Update. *Molecules* 25, 5878. DOI: 10.3390/molecules25245878.

17. Kayaci, F., Sen, H.S., Durgun, E. & Uyar, T. (2014). Functional electrospun polymeric nanofibers incorporating geraniol–cyclodextrin inclusion complexes: High thermal stability and enhanced durability of geraniol. *Food Res. Int.* 62, 424–431. DOI: 10.1016/j.foodres.2014.03.033.

18. Siva, S., Li, C., Cui, H. & Lin, L. (2019). Encompassment of isoeugenol in 2-hydroxypropyl-β-cyclodextrin using ultrasonication: Characterization, antioxidant and antibacterial activities. *J. Mol. Liq.* 296, 111777. DOI: 10.1016/j.molliq.2019.111777

19. Real, D., Leonardi, D., WilliamsIII, R.O., Repka, M.A. & Salomon, C.J. (2018). Solving the Delivery Problems of Triclabendazole Using Cyclodextrins. *AAPS PharmSciTech* 19, 2311–2321. DOI: 10.1208/s12249-018-1057-5.

20. Zhu, G., Jiang, X., Zhu, G. & Xiao, Z. (2020). Encapsulation of difurfuryl disulfde in β-cyclodextrin and release characteristics of the guest from its inclusion complex. *J. Incl. Phenom. Macro. Chem.* 96, 263–273. DOI: 10.1007/s10847-019-00967-x.

21. Zhu, G., Zhu, G. & Xiao, Z. (2021). Study of formation constant, thermodynamics and β-ionone release characteristic of β-ionone-hydroxypropyl-β-cyclodextrin inclusion complex. *Polym. Bull.* 78, 247–260. DOI: 10.1007/s00289-020-03108-4.

22. Zhu, G., Xiao, Z., Yu, G., Zhu, G., Niu, Y. & Liu, J. (2021). Formation and characterization of furfuryl mercaptan-β-cyclodextrin inclusion complex and its thermal release characteristics. *Pol. J. Chem. Technol.* 23(4), 35–40. DOI: 10.2478/pjct-2021-0035.

23. Zhu, G., Xiao, Z. & Zhu, G. (2021). Fabrication and characterization of ethyl acetate-hydroxypropyl-β-cyclodextrin inclusion complex. *J. Food Sci.* 86, 3589–3597. DOI: 10.1111/1750-3841.15835.

24. Saenger, W., Jacob, J., Gessler, K., Steiner, T., Hoffmann, D., Sanbe, H., Koizumi, K., Smith, S.M. & Takaha, T. (1998). Structures of the common cyclodextrins and their larger analoguess beyond the doughnut. *Chem. Rev.* 98, 1787–1802. DOI: 10.1021/cr9700181.

25. He, Y., Fu, P., Shen, X. & Gao, H. (2008). Cyclodextrin-based aggregates and characterization by microscopy. *Micron* 39, 495–516. DOI: 10.1016/j.micron.2007.06.017.

26. Stuart, B. (1996). Modern Infrared Spectroscopy. New York, John Wiley & Sons27.Ning, Y. (2010). Spectroscopy in Organic Chemistry and Spectral Analysis. Beijing, China Science Publishing & Media Ltd.

27. Ning, Y. (2010). Spectroscopy in Organic Chemistry and Spectral Analysis. Beijing, China Science Publishing & Media Ltd.

28. Zhu, G., Zhu, G. & Xiao, Z. (2019). A review of the production of slowrelease favor by formation inclusion complex with cyclodextrins and their derivatives. *J. Incl. Phenom. Macro. Chem.* 95, 17–33. DOI: 10.1007/s10847-019-00929-3.

29. Zhu, G., Jin, Y., Xiao, Z. & Zhu, G. (2022). Preparation and characterization of the dimethyl sulfide-β-cyclodextrin inclusion complex. *J. Food Sci.* 87, 3084–3094. DOI: 10.1111/1750-3841.16216.

30. Zhu, G., Xiao, Z., Zhou, R., Liu, J., Zhu, G. & Zheng X. (2022). (-)-Menthol-β-cyclodextrin inclusion complex production and characterization. *Pol. J. Chem. Technol.* 24(2), 1–7. DOI: 10.2478/pjct-2022-0008.