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Preparation of Chitosan/β-Cyclodextrin and Its Absorption on Puerarin

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A new type of chitosan/β-cyclodextrin was prepared by reversed-phase suspension method with the chitosan and β-cyclodextrin as materials, sodium periodate as oxidant, epichlorohydrin as crosslinking agent in inverse suspension. Its absorption behaviour for puerarin was studied. The structure was characterized by means of IR and SEM. The different affected factors on absorption of puerarin were investigated. Results showed that the adsorption content was optimal when the adsorption time was 4 h, the temperature was 35 ºC, pH = 7 and initial concentration of puerarin was 0.5 mg/mL. The absorptive capability of chitosan/β-cyclodextrin for pueratin was 52.43 mg/g.

Key Words: Chitosan, β-Cyclodextrin, Epuerarin, Absorption.

INTRODUCTION

Chitosan (CS), is natural polymers extract from the shell of crustaceans, with non-toxic, inexpensive, can be biodegradable and good adsorption properties. Chitosan belongs to alkaline polysaccharide which have free amino, as the molecular chain have the amino and hydroxy, groups[1-4]. β-Cyclodextrin is composed of seven glucose units with α-1,4-glycosidic bond linked ring-shape oligosaccharides. It has a unique structure in which the interior is hydrophobic, external is hydrophilic, through van der Waals forces, hydrophobic interactions and match between the host-guest molecule, so β-cyclodextrin with many organic and inorganic molecules form inclusion compounds[5,6]. Chitosan and cyclodextrin cross-linked polymer has property of identification with the performance of cyclodextrin and feature of chitosan such as biodegradable, non toxic, absorption *etc*. So Chitosan/β-cyclodextrin has double features and has better adsorption capacity[7-10].

Puerarin is a separate isoflavones which is extracted from traditional chinese medicine Kudzu. It is the largest proportion in flavonoids of Kudzu. It has been experimentally demonstrated that puerarin can reduce myocardial oxygen consumption, improve the local microcirculation and reduce blood sugar and other pharmacological effects[11].

EXPERIMENTAL

Chitosan (Beijing Huikangyuan Biology Technology Limited Company), β-cyclodextrin (Tianjin Yingdaxigui Chemistry Reagent Company), sodium borohydride, sodium periodate, epichlorohydrin (Tianjin Fuchen Chemistry Reagent Company), epuerarin (Chinese Institute of Drugs and Biological Products Identification); liquid paraffin (Xilong Chemical Technology Limited Company); Tween 80 (Tianjin Guangfu Chemistry Reagent Company). Ethyl acetate (West Long Chemical Factory, Shantou City, Guangdong Province).

UV-1700 spectrophotometer (Shimadzu); Shaking water bath (Jintan city Jingda Electric Appliance Factory); SB-1000 water bath (Shanghai Airo Instrument Co. Ltd.); SHB-B multi-purpose circulating water pump (Zhengzhou Great Wall Industry and Trade Co. Ltd.); Lyophilizer (Christ); scanning electron microscope (FEI Company Netherlands); JJ-1A-Stirrer (Jintan City, Jiangsu Province, Ronghua Instrument Manufacturing Co. Ltd.); NEXUS-470 Fourier transform infrared spectrometer (Nicolet company USA).

Preparation of Chitosan/β-cyclodextrin

β-Cyclodextrin activation: The oxidation products of β-cyclodextrin generate by the reported method[12]. Weigh the amount of β-cyclodextrin dissolved in 100 mL of distilled water, adding 25 mL of 12 % solution of sodium periodate at 10 ºC, with constant stirring for 20 min under dark conditions, save in the dark place at 5 ºC overnight. With 5 % sodium bisulfite solution titrate the unreacted sodium periodate after 24 h. Then adsorption 0.5 h by 717 resin which has been pretreated, then use 732 resin adsorption 0.5 h to remove impurities of anions and cations, respectively. After freeze-dried the solution, cyclodextrin oxidation products was obtained.

Chitosan/cyclodextrin cross-linking reaction: Adding a small amount of Span 80 and Tween 80 to liquid paraffin, stir into the emulsion. Adding small amount of ethyl acetate to 2 % concentration of chitosan in acetic acid solution, then added to liquid paraffin emulsion, oil-water ratio is 1:2. Adding cyclodextrin aldehyde derivative stir 2 h at 40 ºC. We can get a stable intermediate by reduction with sodium borohydride. Under alkaline conditions with epichlorohydrin, cross-linking reaction occur at 60 ºC after 2 h. Synthesis products was washed by petroleum ether, methanol, distilled water, filtered to get chitosan/cyclodextrin (Fig. 1).

UV Spectrophotometer: UV analysis was applied to determine the content of epuerarin. Epuerarin concentration was calculated using the standard sample as the calibration standard. A good linear relationship was obtained over the range of 2 μg/mL < C < 14 μg/mL and the regression was y = 75.5x + 0.0036 (R^2 = 0.9998), where y is the absorbance at 250 nm, x is the concentration of epuerarin (mg mL^{-1}) and R is the regression coefficient.

Aldehyde content analysis: Using semi-micro hydroxylamine hydrochloride method[13] to analysis aldehyde content, hydroxylamine hydrochloride methanol solution and aldehyde

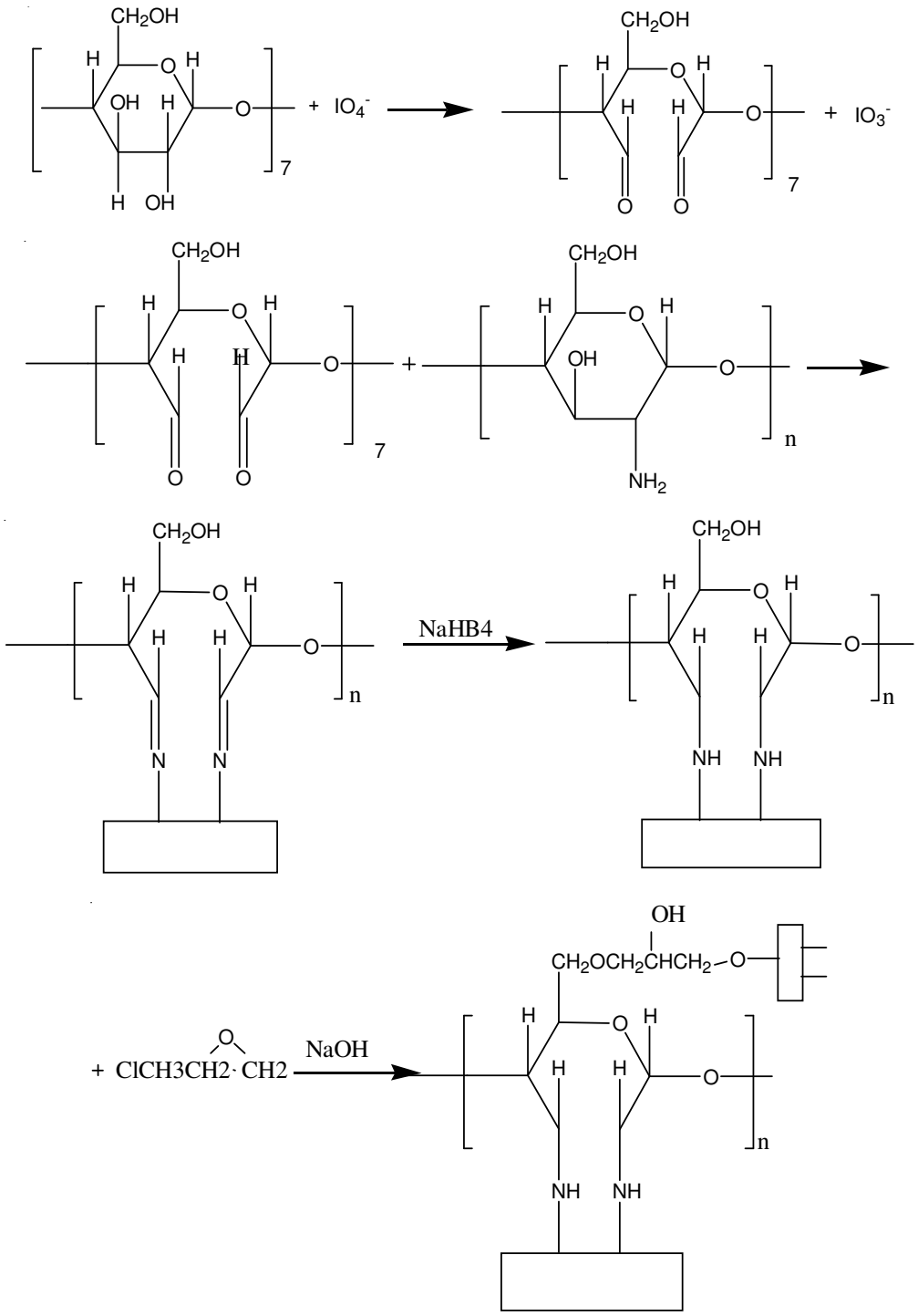

Fig. 1. Reaction scheme

can react quantitative, generate Schiff base, hydrochloric acid titrate with sodium hydroxide methanol solution. Weigh 0.1 g dry samples, mixed with 20 mL concentration of 0.3 mol/L hydroxylamine hydrochloride methanol solution, add 15 drops of 0.04 % methanol solution of thymol blue, reflux 2 h in water bath under 70 ºC, cool to room temperature, titration with 0.03 mol/L sodium hydroxide methanol solution which has been calibrated, until the solution colour from pink to yellow and make a blank experiment at the same time.

The formula is used to quantify aldehyde content:

$$H = 30X/1000G$$

where H is the aldehyde content (mol/g); X is volume consumption of sodium hydroxide (mL); G is sample quality (g).

Determination of chitosan/β-cyclodextrin absorption behaviour of puerarin: Chitosan/β-cyclodextrin are put into flask with stopper, some concentration of puerarin are added, the flask is then shaken for a while in water bath shaker. After the adsorption process, filter, measured the concentration of puerarin by UV at 250 nm absorbance. The following formula is used to quantify the adsorption capacities:

$$\text{Adsorption capacity } Q = \frac{V(C_0 - C_e)}{m}$$

where Q is the equilibrium adsorption capacity (mg/g); C_0 and C_e are the initial and equilibrium concentrations of puerarin in the solution, respectively (mg mL^{-1}); V is the volume of the initial solution of puerarin (mL) and m is the quality of the chitosan/β-cyclodextrin (g).

RESULTS AND DISCUSSION

Cyclodextrin C2, C3 selectively oxidized to aldehyde by sodium periodate. Cyclodextrin aldehyde derivatives under the appropriate conditions form Schiff base by reaction of chitosan of the amino. A stable chitosan/cyclodextrin intermediate is formed by reduction with sodium borohydride. Under alkaline conditions, cross-linking reaction use bifunctional cross-linking agent epichlorohydrin, cross-linking reaction occurs mainly on the C6 primary hydroxyl, obtain chitosan/cyclodextrin cross-linked material.

Structural analysis: FTIR diagram measured cyclodextrin, cyclodextrin aldehyde derivatives, chitosan/cyclodextrin and chitosan by Fourier transform infrared spectrometer (Fig. 2). Compare a, b shows that, due to open ring oxidation by $NaIO_4$ damage of the cyclodextrin structure, the fingerprint region of 'a' is different from the fingerprint region of 'b'. In addition, it shows the carbonyl region of 1650 cm^{-1} has a new absorption band , that means a new aldehyde produced. On comparing FTIR diagram c, d shows that as the chitosan amino substituted for the N-cyclodextrin derivatives, 1157 cm^{-1} peak intensity increases, C-N peak enhancement, in the 1560 cm^{-1} -NH_2 absorption peak significantly reduced and near 1652 cm^{-1} the C=O absorption peak has been enhanced. This indicates that part of -NH_2 and the C=O aldehyde reaction occurred. Other absorption peaks have little change. The origin of chitosan C-H stretching vibration of 2879 cm^{-1} and the origin of β-cyclodextrin C-H stretching vibration of 2925 cm^{-1} were significantly enhanced.

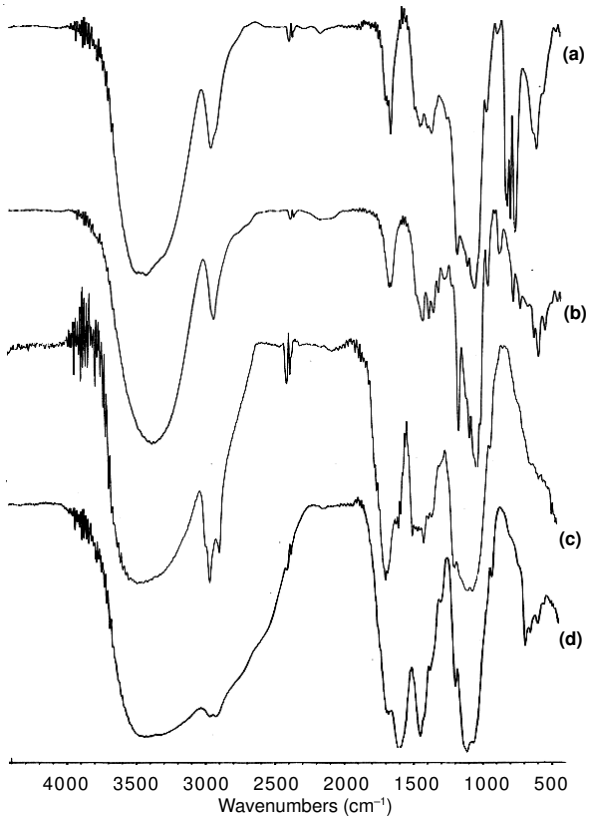

Fig. 2. FTIR of chitosan, cyclodextrin and the reaction product. (a) cyclodextrin, (b) cyclodextrin oxidized by sodium periodate, (c) chitosan/cyclodextrin, (d) chitosan

Scanning electron microscope: As can be seen in Fig. 3, polymer structure is irregular spherical and its surface was uneven honeycomb, which can increase the surface area, since there are some cavity surface structure, so the polymer have a certain degree of adsorption and embedding effect.

Aldehyde content analysis: The amount of aldehyde which is cyclodextrin derivatives is determined using titration. Titration shows that the amount of aldehyde which come from cyclodextrin oxidation by sodium periodate is 5.1 mol/g. It is to open the ring of cyclodextrin, hydroxyl of cyclodextrin C2, C3 selectively oxidized to aldehyde.

Chitosan/cyclodextrin swelling: 0.10 g dry chitosan/cyclodextrin placed in different pH buffer solution immersion 3 h, filtering then weighing. The following formula is used to calculate the swelling rate Sw = (mt – md)/md, where mt is wet chitosan/cyclodextrin; md is dry chitosan/cyclodextrin; Fig. 4 shows that pH values from 3.0-6.0 swelling ratio decreased; pH values from 6.0-9.0 swelling ratio is stable. Swelling ratio is up to a minimum at pH 6 and acid resistance of chitosan/cyclodextrin is superior to that of chitosan.

Effect of time to adsorption: Adding chitosan/cyclodextrin as adsorbent 0.05 g, the concentrate of epuerarin is 6 mg/100 mL, adsorption temperature is 30 ºC, the solution pH is 7, Oscillation rate is 150 times/min, the chitosan / cyclodextrin adsorption properties was studied under the above conditions. As time increases, the adsorption capacity increases, the adsorption is faster within 2 h, at 4 h, chitosan/cyclodextrin adsorb puerarin reach maximum, after 4 h the basic balance. The best adsorption time is 4 h (Fig. 5).

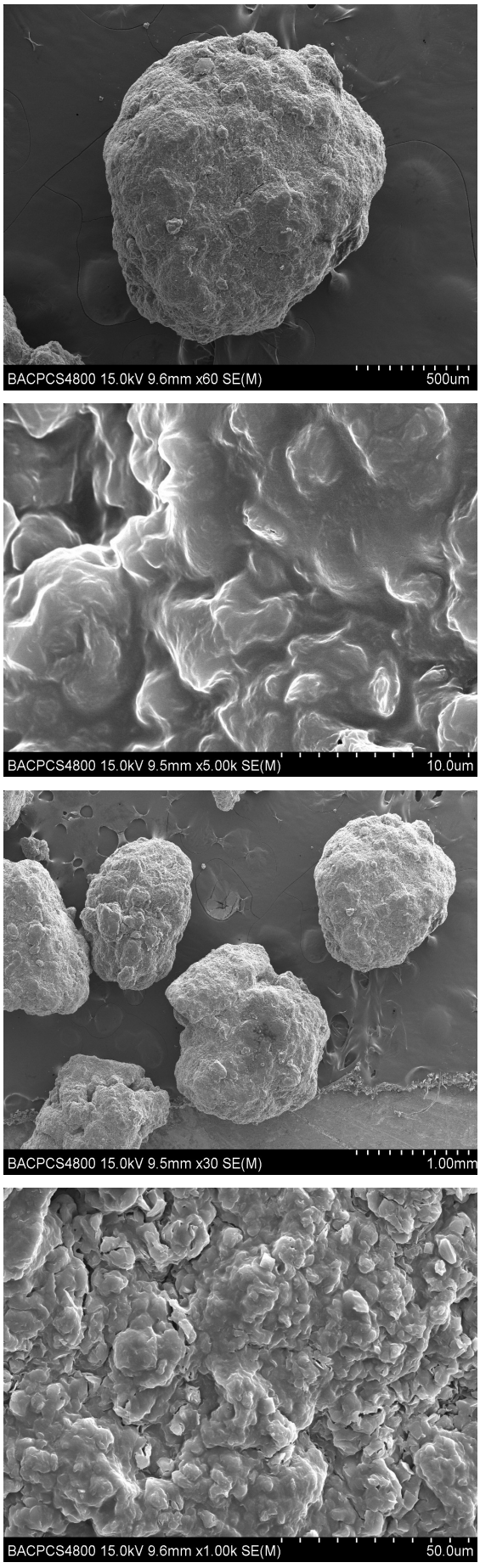

Fig. 3. SEM images of the chitosan/cyclodextrin

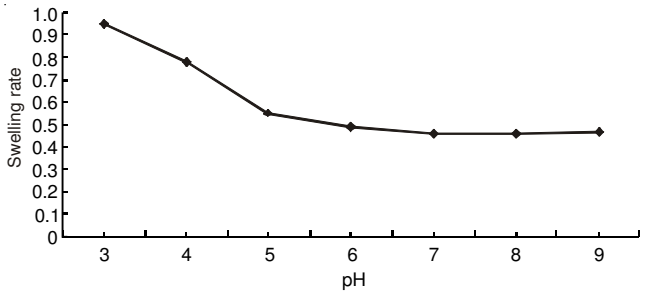

Fig. 4. Swelling rate of the chitosan/cyclodextrin

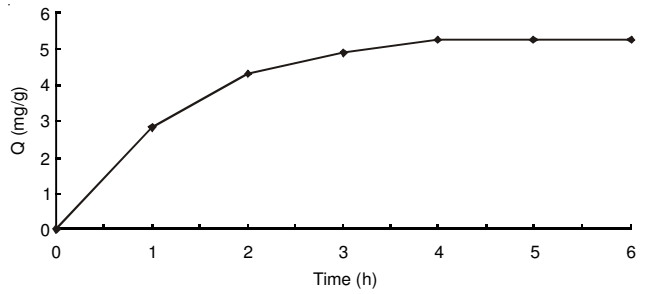

Fig. 5. Effect of time on adsorption

In the solid-liquid adsorption process, the adsorbent can adsorb solute and solvent. In the initial stages of adsorption, the adsorbent for the solute adsorption dominated, resulting in rapid increase in adsorption capacity, when the adsorbent for the solute adsorption is saturated, the adsorbent adsorb solvent relatively rapid, resulting in adsorption stabilized, when the two adsorption rate is the same, the concentration of the solution does not change.

Effect of temperature to adsorption: Adding 0.05 g chitosan/cyclodextrin sample to a certain concentration of puerarin 10 mL solution, after 4 h shaking, filter and measure absorbance values, calculate the adsorption capacity. Fig. 6 shows that adsorption capacity reaches the maximum when the temperature is at 35 ºC. Activation energy of the two processes adsorption and desorption are required and the adsorption activation energy is less than the desorption activation energy, so in the low temperature range, adsorption capacity increases with increasing temperature; but in the high temperature range, as the desorption process occur, adsorption capacity decreases with increasing temperature. Adsorption at 35 ºC is best.

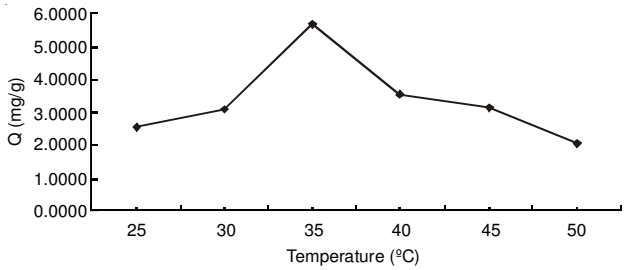

Fig. 6. Effect of temperature on adsorption

Effect of pH to adsorption: Adding 0.05 g chitosan/cyclodextrin sample to a certain concentration of puerarin 10 mL solution, after 4 h shaking, filter and measure absorbance values, calculate the adsorption capacity. Fig. 7 shows that adsorption capacity reaches the maximum when the pH is 7.

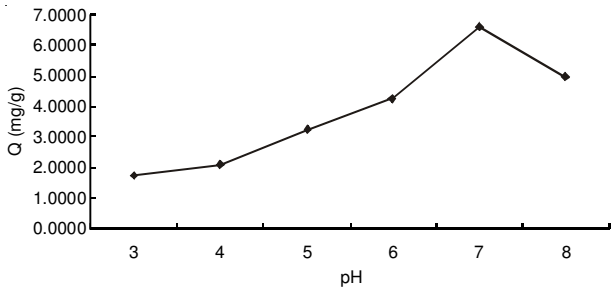

Fig. 7. Effect of pH on adsorption

When the pH value is small, the solution has a large number of H^+, inhibit the ionization of puerarin solution, which the solution is too acidic not conducive to the adsorption of puerarin. When the pH increases, OH^- concentration increases, it is not conducive to the hydrogen bonding of chitosan/cyclodextrin and the hydrophobic inclusion characteristic of cyclodextrins, so that the adsorption decreased. The force between adsorbent and puerarin may be mainly hydrogen bonding and hydrophobic interactions, when the solution is too acidic or too alkaline will weaken this force.

Effect of initial concentration to adsorption: Adding 0.05 g chitosan/cyclodextrin sample to the conditions of different concentrations of puerarin 10 mL solution, under the conditions at pH = 7, 40 ºC, after shaking 4 h, filter and measure absorbance values, calculate the adsorption capacity. It can be seen from Fig. 8, with the initial concentration of puerarin increases, the adsorption capacity of chitosan/cyclodextrin to puerarin increased gradually, when the initial concentration of puerarin increased to 0.5 mg/mL, the adsorption capacity reached 52.43 mg/g, chitosan/cyclodextrin adsorption reached saturation. Therefore, the best of puerarin initial concentration is 0.5 mg/mL.

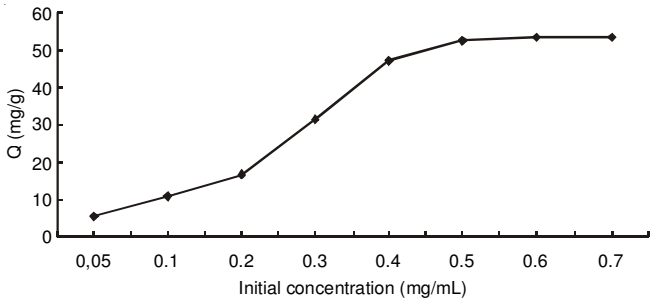

Fig. 8. Effect of initial concentration on adsorption

Conclusion

Chitosan/cyclodextrin has good adsorption performance for puerarin, when cyclodextrin is oxygenated by sodium periodate and combined with chitosan. The surface of chitosan/cyclodextrin had uneven cavity and there was honeycomb structure, temperature, time, pH and initial concentration of puerarin can affect the adsorption. According to the literature the adsorption capacity useing macroporous resin to adsorb pueraria flavonoid is 82.0 mg/g, ratio of puerarin adsorption capacity is 31.96 mg/g[14]. In this experiment, the absorptive capability of chitosan/β-cyclodextrin for pueratin is 52.43 mg/g, has good adsorption effect.

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