

Study on the Adsorption of Bile Acid by Bentonite in Vitro

Fengxian Yan², Xiaolai Ma¹, Zhifu Wu^{1*}

1 School of Pharmacy, Guilin Medical University, Guilin, Guangxi, 541199, China

2 Shenzhen Hepalink Pharmaceutical Co. Ltd. Shenzhen, Guangdong, 518057, China

Date of Submission: 01-08-2021

Date of Acceptance: 18-08-2021

ABSTRACT: Objective: To investigate the adsorption efficiency of sodium bentonite, acid bentonite and calcium bentonite. So as to explore the effective domestic drugs to reduce bile acid in vivo. **Methods:** The maximum absorption of bile acid at 387 nm after dehydration of sulfuric acid solution was obtained. The standard curve was obtained by measuring absorbance and concentration of bile acid by UV spectrophotometry. The method was sensitive, accurate and stable. The absorbance of bentonite for bile acid adsorption was measured, and the corresponding concentration of the corresponding bile acid was obtained from the standard curve, and then the adsorption rate of bentonite on bile acid was calculated in vitro. **Results:** The adsorption capacity of bentonite to bile acid was higher than that of sodium bentonite than that of calcium bentonite and acid bentonite.

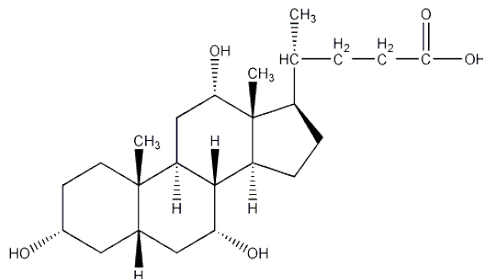
Conclusion: Sodium bentonite has the best adsorption efficiency for bile acid. It has strong adsorption and good clinical application prospect.

Key Words: bile acid ; UV-Spectrophotometry ; adsorption ; bentonite

I. INTRODUCTION

Bentonite (also known as microcrystalline kaolinite) is a non-metallic mineral with montmorillonite as the main mineral component, and a silicate clay mineral with laminated structure and flake crystal. It was named for its first discovery in Montmorillonite, France^[1-2]. It has fine particle size, large specific surface area, and non-uniform charge distribution, so it has a strong covering and adsorption capacity. The mineral layers of bentonite

are negatively charged and can absorb cations such as calcium, sodium, potassium and magnesium, with cation exchange properties^[3]. Bentonite has many medicinal properties, treating acute and chronic diarrhoea, colitis, reflux esophagitis, gastritis, irritable bowel syndrome and mouth ulcers. Bile acids (3A, 12A, 7A-trihydroxyl-cholestane-24-acid) are the main components of animal bile and one of the effective components of artificial bezoar. More than 100 species of bile acids have been discovered, mainly including bile acids, deoxygenated bile acids, goose deoxygenated bile acids, bear deoxygenated bile acids, pig deoxygenated bile acids, etc.^[4]. Bile acids in the human body are synthesized by the liver and discharged into the duodenum with bile. As a component of digestive juice, they can promote the digestion and absorption of lipids. When pyloric dysfunction occurs, bile acid will regurgitate into the stomach, causing damage to the gastric mucosa together with gastric acid and causing discomfort symptoms such as stomachache. Therefore, bile acid is one of the chemical factors that cause gastric mucosa injury^[5]. It has been reported that attapulgit powder can adsorb bile acids, thereby reducing the concentration of acid in gastric juice and reducing various gastroesophageal lesions. If bentonite and bile acid containing drugs are taken together, the adsorption property of bentonite may affect its efficacy, but this effect may also be applied to drug slow release. In this experiment, sodium bentonite, acid bentonite and calcium bentonite were used to adsorb bile acids. The adsorption efficiency of these bentonite on bile acids was studied by ultraviolet spectrophotometry. The following is the formula for bile acids.



Bile acids are steroidal structures and bile salts are emulsifiers that assist in the hydrolysis and absorption of fats in the intestine. Some bile acids also have the functions of relieving spasmodic, invigorating stomach, and reducing the content of cholesterol in blood [6].

II. EXPERIMENT SECTION

2.1 Experimental materials

UV-722 UV Spectrophotometer (Shanghai Science and Precision Instrument Co., Ltd.);KQ-3200 Ultrasonic Cleaner (Kunshan Instrument Company);Shanghai Analytical Instrument Factory for Optical Reading Analytical Balance);Centrifugal precipitator (Shanghai Surgical Instrument Factory);HH-4 type digital display constant temperature water bath box;60% glacial acetic acid;43.6% sulfuric acid solution (50ml concentrated sulfuric acid +65ml distilled water);Bile acid; Sodium bentonite (Anyang hydrometallurgy);Calcium-type bentonite (produced in Lin 'an, Zhejiang);Acid bentonite (produced in Anyang hydrometallurgy).

2.2 Experimental method

2.2.1 Standard curve:

(1) Preparation of bile acid standard solution: Bile acid solution with a concentration of 1mg/mL was obtained by accurately weighing the bile acid standard 50mg dried to constant weight at 105°C and placing it in a 50mL volumetric flask, adding 60% glacial acetic acid to dissolve it, and adding it to the scale and shaking it evenly.

(2) Standard curve drawing:

Precise absorption of 1mg/mL bile acid standard solution 0.1ml, 0.2ml, 0.4ml, 0.6ml, 0.8mL and 1.0mL into the calibrated tube with plug, add 60% acetic acid to 1mL, and then add 14ml of 43.6% sulfuric acid solution, shake well, place in a water bath at 70°C and heat for 40min, take out and cool. The absorbance was determined by spectrophotometry at 387nm. The same was done in the water bath for 60min and 90min. The experimental results are shown in the table below:

Table 1 Bile acid concentration and absorbance value table after heating for different time

The sample	0.1ml	0.2ml	0.4ml	0.6ml	0.8ml	1.0ml
absorbance (40min)	0.015	0.034	0.076	0.111	0.143	0.203
absorbance (60min)	0.042	0.085	0.169	0.249	0.336	0.410
absorbance (90min)	0.059	0.103	0.209	0.241	0.349	0.501

There is no significant difference between the absorbance at 90min and that at 60min. Seen from the experimental data, when the concentration of bile acid solution at the same time, the heating time prolonged, the absorbance of bile acid, that is to say, the heating time extension of bile acid and sulfuric

acid to generate double bond conjugate product quantity increased, absorbance increase, but the time is too long, some water and acetic acid can evaporate, resulting in a decrease of the volume, but still press before heating volume calculation, measuring result is larger, Therefore, the heating time of 60min was

adopted. In addition, when the standard concentration is 0.2mg/ mL, the maximum absorbance measured is not more than 0.3, so the standard solution of this concentration is not used, but the concentration of 1mg/ mL is used as the standard solution. Below is the standard curve of bile acid absorbance - concentration. The results showed that there was a good linear relationship between the absorbance and the concentration of cholic acid in the range of 0.1mg/ mL-1 mg/ml.

(3) Reproducibility test: the standard bile acid solution of 1mg/ml (0.1 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL) was absorbed in two portions, respectively, and the reproducibility was good when operated according to the standard curve.

(4) Solution stability test: the bile acid solution under the standard curve was taken and the absorbance was measured every 20min according to the above conditions. The results showed that the absorption value was stable within 3 hours.

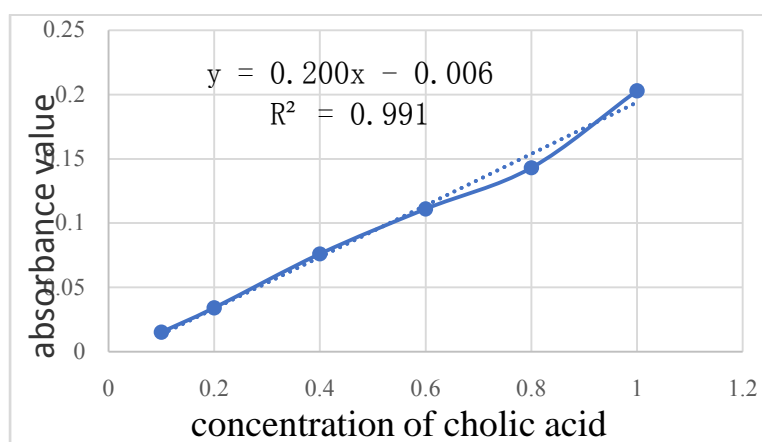


Figure 1. Standard curve of bile acid absorbance after 40min with 70 °C water bath heating

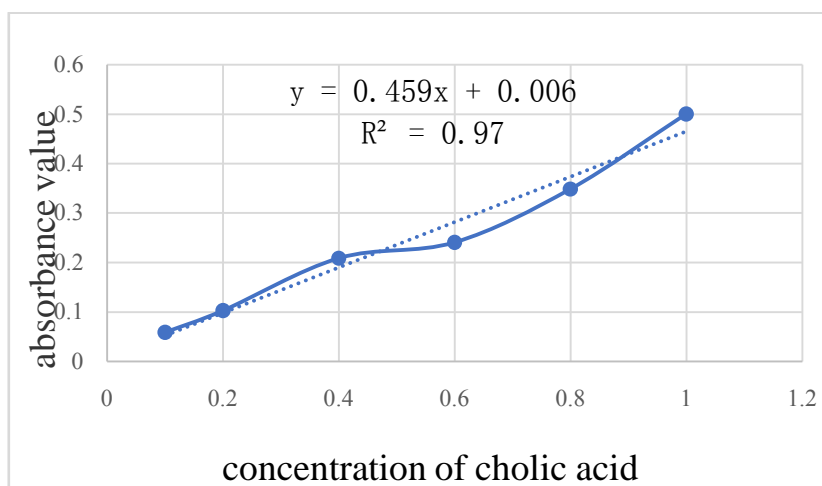


Figure 2. Standard curve of bile acid absorbance after 60min with 70 °C water bath heating

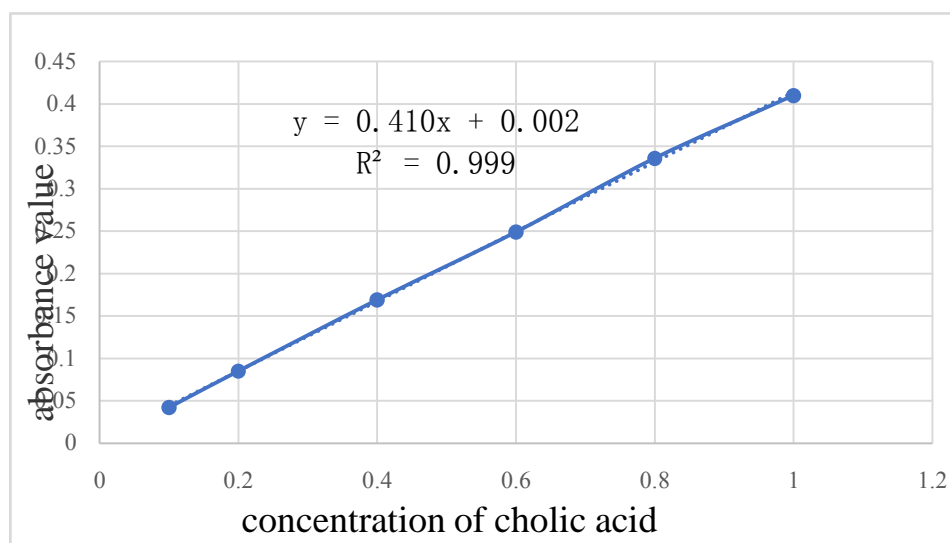


Figure 3. Standard curve of bile acid absorbance after 90min with 70 °C water bath heating

The standard curve obtained by setting three times is shown in the figure above. Comparing the figures, it can be seen that the standard curve reflecting 60 minutes' time is the most linear; The linearity of 40min and 90min reactions is not good. In addition, from the experimental data, when the concentration of bile acid solution is the same, the heating time prolongs, the absorbance of bile acid increases. When the heating time prolongs, the amount of double bond conjugated products generated by the interaction between bile acid and sulfuric acid increases, and the absorbance increases. However, part of the water and acetic acid will volatilize over a long period of time, resulting in a reduction in the volume. However, the measurement results are still too large according to the pre-heating volume, so the reaction time of 60min heating is adopted.

2.2.2 Determination of bentonite adsorption cholic acid sample:

Preparation of the sample: accurately weigh 0.05g of pure bentonite and 20ml of 0.2mg/ml bile acid in a 50ml volumetric flask with 60% acetic acid, and shake well. It was oscillated in a water bath at 37 °C for 5min, placed overnight, and centrifuged in a centrifuge at a rotating speed of 3000 RPM for 30 min. Bentonite was deposited on the bottom of the

centrifuge tube. Conjugate product generation: 1ml supernatant was taken into the tube with plug, and 14ml 43.6% sulfuric acid solution was added, respectively, and shaken evenly. Then 1ml 60% acetic acid solution was put into the stopper tube, and 14ml 43.6% sulfuric acid solution was added, shaken well and used as the reference solution. The above three were heated in a water bath of 70°C for 60min, then cooled to room temperature in cold water, and tested. Absorbance determination: the absorbance of the sample solution and bentonite solution were measured respectively. After adsorption, the absorbance of the sample solution = the absorbance of the sample solution - the absorbance of bentonite solution. The concentration of the sample solution after adsorption can be calculated from the standard curve.

III. RESULTS AND DISCUSSION

Adsorption rate $\eta = \frac{1-C}{C_0} \times 100\%$ (η represents adsorption rate, C represents the concentration of sample solution after adsorption, and the concentration of C_0 sample solution before adsorption) was calculated according to the following formula. The adsorption rates of three different bentonites for bile acids were shown in the table below:

Table 2 Adsorption rates of the three bentonites

Types of bentonite	Initial drug concentration (mg/ml)	Post-adsorption concentration (mg/ml)	The adsorption rate
Sodium -based bentonite	0.4	0.275	31.25%
Calcium-based bentonite	0.4	0.305	23.75%
Acid -based bentonite	0.4	0.315	21.25%

According to the above table, the adsorption rates of the three bentonites can be seen that the sodium bentonite has the best adsorption performance for bile acid, followed by the calcium bentonite, and the acid bentonite has the worse adsorption performance.

Bile acids are steroidal compounds. Although they have polar groups -OH and -COOH, their solubility in water is very low. Therefore, 60% acetic acid is used as the solvent instead of water, so the preparation of relevant samples also takes 60% acetic acid as the solvent. Using acetic acid and sulfuric acid as reference fluid measuring the absorbance of the bentonite solution, because the maximum absorption wavelength of bentonite in bile acid also has the absorbance, using acetic acid and sulfuric acid as reference fluid absorbance of bile acid + + acetic acid sulfate, actually measured the absorbance of bile acid bentonite +, asking too absorbance of bile acid also need to minus the bentonite absorbance. The bentonite solution is colloidal and cannot be filtered to remove the bentonite. If not placed, the absorbance cannot be measured because the centrifugation time is too long to clarify. In large quantities of bentonite, precipitation and centrifugation require a long time, so the amount of bentonite is reduced to 0.05 g. Bentonite is a naturally formed, expandable layered silicate inorganic substance with an alternating arrangement of aluminum oxygen octahedral layer and silicon oxygen tetrahedral layer. Because part of the aluminum ion in the octahedral layer is replaced by the plasma of magnesium, sodium, potassium, calcium and hydrogen and is negatively charged, it has the ability to adsorb positive charge and strong cation exchange characteristics. Bile acid and 60% acetic acid solutions, the hydroxyl and carboxyl groups have protonated, cationic properties, so it can be adsorbed by bentonite.

IV. CONCLUSION

Bile acids are usually synthesized from the liver and discharged into the duodenum by bile as part of digestive juices that facilitate the digestion and absorption of lipids. When pyloric dysfunction, bile acid will flow back to the stomach, together with gastric acid to cause gastric mucosa damage, and cause stomach pain and other uncomfortable symptoms, therefore, bile acid is one of the chemical factors that cause gastric mucosa damage. Clinical commonly used aluminum magnesium carbonate can not only neutralize gastric acid, but also combine with bile acid, thus reducing the damage to gastric mucosa, which is conducive to the elimination of inflammation and the healing of ulcers. Many stomach pain in patients after drug treatment, stomach symptoms should ease in the short term, however, to mucous membrane of damage and repair, need a certain amount of time, such as most of the ulcer healing need four to six weeks of formal treatment, that is to say, eliminate symptoms is not equal to lesions subsided, it is necessary to offer certain time a continuation of the drug. At the same time, it is necessary to treat the causes of bile reflux. The results of this study show that it is an ideal method to use non-toxic and harmless bentonite to adsorb bile acids.

REFERENCES

- [1] Lin Tao, Wang Jun, Yin Xuefeng, Wei Xiaoyao. Research progress on application of bentonite in traditional fields and antibacterial materials. *Journal of Shanxi University of Science and Technology*, 2020, 38(6): 124-132.
- [2] Han Hongqing, Zhu Yue. Study on modification and application of bentonite. *Inorganic salt industry*, 2011, 10(10): 5-8.
- [3] Liu Yuqin. Discussion on preparation technology and performance evaluation index of prehydrated bentonite impervious material. *China Non-metallic Ore Industry Guide*, 2020



- (4) : 15-17.
- [4]. Ma Jie, Liu Gang, Huang Xingguo, et al. Recent advances in the interaction between gut microbiota/metabolites and host lipid metabolism. Science China Press, 2012 , 1,51
- [5]. Huang Xifeng, Huang Jie, Ma Xianguan, Wen Shangkai. The content of total bile acid in snake bile was determined by ultraviolet spectrophotometry. Chinese Medicine in Guangxi , 1996,19 (1) : 42
- [6]. He Nianmin. Determination of bile acid content in dissolvable gallstone tablets by ultraviolet spectrophotometry. Chinese patent medicine , 1995,17 (1) : 18