Notice

DESCRIPTION CN112813123A

A method for preparing copper-containing complexes

[0001]

Technical Field

[n0001]

This invention belongs to the field of biomaterials and relates to a method for preparing copper-containing complexes.

[0003]

Background Technology

[n0002]

Copper is an essential trace element for the human body. It is a cofactor for many enzymes and a necessary condition for the synthesis of collagen and elastin. It can tighten the skin, resist free radical damage, and even work synergistically with vitamin E to repair the skin.

Free copper ions are difficult to penetrate the skin and exert their effects through topical application. The main way to achieve the desired effect is by combining copper with biomass molecules.

For example, GHK-Cu is a complex product of copper and tripeptide. Experiments have shown that by binding copper with peptides, it can stimulate the formation of collagen and elastin, increase the production of intercellular mucus in skin cells, assist the antioxidant enzyme SOD, expand hair follicles and accelerate hair growth, among many other effects.

[n0003]

Currently, GHK-Cu is mainly prepared through artificial synthesis, but the existing methods are costly, which limits its application in cosmetic products.

Experiments have shown that GHK-Cu mainly achieves the above-mentioned functions by

inducing fibroblasts to generate matrix metalloenzymes (MMPs), while other materials can achieve similar effects when used as copper ion carriers.

[n0004]

This invention provides a method for generating small molecule peptides by hydrolyzing collagen and then complexing them with copper ions. This method produces small molecule peptides with a molecular weight of 500-2000 at a relatively low cost. Small molecule peptides in this molecular weight range have high bioavailability and can replace existing synthetic small peptides as copper ion carriers.

[0007]

Summary of the Invention

[n0005]

The technical solution of the present invention is as follows:

[n0006]

A method for preparing a copper-containing complex, the method is as follows:

[n0007]

(1) Add edible gelatin to 85°C 1% sodium hydroxide aqueous solution, stir for 24h, filter out insoluble matter with filter cloth, and cool the filtrate to 50°C;

[n0008]

(2) Adjust the pH of the gel solution to 9.5 with hydrochloric acid, add alkaline protease, and react at 60°C for 4 hours;

[n0009]

(3) Heat the adhesive to 90°C and keep it at that temperature for 1 hour, then cool it to 40°C;

[n0010]

(4) Adjust the pH of the gel solution to 7.8 with hydrochloric acid, add neutral protease, and react at 40°C for 4 hours;

[n0011]

(5) Adjust the pH of the gel solution to 3.0 with hydrochloric acid, add acidic protease, and react at 40°C for 4 hours;

[n0012]

(6) Adjust the pH of the glue solution to 7.0 with sodium hydroxide, add copper hydroxide, stir for 8 hours, and then remove the insoluble matter with filter cloth;

[n0013]

(7) Add anhydrous ethanol at a ratio of 1:4 of the volume of the adhesive solution, stir for 1 hour, and then remove the insoluble matter with a filter cloth.

[n0014]

(8) Continue adding anhydrous ethanol until the volume ratio of ethanol to the gel reaches 1:1. Stir for 1 hour, then filter with a filter cloth to collect the insoluble matter.

[n0015]

Rinse with ethanol and then vacuum dry;

[n0016]

(9) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0017]

Furthermore, in step (1), the mass percentage of edible gelatin added is 3-5%.

[n0018]

Furthermore, in step (2), the pH range of the adhesive solution is 8.0-11.0, preferably 9.0-10.0, and the mass percentage of alkaline protease added is 0.1-0.3%.

[n0019]

Furthermore, in step (4), the pH range of the adhesive solution is 6.5-8.5, preferably 7.5-8.0, and the mass percentage of neutral protease added is 0.2-0.35%.

[n0020]

Furthermore, in step (5), the pH range of the adhesive solution is 2.0-4.0, preferably 2.5-3.5, and the mass percentage of acidic protease added is 0.2-0.4%.

[n0021]

Furthermore, in step (6), the amount of copper hydroxide added is 1-2% of the total weight of the adhesive solution.

[0025]

Attached Figure Description

[n0022]

Figure 1 compares the effects of copper acetate, GHK-Cu, and the product from Experiment 1 containing the same amount of copper ions (10 NER1 mol/L) on promoting the release of matrix metalloenzymes (MMPs) from fibroblasts.

[0027]

Detailed Implementation

[n0023]

The present invention will be further illustrated by the following examples.

[n0024]

Example 1

[n0025]

(1) Add 500g of edible gelatin to 10L of 1% sodium hydroxide aqueous solution at 85°C, stir for 24h, filter out insoluble matter with filter cloth, and cool the filtrate to 60°C;

[n0026]

(2) Adjust the pH of the gel solution to 9.0 with hydrochloric acid, add 30g of alkaline protease (Bacillus licheniformis), and react at 60°C for 4h; heat the gel solution to 90°C and keep it at that temperature for 1h, and then cool it to 40°C;

[n0027]

(3) Adjust the pH of the gel solution to 7.8 with hydrochloric acid, add 35g of neutral protease (Bacillus subtilis), and react at 40°C for 4h;

[n0028]

(4) Adjust the pH of the gel solution to 3.0 with hydrochloric acid, add 40g of acidic protease (Aspergillus niger), and react at 40°C for 4h;

[n0029]

(5) Adjust the pH of the glue solution to 7.0 with sodium hydroxide, add 100g of copper hydroxide, stir for 8 hours and then remove the insoluble matter with filter cloth;

[n0030]

(6) Add 2.5L of anhydrous ethanol, stir for 1 hour, and then remove the insoluble matter with filter cloth;

[n0031]

(7) Continue to add 7.5L of anhydrous ethanol, stir for 1 hour, filter with filter cloth to collect the insoluble matter, rinse with ethanol and then vacuum dry;

[n0032]

(8) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0033]

Example 2

[n0034]

(1) Add 500g of edible gelatin to 10L of 1% sodium hydroxide aqueous solution at 85°C, stir for 24h, filter out insoluble matter with filter cloth, and cool the filtrate to 60°C;

[n0035]

(2) Adjust the pH of the gel solution to 9.0 with hydrochloric acid, add 30g of alkaline protease (Bacillus licheniformis), and react at 60°C for 4h;

[n0036]

(3) Heat the adhesive to 90°C and keep it at that temperature for 1 hour, then cool it to 40°C;

[n0037]

(4) Adjust the pH of the gel solution to 7.0 with hydrochloric acid, add 35g of neutral protease (papain), and react at 40°C for 4h;

[n0038]

(5) Adjust the pH of the gel solution to 3.0 with hydrochloric acid, add 40g of acidic protease (Aspergillus niger), and react at 40°C for 4h;

[n0039]

(6) Adjust the pH of the glue solution to 7.0 with sodium hydroxide, add 100g of copper hydroxide, stir for 8 hours and then remove the insoluble matter with filter cloth;

[n0040]

(7) Add 2.5L of anhydrous ethanol, stir for 1 hour, and then remove the insoluble matter with filter cloth;

[n0041]

(8) Continue to add 7.5L of anhydrous ethanol, stir for 1 hour, filter with filter cloth to collect the insoluble matter, rinse with ethanol and then vacuum dry.

[n0042]

(9) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0043]

Example 3

[n0044]

(1) Add 500g of edible gelatin to 10L of 1% sodium hydroxide aqueous solution at 85°C, stir for 24h, filter out insoluble matter with filter cloth, and cool the filtrate to 60°C;

[n0045]

(2) Adjust the pH of the gel solution to 9.0 with hydrochloric acid, add 30g of alkaline protease (Bacillus licheniformis), and microwave at 60°C for 15min;

[n0046]

(3) Heat the adhesive to 90°C and keep it at that temperature for 1 hour, then cool it to 40°C;

[n0047]

(4) Adjust the pH of the gel solution to 7.8 with hydrochloric acid, add 35g of neutral protease (Bacillus subtilis), and microwave at 40°C for 15min.

[n0048]

(5) Adjust the pH of the gel solution to 3.0 with hydrochloric acid, add 40g of acidic protease (Aspergillus niger), and microwave at 40°C for 15min;

[n0049]

(6) Adjust the pH of the glue solution to 7.0 with sodium hydroxide, add 200g of copper hydroxide, stir for 8 hours, and then remove the insoluble matter with filter cloth;

[n0050]

(7) Add 2.5L of anhydrous ethanol, stir for 1 hour, and then remove the insoluble matter with filter cloth;

[n0051]

(8) Continue to add 7.5L of anhydrous ethanol, stir for 1 hour, filter with filter cloth to collect the insoluble matter, rinse with ethanol and then vacuum dry.

[n0052]

(9) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0053]

Comparative Example 1

[n0054]

(1) Add 500g of edible gelatin to 10L of 1% sodium hydroxide aqueous solution at 85°C, stir for 24h, filter out insoluble matter with filter cloth, and cool the filtrate to 60°C;

[n0055]

(2) Adjust the pH of the gel solution to 9.0 with hydrochloric acid, add 30g of alkaline protease (Bacillus licheniformis), and react at 60°C for 4h;

[n0056]

(3) Heat the adhesive to 90°C and keep it at that temperature for 1 hour, then cool it to 40°C;

[n0057]

(4) Adjust the pH of the gel solution to 7.8 with hydrochloric acid, add 35g of neutral protease (Bacillus subtilis), and react at 40°C for 4h;

[n0058]

(5) Adjust the pH of the glue solution to 7.0 with hydrochloric acid, add 100g of copper hydroxide, stir for 8 hours and then remove the insoluble matter with filter cloth;

[n0059]

(6) Add 2.5L of anhydrous ethanol, stir for 1 hour, and then remove the insoluble matter with filter cloth;

[n0060]

(7) Continue to add 7.5L of anhydrous ethanol, stir for 1 hour, filter with filter cloth to collect the insoluble matter, rinse with ethanol and then vacuum dry;

[n0061]

(8) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0062]

Comparative Example 2

[n0063]

(1) Add 500g of edible gelatin to 10L of water at 85°C, stir for 24h, and cool the filtrate to 60°C;

[n0064]

(2) Adjust the pH of the glue solution to 9.0 with sodium hydroxide, add 30g of alkaline protease (Bacillus licheniformis), and react at 60°C for 4h;

[n0065]

(3) Heat the adhesive to 90°C and keep it at that temperature for 1 hour, then cool it to 40°C;

[n0066]

(4) Adjust the pH of the gel solution to 7.8 with hydrochloric acid, add 35g of neutral protease (Bacillus subtilis), and react at 40°C for 4h;

[n0067]

(5) Adjust the pH of the gel solution to 3.0 with hydrochloric acid, add 40g of acidic protease (Aspergillus niger), and react at 40°C for 4h;

[n0068]

(6) Adjust the pH of the glue solution to 7.0 with sodium hydroxide, add 100g of copper hydroxide, stir for 8 hours and then remove the insoluble matter with filter cloth;

[n0069]

(7) Add 2.5L of anhydrous ethanol, stir for 1 hour, and then remove the insoluble matter with filter cloth;

[n0070]

(8) Continue to add 7.5L of anhydrous ethanol, stir for 1 hour, filter with filter cloth to collect the insoluble matter, rinse with ethanol and then vacuum dry.

[n0071]

(9) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0072]

Comparative Example 3

[n0073]

(1) Add 500g of edible gelatin to 10L of 1% sodium hydroxide aqueous solution at 85°C, stir for 24h, filter out insoluble matter with filter cloth, and cool the filtrate to 60°C;

[n0074]

(2) Adjust the pH of the gel solution to 7.8 with hydrochloric acid, add 35g of neutral protease (Bacillus subtilis), and react at 40°C for 4h;

[n0075]

(3) Heat the adhesive to 90°C and keep it at that temperature for 1 hour, then cool it to 40°C;

[n0076]

(4) Adjust the pH of the gel solution to 3.0 with hydrochloric acid, add 40g of acidic protease (Aspergillus niger), and react at 40°C for 4h;

[n0077]

(5) Adjust the pH of the glue solution to 9.0 with sodium hydroxide, add 30g of alkaline protease (Bacillus licheniformis), and react at 60°C for 4h;

[n0078]

(6) Adjust the pH of the glue solution to 7.0 with hydrochloric acid, add 100g of copper hydroxide, stir for 8 hours, and then remove the insoluble matter with filter cloth;

[n0079]

(7) Add 2.5L of anhydrous ethanol, stir for 1 hour, and then remove the insoluble matter with filter cloth;

[n0080]

(8) Continue to add 7.5L of anhydrous ethanol, stir for 1 hour, filter with filter cloth to collect the insoluble matter, rinse with ethanol and then vacuum dry.

[n0081]

(9) Dissolve the dried product in water and evaporate it under reduced pressure at 60°C to obtain the product.

[n0082]

Experimental results:

[n0083]

1.

The yields of products with different molecular weights obtained from the above experimental examples and comparative examples are as follows:

[n0084]

Molecular Weight Range Example 1 Example 2 Example 3 Comparative Example 1 Comparative Example 2 Comparative Example 3 Mw＝690 3.83% 3.21% 4.64% - 0.14% 1.73% Mw＝940 5.15% 4.90% 6.23% 0.47% 2.39% 3.54% Mw＝1170 9.51% 8.62% 10.85% 1.81% 5.03% 7.21% Mw＝1390 10.24% 11.44% 11.37% 5.12% 7.90% 9.51% Mw＝1710 9.12% 11.67% 12.06% 8.28% 8.61% 8.63% Mw＝1890 7.77% 9.38% 10.23% 8.81% 7.97% 8.15% Mw>2000 12.63% 15.29% 17.05% 15.36% 16.07% 13.45%

[n0085]

2.

The effects of copper acetate, GHK-Cu, and the product from Experiment 1 containing the

same amount of copper ions (10 NER2 mol/L) on promoting the release of matrix metalloenzymes (MMPs) from fibroblasts were compared, and the results are shown in Figure 1.
