IFSCC 2025 full paper (abstract reference number：IFSCC2025 - 1450)

Study on the Preparation ofResveratrol Molecular Inclusion Complexes Assisted by Cellpolypid and Evaluation of Their Skin Whitening Efficacy

Huwu Zhou [1], Lifeng Tang [1*], Wenrong Zhang [1], Weifeng Cheng [2], Mengjie Li [2], Jia Qian [2]
1 Guangzhou Xika Technology Co., Ltd., Guangzhou 510200, Guangdong, P. R. China.
2 Shanghai Oli Industrials Co., Ltd., Shanghai 201401, P. R. China.
*Correspondence: The presenting author.

Abstract: Objective: To investigate the physicochemical characteristics and skin whitening efficacy of Resveratrol (Res) inclusion complexes prepared using Cellpolypid®-PMB (PMB) as an auxiliary material with Cyclodextrin (CD), providing theoretical support for the application of the new bionic material PMB in the cosmetics field.

Methods: The effects of PMB on the encapsulation efficiency and storage stability of Res were determined using High-Performance Liquid Chromatography (HPLC). Transdermal experiments were conducted using pig skin to compare the impact of PMB on the cumulative permeation of Res over 24 hours. In-vitro biochemical methods were used to evaluate the inhibitory effect of PMB/CD/Res on tyrosinase activity. In cellular experiments, spectrophotometry was used to evaluate the inhibitory effect of PMB/CD/Res on melanin synthesis. Further, clinical whitening efficacy tests assessed changes in skin MI values and ITA values in subjects using a whitening cream containing 4% PMB/CD/Res.

Results: The addition of PMB increased the encapsulation efficiency of Res from 90.21% to 98.96%. Within 90 days, the content loss of Res decreased from 13.7% to 4.2%, within 24 hours, while the cumulative permeation of Res increased from 0 to 46.623 ± 0.645 μg·cm^{-2}. Additionally, In-vitro biochemical methods assay demonstrated that PMB/CD/Res exhibits excellent tyrosinase activity inhibitory, with an IC_{50} value of 0.258 mg/mL. The cellular experiments confirmed that PMB/CD/Res has a significant inhibitory effect on melanin synthesis, with an IC_{50} value of 0.165 mg/mL. In clinical evaluations of whitening efficacy, after 4 weeks of using the whitening cream containing PMB/CD/Res, the ITA value increased from 0.7±0.26 to 8.35±0.32, the MI value decreased from -3.08±0.91 to -90.34±3.74.

Conclusion: Through the study of the physicochemical properties of PMB/CD/Res and the evaluation of its in vitro and in vivo whitening efficacy, this research establish a scientific foundation for the use of cellpolypid®-PMB as a novel biomimetic carriers for active ingredients, demonstrating their capacity to improve the stability and bioavailability of these active compounds.

Keywords: resveratrol; cellpolypid; molecular encapsulation; transdermal absorption; skin whitening activity

1. Introduction

Resveratrol (chemical formula $C_{14}H_{12}O_3$) is a non-flavonoid stilbene compound formed by two phenolic groups linked via a vinyl bridge. Its molecular structure features a 3',4',5-trihydroxy-trans-stilbene skeleton, which confers significant antioxidant activity [1]. While resveratrol exhibits high solubility in polar organic solvents (e.g., ether, methanol, ethyl acetate), its extremely low water solubility severely limits bioavailability. Current delivery systems in cosmetics—including liposomes, nanospheres, nanoemulsions, hydrogels, and cyclodextrin encapsulation—aim to improve stability and transdermal efficiency but face industrial challenges: liposomes suffer from surfactant residue-induced storage instability; nanoemulsions are prone to Ostwald ripening; and nanosphere preparation requires complex emulsion-solvent evaporation processes [2,3,4,5,6,7].

This study innovatively constructs a biomimetic bilayer delivery system using cyclodextrin (CD) and Cellpolypid (Cellpolypid®-PMB, PMB) (Fig. 1). As a host for poorly soluble actives, CD encapsulates resveratrol's phenyl rings via hydrophobic interactions and hydrogen bonding. PMB, an amphiphilic phosphorylcholine polymer, forms an electrostatic interaction network with CD through its quaternary ammonium groups, establishing a novel encapsulation architecture. It is demonstrated that the solubility, encapsulation efficiency, stability, and transdermal absorption of resveratrolwas enhanced in this syetem (Fig. 2), overcoming limitations of conventional CD-based technologies and advancing resveratrol's cosmetic applications.

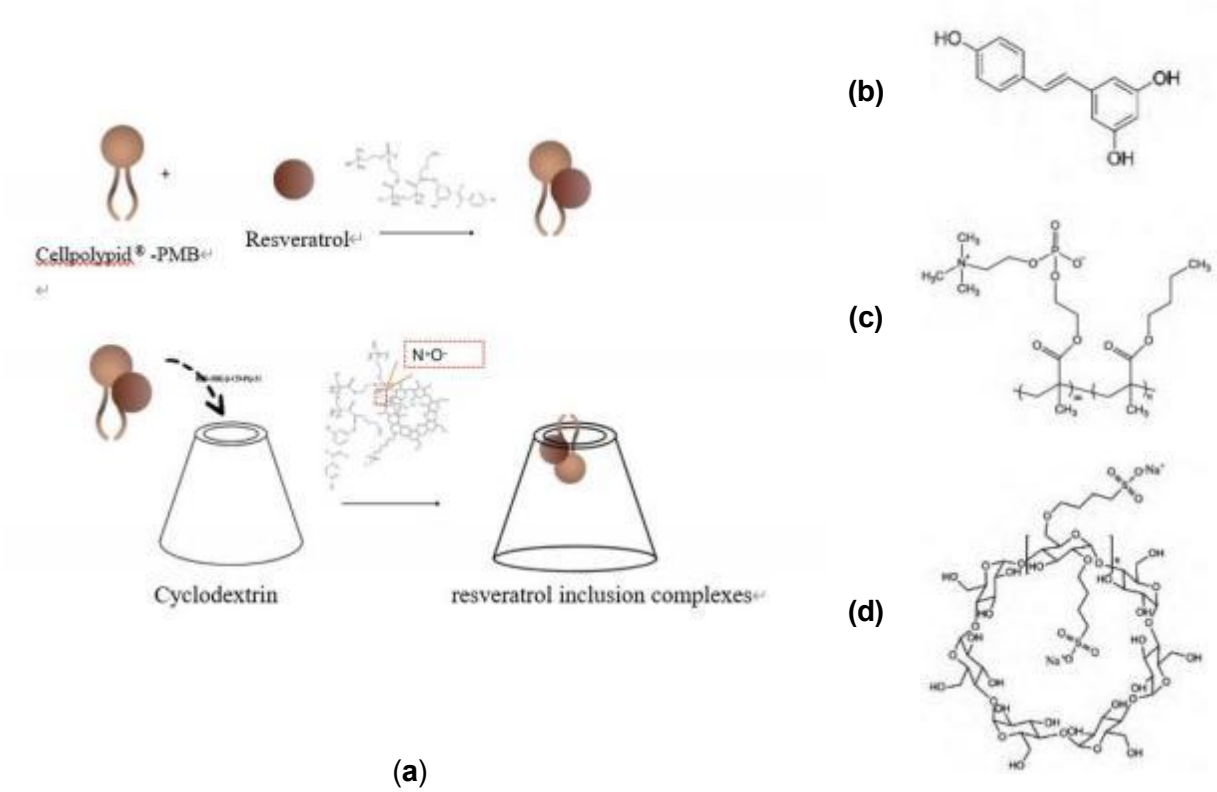


Figure 1. (a) Schematic illustration of the proposed inclusion mechanism of PMB/CD/Res, showing hydrogen bonding (dashed lines) and hydrophobic interactions. (b) Molecular structures of Res, (c) Molecular structures of PMB, (d) Molecular structures of CD.

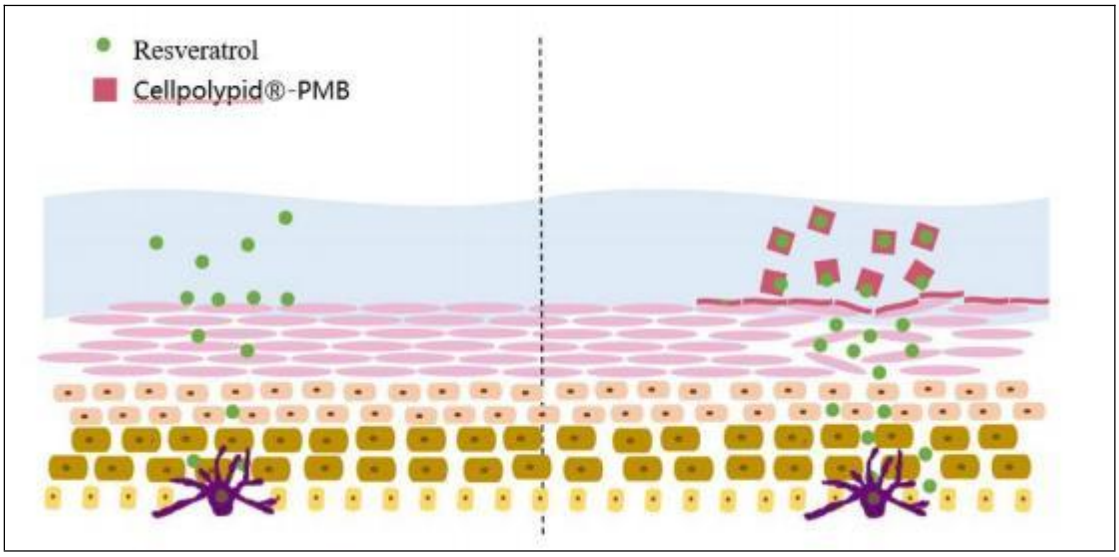


Figure 2. Schematic illustration of PMB-enhanced transdermal delivery of Resveratrol (Res): (left) Conventional Res diffusion vs. (right) PMB-mediated penetration through intercellular routes.

2. Experimental

All experiments in the manuscript were performed in compliance with relevant laws or guidelines.

The clinical trials were from Chinese Academy of Surveillance and Testing(Tianjin) Co., Ltd., and they have all signed the subject's informed consent, the project number was GT00172023596132.

2.1 Materials

Cellpolypid®-PMB, Cyclodextrin, Resveratrol (Shanghai Oli Industrials Co., Ltd.). Murine Melanoma Cells (B16F10) (Shanghai Fuxiang Biotechnology Co., Ltd.). DMSO, Absolute ethanol, Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Butylene glycol (Analytical grade, Sinopharm Chemical Reagent Co., Ltd.). Tyrosinase (Sigma-Aldrich). Acetonitrile (Fisher Chemical). Fetal bovine serum (FBS), DMEM high-glucose medium, Trypsin/EDTA solution, Penicillin-streptomycin (Gibco). Phosphate-buffered saline (PBS), α-Melanocyte-stimulating hormone (α-MSH) (Shanghai Aladdin Biochemical Technology Co., Ltd.). Triton X-100 cell lysis buffer (Shanghai Yeasen Biotechnology Co., Ltd.). Deionized water (Lab-prepared).

Analytical balance (ME204E, Mettler Toledo). Micropipettes (Eppendorf). Vortex mixer, Constant temperature incubator, Constant-temperature water bath (Shanghai Yiheng Scientific Instrument Co., Ltd.). Fluorescence microplate reader (Spark®, Tecan). Biosafety cabinet (AC2-5S1, Esco). CO_2 incubator (CLM-170B-8-CN, Esco). Centrifuge (L420-A, Hunan Xiangyi). Hemocytometer (Marienfeld). Inverted fluorescence microscope (EVOS M5000, Thermo Fisher Scientific). TK-12D transdermal diffusion system (Shanghai Kaikai Technology). Franz diffusion cells (Shanghai Kaikai Technology and Trade Co., LTD.). High-performance liquid chromatography (HPLC) system (Agilent Technologies). Skin-Colorimeter Flex CL440, Mexameter MX18 (Courage & Khazaka, Germany).

2.2 Methods

2.2. 1. Preparation of CD/Res and PMB/CD/Res

1) CD/Res Inclusion Complex

Phase A: Weigh Res and absolute ethanol at a 1:40 (w/w) ratio. Stir vigorously until complete dissolution. Phase B: Weigh CD and sterile water at a 1:5 (w/w) ratio. Stir until homogeneous dissolution. Slowly add Phase A into Phase B under continuous stirring at 25°C. Maintain stirring for 12 h to ensure complete inclusion. Concentrate the mixture via rotary evaporation (40°C, −0.09 MPa, 8 h). Obtain the CD/Res inclusion complex.

2) PMB/CD/Res Inclusion Complex

Phase A: Prepare Res solution as above (Res:ethanol = 1:40 w/w). Stir vigorously until complete dissolution. Phase B: Weigh PMB:CD:sterile water = 1:10:50 (w/w/w). Dissolve at 60°C with stirring, then cool to 25°C and sonicate for 45 min. Combine Phase A and Phase B under stirring at 25°C. Stir for 12 h to facilitate co-assembly. Rotary evaporate (40°C, −0.09 MPa, 8 h) to yield PMB/CD/Res inclusion complex.

2.2.2. Characterization of PMB/CD/Res

The PMB/CD/Res solid, Res solid, and PMB/CD mixed solid were separately applied onto glass slides with double-sided adhesive tape, sputter-coated with gold, and observed under a scanning electron microscope at an operating voltage of 20 kV to examine the surface micromorphology of different sample particles.

2.2.3. Measurement of Res content

1) Chromatographic conditions:

Column: Agilent C18 reversed-phase HPLC column (250 mm × 4.6 mm, 5 μm). Mobile phase: Acetonitrile:water = 40:60 (v/v). UV detector wavelength: 307 nm. Flow rate: 0.6 mL/min. Injection volume: 5 μL. Run time: 15 min. Column temperature: 35°C.

2) Standard curve preparation:

Accurately weigh 0.1 g (to four decimal places) of Res reference standard and transfer to a 100 mL volumetric flask. Add 5 mL ethanol, vortex to dissolve, then dilute to volume with ethanol to prepare the stock solution.

Pipette 1, 2, 3, 4, and 5 mL aliquots of the stock solution into separate 10 mL volumetric flasks (labeled 1–5), dilute to volume with ethanol, and mix thoroughly to obtain standard solutions of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively. Inject each solution for HPLC analysis. Plot concentration (x-axis) against peak area (y-axis) to generate a fourth-order calibration curve. Note: If the correlation coefficient (R^2) is <0.999, repeat preparation.

Result: The Res calibration curve yielded a linear regression equation y = 39,202x + 462 (R^2 = 0.9998), confirming excellent linearity.

3) Encapsulation efficiency (EE) determination:

Accurately weigh 10 mg each of CD/Res and PMB/CD/Res complexes. Freeze-dry at −80°C for 4 h to obtain solid complexes. Add 50 mL ethanol to the solids, ultrasonicate for 3 min to dissolve unencapsulated Res, then centrifuge (10,000 rpm, 10 min) and collect the supernatant.

Quantify free Res in the supernatant via HPLC and calculate EE using the formula:

$$EE\ (\%) = (1 - \frac{M_{Free}}{M_{Total}}) \times 100\% \qquad (1)$$

4) Storage stability testing

CD/Res and PMB/CD/Res complexes were stored in amber light-protected vials under room temperature (25 ± 2°C) conditions.

Initial (Day 0): Measure the Res content (HPLC) and set as the baseline (100%). After 90 days: Reanalyze Res content under identical conditions.

$$\text{Loss rate}\ (\%) = (1 - \frac{M_{Day90}}{M_{Day0}}) \times 100\% \qquad (2)$$

2.2.4. In vitro skin permeation analysis

Due to limited availability of human skin, animal models are commonly used as substitutes for transdermal permeation studies. According to literature reports, commonly used animal models include mice, guinea pigs, rabbits, and pigs [8]. However, studies [9,10] indicate that miniature pig or piglet skin closely resembles human skin in terms of structure and permeability. Therefore, one-month-old piglet skin was selected for this study to evaluate the transdermal permeation of CD/Res and PMB/CD/Res using the Franz diffusion cell method.

Fresh piglet skin was rinsed repeatedly with PBS buffer (pH 7.4, 0.2 M), cut into circular pieces matching the TK-12D transdermal diffusion apparatus, and blotted dry with filter paper. The skin was mounted on Franz diffusion cells with the stratum corneum facing the donor chamber and the dermis facing the receptor chamber. The receptor chamber was filled with 6.5 mL of receptor medium (PBS containing 30% methanol). An additional 1.0 mL was added via a sampling port to eliminate air bubbles, ensuring full contact between the dermis and receptor fluid. The test samples were applied to the skin surface in the donor compartment, with an effective permeation area (S) of approximately 1.77 cm². For each experimental group, 0.2 mL of Res solution (20 mg/mL concentration) was uniformly applied from the center to the periphery of the skin surface in a radial pattern. Three independent replicates were performed for each sample. Magnetic stirring at 300 rpm. Constant temperature water bath maintained at 32±1 。C. Air bubbles were carefully removed from the water jacket. At predetermined time intervals (8 h and 24 h), 2.0 mL aliquots of receptor fluid were withdrawn through the sampling port and collected in centrifuge tubes for subsequent analysis. Immediately after each sampling, an equal volume of fresh, pre-warmed receptor medium was replenished to maintain constant volume conditions.

Cumulative permeability was measured by high-performance liquid chromatography (HPLC) using the following equation:

$$Q_s = C_{sn} \times \frac{V_s}{S} + \sum_{i=1}^{n-1} C_{si} \times \frac{A_s}{S} \qquad (3)$$

Where: Qs: Cumulative permeation per unit area (μg·cm⁻²). Vs: Receptor volume (6.5 mL). Csn: Res concentration at the nth sampling point (μg·mL⁻¹). S: Effective permeation area (1.77 cm²). As: Sample volume (2.0 mL).

2.2.5. In vitro inhibition of tyrosinase activity

The method used in this experiment was modified based on the method for testing the inhibitory effect of cosmetic ingredients on tyrosinase activity (in vitro) [11].

In a 96-well microplate, four types of wells were set up: solvent blank well (Ta), solvent reaction well (Tb), sample blank well (Tc), and sample reaction well (Td). Specifically, the Ta group is the solvent blank group, without the addition of L-tyrosine solution or sample solution; the Tb group is the solvent reaction group, with the addition of L-tyrosine solution but without sample solution; the Tc group is the sample blank group, without the addition of L-tyrosine solution but with sample solution; the Td group is the sample reaction group, with both L-tyrosine solution and sample solution added. Each group contains three replicates. According to the above settings, the total volume in each well is 200 μL, that is, 40 μL of L-tyrosine solution/solvent, 40 μL of sample solution/solvent, and 80 μL of PBS buffer (pH 6.8) are added to each well in sequence, followed by thorough mixing. The plate is then incubated at 37°C for 10 min. Subsequently, 40 μL of tyrosinase solution is added to each well, and the mixture is reacted at 37°C for 10 min ± 5 s before being immediately measured in the microplate reader at a wavelength of 475 nm. The inhibitory rate of tyrosinase activity is calculated using the following formula:

$$\text{inhibitory rate } (\%) = (1 - \frac{Ad - Ac}{Ab - Aa}) \times 100\% \qquad (4)$$

Where: Ad is the absorbance of the sample reaction well. Ac is the absorbance of the sample blank well. Ab is the average absorbance of the solvent reaction well. Aa is the average absorbance of the solvent blank well.

2.2.6. In vitro inhibition of melanogenesis in B16 cells

The method used in this experiment was modified based on the in vitro test for the inhibition of melanin synthesis in B16 cells [12].

1) Test for the effect of samples on relative cell viability

When the cells reached the logarithmic growth phase, they were digested with trypsin and adjusted to a concentration of 1×10^4 cells/mL. A volume of 100 μL was added to each well of a 96-well plate. After 24 h, the test compounds were added. Water-soluble compounds were filtered through a filter membrane, while water-insoluble compounds were dissolved in DMSO. The concentration of DMSO in the culture medium did not exceed 0.2%. The compounds were prepared in five or more concentrations, with three replicates for each concentration. The control group did not receive any compound. The plates were incubated in a 37°C incubator with 5% CO_2 for 72 h. After 72 h of treatment, the supernatant was discarded, and 110 μL of cell culture medium containing CCK-8 reagent (100 μL cell culture medium + 10 μL CCK-8 reagent) was added to each well. The plates were then incubated for an additional 0.5-2 h, and the absorbance at 450 nm was measured using a microplate reader. The relative cell viability was calculated using the following formula:

$$\text{Cell viability } (\%) = (\frac{A_S - A_b}{A_C - A_b}) \times 100\% \qquad (5)$$

Where: A_S is the average absorbance of the experimental wells, which contain cells, culture medium, CCK-8 solution, and the test compound. A_b is the absorbance of the CCK-8 reagent. A_C is the average

absorbance of the control wells, which contain cells, culture medium, and CCK-8 solution but no test compound.

2) Inhibition of melanin synthesis in B16 cells

The concentration of the samples with no significant difference in relative cell viability compared to the control group was selected for the melanin synthesis inhibition experiment.

Cell Culture: B16 mouse melanoma cells, which were cryopreserved at low temperature, were inoculated into cell culture flasks using DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in an environment at 37°C with 5% CO_2. When the cell confluence reached approximately 90%, the cells were digested and resuspended in culture medium to adjust the cell density. The cell suspension was then seeded into a 6-well plate at a density of 40,000 cells per well. After 20-24 h of incubation at 37°C with 5% CO_2, the test compounds and α-MSH (final concentration of 0.025 μg/mL) were added to each well (3 mL per well). The cells were further cultured for 48-72 h until the cell confluence exceeded 90%, at which point melanin detection was performed. The compounds were prepared in three or more concentrations, with three replicates for each concentration. The control group did not receive any compound.

Melanin Detection: The cells were washed with PBS, and the PBS was aspirated. The cells were then digested with 0.25% trypsin, resuspended in PBS by pipetting, and collected into centrifuge tubes. The cells were centrifuged at 4000 rpm for 5 min. The residual PBS in the centrifuge tubes was carefully removed, and 200 μL of melanin extraction solution (10% DMSO, 1M NaOH) was added to each tube. The mixture was vortexed and incubated in a water bath at 80°C for 1 h. After cooling, the mixture was vortexed again. A volume of 150 μL of the solution from each centrifuge tube was transferred to a 96-well plate, and the absorbance at 405 nm was measured using a microplate reader. The inhibition rate of melanin synthesis was calculated using the following formula:

$$\text{inhibitory rate}\ (\%) = \left(\frac{C-T}{C-C_0}\right) \times 100\% \qquad (6)$$

Where: T is the average absorbance of the test sample wells. C is the average absorbance of the control group. C_0 is the average absorbance of the melanin extraction solution background.

2.2.7. Clinical trial efficacy evaluation

The method for testing the skin-lightening efficacy of cosmetics, specifically the first method for evaluating the efficacy of skin-lightening and freckle-removing products using a UV-induced human skin hyperpigmentation model [13], was employed to measure changes in skin color. The whitening efficacy of whitening cream containing 4% PMB/CD/Res was evaluated by measuring the changes in skin brightness (darkness/yellowness) (ITA° value) and melanin index (MI value) before and after use.

Test Samples: Whitening whitening cream containing 4% PMB/CD/Res.

Negative Control: Blank control in the hyperpigmented area.

Positive Control: 7% ascorbic acid (vitamin C), prepared according to the method in Appendix I of the first method for testing the freckle-removing and whitening efficacy of

cosmetics in Chapter 8 of the "Cosmetic Safety Technical Specifications" (2015 edition) (stored refrigerated at 4°C and protected from light in aluminum tubes).

Subjects: A total of 33 participants, including 7 males and 26 females, aged 20 to 60 years, with an average age of 46.6 ± 9.9 years, all meeting the inclusion criteria for volunteer subjects.

Testing Instruments: Skin colorimeter: Skin-Colorimeter Flex CL440 (Courage & Khazaka, Germany). Melanin and hemoglobin tester: Mexameter MX18 (Courage & Khazaka, Germany).

Testing Method: The test was conducted in accordance with the specific requirements of the "Cosmetic Safety Technical Specifications" (2015 edition). The back of each subject was selected as the test site, with each hyperpigmentation test area being no less than 0.5 cm² and located within each application zone. The minimal erythemal dose (MED) of the test site was determined for each subject. Using a solar simulator, the same irradiation point was exposed to a dose of 0.75 times the MED once daily for four consecutive days. The four days following irradiation were designated as the skin hyperpigmentation period, during which no treatment was applied. On the fifth day after irradiation, the skin color of each test area was measured using the instruments, and test areas with inconsistent results were excluded. Starting from the fifth day, the corresponding test substances were applied to each hyperpigmented test area according to a randomization table. A skin area of 6 cm² was selected for application, and the test substances were applied continuously for four weeks at a dosage of 2.00 ± 0.05 mg/cm². Instrumental measurements of skin color were taken and recorded at 1, 2, 3, and 4 weeks after application.

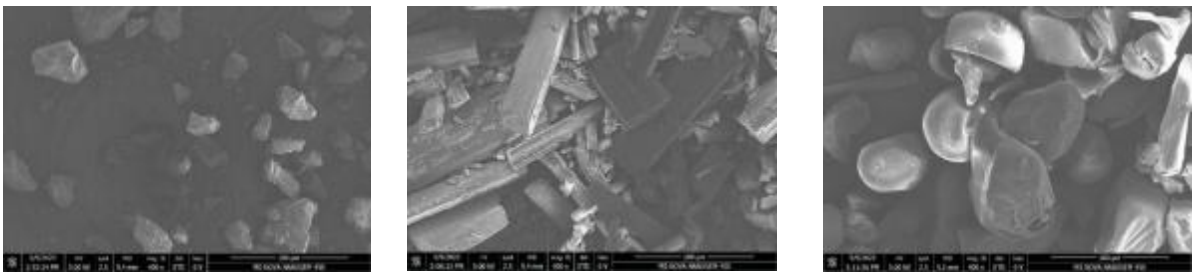
2.2.8. Statistical analysis

Results are presented as mean ± standard deviation. One-way ANOVA was used to determine the significance of differences. Statistical significance was set at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

3. Results and Discussion

3. 1 Characterization of PMB/CD/Res

The SEM (Scanning Electron Microscopy) morphology images of PMB/CD/Res, Res, and PMB/CD are shown in Fig. 3. As seen in Fig. 3(b), raw RES exhibits irregularly sized, rough-surfaced flaky crystal structures. Fig. 3(c) reveals that PMB/CD appears as dispersed hollow spherical structures with micropores on the surface. After the encapsulation process, the resulting composite PMB/CD/Res (Fig. 3(a)) displays a block-like inclusion complex with a continuous, non-porous surface, showing significant morphological changes. The characteristic Res morphology disappears, indicating that PMB and CD cross-linked to form a co-carrier, successfully encapsulating Res. The phosphorylcholine groups in PMB modify Res, which has been proven to enhance the material's adsorption capacity and compactness [14]. Furthermore, the inclusion complexes exhibit regular shapes and uniform particle sizes. This homogeneous structure helps to increase the dissolution rate of the substance and reduce its agglomeration in solution.



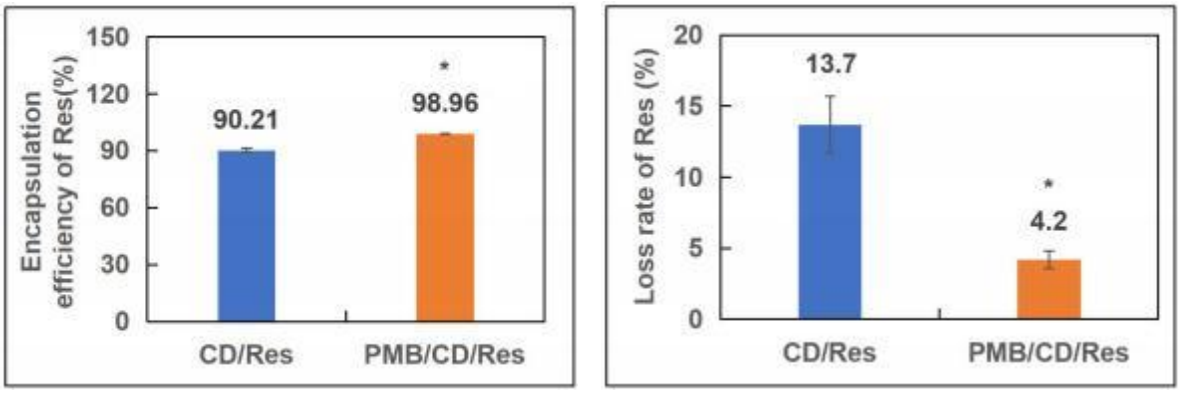
(**a**) (**b**) (c)

Figure 3. Scanning electron micrographs of (a) PMB/CD/Res, (b) Res and (c) PMB/CD.

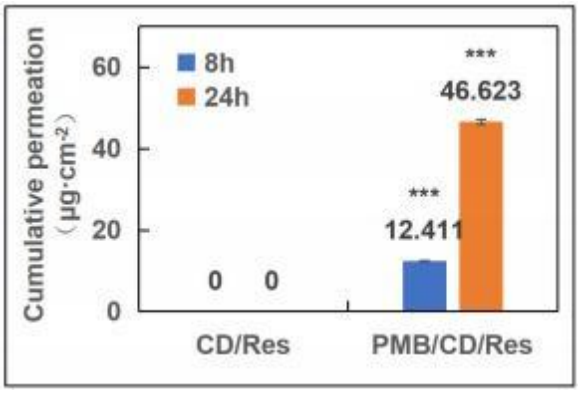
3.2 Physicochemical characterization of PMB/CD/Res

The encapsulation efficiency and storage stability of CD/Res and PMB/CD/Res were calculated and are shown in Fig. 4. The encapsulation efficiency of Res by CD alone was 90.21%, while the introduction of PMB increased the encapsulation efficiency of PMB/CD/Res to 98.96% ($P < 0.05$) (Fig. 4(a)). Compared with CD/Res, the final Res content loss of PMB/CD/Res after 90 days of storage was reduced from 13.7% to 4.2% ($P < 0.05$) (Fig. 4(b)), indicating that the active ingredient was better protected after the inclusion process with PMB. As a special macromolecular polymer, PMB participates in the inclusion process through electrostatic interactions and supramolecular interactions. Its amphiphilic structure, containing both hydrophilic and hydrophobic units, enables it to act as a "bridge" between two phases, forming a denser encapsulation layer with CD and providing a better storage environment for Res [15].

Furthermore, the in vitro transdermal permeation of CD/Res and PMB/CD/Res was compared using Franz diffusion cells on porcine skin. At 8 h and 24 h, the cumulative permeation amounts of CD/Res were 0, respectively. After PMB incorporation, the 8-h and 24-h cumulative permeation of RES increased from 0 to 12.411 ± 0.132 μg·cm^{-2} and from 0 to 46.623 ± 0.645 μg·cm^{-2}, respectively (***P<0.005). (Fig. 4(c)). These data clearly indicate that PMB/CD/Res exhibited superior transdermal penetration performance compared to CD/Res. This enhancement is attributed to PMB's phosphorylcholine headgroup, which resembles the structure of cell membranes [16]. Through molecular mimicry, PMB can specifically interact with the stratum corneum lipid bilayer (primarily composed of ceramides and cholesteryl esters) [14], reversibly modulating skin barrier function and thereby improving the transdermal permeability and bioavailability of resveratrol.



(**a**) (**b**)



(**c**)

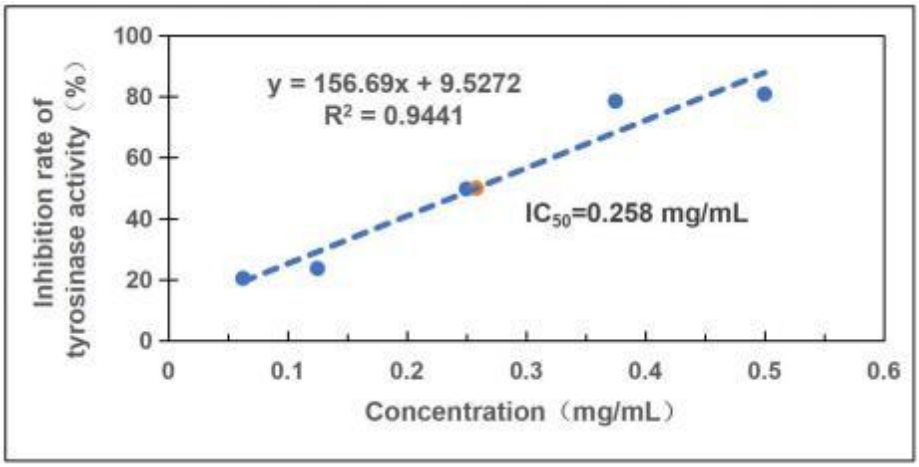
Figure 4. Physicochemical properties study of PMB/CD/Res. (a) The encapsulation efficiency of CD/Res and PMB/CD/Res (n=3). (b) The storage stability of CD/Res and PMB/CD/Res (n=3). (c) The 8-hour and 24-hour cumulative permeation of CD/Res and PMB/CD/Res (n=3). The results are shown as mean±SD. *P<0.05, **P<0.01, ***P<0.005.

3.3 Biological functions of PMB/CD/Res

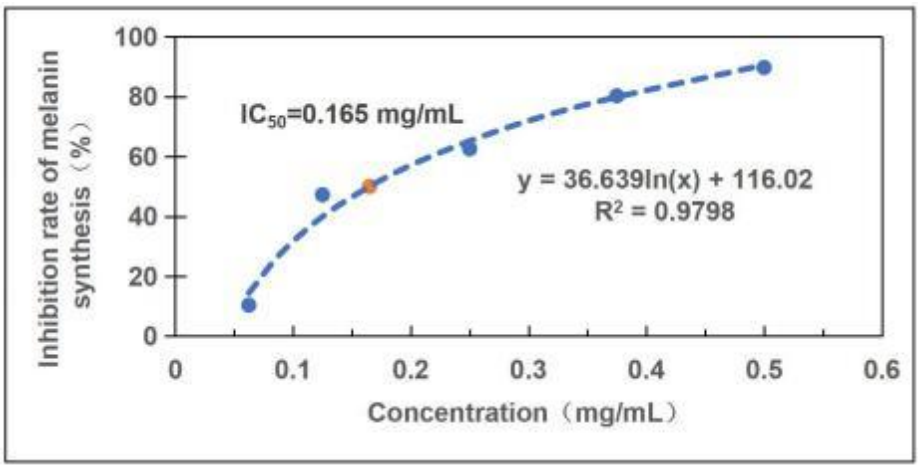
Melanin synthesis is a complex series of oxygen-dependent enzymatic reactions catalyzed by tyrosinase and other enzymes, with tyrosine serving as the substrate. As the rate-limiting enzyme in melanogenesis, tyrosinase participates in multiple reaction steps - its enhanced activity directly correlates with increased melanin production [17]. while biochemical methods typically assess whitening effects through tyrosinase inhibition rates [18].

As shown in Fig. 5(a), PMB/CD/Res exhibits dose-dependent tyrosinase inhibitory effects, with increasing concentrations correlating with enhanced inhibition rates. with a calculated IC_{50} value for tyrosinase inhibition was 0.258 mg/mL, demonstrating its significant whitening potential.

For cellular evaluation, we established a melanogenesis model using α-MSH-stimulated B16 mouse melanoma cells. α-MSH, an endogenous melanocortin receptor agonist, promotes melanin production in B16 cells [12,19]. As presented in Fig. 5(b), PMB/CD/Res showed excellent inhibition of cellular melanin synthesis in a concentration-dependent manner, yielding an IC_{50} value of 0.165 mg/mL. These cellular findings further substantiate the skin-whitening efficacy of the PMB/CD/Res.



(**a**)



(**b**)

Figure 5. Biological functions study of PMB/CD/Res. (a) Inhibition Curve of PMB/CD/Res on Tyrosinase Activity (n=3). (b) Inhibition curve of melanin synthesis in B16 murine melanoma cells by PMB/CD/Res (n=3). The results are shown as mean±SD. *P<0.05, **P<0.01, ***P<0.005.

3.4 Clinical efficacy evaluation ofPMB/CD/Res

Building upon satisfactory results from in vitro tyrosinase inhibition and cellular melanogenesis assays, we further conducted clinical trials to evaluate the skin-whitening efficacy. Thirty-three volunteers were recruited for a 4-week clinical study using a whitening cream containing 4% (w/w) PMB/CD/Res inclusion complex. The evaluation parameters included: (1) skin brightness (ITA° value) and (2) melanin index (MI value) [13].

As shown in Fig. 6(a), the PMB/CD/Res treatment group demonstrated statistically significant improvements ($p<0.05$) in ITA° values compared to the negative control at all follow-up intervals (weeks 2, 3, and 4). After 4 weeks of treatment, the ITA° value increased from 0.7±0.26 to 8.35±0.32. Regression analysis (Table 1) confirmed significantly greater improvement in the treatment group ($p<0.05$). Fig. 6(b) reveals that the MI values decreased significantly ($p<0.05$) in the treatment group compared to controls at all timepoints, with a reduction from -3.08±0.91 to -90.34±3.74 after 4 weeks. The regression coefficients (Table 1) showed statistically superior improvement versus controls ($p<0.05$).

The clinical trial demonstrates that the 4% PMB/CD/Res whitening cream significantly enhances skin brightness and reduces melanin deposition, confirming its efficacy for skin whitening and spot-reduction applications.

Table 1. Results of Regression Coefficients (Slope k-values) for Skin ITA° and MI Values (Statistical analysis was performed using SPSS software, *p < 0.05 vs. NC).

Group	ITA° value	MI value
NC	0.92	-7.09
PC	1.74*	-16.45*
PMB/CD/Res	2.19*	-23.64*

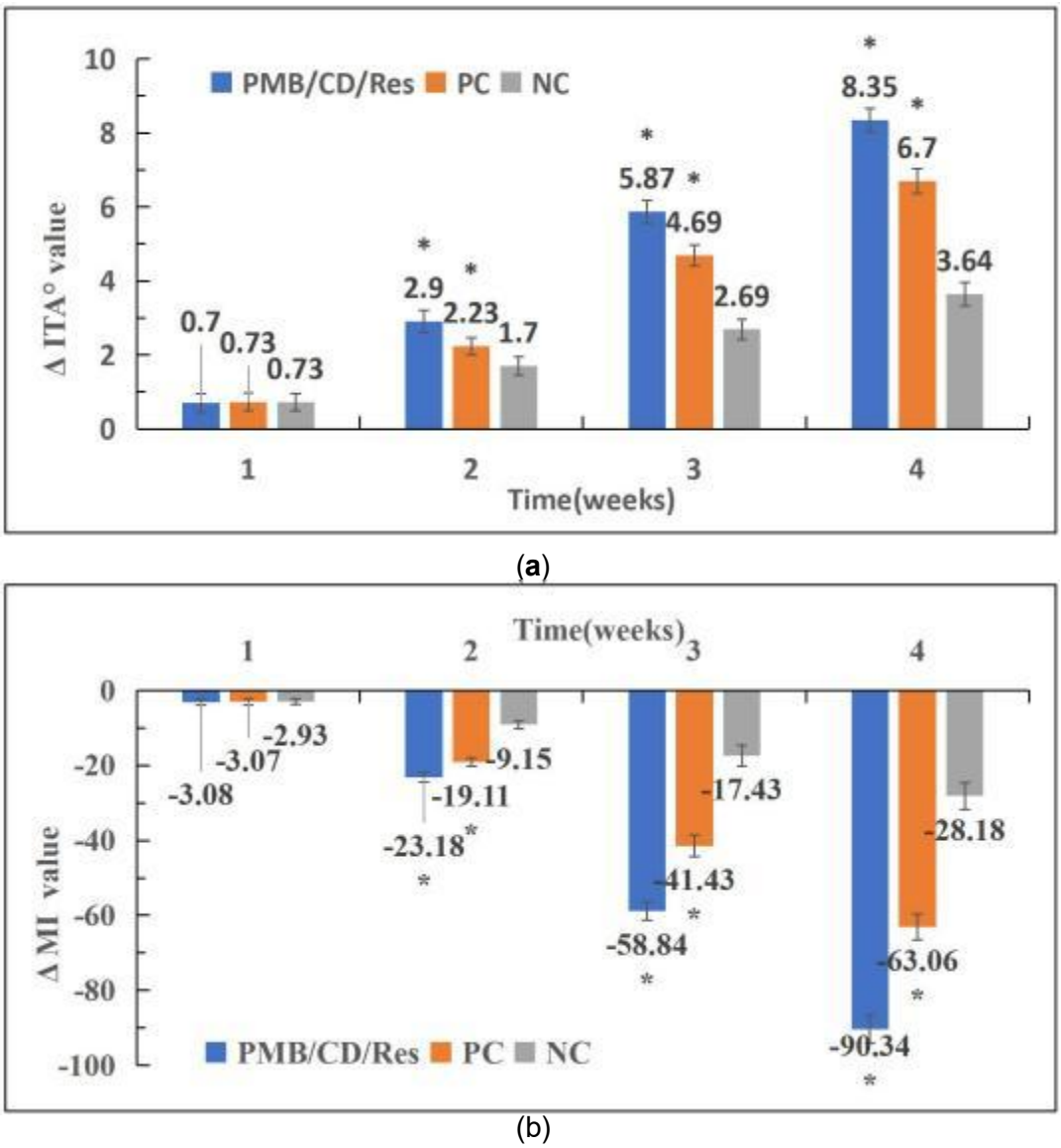


Figure 6. Clinical efficacy evaluation of PMB/CD/Res. (a) ΔITA° values after 4-week application of whitening cream containing 4% PMB/CD/Res. (b) ΔMI values after 4-week application of whitening cream containing 4% PMB/CD/Res. (*p < 0.05 vs. NC, paired t-test, mean±SD，n=33).

4. Conclusion

This study investigated the potential of using Cellpolypid®-PMB as an auxiliary material in combination with Cyclodextrin to prepare Resveratrol encapsulates in terms of physicochemical properties and skin-whitening effects. The results showed that the Res encapsulates exhibited a continuous surface in the form of block-like structures under scanning electron microscopy. The addition of PMB increased the encapsulation efficiency of Res from 90.21% to 98.98% and reduced the content loss over a 90-day storage period from 13.7% to 4.2%. Moreover, transdermal experiments indicated that PMB enhanced the retention and subcutaneous penetration of Res in the skin over a 0-24 h period. Additionally, the tyrosinase inhibition assay (IC_{50}= 0.258 mg/mL) and melanin synthesis inhibition assay in B16 cells both demonstrated the significant whitening effects of the complex (IC_{50} = 0.165

mg/mL). Clinical evaluation also revealed that after four weeks of using a whitening cream containing 4% PMB/CD/Res, the skin brightness (ITA。 value) of participants significantly increased, while the melanin index (MI value) markedly decreased.

The transdermal permeation-promoting mechanism of PMB is attributed to its molecular structural similarity to the phospholipid bilayer of the cell membrane. When involved in the delivery of active substances, PMB enhances transdermal delivery by temporarily reorganizing the intercellular lamellar structure,, thereby facilitating active ingredient penetration without compromising skin barrier integrity.

This study not only provides a solid theoretical basis for the application of PMB as a novel biomimetic carriers in the field of cosmetics but also demonstrates the great potential of enhancing the skin-care efficacy of active ingredients by optimizing their stability and bioavailability. These findings pave the way for the development of safer and more effective whitening skin-care products.

5. Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. Acknowledgements

This work was funded by Guangzhou Xika Technology Co., Ltd. and experimentally conducted by Shanghai Oli Industrials Co., Ltd. We gratefully acknowledge the Chinese Academy of Surveillance and Testing (Tianjin) Co., Ltd. for performing the clinical study and data analysis.

References：

[1] OH W Y, SHAHIDI F. Lipophilization of resveratrol and effects on antioxidant activities[J]. Journal of Agricultural and Food Chemistry, 2017, 65(39): 8617-8625.

[2] ARROYO E, VALDEZ R, CORNEJO-BRAVO J M, et al. Nanogels as controlled drug release systems for Coenzyme Q10 and Resveratrol for cosmetic application[J]. Journal of Nanoparticle Research, 2021, 23: 1-11.

[3] DAS S, LEE S H, CHOW P S, et al. Microemulsion composed of combination of skin beneficial oils as vehicle: Development of resveratrol-loaded microemulsion based formulations for skin care applications[J]. Colloids and Surfaces B: Biointerfaces, 2020, 194: 111161.

[4] MIAO L, DAOZHOU L, YING C, et al. A resveratrol-loaded nanostructured lipid carrier hydrogel to enhance the anti-UV irradiation and anti-oxidant efficacy[J]. Colloids and Surfaces B: Biointerfaces, 2021, 204: 111786.

[5] AMIN D R, HIGGINSON C J, KORPUSIK A B, et al. Untemplated resveratrol-mediated polydopamine nanocapsule formation[J]. ACS applied materials & interfaces, 2018, 10(40): 34792-34801.

[6] DUARTE A, MARTINHO A, LUÍS Â, et al. Resveratrol encapsulation with methyl-β-cyclodextrin for antibacterial and antioxidant delivery applications[J]. LWT-Food

Science and Technology, 2015, 63(2): 1254-1260.

[7] SESSA M, BALESTRIERI M L, FERRARI G, et al. Bioavailability of encapsulated resveratrol into nanoemulsion-based delivery systems[J]. Food chemistry, 2014, 147: 42-50.

[8] Tian Lu, Zhang Xu, Lin Huaqing, et al. Research progress on in vitro transdermal diffusion test methods of transdermal drug delivery preparations [J]. China Pharmacy, 2012, 23(29): 2761-2763.

[9] Daniela K, James D B, Jim E R, et al. Modeling the effect of experimental variables on the in vitro permeation of six model compounds across porcine skin [J]. Int J Pharm，2013, 443(1-2): 58-67.

[10] Michael H Q, Ulla H, Bo K, et al. Evaluation of Gottingenminipig skin for transdermal in vitro permeation studies[J]. Eur J Pharm Sci, 2000, 11(1): 59-68.

[11] T/GDCA 006-2021. Test method for tyrosinase activity inhibition of cosmetic ingredients (in vitro). Guangdong Cosmetics Association Group Standard.

[12] TSHRH 027-2019. In vitro test for melanin synthesis inhibition in B16 cells. Shanghai Daily Chemical Industry Association.

[13] China Food and Drug Administration. Safety and Technical Standards for Cosmetics (2015 Edition) [S]. Beijing: China Food and Drug Administration, 2015.

[14] Wu Xue, Xin Zhong, Yuan Weikang. Review on structure and biochemical characteristic of phosphatidylcholine analogous polymers[J]. Chemical Industry and Engineering Progress, 2008(9): 1375-1383.

[15] Feng Yanju, Li Mengjie, Cheng Weifeng. Preparation and Characterization of Resveratrol Inclusion Complexes Mediated by Biomimetic Cell Membranes[J]. Flavour Fragrance Cosmetice, 2024, (03): 128-134.

[16] Vance JA, Devaraj NK. Membrane mimetic chemistry in artificial cells[J]. Journal of the American Chemical Society. 2021 May 20; 143(22): 8223-31.

[17] Zhao MJ, Hu JJ, Ni H, et al. Research progress in melanogenesis signaling pathway. Chin J Biotech, 2019, 35(9): 1633–1642.

[18] Park S H, Jung J H, Park S H, et al. Design and Synthesis of Novel 6-(Substituted phenyl)-[1,3]dioxolo[4′,5′:4,5]benzo[1,2-d]thiazole Compounds as Tyrosinase Inhibitors: In Vitro and In Vivo Insights[J]. Molecules, 2025, 30(7): 1535-1535

[19] Han Siyin, Zhang Jingjing, Cen Yanhui, et al. Study on the Mechanism of Hydrolyzed Pearl Solution Inhibiting MelaninFormation in Human Skin Cells[J]. Asia-Pacific Traditional Medicine, 2023, 19(12): 26-33.