Prescription Proportion of Pomegranate Extract Gallic Acid Gel by Orthogonal Design

Gaofu Fan¹, Xiushu Liu¹, Jie Tang¹, Jumei Gong¹, Entao Fu¹, Yuhua Cai¹ and Zhenguo Xu¹*

¹Faculty of Bioscience Engineering, Hefei technology college, Hefei, China
chzyjsxy2006@126.com

Abstract. The aim of the present work was to optimize the formulation of pomegranate extract gallic acid gel by orthogonal design. Using orthogonal design, propylene glycol, carbomer-940 and gel pH level as influencing factors, the evaluation key index was external appearance malleability, uniformity, and eccentric for gel, and the optimum formula was selected. The present findings suggest that 10% propylene glycol, 1.5% Carbopol-940, and gel pH in the range of 4.5~5.5, and the indexes of the optimal. The inclusion complexes showed that after the orthogonal design, the preparation process was simple, stable and controllable quality, with production feasibility.

1. Introduction
Pomegranate was one of the special fruits of Huaiyuan area in Bengbu, Anhui Province. It had the functions of food and medicine and health care. It could prevent and treat malignant tumors, infectious diseases and cardiovascular diseases. These effects were derived from polyphenols, riched in pomegranate fruit, and gallic acid was one of the most simple components of polyphenols, and had anti-inflammatory, anti-oxidation, anti-bacterial, anti-virus, anti-allergic and anti-effect and other effects [1-5], especially with the skin Related anti-inflammatory anti-bacterial itching, anti-allergy, wound repair, whitening and other functions [6-8], to provide a theoretical basis for the clinical development of external preparations.

Gels was a more commonly used topical preparations, in the preparation process, need to consider prescription technology and raw materials base, and the preparation of bioavailability is not high. According to the clinical application characteristics, need to consider the physical and chemical properties of pomegranate extract gallic acid, matrix components and skin physiological characteristics, using orthogonal design, propylene glycol, carbomer-940 and gel pH level as influencing factors, the evaluation key index was external appearance malleability, uniformity, and eccentric for gel, and the optimum formula was selected to research and develop external use of new formulations of gels.

2. Material

2.1. Materials and Reagents
Pomegranate peel (Anhui Bengbu Huaiyuan area); gallic acid extract (self-prepared); medicinal grade accessories Kaibuomu -940 (Beijing people Yikang Technology Co., Ltd.); use test analysis level triethanolamine, propylene glycol.

2.2. Instruments and Equipment
RE-2000A rotary evaporator (Shanghai Yarong biochemical instrument factory), SCIENTZ-10ND multi-manifold gland type freeze dryer (Ningbo Xinzhi Biotechnology Co., Ltd.), HH-W0-20 intelligent constant temperature oil bath pot (Shenzhen Sanli Chemicals Co., Ltd.), PHG-3C desktop acidity meter (Shanghai instrument and electrical scientific instrument Limited by Share Ltd.), DHG-9075A desktop electric heating oven (Shanghai Qi Xin Scientific Instrument Co., Ltd.), FA1004 type of electronic Analytical balance (Tianjin Tianma Hengji Instrument Co., Ltd.), TGL-20B high-speed desktop centrifuge (Beijing Jiayuan Industrial Technology Co. Ltd.).

3. Methods

3.1. Preparation of Ellagic Acid Extract
The pomegranate peels in Huaiyuan area of Bengbu, Northern Anhui Province were dried and crushed, and the alcohol was extracted with different concentrations of ethanol (concentration: 90%, 70% and 40%) for 1.5h. The extracts were filtered and concentrated under reduced pressure. Ethanol was recovered and the extract was concentrated and concentrated to a solution of 0.5 g of pomegranate per ml of liquid. Ultrasonic combination of acid hydrolysis method to optimize the extraction, D201 macroporous resin adsorption technology separation and purification of pomegranate extract gallic acid, the separation and purification of gallic acid by freeze-drying was placed in reserve.

3.2. Orthogonal Design
In this study, the propylene glycol, carbomer concentration and pH value were set as the influencing factors of the gel preparation. The experimental design was carried out using L9 (34) orthogonal test table, and 10%, 20% and 30% propylene glycol; 1.0%, 1.5% and 2.0% Carbopol-940; pH is set at 4.5 - 5.5, 5.6 - 6.5 and 6.6 - 7.5 as the table of nine factors were prepared corresponding gel preparation, see Table 1.

Table 1. Factors table
| level | propylene glycol(A)% | carbomer -940(B)% | pH value(C) |
|------|-------------------|------------------|------------|
| 1    | 10                | 1                | 4.5-5.5    |
| 2    | 20                | 1.5              | 5.6-6.5    |
| 3    | 30                | 2                | 6.6-7.5    |

3.3. Gel inspection Standards
The appearance, coating property, uniformity and centrifugation of the gel were examined, and the appearance of the gel was measured by the gelatin surface, and the appearance shape was evaluated by smoothness. The gel was coated with a glass rod, If the coating on the coating evenly distributed, and no adhesion on the glass rod was good for the coating; whether the gel was fine and even, and had no granular micelle, caking exists to evaluate its uniformity; respectively, the group of gel to 3000 r/Min speed for 30 min centrifugal, to observe whether there was stratification to determine its centrifugal traits. Each indicator 0 ~ 2.5 points, 10 was divided into the highest value, see Table 2 [9-11].

Table 2. Scoring criteria list

| index                      | scores                     |
|----------------------------|----------------------------|
|                            | Excellent (2.0-2.5) | Good(1.0-1.5) | Medium(0.8-0.9) | Poor(0.0-0.2) |
| external appearance        | smooth surface             | smooth surface finish | dull surface | Having a surface with no gloss |
| malleability               | easy                       | can, but less delicate | can, but the fineness is poor | The outflow of liquid or viscous difference |
| uniformity                 | fine and smooth            | lower delicate | low ammonia | coarseness |
| eccentric                  | the appearance of the same | no layered | having particles or agglomerates on the surface | layered |
3.4. Statistical analysis Method

This study was analyzed by SPSS19.0 statistical software package. The data were analyzed by single factor analysis. The difference was statistically significant by $P < 0.05$.

4. Results

4.1. The results of Orthogonal Test are Shown in Table 3

| factors | A  | B  | C  | D  | results |
|---------|----|----|----|----|---------|
| 1       | 1  | 1  | 1  | 1  | 9.2     |
| 2       | 1  | 2  | 2  | 2  | 9.5     |
| 3       | 1  | 3  | 3  | 3  | 6.5     |
| 4       | 2  | 1  | 2  | 3  | 8       |
| 5       | 2  | 2  | 3  | 1  | 7.5     |
| 6       | 2  | 3  | 1  | 2  | 8.3     |
| 7       | 3  | 1  | 3  | 2  | 6.3     |
| 8       | 3  | 2  | 1  | 3  | 9       |
| 9       | 3  | 3  | 2  | 1  | 6.3     |
| $K_1$   | 8.4| 7.833 | 8.833 | 7.667 |
| $K_2$   | 7.933 | 8.667 | 7.933 | 8.033 |
| $K_3$   | 7.2 | 7.033 | 6.767 | 7.833 |
| $R$     | 1.2 | 1.634 | 2.066 | 0.366 |

4.2. Analysis of Variance Results

Through the variance analysis, the three main factors of the study, there was significant B and C factors ($P < 0.05$), but there is no obvious correlation between the factors and the effect of A ($P > 0.05$), See Table 4 for details.

4.3. Verification Experiment

Combination with the results obtained in Table 3 and Table 4, it was concluded that the effect of pH value (C) was the highest in the three influencing factors of this study, and that of the adjuvant material carbomer-940 was the second, and propylene glycol (B) did not show correlation, and with the increase in the number of its effect weakened. From the industrial production to reduce energy consumption and control costs and many other considerations, $A_1B_2C_1$ combination of the best, the specific process to take 1.5 g carbomer-940, add appropriate amount of distilled water, put it overnight, until it was fully expanded, weighed 6g pomegranate extract gallic acid and added a certain amount of propylene glycol to dissolve it until it was dissolved and then added to the gel matrix, and to adjust its pH by triethanolamine, adjusted to 4.5 ~ 5.5 stop, then add distilled water to 100 g , well mixed. According to this method, three parallel preparations, showed that the preparation process was highly stable and the operation was simple. The detailed results were shown in table 5.

Table 4. Analysis of variance (n=3)

| sources of variation | sum of squars | n  | MS  | F    | Significance difference |
|----------------------|---------------|----|-----|------|-------------------------|
| A                    | 2.2           | 2  | 1.1 | 10.86| >0.05                   |
| B                    | 4             | 2  | 2   | 19.79| <0.05                   |
| C                    | 6.44          | 2  | 3.22| 31.86| <0.05                   |
| D                    | 0.2           | 2  | 0.1 |      |                         |
| error                | 0.2           |     |     |      |                         |
| total variation      | 13.91         | 8  |     |      |                         |

$F_{0.05(2, 2)} = 19.00$
Table 5. Parallel research results

| No | Overall score | \(\chi^2\) | P value |
|----|---------------|-----------|---------|
| 1  | 9.8           |           |         |
| 2  | 10            | 15.779    | <0.05   |
| 3  | 9.9           |           |         |
| average | 9.9 |           |         |

5. Discussions
In the 2015 edition of the "Chinese Pharmacopoeia" four appendixes has been defined in the gel, refers to the amount of matrix and extract prepared by the gel characteristics of the thick or semi-solid preparation, the quality requirement of gel should be uniform and delicate, and keep the colloid at room temperature, not dry or liquefied. As the preparation of this study was mainly used for skin smear, taking into account its absorption characteristics, so in the preparation of gels in the process, the main inspection for the preparation were the malleability, stability, appearance, uniformity and other items.

Carbomer 900 series was a high polymer acrylic monomer and allyl pentaerythritol allyl sucrose or crosslinked, acidic groups in molecular structure containing 52% to 68%, its 1% aqueous solution of pH is 2.5 ~ 3, adding alkali (sodium bicarbonate) or organic alkali (such as triethanolamine) neutralization. The hydrogels formed after neutralization was stable in the range of pH4.0 to 11, and the viscosity was the most viscous in the range of pH 6.5 to 7.5. When the pH is less than 3 or greater than 12, the viscosity decreased [12]. Carbomer after neutralization had good adhesion, gelling property, but also had good emulsifying properties, thickening, suspending and film-forming properties, widely used in emulsion, cream, and gel preparation. At present, the major domestic carbomer models have 934, 940, and 941. The carbomer 940 was easy to spread, good adhesion, easily cleaning, no greasy feeling, could absorb the tissue exudates, no stimulation of the skin, often used as a gel matrix, was widely used in the mucosa, transdermal drug delivery preparation.

In this study, we found that the pH of the gel and the gel matrix Carbomer-940 were directly related to the effect of the gel formulation during the preparation of the pomegranate extract gallic acid gel preparation (P <0.05) The reason for this phenomenon may be that the pH value directly affects the solubility of pomegranate extract gallic acid in propylene glycol, Its effect was related to the formation of hydrogen bonds between hydroxyl groups and hydroxyl groups of hydroxyl radical in pomegranate extract gallic acid, and the concentration of carbomer-940 was related to the molding of the gel.

6. Conclusions
The product quality inspection found that, at a rate of 3000r/min centrifugal 30min, and then put it into the oven in 24h, no stratification occurred. The results showed that the pomegranate extract gallic acid gel prepared by using the concentration of 10% propylene glycol as the solubilizer, 1.5% carbomer-940 as the gel matrix and controlling the pH of the gel between 4.5 and 5.5, had a very good stability. It was worthy of wide industrial production test further.

7. Acknowledgements
This study was supported by the Key project of Anhui provincial excellent young talent support program (gxydZD2017128); Natural Science Foundation of Anhui Province Key Project (KJ2015A440, KJ2016A616); Key quality engineering project of Anhui Provincial education Department(2014sxzx047,2016msgzs062,2015ckjh157). Gaofu Fan was responsible for the design and writing of the thesis; Zhenguo XU and Xiushu LIU put forward constructive suggestions on this paper and made some modifications; Jie Tang work for part of the experimental; Jumei Gong , Entao Fu and Yuhua Cai are responsible for the literature review of the article. And the authors declare that they have no conflicts of interest.

8. References
[1] Zheng X.H, Yang J and Yang Y 2017 Research progress on pharmacological effects of gallic acid. J. Chin Hosp Pharm 1 94-99
[2] BenSaad L.A, Kim K.H, Quah C.C, Kim W.R and Shahimi M 2017 Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from Punica granatum. J. BMC Complementary and Alternative Medicine 2 15-18
[3] Bhattacharyya S, Ahammed S.M, Saha B.P and Mukherjee P.K 2013 The gallic acid–phospholipid complex improved the antioxidant potential of gallic acid by enhancing its bioavailability. J. AAPS Pharm Sci Tech 3 1025-1033
[4] Subramanian A.P, Jaganathan S.K, Mandal M, Supriyanto E and Muhamad I.I 2016 Gallic acid induced apoptotic events in HCT-15 colon cancer. J. AAPS Pharm Sci Tech 3 1025-1033
[5] Russell Jr L.H, Mazzio E, Badisa R.B, ZhiPing Zhu, Agharahimi M, Oriaku E.T and Carl B.G 2012 Autoxidation of gallic acid induces ROS-dependant death in human prostate cancer LNCaP Cells. J. Anticancer Res 5 1595–1602
[6] Fawole O.A, Makunga N.P and Opara U.L 2012 Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. J. BMC Complementary and Alternative Medicine 2 31-35
[7] Su T.R, Lin J.J, Tsai C.C, Huang T.K, Yang Z.Y, Wu M.O, Zheng Y.Q, Su C.C and Wu Y.J 2013 Inhibition of elastogenesis by Gallic Acid. J. Possible Involvement 4 43-45
[8] Meritxell M, Cristina A, Vanessa M, Manel L, Alfons D, Parra J.L and L Isabella C 2013 Cosmetotextiles with gallic acid: skin reservoir effect. J. Journal of Drug Delivery 17
[9] Luo Y.Q, Ablizl A, Zhong L.H and Aisa H 2013 Formula of Punica granatum peel phenols gel by orthogonal design. J. Xinjiang Med Univ 6 734-736
[10] Fan G.F, Liu X.S, Tang J, Gong J.M and Cai Y.H 2016 Prescription ratio of pomegranate ellagic acid gel by orthogonal design. J. Chin Mod Appl Pharm 12 1523-1525
[11] Yang Y.Y, Jiang X and Wang Y.Q 2017 Formulation optimization of compound tripterygium hypoglaucum gel by orthogonal test. J. Chi Phar 7 961-963
[12] Guo H.Y, Yi B.W, Yan X.P, Wang C.H and Yu L 2013 Application status of carbomer in gel preparations. J. Chinese Journal of Experimental Traditional Medical Formulae 17 371-374