

## Modelling Mechanical Properties of Topical Films Containing *Ganoderma applanatum* using the Design of Experiments

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### Abstract

With the guidance of Design-Expert<sup>®</sup> software version 11, the mechanical properties of topical films containing *Ganoderma applanatum* (*G. applanatum*) extracts were investigated. While ethyl cellulose (EC), *G. applanatum* extracts, and triethyl citrate (TEC) were independent factors, ultimate tensile strength (UTS), elongation at break, and folding endurance were dependent variables. The best relationships between UTS, elongation at break, and folding endurance could be predicted more accurately using quadratic, 2FI, and linear models, respectively. The ratio of EC, *G. applanatum* extracts, and TEC was predicted by the Design of Experiments (DoE) to be 2.6: 2.8: 2.8. The model's suitability for predicting outcomes inside the design space without necessitating extra trials was established by proving that the ratio's value of prediction was more than 4. The experimental values of UTS, elongation at break, and folding endurance were 82.15±7.42 MPa, 87.40±15.56 %, and 79±6 folds, respectively. The percent errors of prediction were 12.27, 15.49, and 11.82 %, respectively. The Design-Expert<sup>®</sup> software's DoE effectively demonstrated a low percent error of the prediction of less than 20 %. This was suitable and acceptable for developing film formulation in drug delivery systems.

**Keywords:** Preparation, Mechanical properties, *Ganoderma applanatum*, Topical films

### Introduction

Mushrooms are an important source among the sources of several natural antioxidants. The extracts produced from mycelium and fruiting bodies of non-toxic mushrooms including *Boletus badius*, *Russula delica*, *Volvariella volvacea*, *Hericiium erinaceus*, *Hypsizigus marmoreus*, *Polyporus squamosus*, *Lepista nuda*, *Termitomyces*, *Lentinula edodes*, *Verpa conica*, and *Agaricus bisporus* have antioxidant activity and has potential for *in vivo* usage [1]. The macrofungus genus *Ganoderma*, which belongs to the family *Ganodermataceae*, has a significant species variability of mushrooms and has historically been used largely as medicinal mushrooms rather than as food to cure a variety of illnesses in traditional Asian remedies [2]. One of the *Ganoderma* species is the widely widespread perennial bracket fungus known as *G. applanatum*. *G. applanatum* is an increased medicinal basidiomycete fungus that works to prevent cancer in a diverse range of ways [3-5]. Due to its strong antioxidant capacity and antimicrobial characteristics, including its antibacterial and antifungal actions against pathogenic microorganisms, *G. applanatum* has been utilized in herbal remedies [6-8]. It has been established that the polysaccharide and total phenolic contents of mushroom extracts are associated with each other and with their antioxidant activity. The numerous polysaccharides, phenolic chemicals, and minor proteins including lectins found in mushroom water extracts each have their potential biological effects [9].

Topical patch formulations are used in the replacement of oral medications. Drugs administered topically offer several advantages over those administered orally, including compliance, the ability to apply treatment to a distant specific location, and more consistent and sustained plasma levels. One method of medication administration is using patch formulations, often known as patch systems. Topical patches are designed to maintain drugs close to the application site for targeted delivery. Topical patches deliver a specific dosage to a region located for a longer period than traditional topical formulations including creams, gels, and sprays. Additionally, using the patch avoids any mess or skin discoloration that may happen when using creams or gels [10-13].

The DoE is the principal tool in the statistical toolkit that is used to establish quality by design in both academic and commercial organizations. It is essential to mention that there are several mathematical modelling methods available for working with pharmaceutical development, more especially under the quality by design and process analytical technology concept. DoE can begin with, as an illustration, multivariate data analysis (MVDA) techniques that concentrate on statistical information. In order to analyze both independent and dependent variables, MVDA and DoE can be utilized together. An organized, systematic strategy known as experimental design may be used to establish the relationships between the inputs and outputs of a procedure. In those other terms, the latter is a strategy for developing process knowledge that involves creating mathematical correlations between the inputs and outputs of a process [14,15].

When forces are applied to the topical film sample, mechanical properties, which are physical characteristics, are shown. The term “mechanical properties” refers to a material’s properties that influence how it responds to applied loads. Predicting how a material will perform in an application is made easier by considering a material’s mechanical properties before selecting one. The important mechanical properties influence the design of topical patches. Therefore, this research investigated the topical films loaded with *G. applanatum*’s mechanical characteristics. To select the appropriate formulation, these properties would be optimized using Design-Expert® software version 11 (Stat-Ease, Inc, USA).

## Materials and methods

### Materials

EC and TEC were bought from Sigma-Aldrich (USA). *G. applanatum* was collected from Surin Province, Thailand. The solvent of the chemical grade was utilized.

### *G. applanatum* extraction

In Thailand, Surin Province (15°18'11.7"N 103°50'52.4"E), *G. applanatum* was taken in the forest area having started in August 2022. Niran Vipunnern, a taxonomist from the Department of Pharmacognosy at the College of Pharmacy, Rangsit University, identified the basis of *G. applanatum*’s morphology. The Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University, managed the processing of the voucher specimen of *G. applanatum*, designated as JS-GA1-08-2022. Fresh *G. applanatum* was carefully cleaned 2 - 3 times with flowing distilled water before being completely cleansed with 99.8 % absolute ethanol to inhibit microbiological contamination. The samples were immediately divided into small chunks and dried for 6 to 7 days at room temperature in the sunlight. Dried materials were ground up into a powder using an electric blender.

The *G. applanatum* powder extraction procedure was based on an unpublished publication that had developed by our team. Briefly, 200 mL of methanol was used in a beaker to extract 5 g of *G. applanatum* powder. For solvent evaporation, the extraction method was subsequently carried out using a microwave oven (MS23F300EEK/ST model, triple distribution system, Samsung Electronics Co., Ltd., Malaysia). Continuous microwave radiation was utilized for 60 sec (“on”), after which there was no heating for 60 s to prevent the extraction solvent from overheating (“off”). As a result, each cycle’s cumulative extraction time was 120 s. There were 4 cycles of microwave-assisted extraction, and the power output was 450 watts. Finally, the temperature of the extraction solution was found to be 76±2 °C using a glass laboratory thermometer. Following the separation approach, the liquid phase was concentrated using a rotary evaporator at 40 - 60 °C under vacuum before being filtered using 0.45 µm of Whatman No. 1 filter paper. When the weight remained stable and shielded from the light, the raw *G. applanatum* extracts were maintained there.

Following the procedures given by Dandapat et al., freshly obtained *G. applanatum* extracts were utilized for preliminary phytochemical screening assays such as carbohydrates, glycosides, proteins, alkaloids, steroids, triterpenes, phenols, flavonoids, tannins, saponins, and lipids [16]. The raw *G. applanatum* extracts were checked.

### Topical films containing *G. applanatum*

After being dissolved in 100 mL of ethanol, the EC, TEC, and *G. applanatum* extracts were well-blended using a mechanical stirrer. **Table 1** lists the components of the topical films containing *G. applanatum*. The mixture solution was sonicated to remove bubbles for 30 min. The combined solution was then put into a 70.88 cm<sup>2</sup> petri dish, where it had to remain overnight drying at 80 °C. Topical films containing *G. applanatum* were removed from petri dish and stored in a desiccator until they were needed for physical characterization.

**Table 1** Properties of topical films containing *G. applanatum*.

Formulas	EC	<i>G. applanatum</i>	TEC	UTS	Elongation at break	Folding endurance
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>			
	(g)	(g)	(g)	(MPa)	(%)	(Folds)
1	2	1	2	81.95±9.26	30.60±4.67	93±8
2	6	1	2	108.10±8.20	59.00±4.38	129±8
3	2	3	2	60.05±6.86	82.35±7.14	73±6
4	6	3	2	102.85±7.71	32.40±7.07	80±10
5	2	2	1	83.20±8.49	47.30±3.54	60±9
6	6	2	1	107.75±13.51	38.45±3.32	77±6
7	2	2	3	69.35±9.97	62.85±4.88	56±4
8	6	2	3	82.75±9.26	44.05±1.20	70±11
9	4	1	1	69.85±10.68	52.50±9.76	60±9
10	4	3	1	57.50±5.23	41.05±5.44	51±3
11	4	1	3	50.10±2.55	81.40±10.04	122±11
12	4	3	3	92.20±6.79	60.10±8.77	81±8
13	4	2	2	61.95±8.98	61.15±10.25	91±4

### Mechanical properties

The mechanical properties were examined using the TA.XT Plus Texture Analyzer (Texture Technologies Corporation and Stable Micro Systems, Ltd., USA). A loaded cell weighing 500 g was employed, and the testing speed was set at 10 mm per min. Each piece of film was cut into a 10×60 mm<sup>2</sup> rectangle. Every formula was checked. By comparing the cross-sectional testing area (width×thickness of the film, mm<sup>2</sup>) and load cell force, the UTS of the film was estimated. Utilizing the difference between the film's starting length (mm) and ending length at the breaking point, the percentage of elongation at break was estimated (mm) [17,18].

### Folding endurance

A film sample's ability to maintain a certain number of folds before breaking was investigated. The number of folds a film sample could withstand before cracking was used to calculate its folding endurance [19,20].

### Optimization of properties of topical films containing *G. applanatum*

DoE approach used the Design-Expert<sup>®</sup> program version 11 (Stat-Ease, Inc., USA) predicted the UTS, elongation at break, and folding endurance of the films to estimate the optimal ratio of the amounts of the EC, *G. applanatum*, and TEC.

### Results and discussion

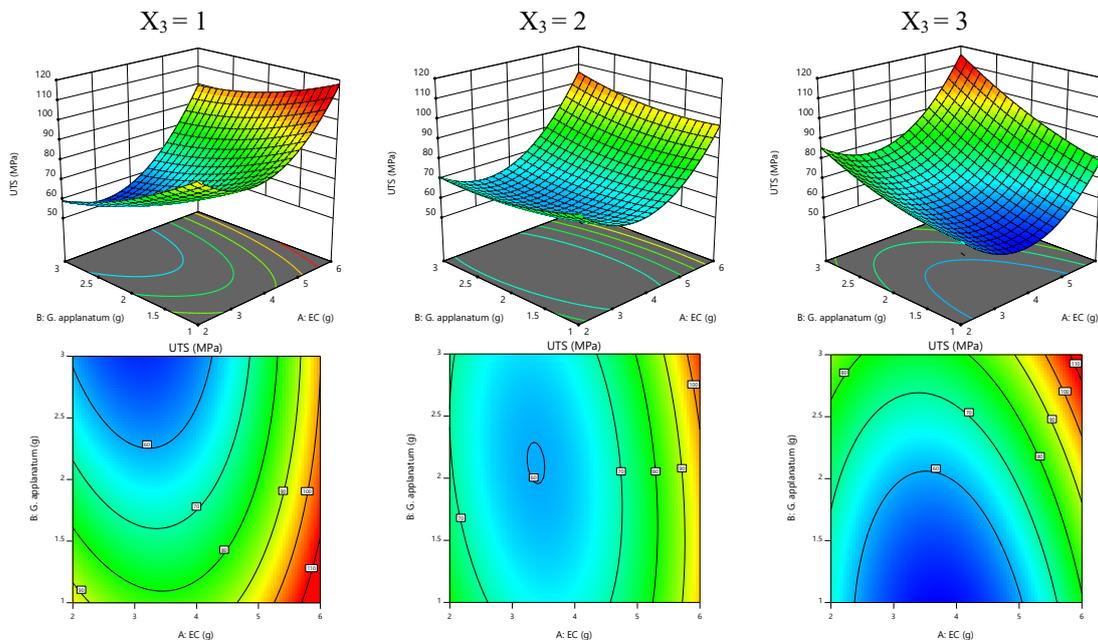
*G. applanatum* methanol extract has the greatest inhibition zone against *Staphylococcus aureus*, with an inhibition zone of 18.50 mm, and the greatest inhibition zone against *Colletotrichum capsici*, with an inhibition zone of 75.49 % [2]. Methanol was chosen as the extraction solvent for *G. applanatum*. *G. applanatum* extracts that were produced were a viscous, dark brown liquid. It was soluble in both water and ethanol. Different techniques were used to evaluate the phytochemical screening results, which included the carbohydrates, glycosides, proteins, alkaloids, steroids, triterpenes, phenols, flavonoids, tannins, saponins, and lipids that were identified in the prepared *G. applanatum* extracts.

The main benefit of a DoE analysis offers both a strategy and information on the area surrounding that response. This may lead the researcher to improve the formulation's design or modify an input parameter to improve the formulation's quality. Moreover, the DoE's primary priority is to use a statistical design method to eliminate and cut down the number of testing required to find the factors impacting the study's findings. Varied interactions, variable levels, variable counts, and the number of permissible trials are among the variables taken into account while choosing the DoE processes [21]. The present research demonstrated that EC, *G. applanatum* extracts, and TEC affected topical films' UTS, elongation at break, and folding endurance. The results of UTS, elongation at break, and folding endurance are summarized in **Table 1**. **Figures 1 - 3** show the 3D response surface and contour plot of the modelling parameters for UTS, elongation at break, and folding endurance, respectively, for topical films containing *G. applanatum* extract. The best relationships of UTS, elongation at break, and folding endurance were better predicted using quadratic, 2FI, and linear models, respectively. The following equations were obtained from Design-Expert<sup>®</sup> software version 11 that could be used to predict the design space.

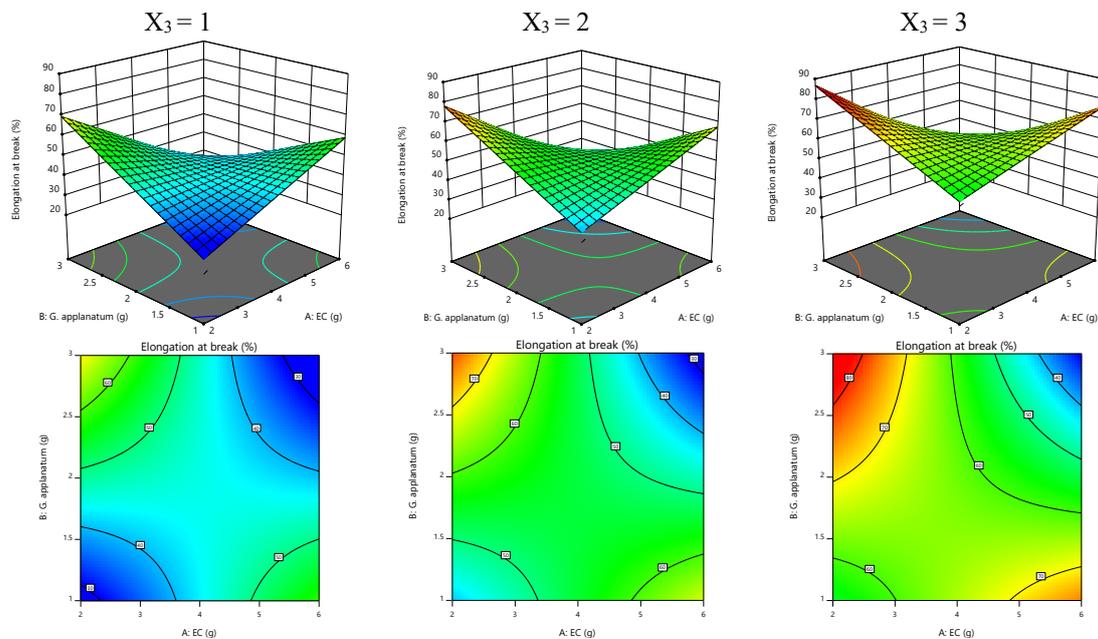
$$UTS = 211.625 - 39.33125(EC) - 51.1(G. \textit{applanatum}) - 30.6125(TEC) + 2.08125(EC * G. \textit{applanatum}) - 1.39375 (EC * TEC) + 13.6125(G. \textit{applanatum} * TEC) + 5.5796875(EC)^2 + 3.96875(G. \textit{applanatum})^2 + 1.49375(TEC)^2$$

$$\text{Elongation at break} = -47.901923076923 + 19(EC) + 43.15(G. \textit{applanatum}) + 18.5375(TEC) - 9.79375(EC * G. \textit{applanatum}) - 1.24375(EC * TEC) - 2.4625(G. \textit{applanatum} * TEC)$$

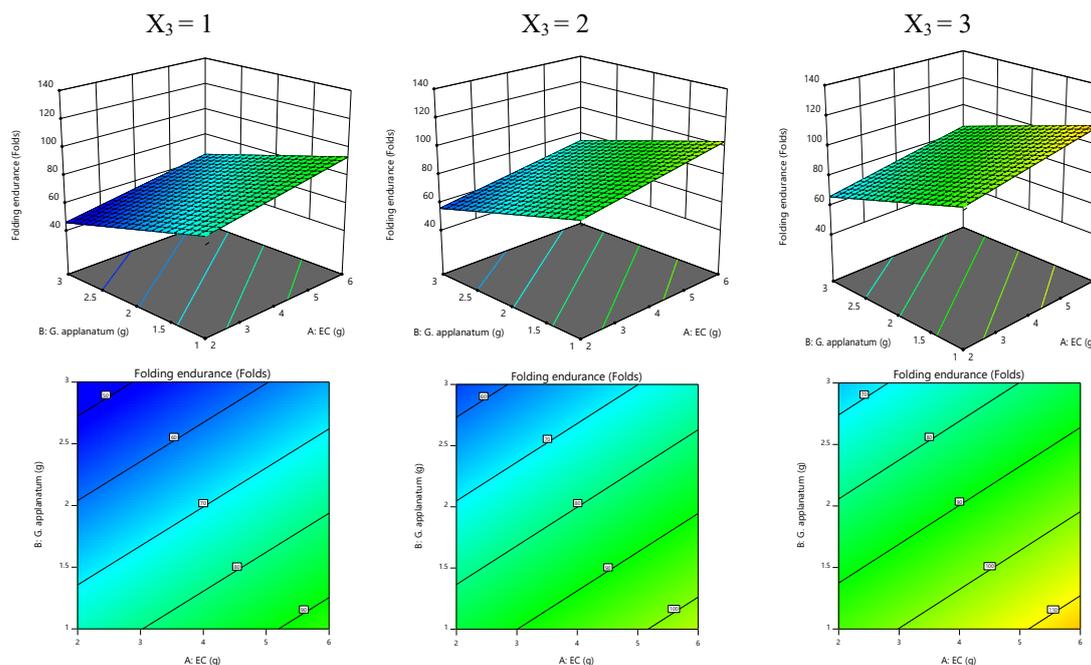
$$\text{Folding endurance} = 70.461538461538 + 4.625(EC) - 14.625(G. \textit{applanatum}) + 10.125(TEC)$$



**Figure 1** (upper) 3D response surface and (lower) contour plot of model conditions for UTS of topical films containing *G. applanatum* formulations.

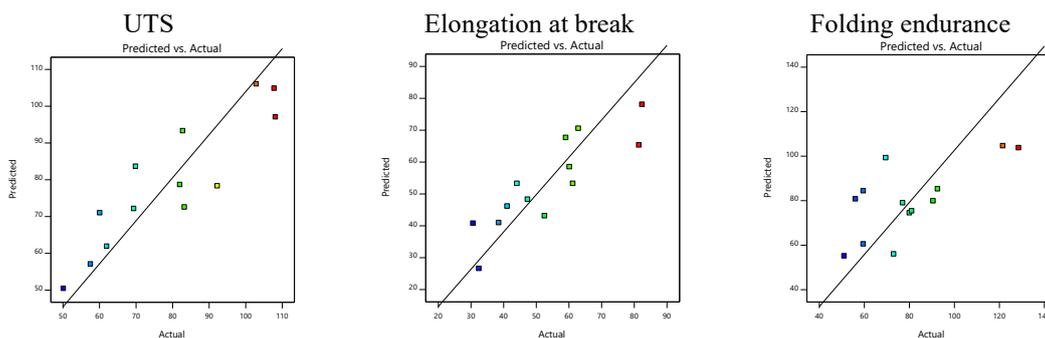


**Figure 2** (upper) 3D response surface and (lower) contour plot of model conditions for elongation at break of topical films containing *G. applanatum*.



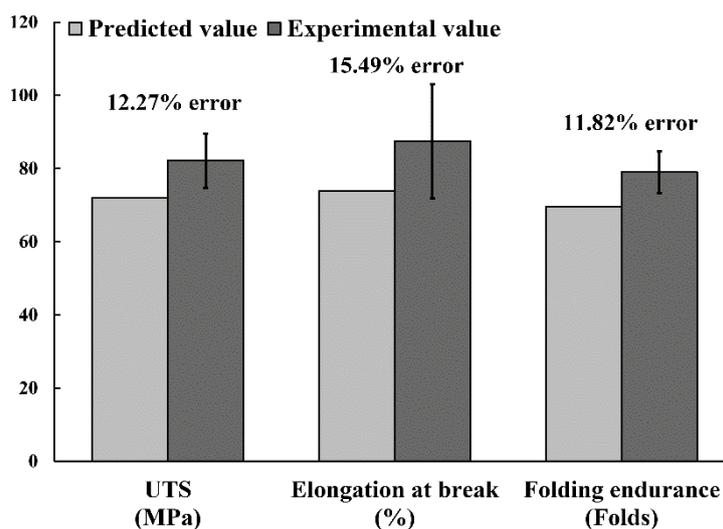
**Figure 3** (upper) 3D response surface and (lower) contour plot of model conditions for folding endurance of topical films containing *G. applanatum*.

For the UTS, a quadratic model was provided. UTS that was inversely associated increased as the concentrations of EC, *G. applanatum* extracts, and TEC were reduced. In this instance, it was important to consider the model parameters “EC \* *G. applanatum* extract”, “EC \* TEC”, “*G. applanatum* \* TEC”, “EC<sup>2</sup>”, “*G. applanatum* extract<sup>2</sup>”, and “TEC<sup>2</sup>”. For elongation at break, a 2FI model was predicted. Elongation at break that was positively associated increased as the concentrations of EC, *G. applanatum* extracts, and TEC. The “EC \* *G. applanatum*”, “EC \* TEC”, and “*G. applanatum* \* TEC” model parameters in this instance were important. The amounts of EC, *G. applanatum* extracts, and TEC were shown to be linearly related to folding endurance. When the amount of *G. applanatum* extracts was increased, folding endurance that was decreased. This was because the herbal extracts might make the film less flexible which was related to the earlier publication of the herbal film [19,22]. When the amount of EC and TEC increased, folding endurance that was positively correlated increased. Because of the high polymer matrix and plasticizer, EC and TEC might also make the film more flexible [23]. According to another report, adding more plasticizers to a plasticized polymer system decreased the force needed to break the films and the resistance to deformation because the presence of plasticizers reduced the interaction between polymer chains [24]. Accordingly, a significant proportion of EC and TEC near level 1 of other variables might result in a high folding endurance, whereas a significant proportion of *G. applanatum* extracts close to level 1 of other factors might result in a low folding endurance. A ratio greater than 4 is normally regarded as satisfactory. The signal-to-noise ratio is evaluated, and the range of the predicted values at the design points is compared to the average prediction error. If the required accuracy is more than 4, the model can predict the outcomes inside the design area without the requirement for additional experiments [25]. The significance of the model was validated by the UTS, elongation at break, and folding endurance ANOVA findings, which had enough precision of 4.6875, 6.0019, and 4.4919, respectively. Therefore, without the need for additional experiments, these models might successfully estimate the design area’s findings. Furthermore, these estimations’ “predicted R-squared” and “adjusted R-squared” values were both higher than 0.8, demonstrating their high degree of accuracy. An F-value larger than 2 indicated that the model was significant, and there was a 0.01 % probability that the “F-value model” would be significant due to noise. **Figure 4** represents the linearity plot of the model ratios of the variables’ predicted values in comparison to actual values, which demonstrated a significant correlation.



**Figure 4** Predicted versus actual plots of model conditions of topical films containing *G. applanatum*.

The ratio of EC, *G. applanatum* extracts, and TEC was predicted by the DoE from Design-Expert<sup>®</sup> software version 11 to be 2.6: 2.8: 2.8. UTS, elongation at break, and folding endurance were all computed to be 72.1 MPa, 73.9 %, and 70 folds, respectively, at this appropriate situation. The factor contributing was then produced once again under the following utilization situation. **Figure 5** shows experimental results for UTS, elongation at break, and folding endurance of  $82.15 \pm 7.42$  MPa,  $87.40 \pm 15.56$  %, and  $79 \pm 6$  folds, respectively. As determined by the formula  $[(\text{Experimental value} - \text{predicted value}/\text{experimental value}) \times 100]$ : The percent errors of the prediction were calculated to be 12.27, 15.49, and 11.82 %, respectively. The DoE from Design-Expert<sup>®</sup> software showed a low percent error of the prediction, was less than 20 %, which was acceptable and suggested for the formulation of *G. applanatum* extract-loaded topical films for drug delivery systems.



**Figure 5** Predicted values, experimental values, percentage error of prediction.

## Conclusions

The mechanical properties of topical films containing *G. applanatum* extracts, including UTS, elongation at break, and folding endurance, were examined and optimized using the Design-Expert<sup>®</sup> program version 11. Dependent variables were UTS, elongation at break, and folding endurance, whereas independent variables included EC, *G. applanatum* extracts, and TEC. Using quadratic, 2FI, and linear models provided better predictions of the best relationships for UTS, elongation at break, and folding endurance, respectively. The DoE expected that EC, *G. applanatum* extracts, and TEC would be present in the ratio of 2.6: 2.8: 2.8. For this ideal situation, UTS, elongation at break, and folding endurance were all estimated to be 72.1 MPa, 73.9 %, and 70 folds, respectively. The model's appropriateness for predicting the outcome inside the design space without the requirement for additional tests was shown by the ratio's value of prediction being higher than 4. Experimental values for UTS, elongation at break, and folding

endurance were  $82.15 \pm 7.42$  MPa,  $87.40 \pm 15.56$  %, and  $79 \pm 6$  folds, respectively. It was found that the errors were 12.27, 15.49, and 11.82 %, respectively. This topical film formulation optimization using *G. applanatum* extract was successful and advised for development in drug delivery systems.

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### References

- [1] M Kozarski, A Klaus, M Nikšić, MM Vrvic, N Todorović, D Jakovljević and LJLD Van Griensven. Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *Ganoderma applanatum*, *Ganoderma lucidum*, *Lentinus edodes* and *Trametes versicolor*. *J. Food Compos. Anal.* 2012; **26**, 144-153.
- [2] S Jogaiah, M Kurjogi, M Abdelrahman, N Hanumanthappa and L-SP Tran. *Ganoderma applanatum*-mediated green synthesis of silver nanoparticles: Structural characterization, and *in vitro* and *in vivo* biomedical and agrochemical properties. *Arab. J. Chem.* 2019; **12**, 1108-20.
- [3] YT Jeong, BK Yang, SC Jeong, SM Kim and CH Song. *Ganoderma applanatum*: A promising mushroom for antitumor and immunomodulating activity. *Phytother. Res.* 2008; **22**, 614-9.
- [4] X Sun, C Zhao, W Pan, J Wang and W Wang. Carboxylate groups play a major role in antitumor activity of *Ganoderma applanatum* polysaccharide. *Carbohydr. Polym.* 2015; **123**, 283-7.
- [5] WA Elkhateeb, GM Zaghlol, IM El-Garawani, EF Ahmed, ME Rateb and AE Abdel Moneim. *Ganoderma applanatum* secondary metabolites induced apoptosis through different pathways: *In vivo* and *in vitro* anticancer studies. *Biomed. Pharmacother.* 2018; **101**, 264-77.
- [6] O Tsvileva, A Pankratov, V Misin, A Zavyalov, V Volkov, O Tsybal, N Yurasov and VE Nikitina. Antioxidant properties of the artist's conk medicinal mushroom, *Ganoderma applanatum* (Agaricomycetes), upon cultivation with *para*-substituted phenolic compounds and tea leaf extracts. *Int. J. Med. Mushrooms* 2018; **20**, 549-60.
- [7] W Elkhateeb, G Daba, A El-Dein, D Sheir, W Fayad, M Shaheen, E Elmahdy and T-C Wen. Insights into the *in-vitro* hypocholesterolemic, antioxidant, antirotavirus, and anticolon cancer activities of the methanolic extracts of a Japanese lichen, *Candelariella vitellina*, and a Japanese mushroom, *Ganoderma applanatum*. *Egypt. Pharmaceut. J.* 2020; **19**, 67-73.
- [8] S Mohammadifar, S Fallahi Gharaghoz, MR Asef Shayan and A Vaziri. Comparison between antioxidant activity and bioactive compounds of *Ganoderma applanatum* (Pers.) Pat. and *Ganoderma lucidum* (Curt.) P. Karst from Iran. *Iran. J. Plant Physiol.* 2020; **11**, 3417-24.
- [9] LM Cheung, PCK Cheung and VEC Ooi. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* 2003; **81**, 249-55.
- [10] DA Williamson, GP Carter and BP Howden. Current and Emerging Topical Antibacterials and Antiseptics: Agents, Action, and Resistance Patterns. *Clin. Microbiol. Rev.* 2017; **30**, 827-60.
- [11] TJ Coderre. Topical drug therapeutics for neuropathic pain. *Expert. Opin. Pharmacother.* 2018; **19**, 1211-20.
- [12] SC Yeoh and CF Goh. Topical delivery of salicylates. *Drug Deliv. and Transl. Res.* 2022; **12**, 981-1001.
- [13] RK Thapa, KG Grønlien and HH Tønnesen. Protein-based systems for topical antibacterial therapy. *Front. Med. Tech.* 2021; **3**, 1-14.
- [14] SN Politis, P Colombo, G Colombo and DM Rekkas. Design of experiments (DoE) in pharmaceutical development. *Drug Dev. Ind. Pharm.* 2017; **43**, 889-901.
- [15] IM Fukuda, CFF Pinto, CdS Moreira, AM Saviano and FR Lourenço. Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). *Braz. J. Pharm. Sci.* 2018; **54**, 1-16.
- [16] S Dandapat, M Kumar, R Ranjan and MP Sinha. *Ganoderma applanatum* extract mediated synthesis of silver nanoparticles. *Braz. J. Pharm. Sci.* 2022; **58**, e19173.
- [17] Y Sampaopan and J Suksaeree. Formulation development and pharmaceutical evaluation of *Lysiphyllum strychnifolium* topical patches for their anti-inflammatory potential. *AAPS PharmSciTech* 2022; **23**, 116.
- [18] Y Sampaopan and J Suksaeree. Effect of Eudragit® NE 40D on the properties of pectin film-based polymer blends. *J. Polym. Environ.* 2021; **29**, 2881-7.

- [19] C Monton, Y Sampaopan, W Pichayakorn, K Panrat and J Suksaeree. Herbal transdermal patches made from optimized polyvinyl alcohol blended film: Herbal extraction process, film properties, and *in vitro* study. *J. Drug Deliv. Sci. Tech.* 2022; **69**, 103170.
- [20] W Pichayakorn, C Monton, Y Sampaopan, K Panrat and J Suksaeree. Fabrication and characterization of buccal film loaded self-emulsifying drug delivery system containing *Lysiphyllum strychnifolium* stem extracts. *AAPS PharmSciTech* 2022; **23**, 194.
- [21] R Gottipati and S Mishra. Process optimization of adsorption of Cr(VI) on activated carbons prepared from plant precursors by a two-level full factorial design. *Chem. Eng. J.* 2010; **160**, 99-107.
- [22] J Suksaeree. Mechanical properties of herbal patches from chitosan-based polymer blends for medical applications. *Mater. Sci. Forum* 2018; **917**, 52-6.
- [23] L Huo, Y Wei, H Zhang, Y Wang, B Deng, Y Wang and L Jin. Preparation and properties of triethyl citrate plasticized chitosan-based membranes for efficient release of curcumin. *J. Appl. Polym. Sci.* 2022; **139**, 51908.
- [24] SANA Zuber, A Rusli and H Ismail. Effectiveness of triacetin and triethyl citrate as plasticizer in polyvinyl alcohol. *Mater. Today: Proc.* 2019; **17**, 560-7.
- [25] S Costa, M Barroso, A Castañera and M Dias. Design of experiments, a powerful tool for method development in forensic toxicology: application to the optimization of urinary morphine 3-glucuronide acid hydrolysis. *Anal. Bioanal. Chem.* 2010; **396**, 2533-42.