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DEVELOPMENT AND EVALUATION OF GEL CONTAINING PHOSPHATIDYLCHOLINE COMPLEXES OF ARBUTIN AND CURCUMIN AS SKIN WHITENING AGENT

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ABSTRACT

Arbutin and curcumin are widely used for the treatment of hyperpigmentation. A very hydrophilic drug (arbutin) is unable to penetrate the skin, while a very lipophilic drug (curcumin) has the propensity to remain in the layers of the stratum corneum; so, there is need for enhancement of penetration for both drugs. Gel containing phosphatidyl complexes of drugs have emerged as one of the most interesting topical delivery systems as it has dual control release systems i.e., gel and complexes. One side the topical applications of the drugs offers the potential advantages of delivering the drug directly to the site of action and secondly delivering the drug for extended period of time at the affected site. The major objective behind this formulation was enhancing the topical delivery of both drugs by formulating gel containing phosphatidylcholine complexes. Optimized formulation of complexes of both drugs incorporated into gel base and evaluated for visual examination, spreadability, pH, and viscosity. The prepared gel was light yellowish in color and showed good homogeneity with the absence of lumps and syneresis. Spreadability, pH, and viscosity of prepared gel was found 5.86 ± 0.057, 5.53 ± 0.115, and 17535.66 ± 5.859, respectively. The in-vitro drug release was found 81.05 ± 2.62 and 77.67 ± 3.60 after 300 minutes for arbutin and curcumin, respectively from prepared gel. Recovery of arbutin and curcumin from the prepared gels was 99.66% and 98.48%, respectively. No change in color, odor, and

homogeneity, as well as a negligible change in pH and net content of the prepared formulation, was observed during and completion of stability testing.

Keywords: Hyperpigmentation, Arbutin, Curcumin, Phospholipid complexes, Topical gel

INTRODUCTION

Skin pigmentation is one of the most strikingly variable phenotypes in humans. The most common among them include lentigines, postinflammatory hyper-pigmentation, dark eye circles, and melasma. Variability of skin tones throughout the world is well-documented, some skin tones being reported as more susceptible to pigmentation disorders than others, especially in Asia and India. Furthermore, exposure to ultraviolet radiation is known to trigger or exacerbate pigmentation disorders. Preventive strategies for photoprotection and treatment modalities including topical and other medical approaches have been adopted by dermatologists to mitigate these disorders. There are various pharmaceutical issues relating to available marketed formulation of synthetic compounds **[1]**.

For the treatment of the hyperpigmentation without causing undesirable hypo-pigmentation or irritation in the surrounding normally pigmented skin, the commonly used phytoconstituents are arbutin, curcumin **[1]**.

The stratum corneum (SC) is the most important barrier to skin permeation and therefore the rate limiting factor for drugs to cross the skin. An awareness of the importance of drug solubility in the SC emerged from reports during the 1980s that drugs with biphasic (water and lipid) solubility better permeated than those with high monophasic (water or lipid) solubility. A very hydrophilic drug (e.g., arbutin) is unable to penetrate the skin, while a very lipophilic drug (e.g., curcumin) has the propensity to remain in the layers of the SC. While the SC is lipophilic in nature and favors the permeation of lipophilic drugs, the aqueous nature of the layers beneath the SC dictate that drugs should embody some hydrophilic properties to pass through them **[2-5]**.

Figure 1 shows complete plan of research work, from which development of drugs complexes already completed and this article focused on incorporation of prepared complexes in gel, and evaluation of final formulation **[10, 14-17]**.

Table 1: Tabulated description of arbutin [6-9]

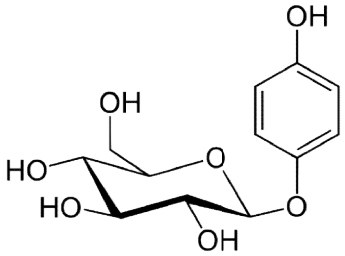
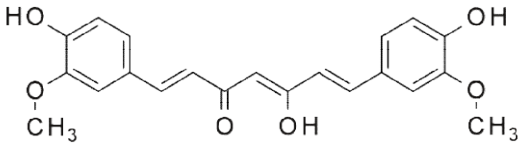
Parameter	Information
Name	**Arbutin**
CAS No.	**497-76-7**
PubChem CID	**440936**
Chemical Name	**(2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-(4-hydroxyphenoxy)oxane-3,4,5-triol**
Molecular Formula	**$C_{12}H_{16}O_7$**
Molecular Weight	**272.253 g/mol**
Physico-chemical properties	**Melting Point: 199.5 °C** **log P (octanol-water): -1.35** **Water solubility: 39.1 mg/mL at 25°C**
Structure	
Mode of action	**Tyrosinase inhibition**
Source	***Arctostaphylos uva-ursi*(Ericaceae)** **Common bearberry plant**

Table 2: Tabulated description of curcumin [10-13]

Parameters	Information
Name	**Curcumin**
CAS No	**458-37-7**
Pubchem CID	**CID 969516**
Chemical Name	**(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione**
Molecular Formula	**$C_{21}H_{20}O_6$**
Molecular Weight	**368.3799 g/mol**
Physico-chemical properties	**Melting point: 183 °C** **log P (octanol-water): 3.29 Predicted** **Water solubility: Insoluble**
Structure	
Mode of action	**Tyrosinase inhibition**
Source	***Curcuma longa* (Zingiberaceae)** **Turmeric**

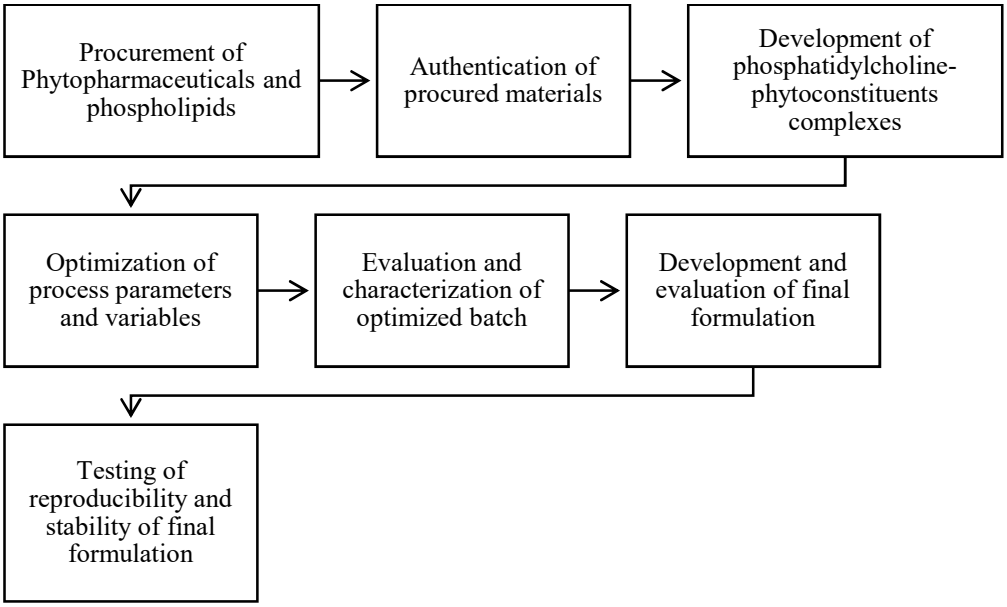
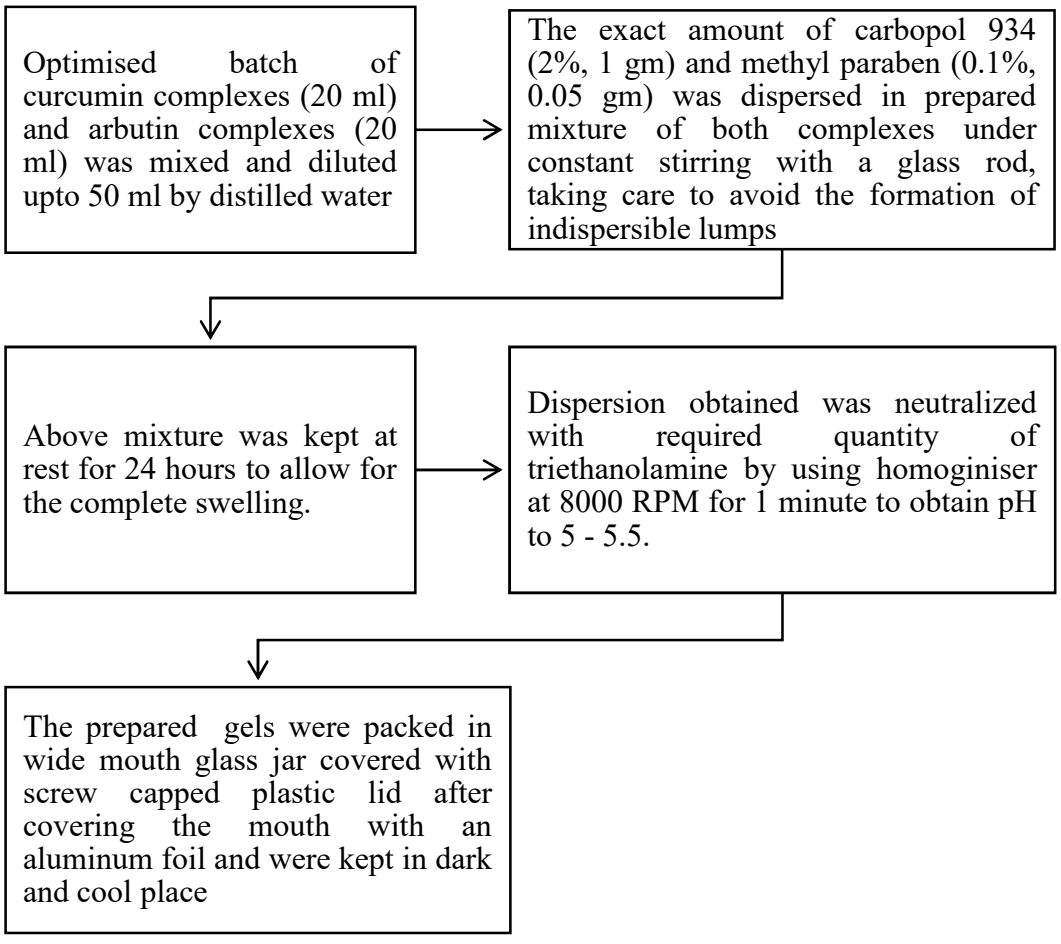


Figure 1: Plan of research work

MATERIALS AND METHODS

Development of gel: **[18]**



Evaluation of gel:

Visual examination: The gel was inspected for its homogeneity, color, syneresis, and presence of lumps by visual inspection after the gel have been set in the container **[19]**.

Spreadability: The spreadability of the gel was measured by spreading of 0.5 g of the gel on a circle of 2-centimeter diameter premarked on a glass plate and then a second glass plate was employed. A half kilogram of weight was permitted to rest on the upper glass plate for 5 minutes. The diameter of the circle after the spreading of the gel was determined **[18]**.

pH measurement: The pH of the gel was determined by using a digital pH meter. The glass electrode was calibrated with the solutions determined for the equipment (pH of 4.00 and 7.00). The preparation was left for about 15 min for attaining equilibrium while measuring **[18]**.

Viscosity study: The viscosity measurement of the prepared gel was performed with a Digital Rotational Viscometer **[18-20]**.

In-vitro drug release study of arbutin and curcumin from the prepared gel:

In vitro release studies were performed using modified Franz diffusion cells having a surface area of 2.84 cm^2 and 20 ml of capacity. Dialysis membrane-70 (HIMEDIA, Mumbai, India) was used. Before the start of a study, the membrane was continuously washed with running water for 3 hours. Phosphate buffer solution of pH 7.4 (saline) used as receptor medium. The receptor phase was continuously stirred and kept at a temperature of 37°C ± 0.5°C during the experiments. The prepared gel was placed in the donor compartment. At fixed time intervals, 1 ml of the sample was withdrawn from the receiver compartment and the same amount of fresh solution was added to keep the volume constant. Each experiment was run in three independent cells. The samples were analyzed spectrophotometrically at a wavelength of 221 nm and 420 nm, for arbutin and curcumin, respectively, and the concentration of both drugs in each sample was determined from a standard curve. Study of various release kinetics performed by the application of mathematical models **[21]**.

Simultaneous estimation of arbutin and curcumin by RP-HPLC [22]

Instrumentation:

Table 3: Specification of HPLC

Make	**SHIMADZU**
Model	**LC 2010**
Type	**Isocratic elution mode**
Detector	**UV detector**
Software	**LC Solution**
Column	**Hypersil ODS C_{18} (250*4.6 mm, 5 µm)**
Pump	**High Pressure Gradient (Reciprocating pump)**

Chromatographic conditions: Analysis was performed on packed silica column Hypersil ODS C_{18} (250*4.6 mm, 5 µm). After optimization, mobile phase consisting of acetonitrile: water (10:90 v/v) was used at flow rate of 0.5 ml/min.

Selection of analytical wavelength: The spectra taken at 280nm of arbutin and curcumin in proposed mobile phase was found to be linear. So 280nm of arbutin and curcumin were chosen as detection wavelength in HPLC.

Preparation of mobile phase: A blend of 10 ml acetonitrile, 90 ml water was filtered through 0.45 µm filter paper.

Validation of proposed method: The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines Q2 (R1) was use to validate the proposed method.

The method was validated for the following parameters: accuracy, repeatability, inter-mediate precision, specificity, linearity and range.

Assay of prepared gel formulation: The content of drugs in the gel is 0.0572 % w/w and 0.592 % w/w respectively as curcumin and arbutin i.e., 100 gm of gel contains 57.2 mg curcumin and 592 mg arbutin. To determine curcumin and arbutin content of the gel formulations, 0.5 g of the gel was taken in a 100 ml volume flask. To this, 5 mL of acetonitrile was added (to dissolve both drugs), and the volume was made up to 100 ml with acetonitrile (HPLC grade). The solution was sonicated for 20 minutes to ensure complete dissolution of the curcumin and arbutin. The final concentration of arbutin and curcumin was 29.6 µg/ml and 2.86 µg/ml, respectively. The solution was filtered by a 0.45-micron filter. The solution obtained was injected for HPLC analysis. The analysis procedure was repeated three times for formulation **[23]**.

Stability testing of formulation: The accelerated stability testing for the prepared topical gel formulation was done as per ICH guidelines [Q1A(R2)] in a stability chamber for 6 months. The prepared topical gel formulation was loaded in a humidity chamber at 40°C ± 2°C/75% RH ± 5% RH. Samples were withdrawn at an initial, first, second, third, and sixth months and evaluated for change in color, odor, homogeneity, pH, viscosity, and net content **[24]**.

RESULTS AND DISCUSSION

Visual examination:

The prepared gel was light yellowish in color and showed good homogeneity with the absence of lumps and syneresis.

Simultaneous estimation of arbutin and curcumin by RP-HPLC

Optimizations of chromatographic conditions were performed to obtain a good

peak shape and peak parameter (tailing factor, theoretical plates). For the selection of the mobile phase initially, the different concentrations of acetonitrile, methanol, and water with different pH have been tried, but it gave poor peak shape and also poor system suitability parameters. Finally, acetonitrile and water (10:90) mixture tried at a flow rate of 0.5 ml/min was found to be satisfactory and good system suitability parameter. Using these experimental conditions, all peaks were well resolved with good peak shape. Therefore, this mobile phase provided the best chromatographic response and was used for further studies.

The average retention time (R_t) for arbutin and curcumin were found to be 3.026 ± 0.0.473% and 6.343 ± 0.190%, respectively. Values are as average ± percentage relative standard deviation (%RSD).

Table 4: Evaluation parameters for prepared gel (mean ± SD, n=3)

Spreadability (cm)	**5.86 ± 0.057**
pH	**5.53 ±0.115**
Viscosity (cp)	**17535.66 ± 5.859**

Table 5: In-vitro release of arbutin from prepared gel

Time (Minutes)	% Drug release from the gel (Avg. ± SD, n=3)
15	**5.71 ± 0.42**
30	**17.20 ± 0.81**
60	**35.83 ± 1.32**
120	**51.02 ± 1.69**
180	**66.25 ± 1.74**
240	**73.65 ± 2.21**
300	**81.05 ± 2.62**

Table 6: In-vitro release of curcumin from prepared gel

Time (Minutes)	% Drug release from the gel (Avg. ± SD, n=3)
15	**4.19 ± 0.21**
30	**17.41 ± 0.59**
60	**34.80 ± 0.52**
120	**49.85 ± 1.64**
180	**63.54 ± 1.97**
240	**70.15 ± 2.86**
300	**77.67 ± 3.60**

Table 7: Summary of validation parameter and stability testing for RP-HPLC

Parameters	Arbutin	Curcumin
Linearity		
(Concentration range) (µg/ml)	**10-50**	**1-5**
(Linear equation)	**y = 13766x + 167413**	**y = 71038x + 78915**
(Linear R-squared value)	**0.9979**	**0.9957**
Repeatability (Intra-day precision) (% RSD)	**0.13 - 0.28**	**0.35 - 0.54**
Intermediate precision (inter-day precision) (% RSD)	**0.13 - 0.31**	**0.79 - 0.94**
Accuracy		
(% Recovery)	**96.36 - 102.03**	**95.56 - 97.78 %**
(% RSD)	**1.42-176**	**0.56-1.45**
Robustness (% RSD)	**1.01-1.06**	**0.62-1.54**
Assay (%)	**99.66**	**98.48**
Stability testing	**Stable**	**Stable**

Table 8: Assay of the prepared gel formulation

Drug	Amount taken (µg/ml)	Amount found (µg/ml) (Mean ± SD, n=3)	% Assay (Mean ± SD, n=3)	% RSD
Arbutin	29.6	29.5 ± 0.05	99.66	0.16
Curcumin	2.86	2.82 ± 0.03	98.48	0.89

Table 9: Stability testing data for prepared gel

	Storage condition: 40°C ± 2°C/75% RH ± 5% RH						
	Months						
Parameters	0	1	2	3	4	5	6
Color	Ligh yellow	No change in color					
Odor	Odorless	No change in odor					
Homogeneity	Smooth	No change in homogeneity					
pH	5.64	5.60	5.58	5.53	5.51	5.51	5.48
Viscosity (cp)	17535.66	17520	17485	17469	17432	17413	17386
Net content (%)	99.08	98.95	98.64	98.49	98.40	98.37	98.31

CONCLUSION

During research project, successful development of phosphatidylcholine complexes of arbutin and curcumin carried out. Encouraging results of higher percentage entrapment efficiencies and in-vitro drug release with phosphatidylcholine may useful to overcome issues of poor skin penetration of both drugs. Even drugs which are having same physical and chemical characteristics of arbutin and curcumin can be selected for further research for improvement in skin penetration.

Further satisfactory development of gel containing complexes of arbutin and curcumin with satisfactory reproducibility and stability.Overall conclusion from entire research work is that, one can achieve improvement in skin penetration of topical preparation by development of complexes of suitable drug/s with phosphatidylcholine.

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Conflict of interest: We have no conflict of interest to declare.

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