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DEVELOPMENT AND EVALUATION OF PHOSPHATIDYLCHOLINE COMPLEXES OF ARBUTIN AS SKIN WHITENING AGENT

R. K. Patel and M. B. Jadeja *

Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mahesana - 384012, Gujarat, India.

Keywords:

Phosphatidylcholine complexes, Arbutin, Box-Behnken design, Skin whitening agents, Rotary flask evaporation method

Correspondence to Author:
M. B. Jadeja

Ph.D. Scholar,
Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mahesana - 384012, Gujarat, India.

E-mail: jadeja.mukesh@gmail.com

ABSTRACT: Introduction: Disorders of hyperpigmentation are difficult to treat, particularly in dark skin individuals. There is a dramatic enhancement of skin permeability of phytoconstituents due to their complexation with phospholipids. Complexation leads to better permeation through the skin, which is otherwise not possible. Research work focused on the development and evaluation of phosphatidylcholine complexes of arbutin, having the issue of poor skin permeability as skin whitening agents. **Materials and Methods:** Arbutin, phosphatidylcholine, and petroleum ether have been used for the development of complexes. Authentication of arbutin has been performed by Fourier Transform Infrared study. UV–visible spectrophotometric method has been developed for estimation of arbutin followed by the development of complexes by rotary flask evaporation technique, preliminary trials for selection of dependent and independent variables, optimization of complexes by Box- Behnken reduced surface response design, evaluation of optimized batch by percentage entrapment efficiency, particle size, zeta potential, and *in-vitro* drug release study by Franz diffusion cell. **Result and Discussion:** Arbutin sample FTIR data and standard FTIR data are matched. Final polynomial equation is, % Entrapment Efficiency = + 161.924 - 25.954 * Arbutin: PC ratio - 3.8654 * Film formation temperature 0.991 * Hydration temperature +0.29737654320988 * Hydration time +17.0566 * Arbutin: PC ratio 2 + 0.03880416666667 * Film formation temperature 2 + 9.941E-003 * Hydration temperature 2 -1.499E-003 * Hydration time 2. Results show that there is ≥ 92% similarity between predictive and actual values. Developed complexes show a better *in-vitro* drug release profile compared to an aqueous solution of arbutin, which indicates optimized complexes improve permeability.

INTRODUCTION: 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.

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Preventive strategies for photoprotection and treatment modalities, including topical and other medical approaches, have been adopted by dermatologists to mitigate these disorders [12]. There are various issues relating to the available marketed formulation of synthetic compounds.

So, there is a clinical need for the development and optimization of the formulation, which may provide better absorption of the drug over the skin and treat the diseases related to hyperpigmentation. A phytosomes is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex result from the reaction of stoichiometric amounts of phospholipid

with the selected phytoconstituents. On the basis of their physicochemical and spectroscopic data, it has been shown that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (*i.e.*, phosphate and ammonium groups) and the polar functional groups of the substrate. They are lipophilic substances with a clear melting point, freely soluble in non-polar solvents (in which the hydrophilic moiety was not), and moderately soluble in fats.

When treated with water, phytosomes assume a micellar shape is forming liposomal-like structures. In liposomes, the active principle is dissolved in an internal pocket or floats in the layered membrane, while in phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane. Molecules are anchored through chemical bonds to the polar head of the phospholipids, as can be demonstrated by specific spectroscopic techniques [3-10].

TABLE 1: COMMERCIAL PRODUCTS [4, 11, 12]

Trade name	Phytochemicals	Indication
18ß-glycyrrhetinic acid Phytosome®	18ß-glycyrrhetinic acid from liquorice rhizome	Soothing
Centella Phytosome®	Triterpenes from *Centella asiatica* leaf	Cicatrizing, trophodermic
Crataegus Phytosome®	Vitexin-2″-O-rhamnoside from Hawthorn flower	Antioxidant
Escin ß-sitosterol Phytosome®	Escin ß-sitosterol from horse chestnut fruit	Anti-oedema
Ginkgoselect® Phytosome®	Ginkgoflavonglucosides, ginkgolides, bilobalide from *Ginkgo biloba* leaf	Vasokinetic
Ginselect® Phytosome®	Ginsenosides from *Panax ginseng* rhizome	Skin elasticity improver, adaptogenic
Ginkgo biloba terpenes Phytosome®	Ginkgolides and bilobalide from *Ginkgo biloba* leaf	Soothing
Ginkgo biloba Dimeric Flavonoids Phytosome®	Dimeric flavonoids from *Ginkgo biloba* leaf	Lipolytic, vasokinetic
Greenselect® Phytosome®	Polyphenols from green tea leaf	Prevention of free radical mediated tissue damages and weight management
Leucoselect® Phytosome®	Polyphenols from grape seed	Antioxidant, capillarotropic
Meriva®	Curcuminoids from turmeric rhizome	Potent antioxidant, anti-inflammatory
PA_2 Phytosome®	Proanthocyanidin A_2 from horse chestnut bark	Anti-wrinkles, UV protectant
Sericoside Phytosome®	Sericoside from *Terminalia sericea* bark	Anti-wrinkles
Siliphos®	Silybin from milk thistle seed	Hepatocyte protection
Silymarin Phytosome®	Silymarin from milk thistle seed	Antihepatotoxic
Virtiva®	Ginkgoflavonglucosides, ginkgolides, bilobalide from *Ginkgo biloba* leaf	Vasokinetic
Visnadex®	Visnadin from *Ammi visnaga*	Vasokinetic
Mirtoselect® phytosome	Anthocyanosides of bilberry	Potent antioxidants
Sabalselect® phytosome	Saw palmetto berries	Benefit non-cancerous prostate enlargement
Lymphaselect™ phytosome	*Melilotus officinalis*	For venous disorders, including chronic venous insufficiency of the lower limbs
Oleaselect™ phytosome	Olive oil polyphenols	Anti-oxidant, anti- inflammatory, anti hyperlipidemic
Polinacea™	*Echinacea angustifolia*	Neutraceutical, immuno-modulator.

Proposed research work focused on development and evaluation of phosphatidylcholine complexes of some phytopharmaceuticals having an issue of poor skin permeability as skin whitening agents. Selection of phytoconstituents or standardized herbal extracts having skin whitening activity and poor skin permeability for a dermatological disease like hyperpigmentation. Selection of a suitable method of preparation for the development of phosphatidylcholine-phytoconstituents complexes by preliminary screening or trials. Development of phosphate-dylcholine-phytoconstituents complexes by the selected method. Optimization of process parameters and variables for the selected method of

preparation and selection of optimized batch by suitable statistical design and experimental design. Evaluation and characterization of optimized batch on selected criteria.

TABLE 2: PATENT ON PHOSPHOLIPID-PHYTOCONSTITUENTS COMPLEXES [13-20]

S. No.	Content	Description
1	Title of patent:	Phospholipid complexes of olive fruits or leaves extract having improved bioavailability
	Patent no.	EP1844785
	Abstract	Phospholipids complexes of olive fruits or leaves extracts or compositions containing it having improved bioavailability.
2	Title of patent:	Compositions comprising *Ginko biloba* derivatives for the treatment of asthmatic and allergic conditions
	Patent no.	EP1813280
	Abstract	Compositions containing fractions deriving from *Ginkgo biloba*, useful for the treatment of asthmatic and allergic conditions.
3	Title of patent:	An anti-oxidant preparation based on plant extracts for the treatment of circulation and adiposity problems
	Patent no.	EP1214084 and US6756065B1
	Abstract	preparation based on plant extracts, with an anti-oxidant action which is particularly useful in the prevention and treatment of circulation problems and in the prevention and treatment of surplus fat deposits, characterized in that its active ingredients comprise, in association, Ginkgo bilobabiflavones, catechine and/or epicatechine, iodine and a component selected from among madecassic acid, asiatic acid, asiaticoside or combinations thereof.
4	Title of patent:	Complexes of saponins with phospholipids and pharmaceutical and cosmetic compositions containing them
	Patent no.	EP0283713
	Abstract	Complexes of saponins with natural or synthetic phospholipids have high lipophilia and improved bioavailability and are suitable for use as active principle in pharmaceutical, dermatologic and cosmetic compositions.
5	Title of patent:	Phospholipid complexes of Extracts of *Vitis vinifera*, their Preparation process and Pharmaceutical and cosmetic Compositions containing them
	Patent no.	US4963527
	Abstract	Complexes resulting from the reaction of phospholipids, synthetic or of vegetable or animal origin, with flavonoids extracted from *Vitis vinifera*, their use in therapeutics and in cosmetics
6	Title of patent:	Method for the improvement of transport across adaptable semi-permeable barriers
	Patent no.	US20030099694A1
	Abstract	The invention relates to a method, a kit and a device for controlling the flux of penetrants across an adaptable semi-permeable porous barrier, the method comprising the steps of: preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds of forms of amphiphilic substances with a tendency to aggregate, said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores, selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.
7	Title of patent:	Composition for applying active substances to or through the skin
	Patent no.	US5716638A
	Abstract	A cosmetic or medical composition for topical application to the skin. It results in the transdermal passage of an active ingredient, or in the introduction of such agent into the skin. The essential components of such compositions are phospholipids, an aliphatic alcohol of three or four carbon atoms or a combination of these alcohols, water and a compatible active ingredient, optionally with propylene glycol. Compositions advantageously comprise from 0.5% to 10% phospholipids, from 5% to 35% of a C_3 - or C_4 -alcohol, 15 to 30% ethanol, which contain together at least 20% but not more than 40 wt. % of ethanol and the C_3 -alcohol; up to 20 wt. % propylene glycol, at least 20% water and at least one active ingredient. The compositions are suitable for the topical application of a wide variety of cosmetic and pharmaceutically active compounds. Phospholipids of choice are phosphatidylcholine, (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine(PS), phosphatidylethanolamine(PE), phosphatidylglycerol(PPG) and phosphatidylinositol (PI)
8	Title of patent:	A composition based on natural extracts useful in the prevention and treatment of cutaneous wrinkles
	Patent no.	WO2001078674A1
	Abstract	A composition based on natural extracts useful in the prevention and treatment of cutaneous ageing and particularly wrinkles, which comprises in combination: leucocyanadines in the form of extract of *Vitis vinifera*; triterpenes in the form of an extract of *Centella asiatica*; fish cartilage extract.
9	Title of patent:	Tea extract-phytosomes composite and preparation method thereof

	Patent no.	CN101554192A
	Abstract	The invention relates to a tea extract (TE)-phytosomes (ph) composite and a preparation method thereof. The composite takes TE and ph as raw materials. By means of pH encapsulation of the TE, the physical and chemical properties of the TE are changed and the antioxygenic property of the TE in different environments is maintained or enhanced. The invention takes advantage of the ph encapsulation of the TE to change the physical and the chemical properties of the TE and maintain or enhance the environments, thereby expanding the application approach of the TE and enhancing the functions of the TE in certain environments.antioxygenic property of the TE in different
10	Title of patent:	Topical compositions for the prevention and treatment of inflammatory and/or infective conditions of the genital area
	Patent no.	EP2014295A2
	Abstract	The present invention relates to topical compositions containing Zanthoxylum bungeanum extract, 18-beta glycyrrhetic acid, Matricaria chamomilla essential oil, Melaleuca alternifolia essential oil, Curcuma longa or curcumin extract and lactic or propionic acid, for the prevention and treatment of inflammatory and/or infective conditions affecting mainly the genital area, in particular vaginosis, vaginitis and vulvo-vaginitis, also those which are recurring.
11	Title of patent:	An antioxidant preparation based on plant extracts for the treatment of circulation and chronic degenerative problems and of hypertension
	Patent no.	EP1214085B1
	Abstract	A preparation based on plant extracts, with an antioxidant action which is particularly useful in the prevention and treatment of circulation and chronic degenerative problems and in the prevention and treatment of hypertension, characterised in that its active ingredients comprise, in association, Ginkgo biloba biflavones, catechine and/or epicatechine, cumarine and derivatives thereof and a component selected from among madecassic acid, asiatic acid, asiaticoside or combinations thereof.
12	Title of patent:	Compositions for the treatment and prevention of vertigo and tinnitus including citicoline, ginkgo biloba extract and dimeric flavones of Ginkgo biloba
	Patent no.	EP2018868A1
	Abstract	Disclosed are compositions containing: a) citicoline, b) Ginkgo biloba extract; and c) dimeric flavones of Ginkgo biloba; for the treatment and prevention of vertigo and tinnitus.
13	Title of patent:	Compositions for the prevention and treatment of erectile dysfunction and impotence and for improving sport performance
	Patent no.	WO2012063198A1
	Abstract	The present invention relates to compositions comprising active principles of vegetable origin whether or not combined with nitric oxide promoters and/or protectors for the prevention and treatment of erectile dysfunction and impotence and for improving sports performance.
14	Title of patent:	A product for topical administration
	Patent no.	ES2525245T3
	Abstract	A product for topical administration, wherein the product comprises *Melia Azadirachta* leaf extract, root extract *Withania Somnifera* leaf juice and *Aloe Barbadensis* for use in the treatment of eczema or psoriasis.
15	Title of patent:	Preparation method for berberine-loaded phospholipid composite nanoparticles
	Patent no.	CN104940167A
	Abstract	The invention discloses a preparation method for berberine-loaded phospholipid composite nanoparticles. The preparation method is characterized by comprising the following steps: (1), preparing a soybean phospholipid solution; (2), preparing a berberine ethanol solution; (3), adding the berberine ethanol solution into the soybean phospholipid solution, and carrying out rotary evaporation so as to obtain a phospholipid single-layer film; (4), redissolving the phospholipid single-layer film with an aprotic reaction reagent, and adding ultrapure water for secondary rotary evaporation so as to obtain nanoparticle suspension liquid; (5), adding a spray-drying protective agent into the nanoparticle suspension liquid to obtain an original medical solution; (6), atomizing the original medical solution through a single-line micro jet atomizer under a certain pressure, and drying in a spray-drying tower so as to obtain the berberine-loaded phospholipid composite nanoparticles. The entrapment efficiency of the berberine-loaded phospholipid composite nanoparticles prepared through the preparation method provided by the invention is 85% or above, the berberine-loaded phospholipid composite nanoparticles are uniform in particle size, the particle size is changed slightly after the berberine-loaded phospholipid composite nanoparticles are redissolved in water, *in-vitro* release of berberine is not influenced, the bioavailability is high and long-term storage stability is high.

MATERIALS AND METHODS: Chemicals and instruments used for research work are in tabulated form as **Table 3** and **Table 4**. Research work performed from January 2016 to January 2019.

TABLE 3: CHEMICALS

Ingredients	Supplier
Arbutin	Hangzhou Reb Technology Co., Ltd., China
Phosphatidylcholine	Sant Neutraceuticals, Anand, India
Petroleum ether	Chemdyes Corporation, Vadodara, India

TABLE 4: EQUIPMENT AND INSTRUMENTS

Instrument	Supplier
UV-Visible spectrophotometer	Shimadzu UV-1601, Japan.
Analytical balance	Mettler Toledo India Pvt. Ltd, Vadodara, India
Rotary flask evaporator	lalco scientific instruments, Maninagar, India
Digital microscope	Almicro instruments, Haryana, India
Franz diffusion cell	Durga scientific, Vadodara, India
pH meter	Welltronix Instruments, Ahmedabad, India
Magnetic stirrer	lalco scientific instruments, Maninagar, India

Authentication of Active Pharmaceuticals: The Fourier Transform Infrared (FTIR) spectrum of Arbutin was recorded on Shimadzu FTIR spectrophotometer.

The sample was prepared by using the press pellet technique and scanned for transmission in the wavenumber. Then it was compared with the standard FTIR spectrum of Arbutin.

UV–Visible Spectrophotometric Method Development for Estimation of Arbutin: All solutions utilized for sample preparation were of analytical grade. A stock solution of Arbutin at a concentration of 1000 ppm was prepared by dissolving 100 mg Arbutin in 100 ml distilled water. Working solution of Arbutin at concentration of 100 ppm was prepared by 10 times dilution of stock solution. Calibration sample solutions were prepared by serial dilution from the working solution at a concentration of 10, 15, 20, 25, 30 ppm. The baseline was corrected across 400 to 200 nm, using distilled water as a sample and blank. λ_{max} of Arbutin was observed in spectrum mode, using 30 ppm calibration solution in the sample holder. Absorbance was recorded for rest of the calibration samples in photometric mode at 221 nm as λ_{max}. A calibration curve was prepared by plotting absorbance versus concentration of arbutin. From plot, slope and intercept were determined.

Method for Development of Complexes by Rotary Flak Evaporation Technique: Phosphatidylcholine dissolved in petroleum ether in a round bottom flask of rotary flask evaporator. Applied defined temperature for formation of a film of phosphatidylcholine by rotation of flask. For the hydration of film, a previously prepared aqueous solution of Arbutin in a defined concentration was poured into the flask at a defined temperature and time. At the end of defined time, development of complexes in the form of suspension [21].

TABLE 5: PRELIMINARY TRIALS

Batch no.	Arbutin: PC	FFT ºC	HTe ºC	HTi Minute
PT1	0:1	RT	RT	30
PT2	0:1	RT	RT	60
PT3	0:1	RT	RT	120
PT4	1:1	40	40	30
PT5	1:1	50	60	120
PT6	1:2	60	80	30
PT7	1:2	40	40	120
PT8	2:1	50	60	30
PT9	2:3	60	80	120

Optimization of Arbutin – PC Complexes: Based on results of Preliminary trials and literature reviews, selection of independent and dependent factors was carried out. Box–Behnken design was selected for optimization.

TABLE 6: EXPERIMENTAL DESIGN DETAIL FOR OPTIMIZATION OF ARBUTIN – PC COMPLEXES

Independent Factor	Coded Level			Uncoded Level		
	Low	Medium	High	Low	Medium	High
X_1 = Arbutin: PC ratio	-1	0	+1	0.5:1	1:1	1.5:1
X_2 = Film formation temperature (ºC)	-1	0	+1	40	50	60
X_3 = Hydration temperature (ºC)	-1	0	+1	40	60	80
X_4 = Hydration time (Minute)	-1	0	+1	30	75	120
Dependent Factors						
Y= Percentage Entrapment Efficiency (% EE)						

TABLE 7: BOX-BEHNKEN DESIGN POINT

Run	X_1(D:P)	X_2(FFT)	X_3(HT)	X_4(Time)
AR1	0.5	40	60	75
AR2	1.5	40	60	75
AR3	0.5	60	60	75
AR4	1.5	60	60	75
AR5	1	50	40	30
AR6	1	50	80	30
AR7	1	50	40	120
AR8	1	50	80	120
AR9	0.5	50	60	30
AR10	1.5	50	60	30
AR11	0.5	50	60	120
AR12	1.5	50	60	120
AR13	1	40	40	75
AR14	1	60	40	75
AR15	1	40	80	75
AR16	1	60	80	75
AR17	0.5	50	40	75
AR18	1.5	50	40	75
AR19	0.5	50	80	75
AR20	1.5	50	80	75
AR21	1	40	60	30
AR22	1	60	60	30
AR23	1	40	60	120
AR24	1	60	60	120
AR25	1	50	60	75
AR26	1	50	60	75
AR27	1	50	60	75

Drug: phosphatidylcholine ratio, 0.5:1 = 100 mg of arbutin drug and 200 mg of phosphatidylcholine, 1:1 = 200 mg of arbutin drug and 200 mg of phosphatidylcholine, 1.5:1 = 300 mg of arbutin drug and 200 mg of phosphatidylcholine

TABLE 8: DETAIL OF CHECK POINT BATCHES

Check point batch	Coded Level				Uncoded Level			
	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4
1	-0.5	-0.5	-0.5	-0.5	0.75	45	50	52.5
2	0.5	0.5	0.5	0.5	1.25	55	70	97.5

TABLE 9: FORMULA FOR OPTIMIZED BATCH OF ARBUTIN – PC COMPLEXES

Ingredients	Amount
Phosphatidylcholine	200 mg
Arbutin	296 mg
Petroleum ether	20 ml
Distilled water	20 ml

Evaluation of Optimized Batch of Arbutin – PC Complexes: [21]

% Entrapment Efficiency: Take 10 ml of a suspension of complexes in a centrifugal tube. Centrifuge the suspension at 10000 RPM for 3 hours using an ultracentrifuge. Then collect the 1ml of supernatant, and dilute with the desired quantity of distilled water. If the supernatant not clear, use a membrane filter. Then absorbance was measured at 221 nm wavelength in UV- Visible Spectrometer.

% EE = (Total amount of arbutin taken – Free arbutin measured) / Total amount of arbutin taken × 100

Particle Size: The average diameter (Z-AVE), poly dispersity index (PDI), and zeta potential of suspension was determined by photoncorrelation spectroscopy (PCS) (Zetasizer Nano-ZS, Malvern Instruments, UK) at room temperature. PCS follows the principle of LASER light diffraction, and it is based on the measurement of the Brownian motion of particles. The Brownian motion is the random movement of particles in suspension. The smaller the particle, the faster the Brownian motion. When the incident laser beam reaches the sample, the light was scattered in such a way, depending on the Brownian motion, and then detected by a photomultiplier positioned at a determined angle. Fluctuations in the intensity of scattered light were converted into output current, which is passed to an autocorrelator.

In this way, a correlation function is generated and analyzed by software. Prepared suspension was added (after suitable dilution) to the sample cell and put into the sample holder unit and measurement was carried out with the help of software of same instrument. The computer can provide the mean size and the distribution width of the nanosomes in the batch (Malvern Zeta Sizer Nano series manual).

Zeta Potential: Diluted suspension was added to the sample cell (quartz cuvette) and put into the sample holder unit, and measurement was carried out with the help of software. The Zeta potential of the optimized formulation was measured using the same instrument used in particle size measurement. The sample was added in specialized zeta cell, and the same procedure was carried out.

***In-vitro* Drug Release Study Using Franz Diffusion Cell:** Franz diffusion cells with a receiver compartment volume of 10 mL and effective diffusion area of 2.84 cm^2 were used to evaluate drug release characteristics of selected compositions.

A dialysis membrane was used for the permeation study. Phosphate buffer solution of pH 7.4 used as receptor medium. The receptor phase was continuously stirred and kept at a temperature of 32 ± 0.5 °C during the experiments. Suspension of

complexes and an aqueous solution of arbutin were placed in the donor compartment. At an appropriate time, 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 221.00 nm, and the concentration of arbutin in each sample was determined from a standard curve.

RESULTS AND DISCUSSION:

Authentication of Active Pharmaceuticals:

Sample FTIR Data:

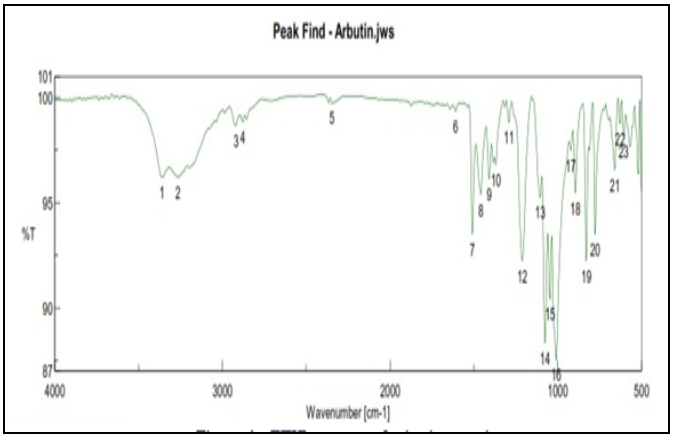

FIG. 1: FTIR SPECTRA OF ARBUTIN SAMPLE

Reported FTIR Data: [22]

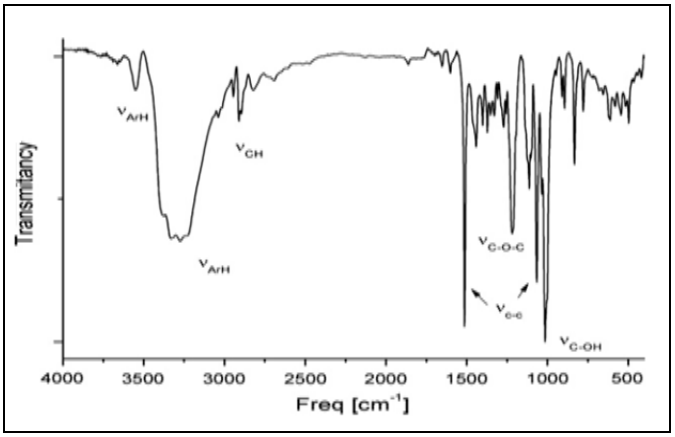

FIG. 2: FT - IR SPECTRUM OF ARBUTIN REFERENCE

Discussion: Arbutin sample FTIR data and standard FTIR reported data were matched, so the arbutin sample was pure.

UV–Visible Spectrophotometric Method Development for Estimation of Arbutin:

Calibration Curve of Arbutin:

TABLE 10: TABULATED RESULT OF CALIBRATION SAMPLES AT 221 nm

Concentration (mcg/ml)	Absorbance Avg. ± SD (n = 3)
10	0.164 ± 0.002
15	0.267 ± 0.002
20	0.369 ± 0.003
25	0.494 ± 0.003
30	0.633 ± 0.004

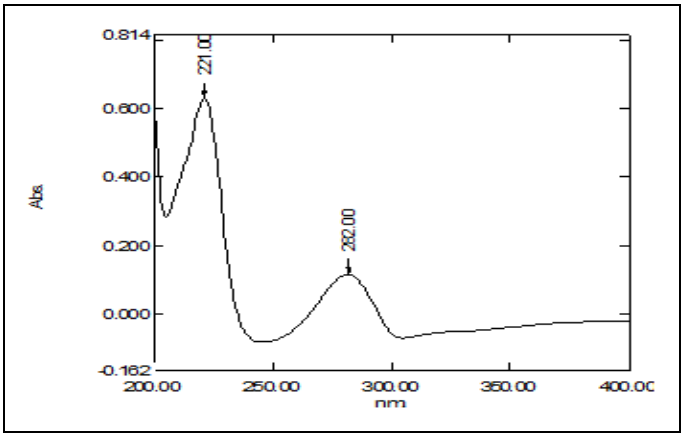

FIG. 3: UV SPECTRA FOR λ_{MAX} DETECTION OF ARBUTIN

Regression Statistics:

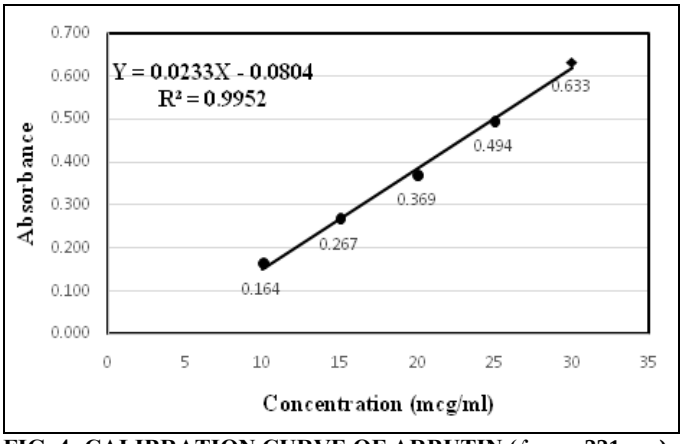

FIG. 4: CALIBRATION CURVE OF ARBUTIN (λ_{max} = 221 nm)

TABLE 11: LINEAR REGRESSION RESULT (ABSORBANCE → CONCENTRATION)

	Coefficients	Standard error	t Stat	P-value
Constant	-0.0804	0.019881482	-4.043964092	0.027217434
Concentration (mcg/ml)	0.023286667	0.000937222	24.84647739	0.000142939

Absorbance = (0.023286667 * Concentration) - 0.0804

Equation 1: Linearity Equation for Calibration Curve of UV Method of Arbutin

TABLE 12: ANOVA OF REGRESSION ANALYSIS

	df	SS	MS	F	Significance F
Regression	1	0.135567211	0.135567211	617.3474389	0.000142939
Residual	3	0.000658789	0.000219596		
Total	4	0.136226			

Development and Evaluation of Arbutin – PC Complexes:

Preliminary Trials:

TABLE 13: % ENTRAPMENT EFFICIENCY OF PRELIMINARY BATCHES

Batch. no.	%Entrapment efficiency (Avg. ± SD, n=3)
PT1	0
PT2	0
PT3	0
PT4	44.23 ± 2.26
PT5	47.26 ± 3.68
PT6	52.46 ± 5.36
PT7	49.52 ± 4.69
PT8	62 ± 2.36
PT9	58.37 ± 3.53

Discussion: Preliminary trials indicate that there is the effect of selected independent factors on the % entrapment efficiency of Arbutin. Literature reviews and preliminary batches results were helpful for a selection of the level of independent factors.

Optimization of Arbutin – PC Complexes:

TABLE 14: RESULTS OF EXPERIMENTAL DESIGN POINTS (AVERAGE ± SD; N = 3)

Run	X1(D:P)	X2(FFT)	X3(HT)	X4(Time)	Y (%EE)
AR1	0.5	40	60	75	52.06 ±2.36
AR2	1.5	40	60	75	59.36 ± 3.21
AR3	0.5	60	60	75	50.9 ± 3.69
AR4	1.5	60	60	75	58.33 ± 3.69
AR5	1	50	40	30	40.03 ± 4.92
AR6	1	50	80	30	49.5 ± 2.23
AR7	1	50	40	120	46.6 ± 3.24
AR8	1	50	80	120	55.7 ± 2.37
AR9	0.5	50	60	30	40.76 ± 4.82
AR10	1.5	50	60	30	48.7 ± 1.01
AR11	0.5	50	60	120	47.12 ± 2.61
AR12	1.5	50	60	120	55.67 ± 3.23
AR13	1	40	40	75	50.76 ± 2.13
AR14	1	60	40	75	50.08 ± 3.12
AR15	1	40	80	75	58.03 ± 1.26
AR16	1	60	80	75	59.9 ± 2.54
AR17	0.5	50	40	75	47.17 ± 3.29
AR18	1.5	50	40	75	56.8 ± 0.23
AR19	0.5	50	80	75	54.26 ± 4.30
AR20	1.5	50	80	75	62.36 ± 2.01
AR21	1	40	60	30	43.8 ± 1.36
AR22	1	60	60	30	45.2 ± 3.98
AR23	1	40	60	120	50.3 ± 1.87
AR24	1	60	60	120	51.7 ± 1.93
AR25	1	50	60	75	47.55 ± 1.65
AR26	1	50	60	75	49.89 ± 3.08
AR27	1	50	60	75	43.32 ± 0.69

TABLE 15: ANOVA FOR RESPONSE SURFACE REDUCED QUADRATIC MODEL

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	878.03	8	109.75	58.41	< 0.0001	significant
X_1	199.68	1	199.68	106.27	< 0.0001	
X_2	0.27	1	0.27	0.14	0.7091	
X_3	194.49	1	194.49	103.51	< 0.0001	
X_4	127.40	1	127.40	67.81	< 0.0001	
X_1^2	96.98	1	96.98	51.61	< 0.0001	
X_2^2	80.31	1	80.31	42.74	< 0.0001	
X_3^2	84.34	1	84.34	44.89	< 0.0001	
X_4^2	49.19	1	49.19	26.18	< 0.0001	

TABLE 16: FIT STATISTICS OF Y:

Reduced Quadratic Model	
R-Squared	0.9629
Adj R-Squared	0.9464
Pred R-Squared	0.9165
Std. Dev.	1.37
Mean	50.96
C.V. %	2.69

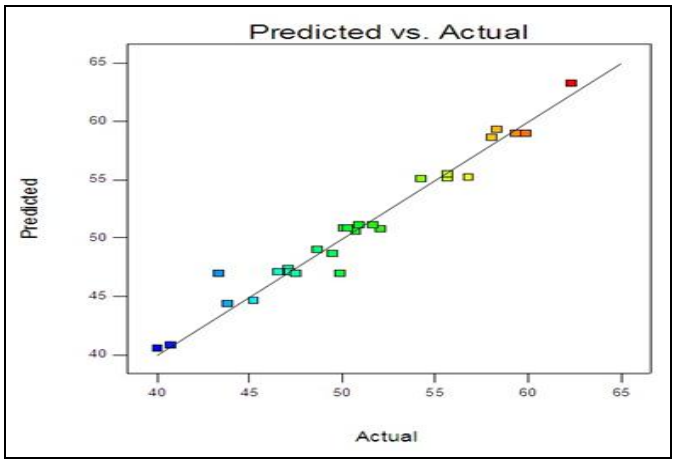

FIG. 5: PREDICTED *VS.* ACTUAL PLOT FOR Y

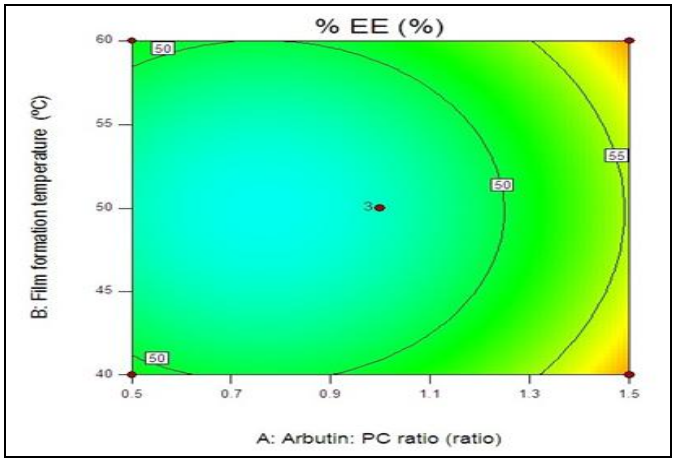

FIG. 6: CONTOUR PLOT OF Y (FIXED LEVEL: X3= 60, X4 = 75)

EE = + 46.92 + 4.079167 × X1 + 0.15 × X2 + 4.02583 × X3 + 3.2583 × X4 + 4.264166 × X12 + 3.8804167 × X22 + 3.9767 × X32-3.037083 × X42

Equation 2: Polynomial equation for Y (Predictive model – code level)

% EE = + 161.924 - 25.954 × Arbutin: PC ratio - 3.8654 × Film formation temperature 0.991 × Hydration temperature + 0.29737654320988 × Hydration time + 17.0566 × Arbutin: PC ratio 2 + 0.03880416666667 × Film formation temperature 2 + 9.941E-003 × Hydration temperature 2 - 1.499E-003 × Hydration time 2

Equation 3: Final Equation for actual factors

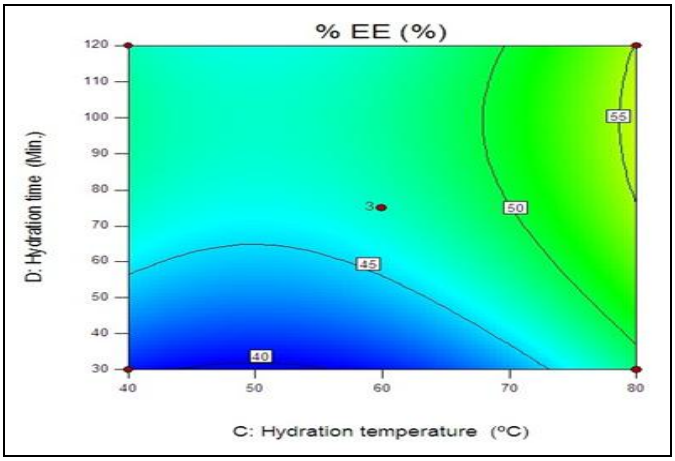

FIG. 7: CONTOUR PLOT OF Y (FIXED LEVEL: X1 = 1, X2 = 50)

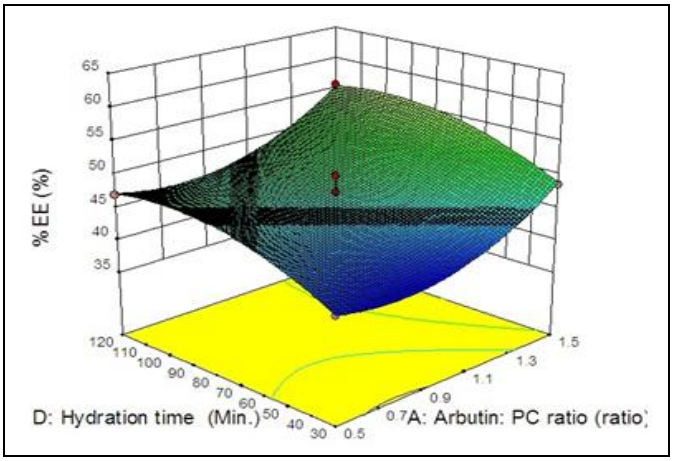

FIG. 8: 3D SURFACE PLOT OF Y (FIXED LEVEL: X2 = 50, X3 = 60)

TABLE 17: CHECKPOINT BATCHES

Check point batch	Predicted	Actual (Avg. ± SD)	% difference
1	54.9477	57.32 ± 2.76	4.317378161
2	43.4344	46.86 ± 1.83	7.88683624

Discussion: Checkpoint batches are helpful to validate polynomial equations. Results show that there is ≥ 92% similarity between predictive and actual values.

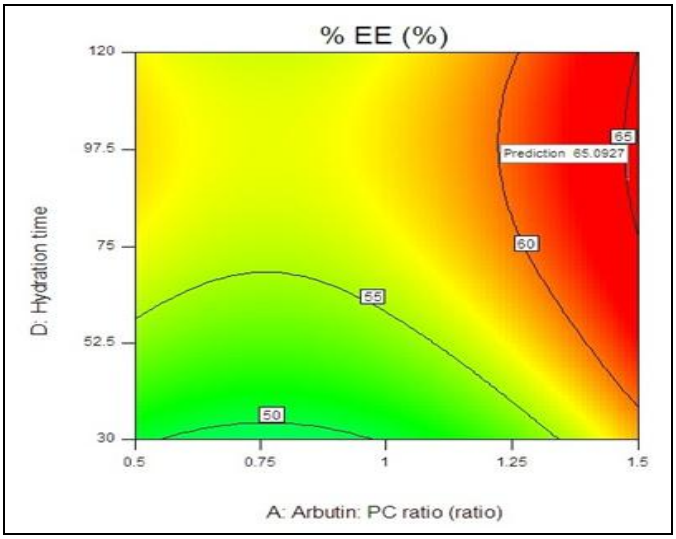

FIG. 9: CONTOUR FOR OPTIMIZATION

TABLE 18: RESULT OF ACTUAL AND PREDICTED FOR OPTIMIZATION USING DESIRABILITY FUNCTION:

X_1	X_2	X_3	X_4	Y	
				Actual	Predicted
1.480	56.591	79.605	90.694	68.73 ± 3.29	65.092

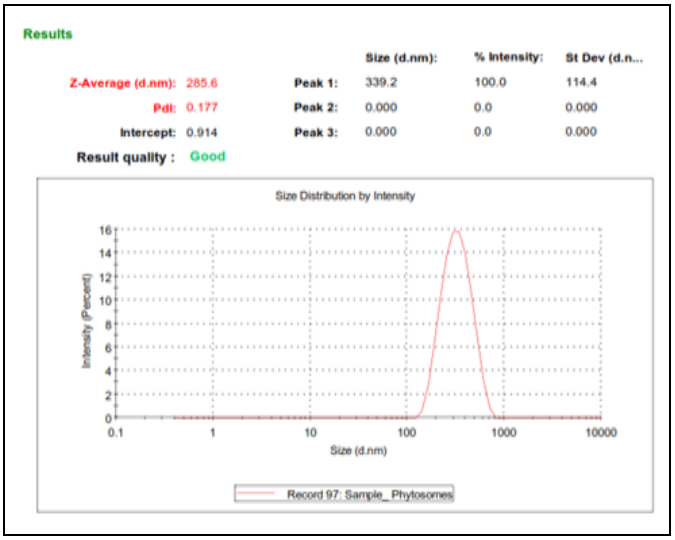

FIG. 10: PARTICLE SIZE OF OPTIMIZED BATCH

Evaluation of Optimized Batch of Arbutin – PC Complexes:

% Entrapment Efficiency: As Shown in Table 18, Particle Size: Here, particle sizes were measured in terms of average particle size diameter, and the uniformity was described in the polydispersity index (PDI).

A PDI value of 0.1–0.3 indicates a fairly narrow size distribution, whereas a PDI value greater than 0.5 indicates a very broad distribution. The particle size of the optimized batch is shown in **Fig. 10**. The average particle size of the optimized batch is 285.6 nm.

Zeta Potential: Zeta potential was found to be –15.8 mV. The Zeta potential of the optimized batch is shown in **Fig. 11**.

In general, a zeta potential value of ± 20 mV is sufficient for stability suspension. Our formulation is -15.8 mV, which means it complies with the requirement of zeta potential for stability.

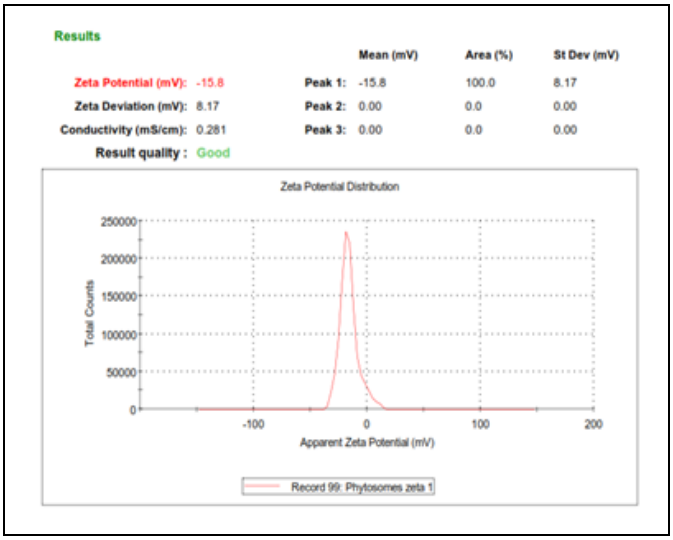

FIG. 11: ZETA POTENTIAL OF OPTIMIZED BATCH

***In-vitro* Drug Release Study Using Franz Diffusion Cell:**

TABLE 19: COMPARISON OF IN – VITRO DRUG RELEASE OF ARBUTIN SOLUTION AND ARBUTIN – PC COMPLEX

Time (Minutes)	% Drug release of complexes (Avg. ± SD, n=3)	% Drug release of arbutin solution (Avg. ± SD, n=3)
15	6.71 ± 1.4	3.68 ± 0.3
30	10.75 ± 1.84	6.75 ± 1.92
60	20.06 ± 2.41	13.49 ± 2.20
120	38.23 ± 4.82	21.39 ± 3.45
180	54.60 ± 4. 70	30.74 ± 3.30
240	70.39 ± 5.84	42.28 ± 3.95
300	84.80 ± 6.06	53.15 ± 4.52

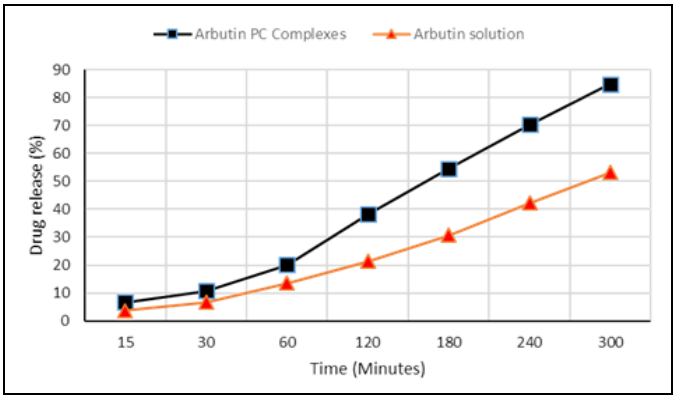

FIG. 12: COMPARISON OF % DRUG RELEASE OF ARBUTIN – PC COMPLEXES AND ARBUTIN SOLUTION

DISCUSSION: As shown in graph percentage, drug release and release rate of complexes are much higher than arbutin aqueous solution. This indicates that complexes of arbutin may improve *in-vitro* performance of Arbutin.

CONCLUSION: Developed complexes show a better *in-vitro* drug release profile compared to an aqueous solution of Arbutin, which indicates optimized complexes improve permeability or absorption of the drug through the skin.

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CONFLICTS OF INTEREST: We have no conflict of interest to declare.

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