Formulation and Development of Vincristine –Vinblastine and Curcumin liposome's incorporate into trance dermal patch (Lipo-VVC-TDP)

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Abstract

Cancer, one of the leading illnesses, accounts for about 10 million deaths worldwide. The treatment of cancer includes surgery, chemotherapy, radiation therapy, and drug therapy, along with others, which is not only put a tremendous economic effect on patients but also develop drug resistance in patients with time. Delivery of active agents to the skin by liposomal carriers has improved topical therapy in the Weld of dermatology. Liposomes are nano-sized spherical vesicles composed of an aqueous core surrounded by one (or more) phospholipid bilayer shells

Transdermal drug delivery systems are polymeric formulations which when applied to skin deliver the drug at a pre determined rate across dermis to achieve systemic effects. Anticancer drugs produces side effect in body and it can be reduces it by applying to skin in form of trance dermal patch for skin cancer. In this article we formulate such kind of formulation liposomes of Vincristine +Vinblastine one liposome's and Curcumin other liposome's incorporates into transdermal patch, by studying of compatibility of patch excipients. We develops F1, F2, F3, F4 F5, and F6 formulation. We also evaluate liposome as well as TDD after formulation. we evaluate thickness drug content, moisture content, moisture uptake, folding endurance, tensile strength diffusion coefficient, permeability coefficient, in vitro permeation, We will formulate such kind of formulation by which liposome of anticancer drugs through skin at skin cancer site then it will may be beneficial for skin cancer patient by avoiding maximum its ADR This study aim are formulate and investigate the anticancer liposome trans dermal patch on skin cancer model.

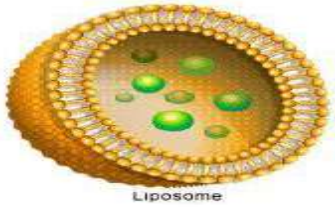
Key word: Liposome, Trance dermal patch, and anticancer

Introduction

Skin cancer can usefully be classified into two types on the basis of biological and clinical differences:? Melanoma skin cancer, arising from malignant transformation of the neural crest-derived melanocytes? Non-melanoma skin cancer (NMSC). The majority of NMSCs are keratinocyte-derived tumours such as basal cell carcinomas (BCC) and squamous cell carcinomas (SCC). Causes of skin cancer The evidence implicating sun expo- sure as the major determinant of NMSC is overwhelming:

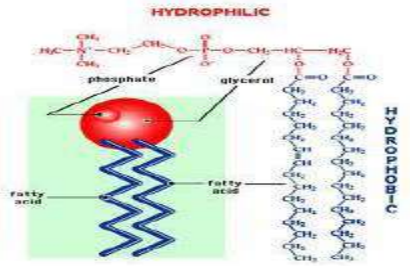
- Tumours are most common on exposed body sites
- They are more common in Caucasian populations living in areas of high ambient ultraviolet radiation (UVR) exposure
- Migrant studies also implicate sun exposure.[1]

Lipid-based nanomedicines are used to 1) protect drugs fromdegradation in vivo, 2) control drug release, 3)modify biodistribution, 4) target drug delivery to the site of disease and 5) enhance solubility and bioavailability. Lipid based delivery systems are also effective as vaccine adjuvants through their ability to protect and deliver antigens (peptide,protein and nucleic acid systems) to the antigen presenting cells and stimulating protective immune responses. Suitable engineering of nanomedicines in terms of their composition, particle size, and surface charge can aid in achieving spatial and temporal delivery of drugs. Liposomes are lipid based spherical shaped vesicular systems, in which a lipophilic bilayer is sandwiched between two hydrophilic layers. The versatility and advantages of liposomes as a drug delivery system for small molecules, peptides, gene, and monoclonal antibodies is well studied and acknowledged in the peer-reviewed scientific literature.[2-6]



Liposome structure formed by phospholipids

Figure No. 1 Structure of liposome



Shape of phospholipids molecule

Figure No. 2 Shape of phospholipids molecule

There are two important layers in skin: Dermis and Epidermis. The outermost layer, the epidermis, is approximately 100 to 150 micrometers thick, has no blood flow and includes a layer within it known as the stratum corneum. This is the layer most important to transdermal delivery

Types of Transdermal Patches[7-13]

Single-layer Drug-in-Adhesive

The Single-layer Drug-in-Adhesive system is characterized by the inclusion ofthe drug directly within the skin-contacting adhesive. In this transdermal systemdesign, the adhesive not only serves to affix the system to the skin, but alsoserves as the formulation foundation, containing the drug and all the excipientsunder a single backing film. The rate of release of drug from this type of systemis dependent on the diffusion across the skin.

Multi-layer Drug-in-Adhesive

The Multi-layer Drug-in-Adhesive is similar to the Singlelayer Drug-in-Adhesive in that the drug is incorporated directly into the adhesive. However, the multi-layer encompasses either the addition of a membrane between two distinct drug-in-adhesive layers or the addition of multiple drug-in-adhesive layers under a single backing film.

Materials and Methods

Vincristin , Vinblaastin , Curcumin are purches from market of cipla Pharmacutical and Himalay puer extract , Mumbai India . eudragit-RL-100, , and hydroxypropyl methylcellulose (HPMC) were procured from Lab CareLtd., Bengaluru, Karnataka, All other chemicals and solvents were of analytical grade, purchased from Merck Pvt. Ltd., Mumbai(MH), India Rotary vacuum evaporator, Buchi Mumbai India made in Japan.

Methods

Preparation of Vincristine and Vinblastine liposome and Curcumin[14]

The lipid membranes of vincristine (VCR-2 mg) and vinblastine (VBL-1mg)-loaded liposomes were composed of Accurately weighed quantities of Soy lecithin, cholesterol, Stearylamine and Dicetylphosphate are dissolved in chloroform and rotated in a rota-vap by applying vaccum of about 25mmHg at 250c, until it forms a thin film. Required quantities of ammonium sulphate and sucrose (0.3%) are dissolved in W.F.I and it is added to the above thin film in R.B flask and rotated until it forms a milky white suspension. The above solution is homogenized for 15 cycles to reduce particle size of liposomes. The above solution is undergone for 25 cycles of dialysis, by using sucrose solution (10%) to remove free ammonia and sulphate from the lipid solution. Drug

solution is prepared by adding the required quantities of Drug and Histidine in a W.F.I and pH is adjusted to 6.4 to 6.7 and this drug solution is added to the solution in a R.B flask (lipid solution) and rotated for 1hr.

In-process Checks:

RPM: 65-70rpm (Film formation), 50-55rpm (Hydration), 60-65rpm (Drug Loading).

Temperature: 40-45°C (Film formation), 65-70°C (Hydration), 65°C (Drug Loading).

The composition and ratios of lecithin, cholesterol and stabilizers for different types of Liposomes. Formulatin Batch of VC-VB and Curcumin should be separately.

Table No: 1 The composition and ratios of Drug, Soy lecithin, Cholesterol, Stearylamine, and Ammonium sulphate for optimized batches.

Formulalation code	Drug (mg/ml) **Curcumin**	Soy lecithin (mg/ml	Cholesterol (mg/ml)	Stearylamine (mg/ml)	Dicetyl phosphate (mg/ml)	Ammonim Sulphate (mg/ml)
F1	2	7	3			30
F2	2	7.5	2.5			30
F3	2	7	3	1		30
F4	2	7.5	2.5	1		30
F5	2	7	3		1	30
F6	2	7.5	2.5		1	30

Table No: 2 The composition and ratios of Drug, Soy lecithin, Cholesterol, Stearylamine, and Ammonium sulphate for optimized batches.

Evaluation of lyophilized liposome's

All the liposomal formulation was evaluated by studying their physicochemical properties like Particle size analysis, Polydispersity index, Zeta potential analysis, SEM analysi Determination

Formulalation code	Drug (mg/ml)		Soy lecithin (mg/ml	Cholesterol (mg/ml)	Stearyla mine (mg/ml)	Dicetyl phosphate (mg/ml)	Ammonim Sulphate (mg/ml)
	Vincristine	**Vinblastine**					
F1	2	1	7	3			30
F2	2	1	7.5	2.5			30
F3	2	1	7	3	1		30
F4	2	1	7.5	2.5	1		30
F5	2	1	7	3		1	30
F6	2	1	7.5	2.5		1	30

of particle size distributionDetermination of average vesicle size of doxorubicin hydrochloride liposomes with carrier was very important characteristic. Polydispersity Index:

Polydispersity was determined according to the equation,

Polydespersity = D (0.9) - D (0.1) / D (0.5)

Where,

D (0.9) corresponds to particle size immediately above 90% of the sample.
D (0.5) corresponds to particle size immediately above 50% of the sample.
D (0.1) corresponds to particle size immediately above 10% of the sample.

Formulalation code	Average vesicular size (nm)	Zeta Potential (mV)	Poly dispersive index (Pdi)
F2	356nm	5.21	0.635
F4	564nm	24.66	0.762
F6	317nm	-23.4	0.645

Formulalation code	Average vesicular size(nm)	Zeta Potential (mV)	Poly dispersive index (Pdi)
F3	82.37	-12.85	0.247
F4	83.13	-11.97	0.261
F6	92.42	-10.39	0.279

Table 3: Physicochemical characteristics of Vincrystin and vinblastine Liposomes for Optimized Batches

Table 4: Physicochemical characteristics of Curcumin Liposome's for Optimized Batches

Liposome's Transdermal Patch Vincristine, Vinblastine and Curcumin DRUG – POLYMER INTERACTION STUDIES

FTIR study

Drug-excipient compatibility studies were performed by Fourier Transform Infrared Spectroscopy. The infrared spectrum of the pure pantoprazole sodium and polymer samples were recorded and the spectral analysis was done. The dry samples of drug and polymers were directly placed after mixing and triturating with dry potassium bromide. The backing membrane was prepared with an aqueous solution of 4%w/v Poly vinyl alcohol (PVA). 4g of PVA was added to 100mL of warm, distilled water and a homogenous solution was made by constant stirring and intermittent heating at 60°C for few seconds. Then 15mL of homogenous solution was poured into glass Petri dishes of 63.5cm2 and was allowed to dry in hot air oven at 60°C for 6h

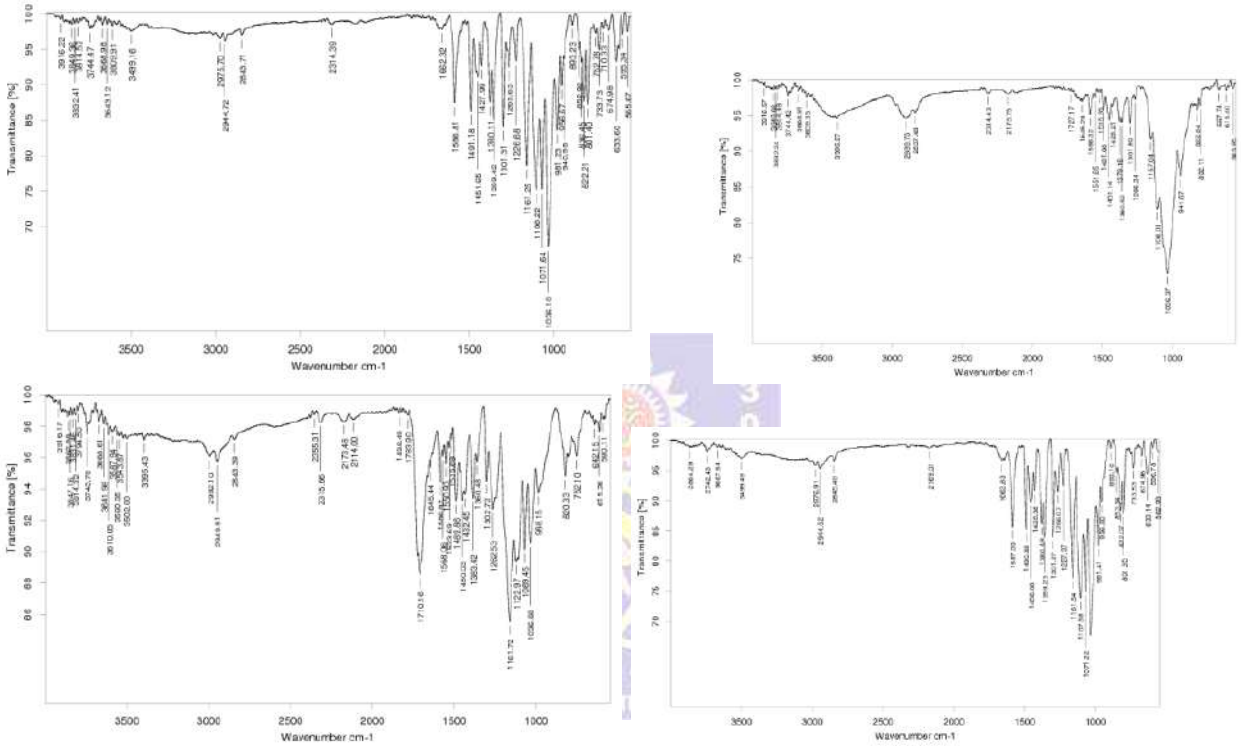


Fig 8 IR Spectrum of (A) VVC (B) physical mixture of VCC and HPMC, E5 (C) VCC sodium and PVP (D) VCC sodium and eudragitL100

FORMULATION OF LIPOSOME'S TRANSDERMAL PATCH VINCRISTINE, VINBLASTINE AND CURCUMIN [15]

Matrix type transdermal patches containing Vincristine, Vinblastine and Curcumin are prepared by solvent casting technique employing a mercury substrate Polymer solutions are prepared using ethanol as solvent To the polymeric solution known weight of drug liposome's Vincristine (2mg/ml), Vinblastine (1mg/mg) and Curcumin (2 mg/ml) is added and mixed slowly with a glass rod for 20 minutes until a homogenous drug polymer solution is formed. Then plasticizer and permeation enhancer of required quantity are added and mixed thoroughly. The resulting homogenous drug-polymeric solution is poured on a mercury substrate (area of 13.86 cm2) in a petridish and dried at room temperature .The rate of evaporation of solvent is controlled by inverting a funnel over the petridish. The film formation is noted by observing the mercury surface after complete evaporation of the solvent. After drying at room temperature for 24 hours, membranes are taken out, packed in aluminium foil and stored in dessicator until further use.

Ingredients	F1	F2	F3	F4	F5	F6
Vincristiin & Vinblastin	2	2	2	2	2	2
Curcumin	2	2	2	2	2	2
HPMC,E5 (mg)	300	150	100	300	450	500

PVP mg	300	450	500	*	*	*
Eudragit L100 (mg)	*	*	*	300	150	100
Ethanol in Drug, Polymer (mL)	10,0	10,0	10,0	10,5	10,5	10,5
Methanol (mL)	8	8	8	*	*	*
Chloroform: Methanol 1:1(mL)	*	*	*	6	6	6
Di butyl phthalate (drops)	15	15	15	15	15	15
DMSO (drops)	2	2	2	2	2	2

Table 5 Formulation of LipoVVC transdermal patches

EVALUATION LIPOSOME'S TRANSDERMAL PATCH VINCRISTINE VINBLASTINE AND CURCUMIN

All the drug-loaded films have uniform thickness. The thickness of the films varied from 0.031 to 0.048 mm and order of the thickness of films is F1 < F 2 < F 4 < F3 < F 5< F 6 (Table 2). The weight of all the patches were found to be uniform. The weight of the films varied from 81.67 to 90.33 mg and order of the weight of films is F 1 < F 2 < F 3 < F 4 < F 5 < F 6 (Table 2). The folding endurance was found to be in the range of 74.67 to 80.33. This data revealed that the patches had good mechanical strength along with flexibility and order of the folding endurance is F 6 < F 5 < F 3 < F2 < F 4 <F 1 (Table 2). This test is important to check the ability of sample to withstand folding, which gives an indication of brittleness; less folding endurance indicates more brittleness. Thumb tack test was performed for all the formulations and it showed optimum tackiness with the thumb and good adherence capacity with human skin. Surface pH study was done and all the formulations show pH in the range of 4.8 to 5.2. So we can expect no irritation. The % flatness in all the patches was found to be 100. This data indicates the smoothness as well as non-constriction nature of the patch. The % moisture content was found to be in the range of 3.80 to 9.166. Patches F 1, F 2, F 3 showed highest loss due to water soluble polymer HPMC and PVP. Patch F3 showed maximum moisture content due to low concentration of HPMC compared to other formulations. The order of the percentage moisture loss is F 4 < F 5 < F 6 < F 1 < F 2 < F 3 (Table 2). From the % moisture uptake study it was found that all the patches showed least % moisture absorption**.** The % moisture content was found to be in the All the drug-loaded films have uniform thickness. The thickness of the films varied from 0.031 to 0.048 mm and order of the thickness of films is F1 < F 2 < F 4 < F3 < F 5< F 6 (Table 2). The weight of all the patches were found to be uniform. The weight of the films varied from 81.67 to 90.33 mg and order of the weight of films is F 1 < F 2 < F 3 < F 4 < F 5 < F 6 (Table 2). The folding endurance was found to be in the range of 74.67 to 80.33. This data revealed that the patches had good mechanical strength along with flexibility and order of the folding endurance is F 6 < F 5 < F 3 < F2 < F 4 <F 1 (Table 2). This test is important to check the ability of sample to withstand folding, which gives an indication of brittleness; less folding endurance indicates more brittleness. Thumb tack test was performed for all the formulations and it showed optimum tackiness with the thumb and good adherence capacity with human skin. Surface pH study was done and all the formulations show pH in the range of 4.8 to 5.2. So we can expect no irritation. The % flatness in all the patches was found to be 100. This data indicates the smoothness as well as non-constriction nature of the patch. The % moisture content was found to be in the range of 3.80 to 9.166. Patches F 1, F 2, F 3 showed highest loss due to water soluble polymer HPMC and PVP. Patch F3 showed maximum moisture content due to low concentration of HPMC compared to other formulations. The order of the percentage moisture loss is F 4 < F 5 < F 6 < F 1 < F 2 < F 3 (Table 2). From the % moisture uptake study it was found that all the patches showed least % moisture absorption**.** The % moisture content was found to be in the

Cumulative % Drug Release

Evaluation of VVC transdermal patches Formulation code	Thickness uniformity (mm)	Weight uniformity (mg)	Folding endurance	Surface pH	% Flatness	% Moisture content	% Moisture uptake	Water vapour transmission rate (gm/cm2/h)	% Drug content
F1	0.031±0.0005	81.67±0.57	80.33±0.57	5.2	100	9.166±1.44	6.90±2.325	0.0044±0.0001	99.21
F2	0.035±0.0010	83.33±0.57	77.33±0.57	5.1	100	9.643±1.51	6.34±1.374	0.0025±0.0005	97.23
F3	0.045±0.0010	84.67±1.52	75.33±1.52	5.2	100	12.5±2.50	4.50±1.558	0.0032±0.0003	93.25
F4	0.042±0.0005	85.67±0.57	79.33±0.57	4.8	100	3.80±1.65	3.63±1.616	0.0036±0.0001	98.36
F5	0.046±0.0005	87.00±1.00	75.33±0.57	5.0	100	5.00±2.50	3.243±1.408	0.0026±0.0001	94.02
F6	0.047±0.0015	90.33±0.57	74.67±0.57	5.1	100	5.23±0.513	2.51±0.623	0.0024±0.0001	92.65

TABLE Evaluation of Liposome's VVC transdermal patches

The percentage of drug release at each time interval was calculated and plotted against time. The drug release from the formulations F1 to F3 which has varying proportion of HPMC and PVP showed release of 71.98 to 93.14 %, the drug release from the formulations F4 to F 6 which has varying proportion of HPMC and Eudragit showed release of 70.12 to 86.92 %, F1 showed maximum release of 93.14 % for 6h (Fig.3). It is well known that the addition of hydrophilic component to an insoluble film former leads to enhance its release rate constant. This is due to the fact that dissolution of aqueous soluble fraction of the polymer matrix leads to the formation of gelaneous pores. The formation of such pores leads to decrease the mean diffusion path length of drug molecules to release into the diffusion medium and hence, to cause higher release rate.

Release kinetics studies

Formulation Code	Kinetic models (r2)			Korsmeyer Peppas	
	Zero order	First order	Higuchi model	r2	n
F1	0.9571	0.958	0.9877	0.9968	0.9289
F2	0.9606	0.992	0.9861	0.9897	0.826
F3	0.967	0.9799	0.961	0.9977	0.8196
F4	0.9518	0.9886	0.9946	0.9978	0.7737
F5	0.9443	0.9918	0.9911	0.9955	0.7553
F6	0.9813	0.9875	0.9665	0.9964	0.7309

Table No VII-4 Release kinetic studies of Liposomal VVC transdermal patch

Stability studies

Parameter	Room Temp	40±20C& RH 70±5%
Visual Appearance	Slightly yellow	Slightly yellow
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2st month	No change	No change
At the end of 3st month	No change	No change
Color	Slightly yellow	Slightly yellow

Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2st month	No change	No change
At the end of 3st month	No change	No change
Texture	Smooth	Smooth
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2st month	No change	No change
At the end of 3st month	No change	No change
Drug Content F1	99.21	99.21
Initial	99.21	99.21
At the end of 1st month	98.26	98.26
At the end of 2st month	97.44	97.49
At the end of 3st month	95.9	95.9

Results and Discussion

The results of the kinetic studies were shown in Table 3. Kinetic studies reveals that majority of the formulations were governed by Peppas model and to see whether the drug release is by diffusion, by swelling or by erosion mechanism, the data was plotted according to Higuchi's equation. The co-efficient of determination indicated that the release data for formulation F1 to F6 followed first order release kinetics with diffusion mechanism. Higuchi equation explains the diffusion release mechanism. The diffusion exponent 'n' values were found to be in the range of 0.5 to 1 indicating Non-Fickian mechanism The stability studies of formulation of aqueous extract of *Lipo VVC* transdermal patch was carried out for 3 months as per the procedure described in the table.No:5. During this period, the formulations were stable and showed no significant changes in visual appearance, colour, texture and drug content.

Conclusion

VVC liposomes were formulated by using solvent evaporation method using poly (SA: RA) where the optimized liposomal formulation F3 showed particle size , drug loading 18., and entrapment efficiency satisfied. Optimized liposomes were formulated into finalized form as transdermal patch using a blend of polymers such as eudragit-RL, HPMC K-50, and ethyl cellulose. Optimized patch formulation F1 was found to have the best release profile when compared to other patches with no skin irritation.

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