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FORMULATION AND EVALUTION OF TRANSDERMAL DRUG DELIVARY SYSTEMS CONTAINING NICOTINE

J. Chakri* and A. Kiran Kumar

Pharmaceutics Sahasra Institute of Pharmaceutical Sciences, Hasanparthy Mandal, Vangapahad Village, Warangal, Telangana. India.

***Corresponding Author: J. Chakri**

Pharmaceutics Sahasra Institute of Pharmaceutical Sciences, Hasanparthy Mandal, Vangapahad Village, Warangal, Telangana. India.

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ABSTRACT

Transdermal drug delivery systems (TDDS), also known as "patches," are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. Nicotine is highly toxic alkaloid. It is the prototypical agonist at nicotinic cholinergic receptors where it dramatically stimulates neurons and ultimately blocks synaptic transmission. Nicotine is both a sedative and a stimulant. was selected for the formulation of transdermal delivery system as it complies with physicochemical properties required to permeate through skin. The preformulation studies involving description, solubility, melting point, partition coefficient of the drug were found to be comparable with the standard.The patches were prepared by solvent casting technique. The patches were subjected to the following evaluation parameters such as physical appearance, weight variation, thickness, folding endurance, drug content, percentage moisture absorption, percentage moisture loss, water vapour transmission rate, tensile strength, diffusion studies and skin irritation studies. All the parameters were within the limits. Based on all these results viz. mechanical properties, compatibility, stability and diffusion studies, formulation A3 was selected as the best formulation. From *in vitro* and skin irritation test, it can be concluded that the developed formulation A3 have great potential for transdermal drug delivery.

KEYWORDS: Nicotine, Transdermal drug delivery system, Sodium alginate, Xanthan gum.

INTRODUCTION

Transdermal drug delivery systems (TDDS), also known as "patches," are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin.[1]Transdermal drug delivery system has been in existence for a long time. In the past, the most commonly applied systems were topically applied creams and ointments for dermatological disorders. The occurrence of systemic side-effects with some of these formulations is indicative of absorption through the skin. A number of drugs have been applied to the skin for systemic treatment. In a broad sense, the term transdermal delivery system includes all topically administered drug formulations intended to deliver the active ingredient into the general circulation. Transdermal therapeutic systems have been designed to provide controlled continuous delivery of drugs via the skin to the systemic circulation.[2]

Nicotine is highly toxic alkaloid. It is the prototypical agonist at nicotinic cholinergic receptors where it dramatically stimulates neurons and ultimately blocks synaptic transmission. Nicotine is also important medically because of its presence in tobacco smoke. Nicotine binds to nicotinic acetylcholine receptors on dopaminergic neurons in the cortico-limbic pathways. This causes the channel to open and allow conductance of multiple cations including sodium, calcium, and potassium. This leads to depolarization, which activates voltage-gated calcium channels and allows more calcium to enter the axon terminal. Calcium stimulates vesicle trafficking towards the plasma membrane and the release of dopamine into the synapse. Dopamine binding to its receptors is responsible the euphoric and addictive properties of nicotine**.** Absorption of nicotine through the buccal mucosa is relatively slow and the high and rapid rise followed by the decline in nicotine arterial plasma concentrations seen with cigarette smoking are not achieved with the inhaler.IUPAC name of nicotine is 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine. Molecular weight of nicotine is 162.33. Nicotine is a hygroscopic, colorless to yellow-brown, oily liquid, that is readily soluble in alcohol, ether or light petroleum. It is miscible with water in its base form between 60 °C and 210 °C. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water-soluble.[3]

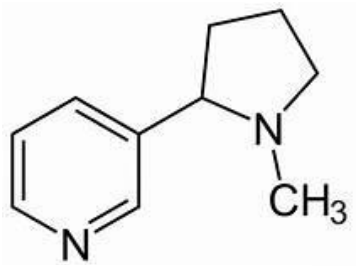

Figure 1: Chemical structure of Nicotine.

MATERIALS

Nicotine was a gift sample from Micro labs, Hyderabad, India, Sodium alginate, Xanthan gum are from Sisco research laboratories, Standard chemical reagents from SD fine chemical Ltd, Hyderabad. Methanol was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical reagent grade

Methodology

Preformulation Studies

Determination of Melting Point

Melting point of drug sample was performed by using capillary tube method. A fine powder of Nicotine was filled in a capillary tube, previously sealed at one end and the capillary tube was tied to the bottom of the thermometer. The thermometer and capillary tube were immersed in to the liquid paraffin taken in the tube. Bottom of the tube was heated gently by means of burner. When the sample starts to melt the reading was recorded.

Solubility studies[4]

The solubility has been determined after shaking a saturated solution of the drug for 2 hrs at 25° C in water, methanol, ether, acetone, acetonitrile and hexane, respectively.

Determination of partition co-efficient[5]

The partition co-efficient study was performed using n-octanol as oil phase and phosphate buffer pH 7.4 as aqueous phase. The two phases were mixed in an equal quantity and were saturated with each other on a mechanical water bath shaker at 32°C for 24h. The saturated phases were separated by centrifugation at 2000 rpm on a Remi Centrifuge. Standard plots of drug were prepared from both the phosphate buffer and octanol. Equal volumes (10ml each) of the two phases were taken in triplicate in conical flask and to each 100mg of weighed amount of drug were added. The flasks were shaken at 32°C for 6h to achieve a complete partitioning at 100 rpm. The two phases were separated by centrifugation at 100rpm for 5min and they were then analyzed for respective drug contents.

Analytical method

Determination of λmax of Nicotinein pH 7.4 phosphate buffer[6]

A stock solution of Nicotine was prepared by dissolving 100mg of drug in 100ml of phosphate buffer of pH 7.4. From this 10ml was taken and diluted to 100ml. From this 4, 8, 12, 16, 20 g/ml dilutions were prepared using phosphate buffer of pH 7.4. The $_{max}$ of the drug was determined using UV-visible spectrophotometer. The absorption maximum of 274nm was selected and at this wavelength, the absorbance of all the other solutions was measured against a blank. The concentration ranges and absorbance data were reported in Table 5.1 Calibration graph was plotted using the data and presented in the Figure 5.1

Drug-excipients compatibility studies by FTIR[7]

FT-IR spectroscopy study was carried to assess the compatibility between Nicotine, Xanthan gum and Sodium alginate. The pure drug and drug with excipients were separately scanned. The pellets were prepared on potassium bromide press. Both the spectra were compared for confirmation of peaks.

Differential Scanning Calorimetry (DSC)[8]

The dynamic DSC studies were carried out on pure drug and drug loaded patch, the obtained thermo grams are presented in Figure 5.3. The data obtained from the DSC scans for the pure drug Nicotine and drug loaded patch are given in terms of onset of melt (To), melting points (Tm) and completion of melt (Tc). The amount of energy consumed for melting or the area under the endothermic peak of DSC curve of the pure drug and drug loaded patch were recorded.

Formulation of Transdermal Patches

In the present study, drug loaded matrix type transdermal films of Nicotine were prepared by molding and solvent casting method. A mould of 5cm length and 5cm width with a total area of 25cm^2 was fabricated.

Preparation of drug-loaded transdermal films

The Transdermal patches were prepared by using solvent casting technique. The bottom of the mold was wrapped with aluminum foil which was used as backing membrane. Drug containing films were prepared by solvent casting method. In brief, the required amounts of a mixture of XG/SA (Table 1) were weighed and polymeric solution was prepared using sufficient quantity of water and kept aside for 2h after stirring.

Accurately weighed Nicotine (2.5 %) and menthol (3% w/w) was dissolved in ethanol (6mL) by stirring for 10 min. The above mixture mixed with different concentrations of glycerin (1–5% w/w) and prepared polymeric solutions for 30 min. Finally mixed soft mass was poured on to cleaned specially designed glass molds with the plastic transparent sheet and kept in a vacuum drier until to get the dried membrane. The cast polymer films with different formulations were then peeled off

covered with aluminum foils and stored in a desiccators until further study.

Table 1: Formulation chart of Nicotine transdermal films.

Formulation code	Metoprolol Tartrate (%)	Xanthan Gum (%)	Sodium Alginate (%)	Glycerin (%)	Menthol (%)
A 1	2.5	10.0	86.0	0.5	1.0
A 2	2.5	20.0	74.5	1.0	2.0
A 3	2.5	30.0	63.0	1.5	3.0
A 4	2.5	40.0	51.5	2.0	4.0
A 5	2.5	50.0	40.0	2.5	5.0
A 6	2.5	60.0	28.5	3.0	6.0
A7	2.5	70.0	17.0	3.5	7.0

Evaluation of transdermal films[9]

The prepared films were evaluated for its uniformity of weight, uniformity of film thickness, tensile strength, percentage elongation, folding endurance, percentage moisture absorption, percentage moisture loss, drug content, scanning electron microscopy, drug diffusion study and primary skin irritation test.

Uniformity of weight[10]

Weight uniformity was done by weighing three different films of the individual batch and the average weight was calculated. Care was taken that the individual weight should not deviate significantly from the average weight of the three. The tests were performed on films, which were dried at 40^0C for 3h prior to testing.

Uniformity of film thickness[11]

The thickness of the films was measured at different points using digital vernier caliper. The averages of three readings of each film at different area were measured.

Tensile strength[12]

Tensile strength of the patches was determined using Hounse field universal testing machine. It consisted of two loaded grips, the upper one was movable and the lower one was fixed. The test patch of specific size ($5x1cm^2$) was fixed between these load grips and force was gradually applied till the patch broke. The tensile strength of the patch was taken directly from the dial reading in Newtons.

Percentage elongation of the patches[13]

Percentage elongation of patches is the ratio of increased length to its original length. It gives information of how much a specimen can elongate before it breaks. It is carried out by Hounse field universal testing machine. The percentage elongation at break point is measured on scale.

Folding endurance[14]

The folding endurance was measured manually for the prepared films. A strip of film 2x2 cm was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance.

Percentage moisture absorption[15]

The moisture absorption studies of various films were studied at 80% relative humidity (RH). Films of $1cm^2$ of all the batches were selected. The films were weighed accurately and placed in a desiccator containing 100ml of fused aluminum chloride, which maintains 80% of RH. After 3days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula.

Percentage moisture Loss[16]

Films of $1cm^2$ of all the batches were selected. The films were weighed accurately and placed in a desiccator containing anhydrous calcium chloride. After 3days, the films were taken out and weighed. The percentage moisture loss was calculated using the formula

Drug content[17]

A formulated film having $1cm^2$ area was cut into small pieces and weighed separately. The films were transferred into a 100ml volumetric flask and 100ml of phosphate buffer solution (pH 7.4) was added. The medium was stirred wit magnetic stirrer for 12h. The contents were filtered using Whatmann filter paper. The filtrate was analyzed at 274 nm spectrophotometrically for drug content against the reference solution containing only placebo films.

***In vitro* Drug Diffusion Study[18]**

Drug diffusion studies were carried out in an open glass diffusion tube. The assembly was placed on a magnetic stirrer and stirred at 100 rpm. The temperature of the system was maintained at 37°C ± 1°C. A known amount of receptor medium (buffer) was withdrawn at regular intervals of time and sink condition was maintained by replacing equal volume of fresh saline. The drug concentration samples were measured spectrophotometrically at 274nm against blank.

Stability of the transdermal films and prepared MT gel[19]

Formulation A3 (2.5 cm^2) and conventional gel were subjected for stability studies at 25 ºC/60% RH, 30 ºC/65% RH, 40 ºC/75% RH for 60 days and the above formulations were evaluated for drug content periodically.

Skin Irritation Study[20,21]

The patches were tested for their potential to cause skin irritation. A primary skin irritation test was performed since skin is a vital organ through which drug is transported. Skin irritation studies were performed on healthy male albino rats (2 Nos.) weighing 125 to132g. The animals were kept under standard laboratory conditions with free access to a standard laboratory diet. On the previous day of the experiment, the hair on the back side area of rats were removed by shaving carefully avoiding peripheral damage. The best formulation (A3) was placed over the skin with the use of adhesive tape on one rat and a placebo film (control) was placed on the other. The patches were removed after 24 hrs and the resulting skin reaction was evaluated as per table 2 and compared with control.

Table 2: Possible score for skin irritation.

Test	Skin Reaction	Score
	Very slight erythema	1
	Well defined erythema	2
Erythema	Moderate to severe erythema	3
	Severe erythema	4
	Total possible erythema score	**4**
	Very slight edema	1
	Well defined edema	2
Edema	Moderate to severe edema	3
	Severe edema	4
	Total possible edema score	**4**
Total score for primary skin irritation		**8**

RESULTS

Analytical Studies

Development of Calibration Curve for Nicotine (λ_{max})

The absorption maximum (λ_{max}) was determined as 274nm. The concentration ranges and data are reported in Table 3. UV Scanning of Nicotinein Phosphate buffer pH 7.4 presented in figure 8 & Calibration curve of Metoprolol Tartrate was plotted using this data and shown in the Fig. 9.

Table 3: Calibration data for Nicotinein pH 7.4 phosphate Buffer.

Sl. No.	Concentration (in µg/ml)	Absorbance ± S.D*	R^2 Value
1.	4	0.170 ± 0.092	
2.	8	0.325 ± 0.075	
3.	12	0.485 ± 0.043	0.9998
4.	16	0.643 ± 0.089	
5.	20	0.815 ± 0.064	

*Standard deviation, n = 3

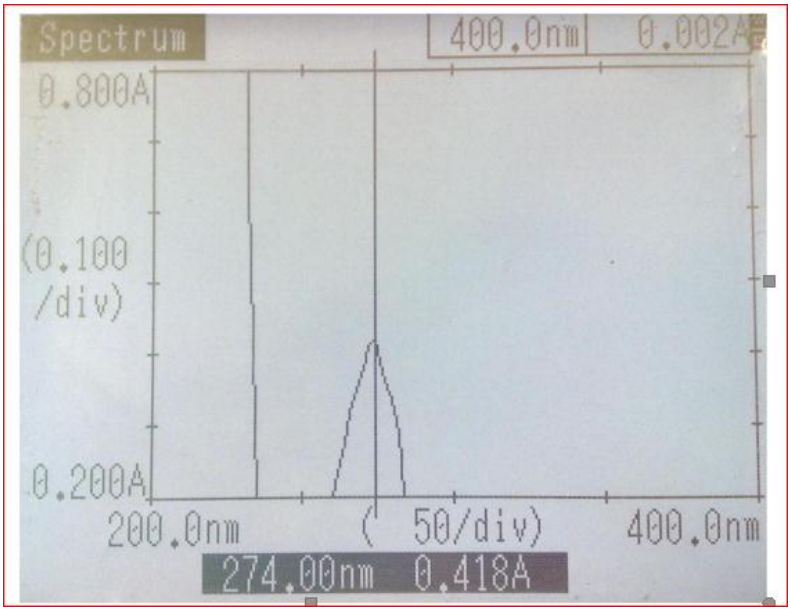

Figure 2: UV Scanning of Nicotinein Phosphate buffer pH 7.4

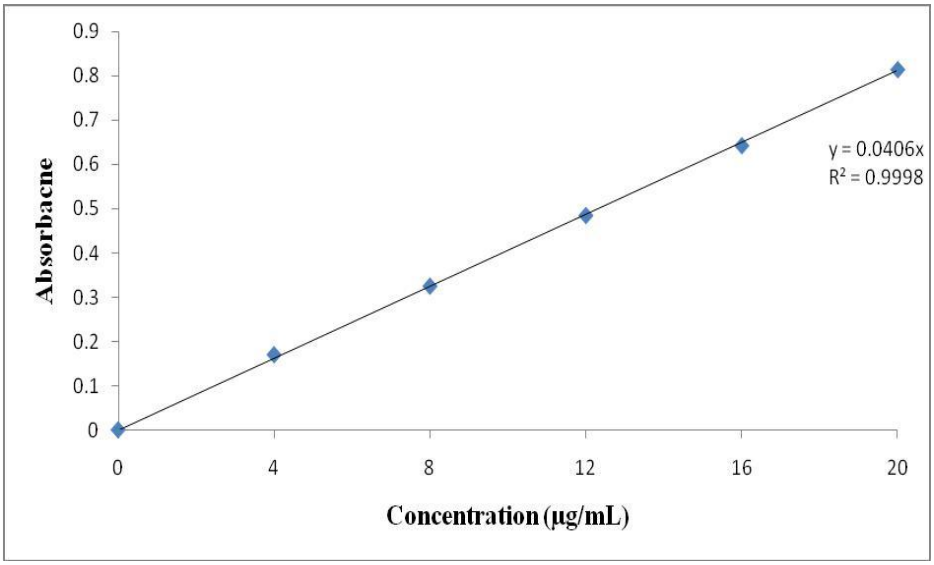

Figure 9: Calibration curve of Nicotinein pH 7.4 phosphate buffer.

Pre Formulation Studies

Determination of Melting Point, Solubility studies[50] & Determination of partition co-efficient

Table 4: Melting point, solubility, partition coefficient and pH of Metoprolol Tartrate.

Melting Point	**121.1 ± 0.87^0 C**
Solubility	water methanol ether acetone acetonitrile
Partition	hexane
coefficient	0.753
Ph	3.46

Drug excipient compatibility

FTIR study

Pure drug Nicotine and polymers were subjected for FTIR spectroscopic analysis for compatibility studies and to ascertain whether there was any chemical interaction between the drug and the polymers used. The FT-IR spectra and data of Nicotine pure drug and Nicotine with polymers are shown in fig. 3-8 and table 5.

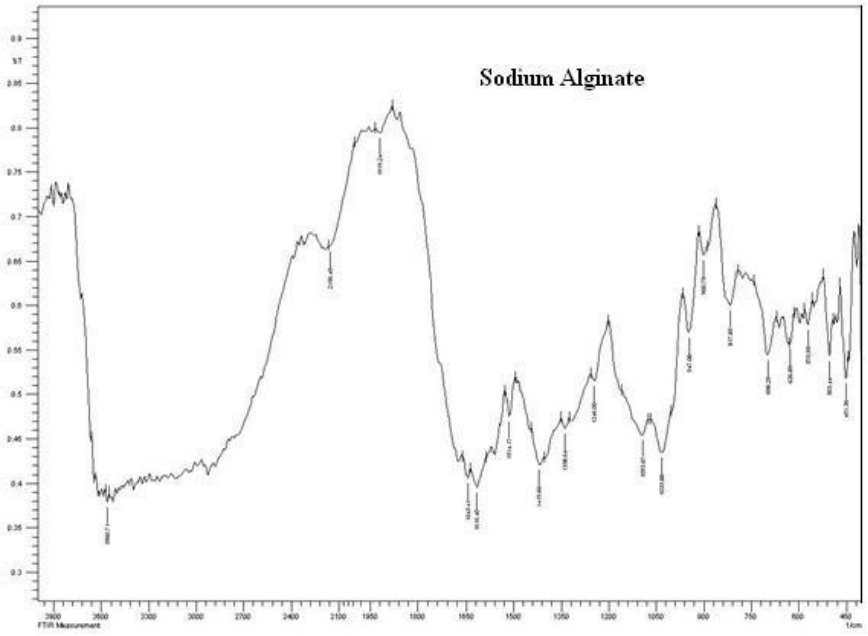

Figure 3: FTIR Spectra of Sodium alginate.

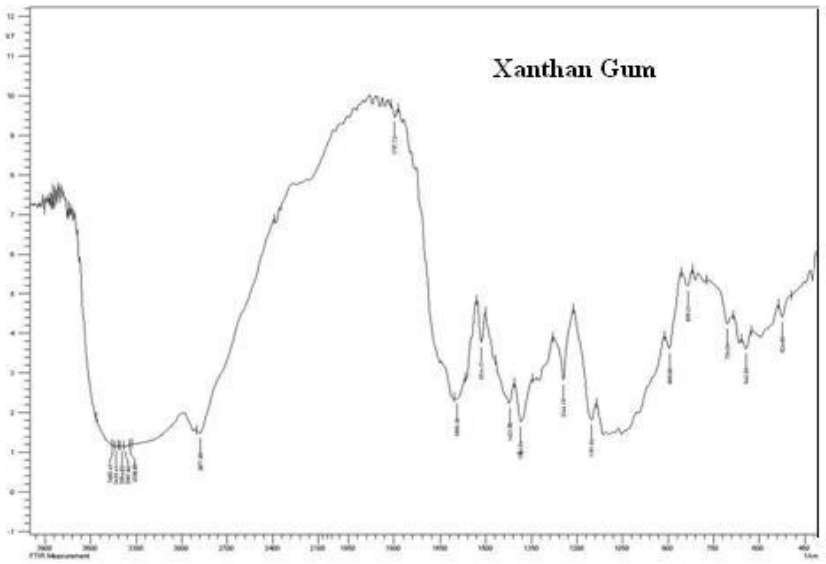

Figure 4: FTIR Spectra of Xanthan Gum.

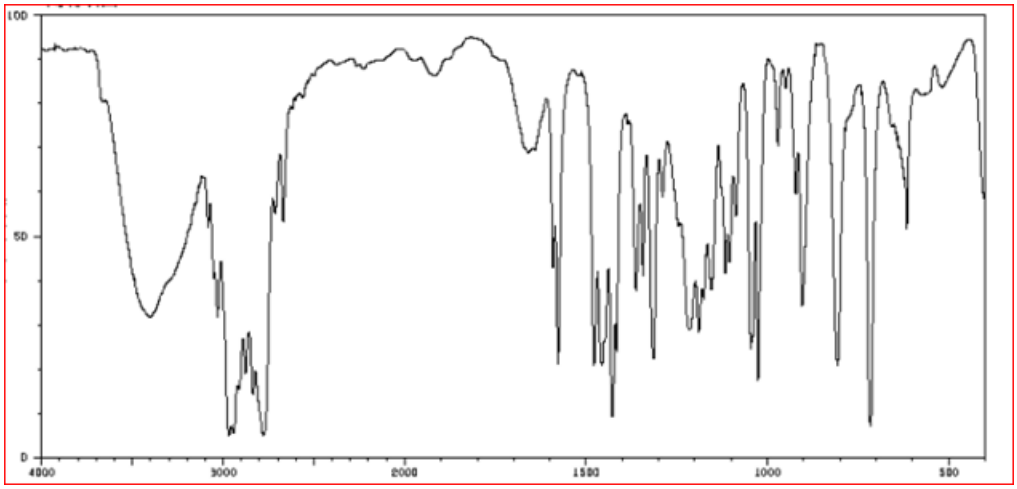

Figure 5: FTIR Spectra of pure Nicotine.

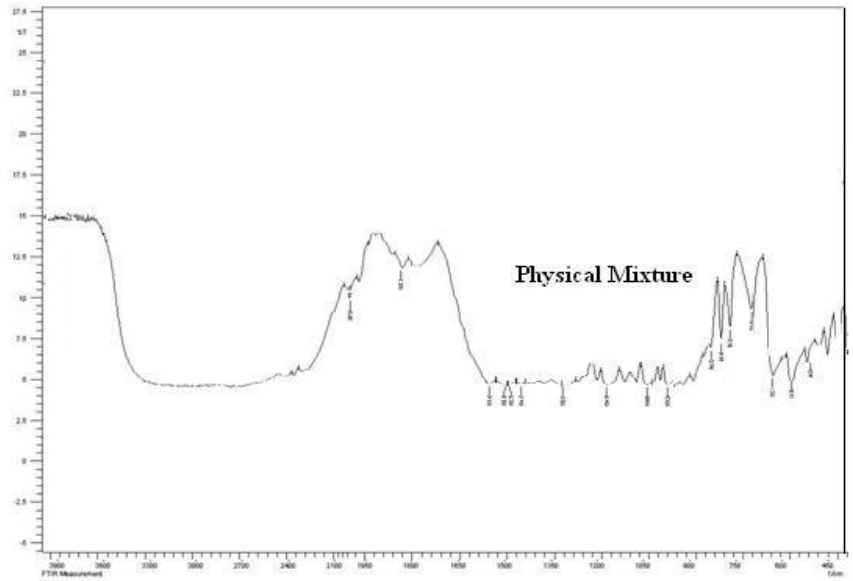

Figure 6: FTIR Spectra of physical mixture of NI, XG and SA.

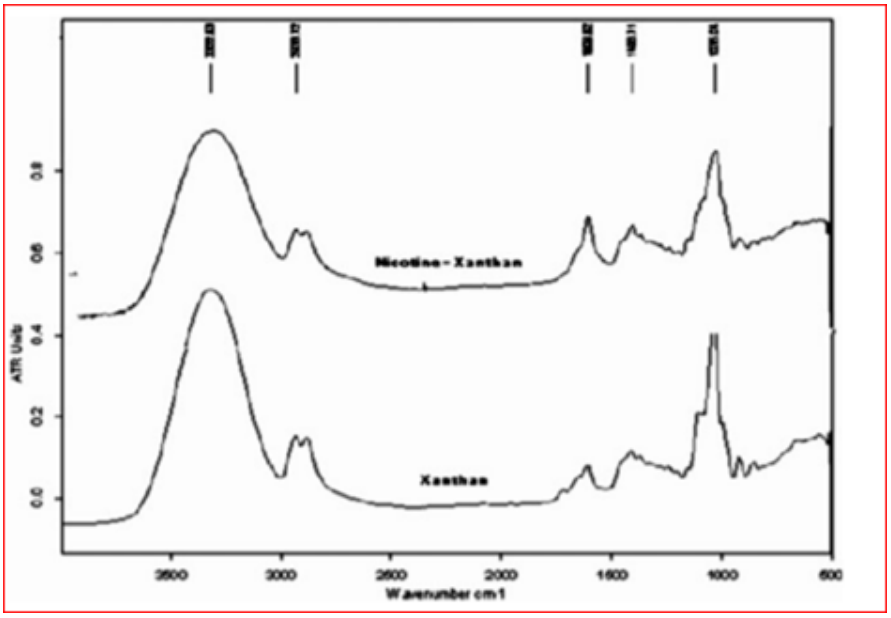

Figure 7: FTIR spectra of Nicotine and Nicotine with polymers.

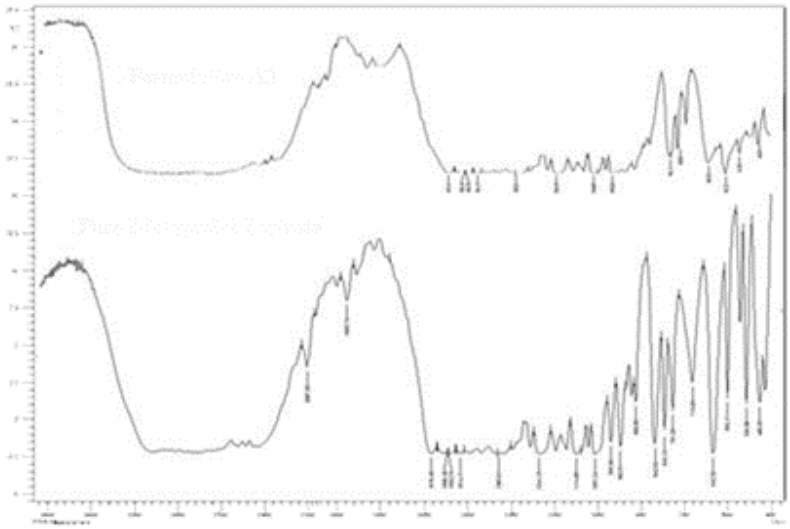

Figure 8: FTIR spectra of Nicotine and Formulation A3.

Table 5: FTIR spectra data of Nicotineand Nicotine and formulation A3.

Assignment	Band position pure drug (cm^{-1})	Band position of formulation (cm^{-1})
Groups	Peak positions in pure drug (cm^{-1})	Peak positions drug with polymers (cm^{-1})
C-H stretch	2934.24	2924.16
Aromatic ether	1242.20	1242.10
Isopropyl group	1189.25	1186.26
R-O-R	1114.89	1112.96
1, 4 disubstituted benzene	844.85	840.70

Differential Scanning Calorimetry (DSC) studies

The DSC Thermogram of xanthan gum. Sodium alginate. Nicotine. Physical mixture. formulation A3 are shown in the figures 9 – 13.

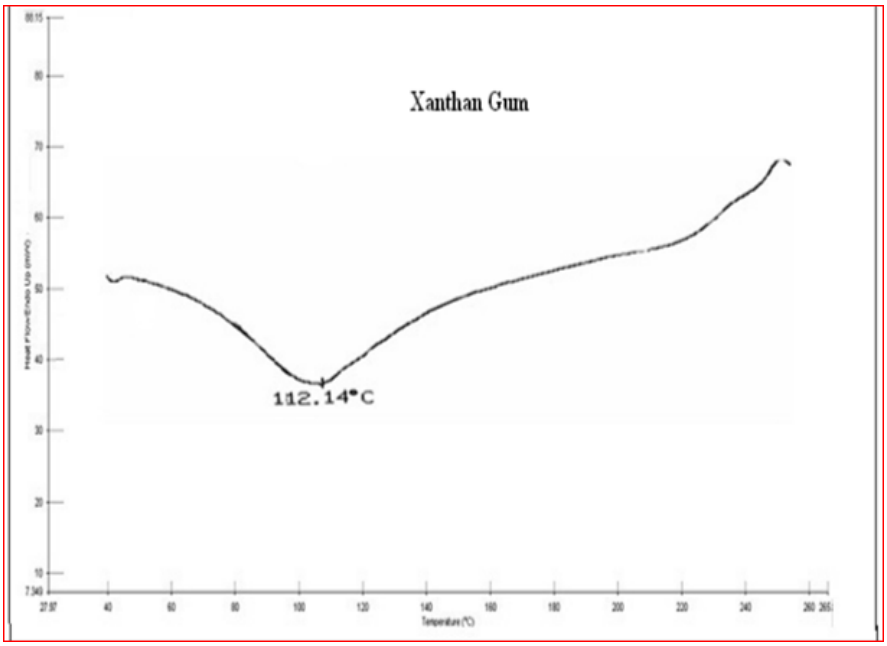

Figure 9: DSC thermogram of Xanthumgum.

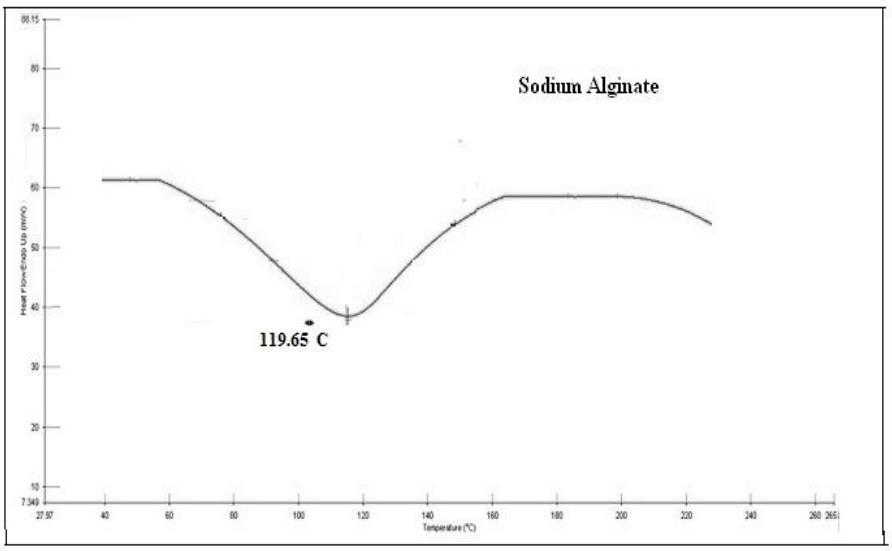

Figure 10: DSC thermogram of pure sodium alginate.

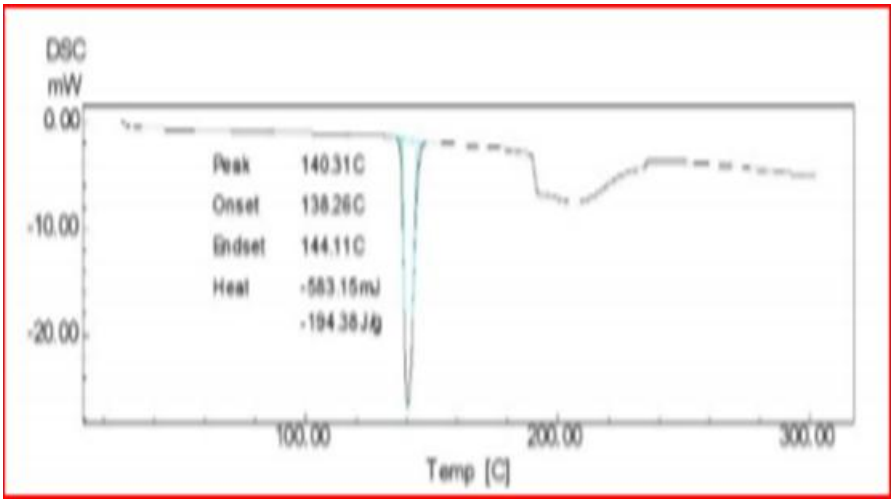

Figure 11: Pure drug Nicotine.

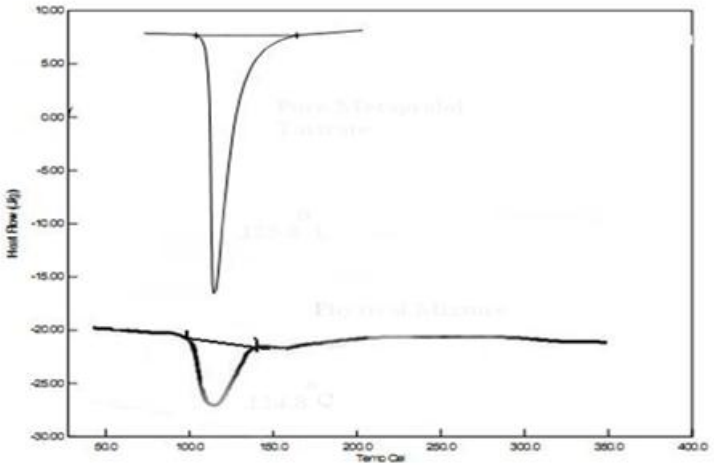

Figure 12: DSC Thermogram of Nicotine and Physical Mixture.

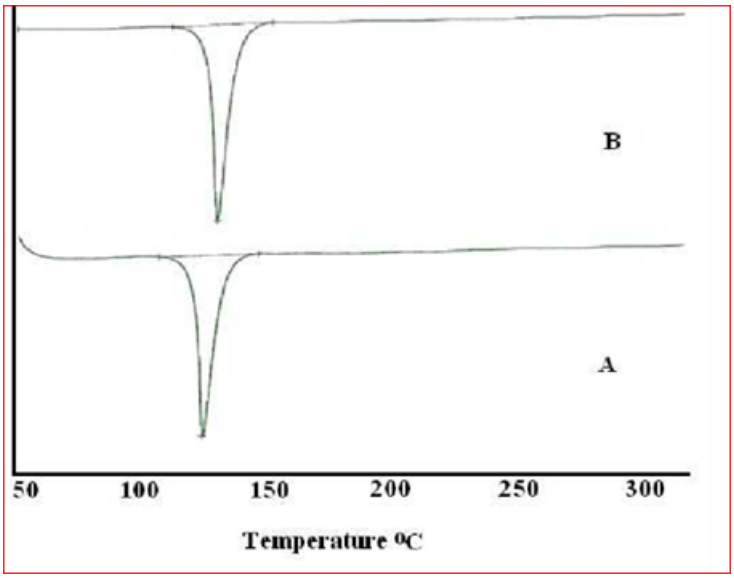

Figure 13: DSC Thermogram of Nicotine and Formulation A3.

Evaluation of the Formulated Transdermal Patches

Five formulations were prepared using solution casting method and dried.

Mechanical properties

Thickness of the prepared films was in the range of 0.24 to 0.27mm is shown in Table 6. Thickness, tensile strength and % elongation of the films increasing by increased ratio of XG and plasticizer in the films. Added glycerin alters the physical and mechanical properties by enhancing the mobility of polymers chains of SA, XG by hydrogen bonding. However it was found that 2% of glycerin gives the best plasticizer effect for Nicotine loaded film.

Evaluation of drug loaded films

The drug loaded films were formulated using drug, polymer (XG/SA), Glycerine used as plasticizer and different concentrations of methanol used as permeation enhancer. The prepared films were evaluated for its uniformity of weight, uniformity of film thickness, tensile strength, percentage elongation, folding endurance, percentage moisture absorption, percentage moisture loss, drug content, scanning electron microscope, *in vitro* drug diffusion study and primary skin irritation test.

Uniformity of weight

Table 6: Uniformity of weight of Nicotine patches (2.5 mg/cm^2).

Sl. No.	Formulation code	Weight of the patch in mg			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	A1	165	167	168	166.66 ± 1.52
2.	A2	167	170	171	169.00 ± 2.08
3.	A3	184	187	180	183.66 ± 3.51
4.	A4	190	196	195	193.66 ± 3.21
5.	A5	193	195	192	193.33 ± 1.52
6.	A6	168	172	172	170.66 ± 2.30
7.	A7	170	174	173	172.33 ± 3.08

*Standard deviation, n = 3

Film thickness

Table 7: Uniformity of thickness of Nicotine patches (2.5 mg/cm^2).

Sl. No.	Formulation code	Thickness of the patch in mm			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	A1	0.26	0.25	0.26	0.25 ± 0.005
2.	A2	0.25	0.22	0.24	0.23 ± 0.015
3.	A3	0.24	0.24	0.26	0.24 ± 0.011
4.	A4	0.28	0.26	0.27	0.27 ± 0.010
5.	A5	0.26	0.25	0.26	0.25 ± 0.005
6.	A6	0.28	0.25	0.25	0.26 ± 0.017
7.	A7	0.27	0.28	0.26	0.27 ± 0.010

Tensile strength Percentage elongation

Table 8: Tensile strength and % elongation of Nicotine patches (2.5 mg/cm^2).

Sl. No.	Formulation code	Tensile strength (kg/mm^2)	Percentage elongation
1.	A1	2.48 ± 0.0036	20.13 ± 0.45
2.	A2	2.67 ± 0.0023	21.79 ± 0.12
3.	A3	2.98 ± 0.0046	19.64 ± 0.19
4.	A4	3.12 ± 0.0053	24.12 ± 0.21
5.	A5	3.29 ± 0.0013	28.98 ± 0.21
6.	A6	3.45 ± 0.0026	31.38 ± 0.11
7.	A7	3.57 ± 0.0011	33.56 ± 0.19

Folding endurance

Table 9: Folding endurance of Nicotine patches (2.5 mg/cm^2).

Sl. No.	Formulation code	Folding endurance			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	A1	265	267	270	267.33 ± 2.51
2.	A2	275	276	277	276.50 ± 1.00
3.	A3	273	275	268	272.33 ± 3.06
4.	A4	268	270	261	267.33 ± 4.72
5.	A5	271	269	270	270.00 ± 1.00
6.	A6	270	265	260	265.00± 5.10
7.	A7	260	271	265	265.33± 5.00

*Standard deviation, n = 3

Percentage moisture absorption & Percentage moisture Loss

Table 10: Data of percentage moisture absorption and moisture loss (2.5 mg/cm^2)

Sl. No.	Formulation code	% Moisture Absorption Mean ± S.D*	% Moisture Loss Mean ± S.D*
1.	A1	1.59 ± 0.12	1.20 ± 0.12
2.	A2	1.58 ± 0.32	1.03 ± 0.10
3.	A3	1.60 ± 0.21	0.96 ± 0.13
4.	A4	1.63 ± 0.42	0.89 ± 0.22

5.	A5	1.68 □ 0.23	0.80□ 0.20
6.	A6	1.71 □ 0.12	0.86 □ 0.17
7.	A7	1.73 □ 0.16	0.84 □ 0.31

*Standard deviation, n = 3

Drug content

Table 11: Drug content uniformity and percentage of drug content (2.5 mg/cm^2).

Sl. No.	Formulation code	Drug content in mg Mean □ S.D*	% of drug content Mean □ S.D*
1.	A1	2.29 □ 0.13	94.56 □ 0.21
2.	A2	2.39 □ 0.11	95.67 □ 0.31
3.	A3	2.45□ 0.09	98.34 □ 0.12
4.	A4	2.38 □ 0.14	95.45□ 0.22
5.	A5	2.36 □ 0.15	95.23□ 0.24
6.	A6	2.34□ 0.16	95.11 □ 0.17
7.	A7	2.32□ 0.13	95.05 □ 0.15

*Standard deviation, n =3

***In vitro* Drug Diffusion Study**

Diffusion studies were carried out in an Franz diffusion tube, using nhydrated cellophane as a diffusion membrane.

Table 12: *In vitro* Diffusion profile of Nicotine Formulation A1 (2.5 mg/cm^2)

Time (hrs)	√ T	Log T	% Cumulative Drug Release	Log %Cum Drug Released	% Cum. Drug Retained	Log % Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	13.72	1.137	86.28	1.935
2	1.214	0.084	16.94	1.228	83.06	1.919
3	1.632	0.212	22.32	1.348	77.68	1.890
4	1.900	0.278	28.82	1.459	71.18	1.853
6	2.149	0.370	35.64	1.551	64.36	1.808
8	2.728	0. 434	42.88	1.632	57.12	1.756
10	3.062	0.485	48.92	1.689	51.08	1.708
12	3.364	0.518	55.74	1.746	44.26	1.646

Table 13: *In vitro* diffusion profile of Nicotine Formulation A2 (2.5 mg/cm^2).

Time (hrs)	√ T	Log T	% Cumulative Drug Release	Log %Cum Drug Released	% Cum. Drug Retained	Log % Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.34	1.156	85.66	1.938
2	1.214	0.084	20.22	1.305	79.78	1.901
3	1.632	0.212	24.48	1.388	75.52	1.878
4	1.900	0.278	32.34	1.509	67.66	1.830
6	2.149	0.370	37.82	1.577	62.18	1.773
8	2.728	0. 434	40.62	1.669	59.38	1.770
10	3.062	0.485	51.20	1.708	49.80	1.679
12	3.364	0.518	58.24	1.720	47.76	1.620

Table 14: *In vitro* diffusion profile of Nicotine Formulation A3 (2.5 mg/cm^2).

Time (hrs)	√ T	Log T	% Cumulative Drug Release	Log %Cum Drug Released	% Cum. Drug Retained	Log % Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	15.22	1.182	84.78	1.928
2	1.214	0.084	22.34	1.349	77.66	1.890

3	1.632	0.212	30.14	1.479	69.86	1.844
4	1.900	0.278	40.82	1.611	59.18	1.772
6	2.149	0.370	47.94	1.681	52.06	1.717
8	2.728	0. 434	53.12	1.725	46.88	1.671
10	3.062	0.485	63.72	1.804	36.28	1.560
12	3.364	0.518	70.28	1.847	29.72	1.473

Table 15: *In vitro* diffusion profile of Nicotine Formulation A4 (2.5 mg/cm^2).

Time (hrs)	√ T	Log T	%Cumulative Drug Release	Log %Cum Drug Released	% Cum. Drug Retained	Log % Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.28	1.155	85.72	1.933
2	1.314	0.084	20.86	1.319	79.14	1.898
3	1.632	0.212	28.74	1.458	71.26	1.853
4	1.900	0.278	38.48	1.585	61.52	1.789
6	2.349	0.370	40.94	1.612	59.06	1.771
8	2.728	0. 434	41.8	1.621	58.20	1.764
10	3.062	0.485	42.70	1.630	57.20	1.758
12	3.364	0.518	50.66	1.704	49.34	1.693

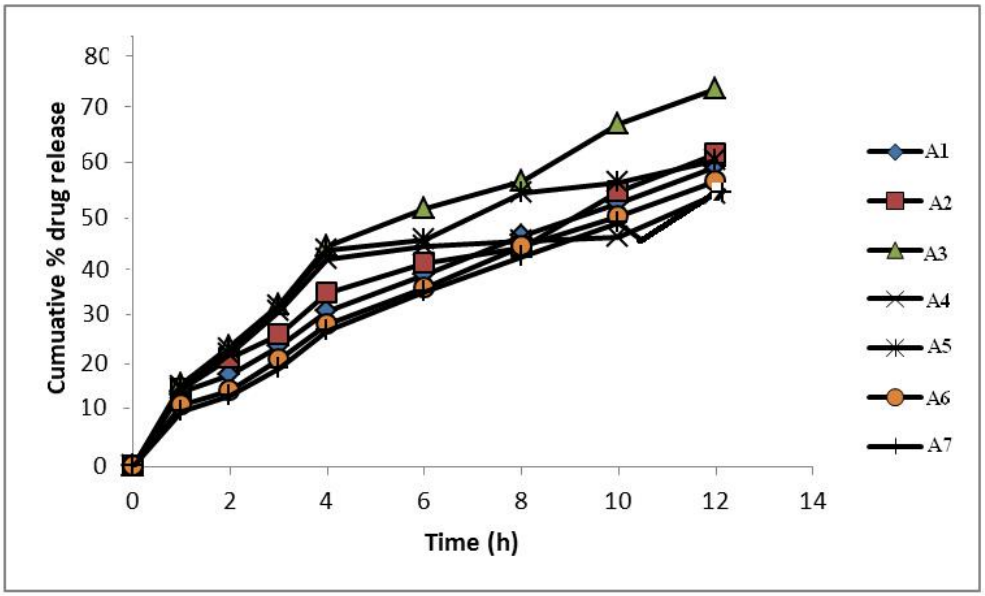

Figure 14: Comparative *in vitro* diffusion profiles of Nicotine according to Zero order kinetics.

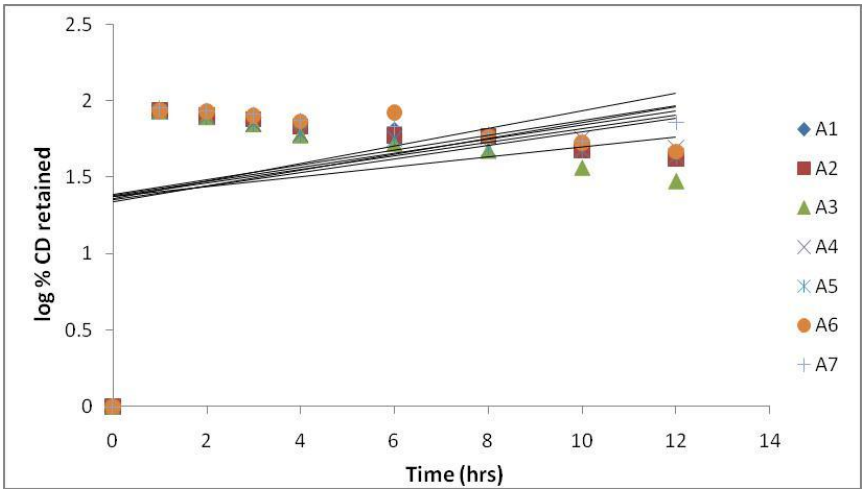

Figure 15: Comparative *in vitro* diffusion profiles of Nicotine patch according to First order kinetics.

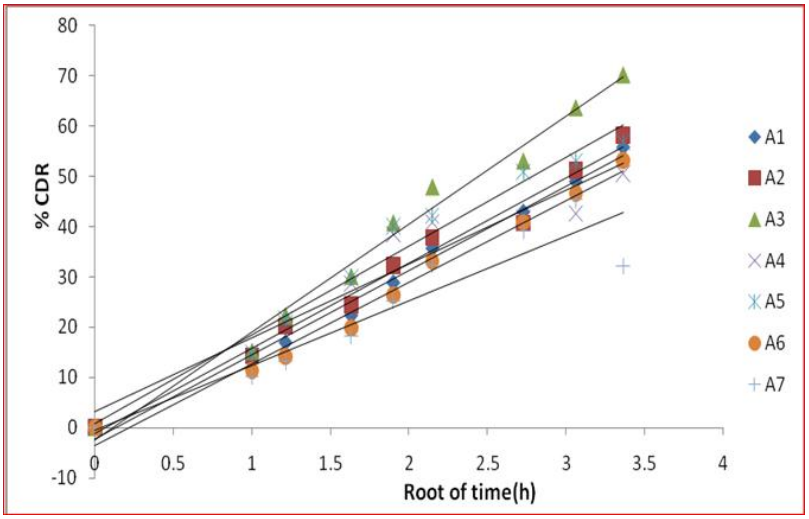

Figure 16: Comparative *in vitro* release profiles of Nicotine patch according to Higuchi matrix.

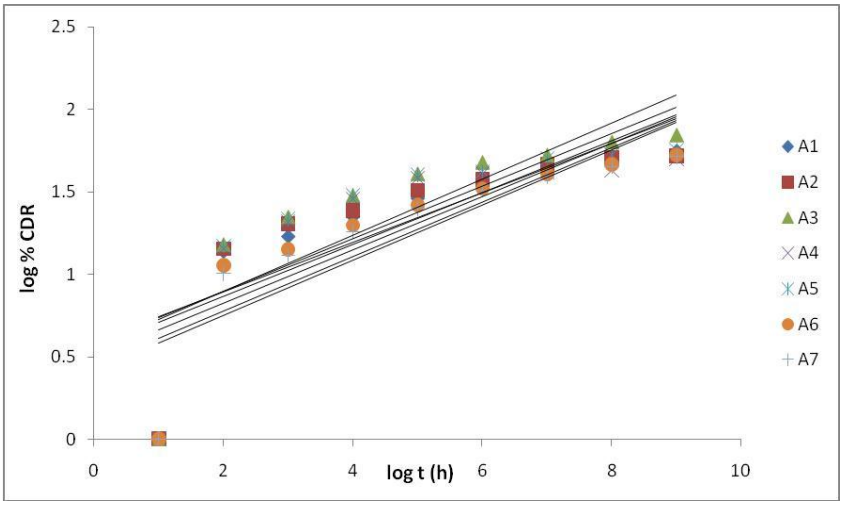

Figure 17: Comparative *in vitro* diffusion profiles of Nicotine patch according to Peppas kinetics.

Table 16: Results of Model Fitting of Nicotine patches.

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	'n' values
A1	0.9654	0.9976	0.9897	0.9969	0.6111
A2	0.9427	0.9935	0.9924	0.9944	0.6013
A3	0.9492	0.9911	0.9871	0.9912	0.6360
A4	0.9447	0.9674	0.9876	0.9899	0.6278
A5	0.9417	0.9654	0.9878	0.9896	0.6211
A6	0.9413	0.9632	0.9853	0.9886	0.6201
A7	0.9401	0.9618	0.9832	0.9879	0.6203

Stability Studies

Table 17: Stability studies of Optimized A3 formulation.

Sampling Intervals in Days	Drug content 25^0C/60 % RH	Drug content 30^0C/65 % RH	Drug content 40^0C/75 % RH
0	98.67	98.67	98.67
15	98.59	98.57	98.51
45	98.46	98.42	98.39
60	98.32	98.29	98.21

Skin Irritation test

Table 18: Score of skin irritation for Nicotine TDDS.

Test	Skin Reaction	Score
	Very slight erythema	0
	Well defined erythema	0
Erythema	Moderate to severe erythema	0
	Severe erythema	0
	Very slight edema	0
	Well defined edema	0
Edema	Moderate to severe edema	0
	Severe edema	0
Total score for primary skin irritation		**0**

DISCUSSION

Analytical Studies

Development of calibration curve for Nicotine (λ_{max})

The absorption maximum (λ_{max}) was obtained as 274nm. This implies the purity of the sample drug Nicotine. The λmax of Nicotinein pH 7.4 phosphate buffer solution was found to be 274 nm which is same as that of literature review. The calibration curve of Nicotinein pH 7.4 phosphate buffer solution shows linearity with R^2 of 0.9998 as show in table 3

Preformulation Studies

Preformulation studies are necessary to understand the physicochemical properties of the drug and the compatibility of the other excipients used in the formulation. The results of the various Preformulation characterizations are given below.

Determination of melting point

Melting point of Nicotinewas found to be 121.1± 0.87^0 C.

Solubility studies

From the result it was found that Nicotineis freely soluble in water, soluble in menthol and ether, slightly soluble in acetone, practically insoluble in acetonitrile and hexane.

Determination of partition co-efficient

The partition co-efficient studies were performed and the partition co-efficient (P) value was experimentally found to be 0.753 as shown in table 4. The results obtained also indicate that the drug possesses sufficient lipophilicity, which fulfill the requirements of formulating the selected drug into a transdermal film.

The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin. The value of partition co-efficient obtained indicates that the drug possesses sufficient lipophilicity, which fulfill the requirements of formulating the selected drug into a transdermal film. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin.

Drug Excipient Compatibility

FT-IR study

The IR spectra of pure Nicotine and polymers were found to be identical. The characteristic IR absorption peaks of Nicotineat 2934.24 cm^{-1} (C-H stretch), 1242.20 cm−1 (Aromatic ether), 1189.25 cm^{-1} (Isopropyl group), 1114.89 cm^{-1} (ether), 844.85 cm^{-1}(1, 4-disubstituted benzene) were present. FTIR spectra of the drug with polymers showed all the Nicotine characteristics absorption bands suggesting there is no chemical interactions between the drug and polymers used in the formulation. FT-IR study was employed to ascertain the compatibility of the Nicotinewith the XG and SA. Both the spectra were compared for confirmation of common peaks. Specific peaks of pure Nicotineand Nicotineloaded patch showed no significant variation in height, intensity and positions of peaks. This confirms that there is no chemical interaction between drug and used polymers as shown in fig. 3-8.

Differential Scanning Calorimetry (DSC)

To understand the compatible state of the drug, DSC studies were carried out on pure drug and drug loaded patch, the thermo grams data obtained are shown in Figures 16 - 20. Nicotineexhibits a sharp endothermic peak at 125.8^0C. It was observed that presence of the endothermic peak at 124.7^0C in the drug loaded patches indicated, that the drug is distributed in the patch without any degradation and compatible with XG/SA. Compared the DSC data and interpretation of this region in our data agrees with their conclusions[38] as shown in Table 6. DSC studies were carried out on pure drug and drug loaded patch. It was understood that the drug and the excipients used were compatible. Also indicated, that the drug is distributed uniformly in the patch without any degradation.

Preparation Of Drug-Loaded Transdermal Patches

Films consist of glycerine as a plasticizer and menthol as permeation enhancer. Drug loaded films were light yellow opaque in colour. All surface of the film was smooth, with elegant appearance, good physical properties. Flatness of the films was observed better when the amount of SA > 50% in the formulated films, might be SA having α- L-guloronic acid, which on interaction with XG produces good flatness to the film.

Thus these formulations can maintain a smooth and uniform surface when applied on skin.

Evaluation of Transdermal Patches

Determination of film forming character

The films prepared using 30.0 % of XG, 63.0 % SA, 1.5 % of glycerin as plasticizer and 3.0% of menthol forms smooth, flexible, transparent and having sufficient mechanical strength.

Evaluation of drug loaded films

The drug loaded films were formulated using polymer, glycerin used as plasticizer, and menthol as permeation enhancer. The prepared films were evaluated for its physicochemical and mechanical properties and the same were discussed individually.

Uniformity of weight

Three different films of the individual batch are weighed and the average weight was calculated. The dried patches were weighed on digital balance and was found exhibit uniform weight. The data of the individual weights are shown in Table 8. Three different dried patches of the individual batch were weighed using digital balance and the average weight was calculated. The films exhibited uniform weight and there was no deviation in the weight of any formulation.

Film thickness

The thickness of the films was measured at different points using digital vernier caliper. The average of three readings were measured and presented in Table 7. The thickness of the films was measured at different points using digital vernier caliper. The films showed uniformity in their thickness. All surface of the film was smooth, with elegant appearance, good physical properties. Flatness of the films was observed better when the amount of SA was greater than 50% in the formulated patches.

Tensile strength

Tensile strength of the patches was determined using Hounse field universal testing machine. The patches have shown reasonable tensile strength and moderate percentage elongation. The data is shown in the Table 8. The tensile strength increased where as percentage elongation decreases with increasing the concentration of Glycerin. The patches have shown reasonable tensile strength and no sign of cracking in the patches were observed, which may be attributed to addition of plasticizer. However it was found that 3% of glycerin gives the best plasticizer effect for MT loaded patches.

Percentage elongation

Percentage elongation of patches gives information of how much a specimen can elongate before it breaks. It was carried out by Hounse field universal testing machine. The percentage elongation at break point is measured on scale and the data of the percentage elongation is presented in the Table 8. The patches have shown moderate percentage elongation and was found satisfactory. The tensile strength and percentage elongation of the formulations increases with increasing the concentration of XG and glycerin.

Folding endurance

The folding endurance was measured manually for the prepared films. A strip of film 2x2 cm was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance. The data are presented in Table 9. The folding endurance was determined manually by repeatedly folding the film at same place until it broke. The formulated film A3 exhibited optimal folding endurance with out any batch variation.

Percentage moisture absorption & Percentage moisture Loss

The moisture absorption studies carried out at 80% relative humidity. All the patches showed least percentage moisture absorption. The data of the same is presented in Table 10. Low moisture uptake was found in films with less percent of plasticizer, after stored in the above conditions. Films with low percent of plasticizer showed a lower capacity to absorb water compared to those with higher amount of plasticizer. As the ratio of plasticizer and RH increases, moisture uptake was increased. This effect was more pronounced on films containing more amount of plasticizer and more amount of plasticizer showed a significant increase in moisture up take at increased RH.The moisture absorption studies carried out at 90 % relative humidity. All the patches showed least percentage moisture absorption. This shows that the film protects the materials from microbial contamination and bulkiness of the patch. Low moisture absorption was found in films with less percent of plasticizer. The moisture loss study was carried out in a desiccator containing anhydrous calcium chloride. All the patches showed least percentage moisture loss. Low moisture absorption was found in films with less percent of plasticizer and as the % of plasticizer increases the moisture absorption loss decreases.

Drug content

The drug content was analyzed spectrophotometrically at 274nm and the data is given in the Table 10. The formulations exhibited uniform drug content and minimum batch variability. The drug content was analyzed by spectrophotometer at 274nm. All the formulations had fairly uniform drug content ranging from 2.29 to 2.45 mg/cm^2. The drug content analysis of the formulations have showed that the process employed to prepare the patches in this study was capable of giving patches with uniform drug content and minimum batch variability.

***In vitro* Diffusion Study**

Diffusion studies for all the films were carried out for 12 hrs in pH 7.4 phosphate buffer solution. From the

diffusion studies, it was observed that, at the end of 12th h, drug diffusion from formulation A3 (70.28%) was maximum than A1 (55.74 %), A2 (58.24 %), A4 (50.66 %), A5 (56.88 %), A6 (53.12 %) & A7 (32.10 %) presented in Tables 14 – 20 and Fig. 21. The kinetics of drug diffusion profiles were found out by plotting different graphical models. All the profiles are shown in Fig and kinetic data is presented in the respective he matrix diffusion controlled transdermal drug delivery system of Metaprolol Tartrate was studied for their *in vitro* drug diffusion to observe the kinetics of drug diffusion from the formulations.

From the above results, it can be concluded that drug diffusion from the patches was controlled. Increased amounts of XG showed higher swellability of the patches.

The process of drug release in most controlled release devices is governed by diffusion and the polymer matrix has strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. The reason for high release drug from A3 is probably because xanthan gum is hydrophilic in nature, exhibiting hydration and swelling of the patches. Xanthan Gum is known to have larger cavity size in its polymeric network and thus it may involve a faster mode of diffusion of Nicotinefrom the formulation A3 as compared to other formulations leading to higher skin permeation. The % of plasticizer from the film could reduce tortuosity of aqueous pore channels of the films, respectively.

In order to understand mechanism of drug release, *in vitro* release data were treated to kinetic models and linearity was observed with respect to Higuchi equation. The correlation coefficient obtained from Higuchi plot was found to be in the range of 0.9832 to 0. 9924. This indicates that mechanism of drug release was diffusion type. As indicated by higher R^2 values, the drug release from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the diffusion mechanism was swelling and diffusion controlled.

The Peppas model is widely used to confirm whether the release mechanism is fickian diffusion, non-fickian diffusion or zero order. 'n' value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be more than 0.50. Hence, this indicates that the diffusion approximates Fickian diffusion mechanism.

Stability studies

Stability studies of Metaprolol Tartrate loaded transdermal patches were carried out to determine the amount of drug content as presented in Table.The optimized formulation A3 was subjected for stability studies and estimated drug content at the end of 60 days. However there was no significant change in drug content from formulation A3.

Skin irritation test

The skin irritation studies showed no presence of erythema and edema after application of films. Thus transdermal patches are free from significant skin irritation.

CONCLUSION

Nicotine is both a sedative and a stimulant. was selected for the formulation of transdermal delivery system as it complies with physicochemical properties required to permeate through skin. The preformulation studies involving description, solubility, melting point, partition coefficient of the drug were found to be comparable with the standard.

The patches were prepared by solvent casting technique. The patches were subjected to the following evaluation parameters such as physical appearance, weight variation, thickness, folding endurance, drug content, percentage moisture absorption, percentage moisture loss, water vapour transmission rate, tensile strength, diffusion studies and skin irritation studies. All the parameters were within the limits.

Based on all these results viz. mechanical properties, compatibility, stability and diffusion studies, formulation A3 was selected as the best formulation. From *in vitro* and skin irritation test, it can be concluded that the developed formulation A3 have great potential for transdermal drug delivery.

From the above studies, it is revealed that the present work was a satisfactory preliminary study of improving patient compliance by development of transdermal drug delivery system of Nicotine.

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