FORMULATION AND EVALUATION OF AMIKACIN-LOADED PLGA NANOPARTICLES-BASED TRANSDERMAL PATCH FOR THE TREATMENT OF WOUND HEALING

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Introduction

Any physical harm to an organism's living tissue, whether in a human or an animal, is referred to as a wound. resulting from recent physical strain. Humans can sustain injuries through burning, asphyxia, penetrating trauma, overexertion, blunt trauma, or toxic exposure. These injuries can happen accidentally or on purpose. Wounds must heal properly to restore the skin's disrupted anatomical continuity and disturbed functional status. The intricate process of healing, which is brought on by an injury, aims to restore the integrity and functionality of damaged tissues. The three overlapping phases of wound healing are cellular proliferation (3–12 days), remodelling (3-6 months), and inflammation (0–3 days). These phases are made possible by continuous interactions between cells and the matrix. To maintain the anatomical continuity of the impacted area (James R. Hanna DPM and Joseph A. Giacopelli 1997).

Innovative drug delivery techniques have been developed as a result of recent technological advancements. Transdermal delivery is one of the most important routes for novel drug delivery systems (NDDS). Transdermal administration is a convenient, painless, and non-invasive method. It can stop the hepatic first-pass metabolism and gastrointestinal toxicity (peptic ulcer disease)1,7. Traditionally, a patch that contains medication is applied to the skin. Transdermal drug delivery has been the subject of numerous studies and is a good alternative due to its improved drug bioavailability[10]. Enhancements in safety, efficacy, and patient compliance are just a few advantages it provides.

Transdermal drug delivery can administer drugs through the skin to the systemic circulation at a predetermined rate and maintain therapeutic concentration for a prolonged period of time[12]. Transdermal drug delivery has been explored and developed for a variety of medications, including those with anti-inflammatory, antihypertensive, anti-anginal, antibiotic, analgesic, and anti-arthritic properties. In the past, people used to apply different substances to their skin

for medicinal reasons. Different topical formulations are now available to treat local indications. The patient can remove the patch that is applied to deliver the drug to his skin.

Some topical amikacin preparations are available as amikacin sulfate; however, patients may find it difficult to administer these as directed by their physician. Transdermal patches enhance patient compliance by providing a controlled release of medication with a single application, all while avoiding the need for needles and being reasonably priced. Nanotechnology is a young field that is significantly changing the way many diseases are treated. The drug's transdermal delivery was deemed impracticable due to amikacin's compromised permeability through the dermis. Amikacin can, however, enter the body through increased skin permeability. Studies have shown that amikacin can be administered in a variety of ways, including injections and transdermal patches. On the other hand, PLGA can efficiently encapsulate amikacin[15].

Using PLGA nanoparticles loaded with amikacin, amikacin transdermal patches were prepared and evaluated as part of this study. To attain systemic therapeutic benefits and concentration, the study aimed to develop a more efficacious therapeutic alternative delivery method for amikacin. The drug can be released at a regulated rate and protected from deterioration by being encapsulated in the nanoparticles.

Preformulation Studies

Solubility

The solubility of the drug is determined by taking various solvents including methanol, ethanol, acetone, chloroform, and distilled water Adding a small quantity of drug in these solvents and observing.

Melting Point

The melting point of the drug was determined by using a capillary tube. Closed one end of capillary and filled with drug (1/4) and observed in lab melting point apparatus.

Calibration Curve

By comparing an unknown sample to a set of standard samples with known concentrations, one can generally determine the concentration of a substance in the unknown sample using a calibration curve, also called a standard curve. One method for solving the instrument

calibration problem is to create a calibration curve; alternative standard methods involve combining the known and unknown to create an internal standard. The calibration curve is a graph that shows how the concentration of the analyte—the material to be measured—affects the instrumental response, also known as the analytical signal[14].

Fourier Transform Infrared Spectroscopy (FTIR)

The Michelson interferometer consists of a beam splitter, a moving mirror, and a stationary mirror. The beam splitter divides the light beam into two halves, which are reflected by the moving and fixed mirrors before being recombined by the beam splitter. As the moving mirror makes reciprocating movements, the optical path difference to the fixed mirror changes, causing the phase difference to shift over time. Interference light is created in the Michelson interferometer by recombining the light beams. An interferogram records the intensity of the interference light, with the optical path difference recorded along the horizontal axis[14].

Differential Scanning Calorimetry (DSC)

The sample and reference holder, the heater, the heat resistor, and the heat sink make up the Heat Flux DSC. Heat resistors and heat sinks allow the heater's heat to enter the sample and the reference. The ratio of heat flow to the heat differential between the heat sink and holders is one. Comparing the heat sink to the sample, it has an adequate heat capacity. If the sample experiences endothermic or exothermic phenomena, such as reaction and transition, a heat sink is used to counteract these events. As a result, the sample's and the reference's temperature differences remain constant. The temperature differential of both holders determines the difference in heat supply to the sample and the reference. Through standard material calibration, the unknown[14].

Materials and Methods

Materials

Amikacin was gifted by BLD Pharma India Pvt. Ltd., Poly lactic Co-glycolic acid (PLGA) and Hydroxy Propyl Methyl Cellulose (HPMC) E5 polymers procured from lab. Sodium alginate, Polyvinyl Alcohol (PVA), Eudragit, Acetone, Methanol and Glycerol were obtained from Loba Chemical Pvt. Ltd.

Preparation of Amikacin-loaded PLGA Nanoparticles

Using a magnetic stirrer, a room-temperature 2% w/v solution of polyvinyl alcohol (PVA) was made in distilled water. 20 mg of amikacin was dissolved in 5 ml of distilled water to create an amikacin solution (4 mg/ml) in a beaker. The magnetic stirrer was used to completely dissolve all of the solutions. In a beaker, 50 mg of PLGA was dissolved in 2 ml of acetone to create PLGA solution (25 mg/ml). The magnetic stirrer was used to completely dissolve all of the solutions.

The process of solvent evaporation was employed to create Amikacin PLGA nanoparticles. Using ultrasound sonication, amikacin and PLGA solutions were combined for 30 seconds in an ice bath. After that, the resultant solution was gradually added, while stirring constantly, for two hours, to 10 millilitres of PVA solution[19]. A summary of formulation ingredients was given in Table 1.

Table No. 1 Quantities of different formulation ingredients for optimization of nanoparticles.

Formulation	Drug(mg)	PVA 2%w/v solution (ml)	PLGA(mg)	Acetone(ml)	Distilled water(ml)
ANF1	20	10	50	2	5
ANF2	20	10	100	2	5
ANF3	20	10	150	2	5

Evaluation of Nanoparticles

Drug Content

The drug content of the prepared nanoparticles was determined by the formula;

Drug content % = weight of the drug in nanoparticles/ Weight of nanoparticles × 100

Entrapment Efficiency

There are two ways to load drugs: the incorporation method, which involves adding the drug during the preparation of the nanoparticles, and the adsorption method, which involves adding the drug after the nanoparticles are formed by incubating the carrier with a concentrated

drug solution. Following drug loading, the entrapment efficiency was determined using the provided formula[19].

Entrapment efficiency = Total drug- Free drug/ Total drug × 100

Particle Size

The particle size of nanoparticles of different batches was measured by particle size analyzer. Particle size of each batch was determined and mean value was taken.

Scanning Electron Microscopy

A scanning electron microscope was used to examine the optimized formulation F3's results. On an adhesive plate with two sides, which was adhered to a glass slide on one side, nanoparticles were applied. After removing any extra nanoparticles, the slide was placed on a sample holder, and an electron microscope was used to take a scanning electron nanoparticle picture[19].

Preparation of Transdermal Patch

Preparation of backing layer

A 4% solution of polyvinyl alcohol (PVA) was utilized to prepare the baking layer. The solution was made using a hot plate magnetic stirrer in deionized water at 80 °C. To ensure even mixing, the precisely weighed quantity of PVA was gradually added in small portions to the deionized water over two hours. Eight millilitres of the baking solution were added to each petri plate once the solution had cooled. After that, it was left to dry for a day at room temperature. On petri plates, a transparent baking layer developed after drying[19].

Preparation of matrix type transdermal patch

Table 2 provides an overview of the ingredients used in the formulation. To ensure full dissolution, 20 millilitres of methanol were mixed with precisely weighed hydroxy propyl methyl cellulose (HPMC) and heated to 32 degrees Celsius with a speed of 250 revolutions per

minute using a hot plate magnetic stirrer. Aluminium foil was placed over the container to keep the solvent from escaping. Following the full dissolution of HPMC, eudragit was added and mixed for an additional 30 minutes to ensure complete dissolution. For a further fifteen minutes, the nanoparticle solution was added gradually and mixed. The medication solution containing polymers was sonicated for 20 minutes after an additional amount of plasticizer was added.

The baking layer (found in petri plates) was then gradually covered with this solution. On the Petri plate, a funnel was inverted to prevent solutions from drying out quickly. Petri plates were baked at 35 °C for 48 hours to dry them out. Following drying, the patches were taken off of the petri plates, placed between two sheets of aluminium foil, and refrigerated[19].

Table No. 2 Quantities of different formulation ingredients for optimization of patch based on Amikacin loaded PLGA NPs.

Formulation	Amikacin loaded (NPs) Contain drug (mg)	HPMC (mg)	NaAl (mg)	PEG400 40%w/w	Methanol (ml)
F1	20	100	400	400	20
F2	20	200	300	400	20
F3	20	300	200	400	20
F4	20	400	100	400	20

Evaluation of Transdermal Patches

Weight Variations

Weight variation test was performed by the taking weight of each nanoparticle based transdermal patch.

Flatness

In order to test for flatness, a clear length band measuring 4 cm was cut off from the patch's center and each of its planes. The percentage flatness was calculated using the following equation:

Flatness %=(Initial – final length)/(Final length) × 100

Folding Endurance

To ascertain the folding strength, a 2 × 2 cm patch was folded until it broke. The fragment was randomly selected from the middle of each transdermal patch.

Thickness

Thickness of the nanoparticle based transdermal patch was determined with the help of vernier calliper from patch boundaries and the middle part.

Surface pH Determination

The films were kept in contact with 0.5 ml of distilled water for 1 hr. the surface pH was measured using pH paper placed on the surface of the swallow patch. It was measured by pH meter also. The means of three readings was recorded.

Percentage Moisture Content

After each prepared film was weighed, it was stored for 24 hours at room temperature in a desiccator filled with fuse calcium chloride. The films were reweighed after a 24-hour period, and the percentage moisture content was calculated using the given formula.

MC = M wet – M dry/ M dry ×100

In-vitro Release Studies

The patch was fastened to the diffusion cell using an open-ended test tube so that the drug-releasing surface of the cell faced the receptor compartment, which held approximately 100 milliliters of a pH 6.8 phosphate buffer solution at 37±10C. At standard rpm, a magnetic stirrer was used to mix the elution medium. At prearranged intervals, the 2 ml aliquot sample was removed and replaced with the same volume of pH 6.8 phosphate buffer. After the samples were diluted with equal parts PBS, the absorbance at 524 nm was measured[19,20].\

Results

Preformulation Studies of Amikacin

Characterization of Amikacin Drug

The physical appearance of Amikacin was amorphous powder in nature, white colour and odourless.

Melting Point

The melting of the drug Amikacin in triplet manner and the average melting point of drug was found to be 227oC.

Solubility

It was concluded that solubility of Amikacin are completely soluble in water, and slightly insoluble in chloroform but insoluble in other solvents.

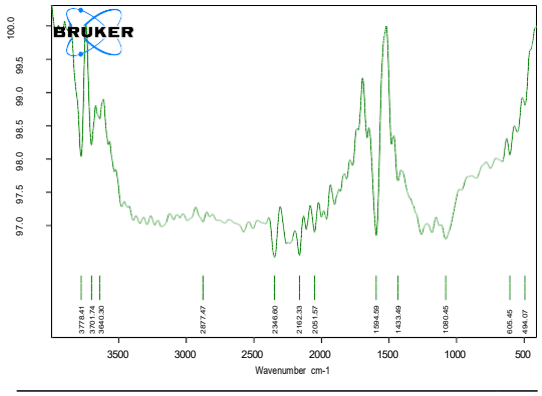


Fig. No.2 FTIR Spectrum of Amikacin

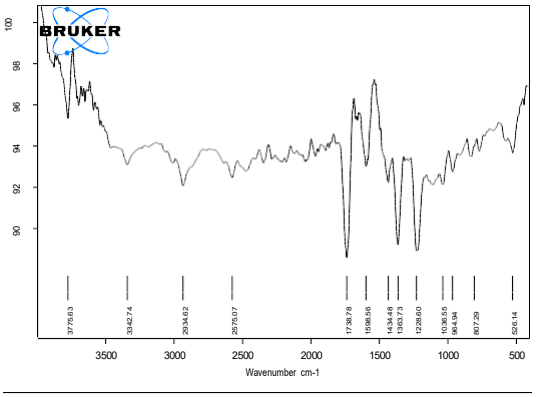


Fig. No.3 FTIR Spectrum of HPMC

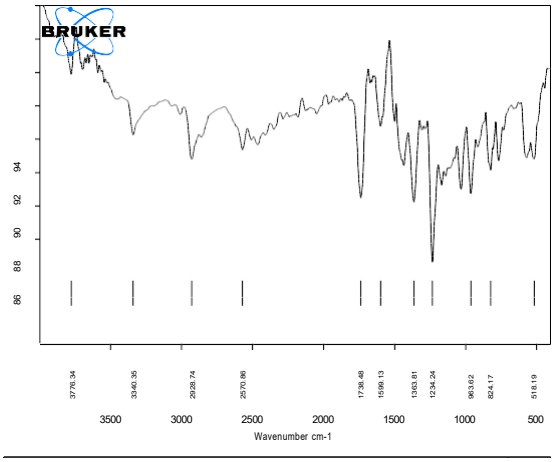


Fig. No.4 FTIR Spectrum of PLGA

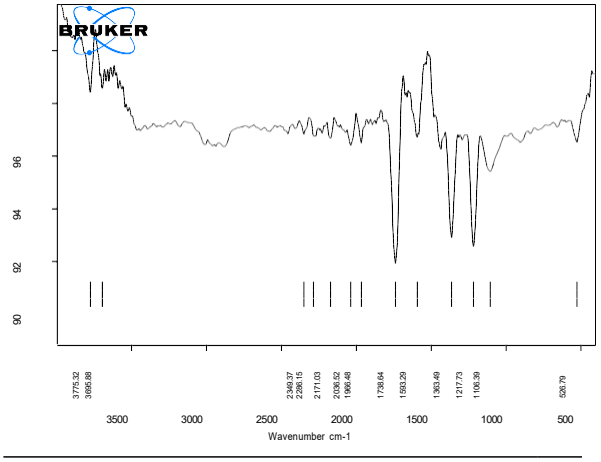


Fig. No.5 FTIR Spectrum of Both Drug & Polymer

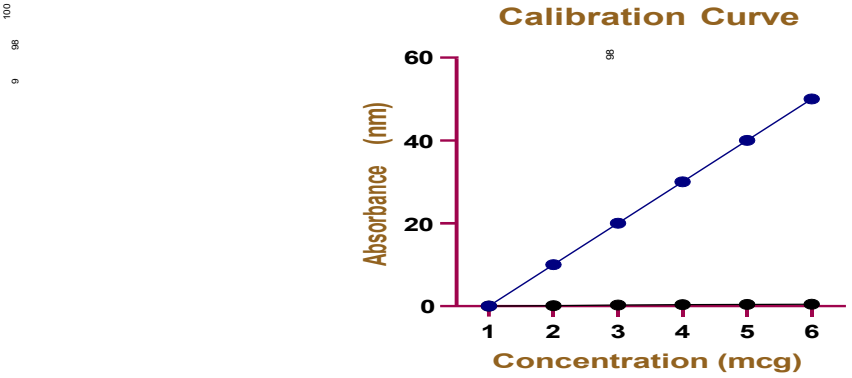


Fig. No.1 Calibration Curve of Amikacin at 524nm

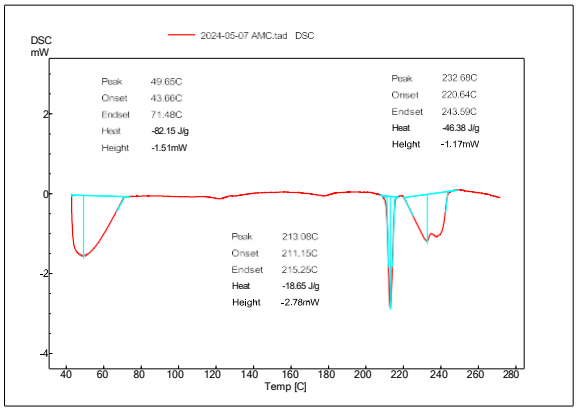


Fig. No.6 DSC Thermogram of Amikacin

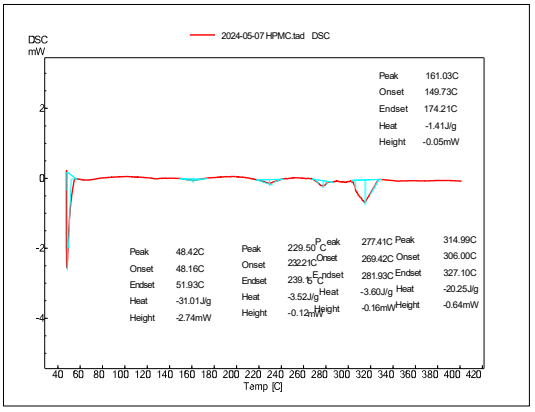


Fig. No.7 DSC Thermogram of HPMC

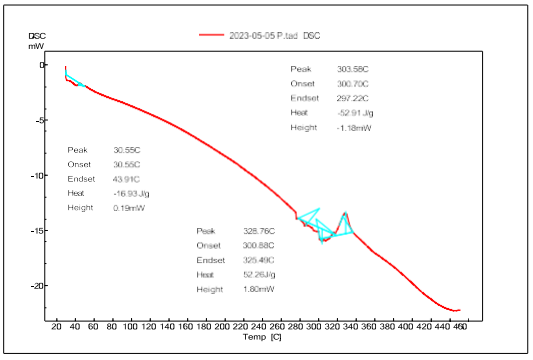


Fig. No.8 DSC Thermogram of PLGA

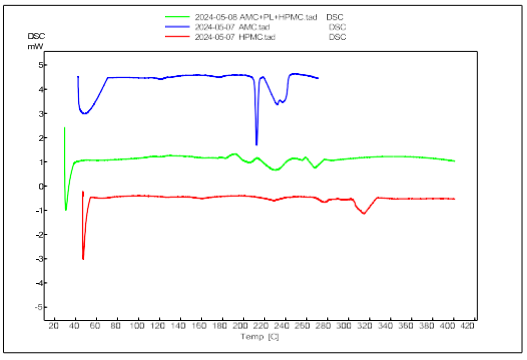


Fig. No.9 DSC Thermogram of Both drug and Polymer

Preformulation characterization of Amikacin is shown in **Fig. 1** Calibration curve of Amikacin at 524nm, **Fig. 2** FTIR Spectrum of PLGA, **Fig. 3** FTIR Spectrum of HPMC, **Fig. 4** FTIR Spectrum of Amikacin, **Fig. 5** FTIR Spectrum of Both and Polymer, **Fig. 6** DSC Thermogram of Amikacin, **Fig. 7** DSC Thermogram of PLGA, **Fig. 8** DSC Thermogram of HPMC, **Fig. 9** DSC Thermogram of Both Drug and Polymer.

Evaluation of Nanoparticles

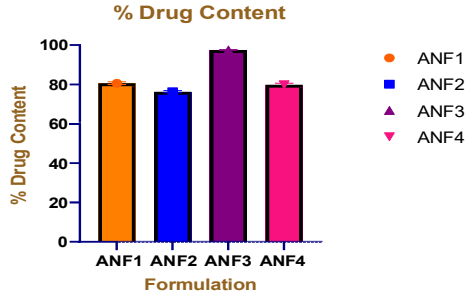


Fig. No. 10 % Drug content

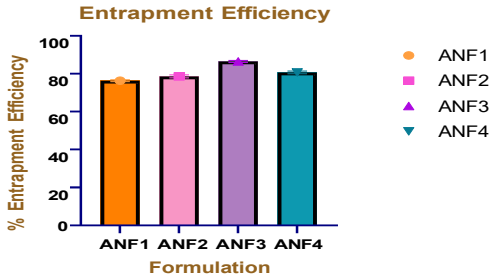


Fig. No. 11 Entrapment efficiency

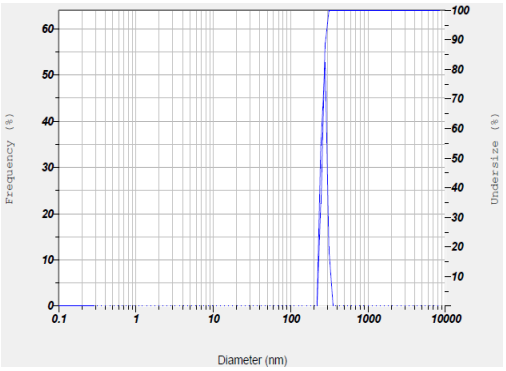


Fig. No. 12 Particle size

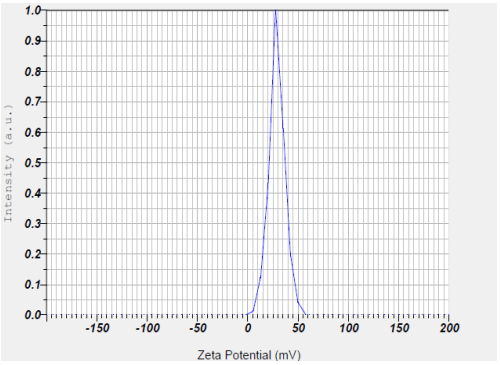
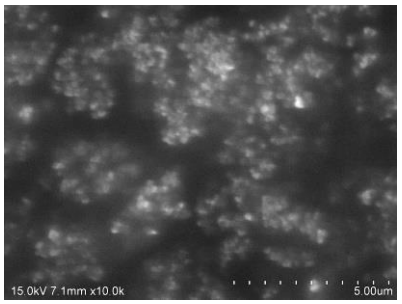
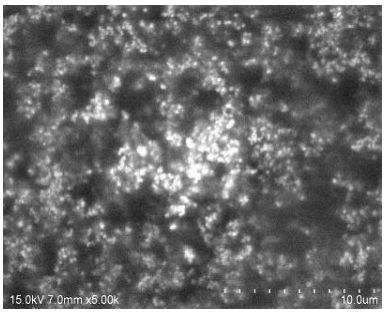


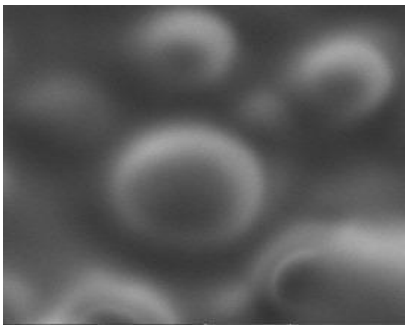
Fig. No. 13 Zeta potential



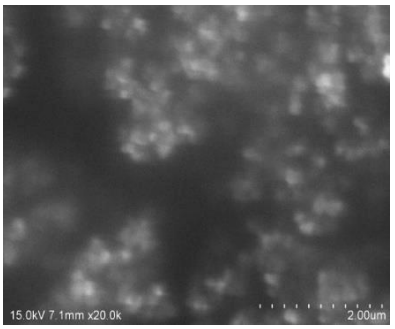
(a) 4-5µm



(b)-10µm



(c) Single particle



(d) 1-2µm

Fig. No. 14 Scanning Electron Microscopy

Evaluation of Amikacin NPs were shown in Fig. 10 % Drug content, Fig. 11 % Entrapment efficiency, Fig. 12 Particle size of Amikacin-PLGA NPs was 264.0nm, Fig. 13 Zeta potential was 34.1, Fig. 14 Scanning Electron Microscopy was range in 1-10µm.

Fig. No. 15 Formulations of Transdermal Patches

Evaluation of Transdermal Patch

Physicochemical characterization of transdermal patches was conducted.

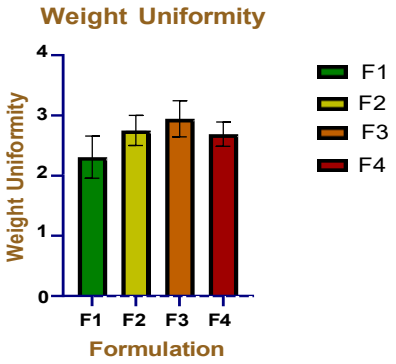


Fig. No. 16 Weight uniformity

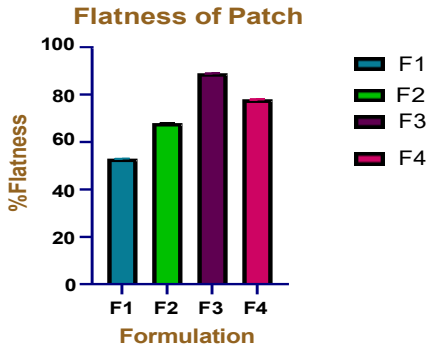


Fig. No. 17 Flatness of Patch

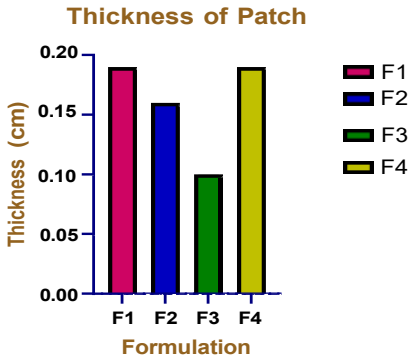


Fig. No. 18 Thickness of patch

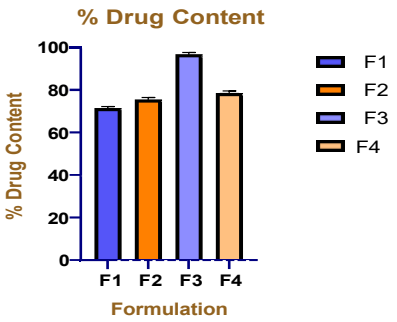


Fig. No. 19 % Drug content

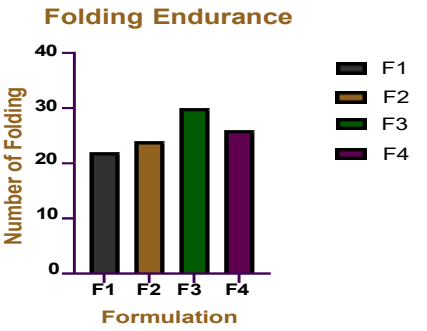


Fig. No. 20 Folding Endurance

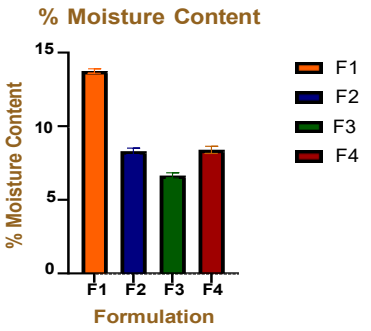


Fig. No. 21 %Moisture content

Time (hrs)	F1	F2	F3	F4
0	0	0	0	0
2	25.4 ± 0.4	25.4 ± 0.3	28.5 ± 0.5	31.1 ± 0.4
4	42.8 ± 0.5	42.8 ± 0.4	48.8 ± 0.4	46.1 ± 0.3
8	48.1 ± 0.4	48.1 ± 0.3	57.8 ± 0.6	59.4 ± 0.1
12	64.8 ± 0.6	64.8 ± 0.3	69 ± 0.3	67.5 ± 0.4
16	72.9 ± 0.4	72.9 ± 0.5	78.9 ± 0.6	73.4 ± 0.6
20	76.2 ± 0.2	76.2 ± 0.1	88.1 ± 0.7	78.4 ± 0.3
24	88.4 ± 0.7	88.4 ± 0.2	98.6 ± 0.1	81.8 ± 0.4

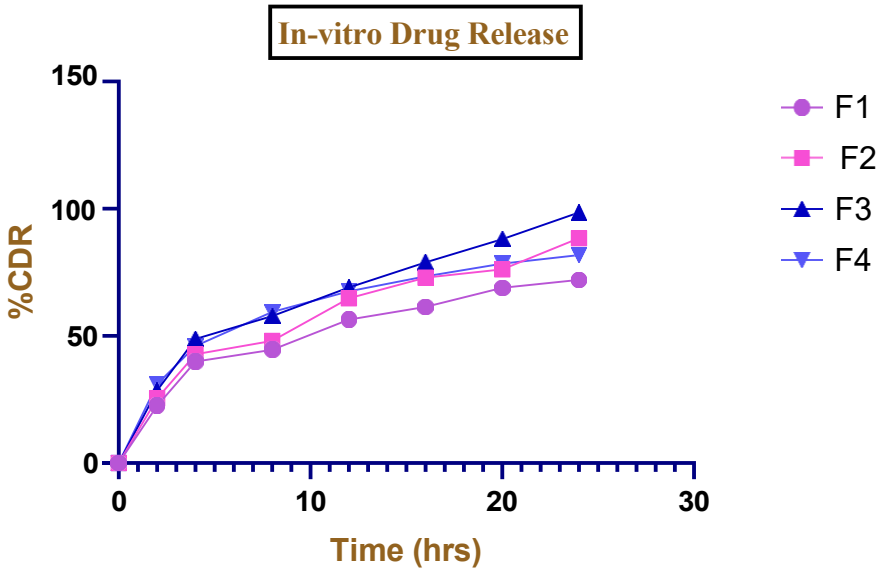


Fig. No. 22 In-vitro Drug Release

According the table no. 20 the In vitro drug release the best formulation has taken out for the in vitro drug release, in that formulation the F3 has shown the maximum drug release of (84%) in 24 hrs, as compared to other formulations.

Discussion

The melting of the drug Amikacin in a triplet manner and the average melting point of drug was found to be 227ºC. Solubility of Amikacin was concluded that it was completely soluble in water, and slightly insoluble in chloroform but insoluble in other solvents

The FTIR spectra of Amikacin showed typical characteristic bands at 3340.35 corresponding to the presence of Aliphatic N-H Stretching. The absorption peak at 2570.86 represented Sulfonyl-chloride S=O Stretching (Fig. 2). FTIR of PLGA showed 3778.4 corresponded to the presence of Alcohol O-H Stretching. The absorption peak at 1594.59 represented Nitro-compound N-O Stretching (Fig. 3). FTIR Spectra of HPMC showed typical characteristic bands at 3695.88 corresponding to the presence of Alcohol O-H Stretching. The absorption peak at 2036.52 represented Isothiocyanate N=C=S Stretching (Fig. 4). All the absorption peaks suggested that there is no significant bond shift and hence no interaction of the individual Drug and Polymers (Fig. 5)[19].

Differential Scanning Calorimetry (DSC) DSC Thermogram of Amikacin had a sharp exothermic peak absorbed at 232.68ºC (Fig. 6). DSC Thermograph of PLGA had a sharp exothermic peak absorbed at 48. 314.99ºC (Fig. 7). DS Thermogram of HPMC were sharp exothermic peak absorbed at 328.76ºC (. DSC Thermograph of a combination of Drug and Polymer (or excipients) similar peaks were observed in the formulation thermogram which confirms there are no significant changes in melting point or any change in drug & polymer, thus revealing that there is no physical compatibility between drug and polymer[20].

The drug content of all formulations showed the ANF1 -ANF4. The drug content was found to be in the range of 80.65% and 79.85%, indicating the uniform distribution of the drug. Although drug content expresses the per cent weight of active ingredient entrapped to the weight of nanoparticles. The drug content depends on the polymer-drug combination and method used.

Entrapment efficiency is the ratio of the experimentally determined percentage of drug content compared with the actual, or theoretical mass, of drug used for the preparation of the nanoparticles. The values were in the range of 76.30 % TO 80.65 % respectively[19].

The hydrodynamic size of GM-PLGA NPs were in the range of 264.0 nm-278.3 nm (Fig. 12) which was ideal for the nanoparticle based drug delivery systems[19]. The minimum polydispersity index was in the range of 0.419-0.486 confirming the homogenous dispersion of NPs. Zeta potential was in the range of 29.1-34.1 (Fig. 13). In SEM, the lyophilized NPs showed porous morphology which is ideal for maximum drug entrapment (Fig. 14)[19]. The physical appearance of Amikacin -TDP is shown in (Fig. 15).

They were subjected to a battery of various physiochemical tests i.e. weight variations, thickness, folding endurance, flatness, and % moisture content. In case of weight variations, patches showed minor variations in weight hence showed that they were of almost equal weights. Weight variation were minimum with formulation F3 (2.945 ± 0.054 g) while maximum variation was shown by F1 (2.309 ± 0.030 g) as shown in (Fig. 16). All the formulations of Amikacin-TDP showed good values of flatness in the range of 53.78 ± 1.43 to 78.34 ± 0.89% as shown in (Fig. 17). In case of thickness testing, Amikacin-TDP showed a thickness range of 0.1944 ± 0.008 cm to 0.0145 ± 0.049 cm as shown in (Fig. 18). The percentage drug content in all four formulations are similar, but formulation F3 has showed the maximum drug content than other remaining formulations was showed in the range of 71.52±0.31 – 78.63±0.23 (Fig. 19). In case of folding endurance, maximum folding times were shown by F3 (30.1 ± 4.69) while minimum by F1 (21.2 ± 2.74) as shown in (Fig. 20). In case of % moisture content was showed maximum moisture content in F1 (13.75±0.15) and minimum moisture content in F3 (6.66±0.18) (Fig. 21).

In the case of a transdermal patch, “F3” showed the best release (98.6%) while “F1” and “F4” exhibited 88.4% and 81.4% respectively till 24th hour of study at pH 6.8 (Fig. 22).

Conclusion

It was concluded that solvent evaporation was a useful method for the successful incorporation of Amikacin with high entrapment efficiency. Prepared nanoparticle loaded transdermal patch of Amikacin using the PLGA and HPMC polymers as showed the promising results for all the evaluated parameters. The developed nanoparticle-loaded transdermal patches increase the therapeutic efficacy and bioavailability of Amikacin.

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