STUDIES ON DELTAMETHRIN LOADED MOSQUITO REPELLENT PATCH

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She had carried out this research based project work independently with proper care and attention. I wish her a bright academic and professional career.

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*I hereby declare that the present thesis entitled "**Studies on Deltamethrin Loaded Mosquito Repellent Patch**" submitted to Gauhati University, is a bona fide and genuine project work carried out by me in Defence R and D Organization, Defence Research Laboratory, Ministry of Defence, Tezpur, Assam under the joint supervision of Dr. Pronobesh Chattopadhyay and Dr. Bipul Nath, GIPS.*

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This thesis is dedicated to the lotus feet of my family members who inspired me to face life with courage and dignity.

Contents…

Page no.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SYMBOLS	ABBREVIATIONS	SYMBOLS	ABBREVIATIONS
%	Percentage	FT-IR	Fourier Transform Infrared
ml	Milliliter	SEM	Scanning Electron Microscopy
% w/w	Percentage weight by weight	DSC	Differential Scanning Calorimetry
% v/v	Percentage volume by volume	EC	Ethyl Cellulose
cm	Centimeter	PVP K-30	Polyvinylpyrrolidine
Kg	Kilogram	DBT	Di-butyl-phthalate
Mg	Milligram	HPLC	High Performance Liquid Chromatography
Mol.	Molar	Vol.	Volume
°C	Degree Celsius	SL.	Serial
µg	Microgram	SD	Standard Deviation
LD	Lethal Dose	ANOVA	Analysis of Variance
hr	Hour	DMSO	Dimethyl Sulfoxide
gm	Gram	IUPAC	International Union of Pure & Applied Chemistry
mv	Millivolt	nm	Nanometer
min.	Minutes	Fig.	Figure
%RH	Percentage Relative Humidity	Sec.	Seconds
rpm	Revolutions Per Minute	No.	Number

Abstract

Despite tremendous advancements of synthetic mosquito repellent preparations, the focus of the present studies is carried out to design and evaluate a deltamethrin loaded patch using blends of two different polymeric combinations of polyvinylpyrrolidone (PVP) and ethyl cellulose (EC) with a view to prolong the repelling action towards mosquito by solvent evaporation method over a backing membrane. Di-butyl-phthalate (DBT) was used as a plasticizer. The study also involves the effect of formulation variables on matrices. The chromatographic technique (HPLC) has been used for quantitative estimation of deltamethrin. All the prepared formulations were subjected to physico-chemical studies like deltamethrin content, thickness, weight variation, percentage moisture content, moisture uptake, surface area, and surface pH determination. The results of physicochemical studies indicate the suitability of patch formulation of deltamethrin with the polymeric combinations of PVP and EC. FT-IR and DSC studies indicated compatibility between the deltamethrin and the excipients employed in the fabrication of patches. SEM study revealed the homogeneous dispersion of the deltamethrin in the polymeric matrices. The best optimized deltamethrin-loaded formulation i.e. A5 shows Fickian diffusion controlled release mechanism governed by Higuchi kinetics. Inhalation pattern of the animals was found within the normal range. Landing repellent activity of deltamethrin-loaded mosquito repellent patches against *A. albopictus* performed under laboratory conditions found that there is no difference in both percent repellency and repellency index of mosquitoes at various time intervals. Similarly, no difference could be observed for percent repellency and repellency index biting of *A. Albopictus* mosquitoes.

Introduction…

CHAPTER NO. 1

Literature Review...

CHAPTER NO. 2

Aims and Objectives…

CHAPTER NO. 3

Profiles of Deltamethrin, Polymers and Plasticizer…

CHAPTER NO. 4

Materials and Methods...

CHAPTER NO. 5

Results...

CHAPTER NO. 6

Discussion. . .

CHAPTER NO. 7

Conclusion…

CHAPTER NO. 8

References...

CHAPTER NO. 9

1. INTRODUCTION

Mosquitoes are found all over the world, except in Antarctica. These two-winged insects belong to the order Diptera. Members of the genera *Anopheles*, *Culex*, and *Aedes* are most commonly responsible for bites in humans. There are approximately 170 species of mosquitoes in North America alone (Fradin & Day 2002). Mosquitoes and other biting arthropods are pests because of their biting activity and their ability to carry and transmit arthropod borne diseases (Olkowski 2001). Arthropod borne infectious diseases pose prime health risk to over billions of people primarily in the tropical countries. Use of personal protection against insect bites and control interventions are currently the most important measures to control insect vector borne diseases (Hazarika *et al*., 2012 & Tawatsin *et al*., 2001).

Malaria affects more than 250 million people and causes more than a million deaths each year (World Health Organization, WHO 1990). One important control strategy against this and other mosquito-borne diseases is mosquito control, which aims to reduce human-mosquito contact. Different control measures are used routinely against mosquitoes and their larvae, including chemical (e.g. insecticide), biological (e.g. larvivorous fish or pathogenic fungi), environmental (e.g. land filling or drainage), and personal protection (e.g. mosquito repellents formulated as pills, coils, ointments, lotions, and sprays; and insecticide-treated or untreated bed nets). Traditionally, early searches for new topical insect repellents for protection of humans against biting of disease vectors relied on the screening of candidate compounds applied to the skin of human volunteers (Smith & Burnett 1948). As late as 1970, Schreck *et al*., described repellent tests in which compounds of unknown toxicity were applied to the hands of human subjects. Today, this is an unthinkable practice

from a human-use safety viewpoint. Although human-biting testing is probably the most effective method to study and characterize repellent compounds (Schreck *et al*.,1989; Barnard *et al*., 1999) limited to study of compounds known to be safe for application to humans. This toxicological limitation severely restricts chemical screening programs for discovery of new and effective arthropod repellents for human use.

1.1. Definition of mosquito repellent:

A mosquito repellent is a substance applied skin, clothing, or other surfaces which discourage insects (and arthropods in general) from landing or climbing on that surface.There is also mosquito repellent products available based on sound production, particularly ultrasound (Mishra *et al*., 2010). Mosquito repellents may be one of the most effective tools for protecting humans from vector-borne diseases, such as dengue hemorrhagic fever, malaria, encephalitis, and filariasis, as well as the nuisance caused by mosquitoes (Barnard *et al*., 2000; Asidi *et al*., 2005). Mosquito repellents are substances that are designed to make surfaces unpleasant or unattractive to mosquitos. An ideal mosquito repellent that provides this long-term duration of efficacy against all mosquito species has not yet been identified (Fradin & Day 2002) despite an extensive research program that was initiated over 60 year ago.

1.2. Classification of mosquito repellents:

1.2.1. Chemical methods

A. Synthetic repellents e.g. DEET (N, N-diethyl-3-methylbenzamide), permethrin and deltamethrin

B. Natural repellents e.g. neem oil, citronella oil

1.2.2. Non-chemical methods

A. Physical method

i. Medicated Net

ii. Non Medicated Net

iii. Mosquito Traps

B. Mechanical methods

i. Electric mosquito zapper

ii. Mosquito Magnet

1.2.3. Biological methods

Biological methods by growing some fish species that feeds on mosquito larvae in the water bodies.

1.3. Introduction to chemical mosquito repellents:

1.3.1. Chemical mosquito repellents:

Repellents are applied to the skin, used to treat clothing, or released into the air. There are a variety of synthetic and plant-derived chemicals known to repel mosquitoes. Few are considered safe enough to be applied repeatedly to the skin. The ideal repellent compound would prevent bites from a broad range of arthropod species, remain effective for at least 8 hour, cause no irritation to the skin or mucous membranes, possess no systemic toxicity or plasticizing effect, be resistant to abrasion and rub off, and be totally greaseless and odorless (Fradin 1998). Repellents may

provide low-dose; specific and low-toxicity augmentations to conventional pesticides applied around the home and workplace (Peterson 2003). They are available in many forms, from creams to lotions to oils, but are most often sold as aerosol products. The synthetic repellents have been proven effective against the insect bites. Several synthetic and natural substances are being used as mosquito repellents and have been registered as insect repellents by the United State (US) environmental protection agency. The most common mosquito repellent formulations available on the market contain DEET (N, N-diethyl-3-methylbenzamide) and deltamethrin which has shown excellent repellency against mosquitoes and other biting insects (Yap 1986; Walker *et al*., 1996).

The repellents are practical and economical means of preventing the transmission of these diseases to humans. One commonly advocated approach for preventing mosquito attack is personal protection. This method allows an individual to select from (or combine) avoidance techniques, exclusion of mosquitoes with physical and chemical barriers, treatment of fabric with toxicants, and the use of topical (skin) repellents (Barnard *et al*., 2001). Application of repellents to the skin is a common personal protection practice. The most common mosquito repellent formulations available on the market contain DEET (N, N-diethyl-3-methylbenzamide) and deltamethrin which has shown excellent repellency against mosquitoes and other biting insects (Yap 1986; Walker *et al*., 1996). The most widely used synthetic chemical mosquito repellent is deltamethrin. This substance remains the gold standard of currently available insect repellents. Therefore, number of synthetic repellents using different technologies has been explored to successful patch with improved performance, safety and efficiency in repelling action. Synthetic

repellents are widely used for personal protection against insect bites. The centers for disease control and prevention (CDC) estimates that approximately 30% of the US population uses an insect repellent each year, and worldwide use exceeds 200 million applications annually (Barnard *et al*., 2001; WHO 1990).

Advantages of synthetic repellents: Synthetic repellents containing DEET or picaridin are more effective than repellents with "natural" active ingredients. All the synthetics gave almost 100% repellency for the first 2 hours, where the natural repellent products were most effective for the first 30 to 60 minutes, and required re-application to be effective over several hours.

1.4. Mechanism of action of mosquito repellents:

Carbon dioxide, excretory products and lactic acid present in sweat in warm-blooded animals act as an attractive substance for female mosquitoes. The perception of the odour is through chemo-receptors present in the antennae of mosquitoes. The repellents block the lactic acid receptors thus destroying upwind flight and as a result the mosquito loses its contact with the host. Usually insect repellents work by masking human scent, or by using a scent which insects naturally avoid (Elissa *et al*., 2004; Mishra *et al*., 2010).

1.5. Sustained release:

There are many definitions of sustained release but the simplest definition is "Any drug or dosage form or medication that prolongs the therapeutic activity of drug" (Gudsoorkar & Rambhau 1993). Among various dosage forms, matrix tablets are widely accepted for oral sustained release (SR) as they are simple and easy to formulate. Matrix system is the release system, which prolongs and controls the

release of drug that is dissolved or dispersed. Sustained release formulations are preferred for such therapy because they maintain uniform drug levels, reduce dose and side effects, better patient compliance, and increase safety margin for high potency drugs. It remains the preferred route of administration investigated in the discovery and development of new drug candidates and formulations (Chein 2005).

In some SR formulations, the drug dissolves into the matrix, and the matrix physically swells to form a gel, allowing the drug to exit through the gel's outer surface. During the last two decades there has been remarkable increase in interest in sustained release drug delivery system. This has been due to various factor viz. the prohibitive cost of developing new drug entities, expiration of existing international patents, discovery of new polymeric materials suitable for prolonging the drug release, and the improvement in therapeutic efficiency and safety achieved by these delivery systems. Now-a-days the technology of sustained release is also being applied to veterinary products. These systems also provide a slow release of drug over an extended period of time and also can provide some control, whether this is of a temporal or spatial nature (Sampath *et al*., 2012). Controlled drug release systems can be assembled from either polymers or pumps. Because of their small size and lower cost, polymers are most widely employed (Langer 1993). As polymer science has developed over the past two centuries with the number of innovative architectures, polymer-based products and pioneering process technologies are playing a very important role in medicine and pharmacy (Kim *et al*., 2007).

1.6. Sustained release patch:

Matrix type patches remain among the most popular, as they are easy to manufacture (Chakkapan *et al*., 1994). Pharmaceutical invention and research are increasingly focusing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects. Patches are new approaches for enhancement of the efficacy of many therapeutic agents and defined as self contained, discrete dosage form which when applied to intact skin delivers the active constituent at a controlled release rate. It offers many advantages over conventional injection and oral methods. It reduces the load that the oral route commonly places on the digestive tract and liver. It enhances patient compliance and minimizes harmful side effects of a drug caused from temporary overdose. Another advantage is convenience, especially notable in patches that require only once weekly application. Such a simple dosing regimen can aid in patient adherence to drug therapy.

Designing and development of controlled release patches can be described as state of the art. Literature survey revealed that use of ethyl cellulose (Someswara Rao 2011) and PVP K 30 (Priyanka Tripathi 2009) as polymers forms matrix patches and gives release for more than 10 hours. They provide a relatively constant release which may lead to decreased side effects. There are two main classes of patches according to their mechanism of drug release (Barry 2002; Uhrich *et al*., 1999). In membrane patches, a polymeric layer modifies the drug release, while in matrix or monolithic patches, a hydrophilic or hydrophobic polymeric matrix controls the release profile (Margetts & Sawyer 2007). An appropriate selection of the polymer matrix is necessary in order to develop a controlled release patches.

1.7. Design of types of patches:

A system designs have been used in development and fabrication of deltamethrin loaded patch. In matrix type patches, drug reservoir is prepared by dissolving the drug and polymer in a common solvent. The insoluble drug should be homogeneously dispersed in hydrophilic or lipophillic polymer. The required quantity of plasticizer like di-butyl-pthalate, triethylcitrate, polyethylene glycol or propylene glycol is then added and mixed properly. The medicated polymer formed is then molded into rings with defined surface area and controlled thickness over the backing membrane on horizontal surface followed by solvent evaporation at an elevated temperature. The film formed is then separated from the rings, which is then mounted onto an occlusive base plate in a compartment fabricated from a drug impermeable backing. Adhesive polymer is then spread along the circumference of the film. Commonly used polymers for matrix are cross linked polyethylene glycol, eudragits, ethyl cellulose, polyvinylpyrrolidone and hydroxypropylmethylcellulose. The dispersion of drug particles in the polymer matrix can be accomplished by either homogenously mixing the finely ground drug particles with a liquid polymer or a highly viscous base polymer followed by cross linking of polymer chains or homogenously blending drug solids with a rubbery polymer at an elevated temperature.

1.8. Aedes (S.) albopictus :

The tiger mosquito or forest day mosquito, *Aedes albopictus* (*Stegomyia albopicta*), from the mosquito (*Culicidae*) family, is characterized by its black and white striped legs, and small black and white striped body. It is native to the tropical

and subtropical areas of Southeast Asia; however, in the past couple of decades this species has invaded many countries throughout the world through the transport of goods and increasing international travel (Scholte *et al*., 2007). It is an important pest mosquito and potential vector of dengue in the Southeastern United states (Moore *et al*., 1988). Because Ae. albopictus larvae are found in both natural and artificial containers, control programs focus on source reduction rather than insecticides. Insecticides are usually avoided for larval control because potentially thousands of containers must be found and treated, making this an extremely labor-intensive undertaking (Nasci *et al*., 1994). This mosquito has become a significant pest in many communities because it closely associates with humans (rather than living in wetlands), and typically flies and feeds in the daytime in addition to at dusk and dawn. The insect is called a tiger mosquito because its striped appearance is similar to a tiger.

Aedes albopictus is an epidemiologically important vector for the transmission of many viral pathogens, including the West Nile virus, Yellow fever virus etc. A test was made using adult Aedes (S.) albopictus (an exotic species). Aedes albopictus is a known vector of dengue (Hawley 1988). It is persistent biters and common pests of humans, mammals, and birds. This species are known for contact with the WN virus transmission cycle in North America (Turell *et al*., 2001) and together support virus transmission in more than three dozen states in the United states (Moore & Olivia 1997).

2. LITERATURE REVIEW

Recently, several new commercially developed mosquito control devices have become available to control mosquitoes and other biting arthropods. These products claim that they can significantly reduce or even eliminate the number of mosquitoes and other biting arthropods present by effectively trapping or repelling them from residential properties (American Biophysics Corporation 2004, Bio Sensory 2004, Schawbel Corporation 2004). The removal of mosquito breeding areas, chemical treatment of mosquitoes using pesticides and repellents, or avoidance of outside activities when mosquitoes are active are often the only effective options a homeowner has to prevent from being bitten by mosquitoes. Although the use of pesticides works to reduce or eliminate mosquitoes from a treated area, it is often a temporary measure and not always environmentally safe. In the past, homeowners relied heavily on pesticides as the primary tool for controlling mosquitoes and other biting arthropods from their yards.

Khoobdel *et al*., (2011) evaluate the efficacy of deltamethrin and find a relation between persistence and residue of this insecticide on the prevalent surfaces against malaria vectors in Southeastern Iran and found there was no significant differences between mortality rate of *An. stephens* in bio-assay tests on prevalent surfaces (plaster, mud and wood surfaces). This difference is only about times of tests and after 120 days of spraying, this insecticide kept its utility in the malaria vector control. Thus after 120 days after spraying, average of mortality rate on these surfaces reached to 60%.

Sunil *et al*., (2012) evaluated the repellent activity of mixture of *Curcuma longa*, *Zanthoxylum limonella* and *Pogostemon heyneanus* essential oils in 1:1:2 ratio at 5%, 10% and 20% concentration against blackflies in Northeastern India. Initially the essential oil mixture tested here has been found effective against *Aedes albopictus* mosquitoes. No appreciable clinical and behavioral signs were observed in the acute dermal toxicity using rat model. No changes were observed in biochemical profiles of treatment group animals.

Larviciding is often done by professionals and has been shown to provide various levels of control depending on the chemical formulation. For example, one study conducted by Nasci *et al*., (1994) indicated that the Abat pellet (temephos, active ingredient), Altosid pellet (methoprene, active ingredient) and Altosid and formulations were able to provide excellent control of *Aedes albopictus skuse* for up to 150 days when applied at 2 grams per container to small breeding sites in Lake Charles, Louisiana.

In field studies, (Olkowski 2001) has been shown Bti (*Bacillus thuringiensis* var. *israelensis*) be effective against several mosquito species in widely differing water quality conditions, including irrigated pastures, storm drains, ponds, dairy lagoons, and salt marsh potholes. Similarly, no prominent lesions were observed in vital organs of treatment in both the sexes. The study concludes that tested repellent is safe for use and has multi-insects repellent property.

Winner *et al*., (1989) demonstrated the effectiveness of using Raid yard guarda readily obtainable consumer product, to control *Wyeomyia* in Bromeliads for up to 35 days. Burning mosquito-coils containing pyrethrin or a synthetic pyrethroid

have also been used by consumers as a means to reduce numbers of biting mosquitoes However, health problems resulting from exposure to allethrin-containing mosquito-coil smoke and possible ineffectiveness make this control method unreliable (Chang & Lin 1998). Another control uses outdoor foggers or sprayers with insecticides such as carbaryl, malathion, resmethrin or other pesticide formulations approved for residential use to treat the yard around the home. Outdoor spraying and fogging can be effective, but limiting factors such as coverage of areas treated or rapid mosquito reinfestation of the treated area can reduce treatment effectiveness (Mount *et al*.,1998).

Hao *et al*., (2012) assessed the effectiveness of mosquito attractants and repellents against *Aedes albopictus* using an olfactometer. The results of the tests indicate that the L-lactic acid/dichloromethane mixture may be used as an effective attractant to evaluate the effect of possible spatial repellents on *Aedes albopictus.*

Perich *et al*., (2003) in another study, a lethal ovitrap designed and developed to kill dengue vectors via an impregnated insecticide-treated ovistrip was evaluated in Rio de Janeiro, Brazil, and shown to be effective in reducing *Aedes aegypti* (L.) populations in and around homes. However, further evaluations are required to determine if the lethal ovitrap is effective in reducing artificial container breeding mosquito species in other regions of the world where they are a problem.

Homeowners may be able to rely on commercially available repellents instead of pesticides to reduce the biting pressure and nuisance of mosquitoes and other biting arthropods when engaging in activities outside the home. Of these, DEET (N, N-diethyl-3-methylbenzamide) is the most widely used with 230 DEET-containing

products from nearly 70 companies. Repellents also provide an excellent alternative protection to homeowners who are sensitive, allergic or concerned about the use and toxicity of pesticides (Peterson 2003). Other repellents such as KBR (picaridin), citronella (p-methane-3,8-diol), IR3535 based on the structure of the amino acid alanine, registered on February 1999.

Rowland *et al*., (2004) worked over DEET mosquito repellent that provides personal protection against malaria a household randomized trial in an Afghan refugee camp in Pakistan tropical medicine internatioanl health. Results test indicating DEET as an effective repellant among synthetic mosquito repellant and also study on DEET mosquito repellent sold through social marketing provides protection against malaria in an area of all-night mosquito biting and partial coverage of insecticide-treated nets:a case-control study of effectiveness.

` Hougard *et al*., (2006) study on lethal and behavioral effects of three synthetic repellents (DEET, IR3535 and KBR 3023) on *Aedes aegypti* mosquitoes in laboratory assays medical and veterinary entomology and they discuss on repellent can refer to molecules that may alter the functioning of sensory motor systems and have neurotoxin effects and to determine the exact mode of action of repellent and their physiological target sites in order to provide chemists with a rational guide for the development of more effective insect repellents that are safer in terms of public health.

Veltri *et al*., (2002) study human exposures to diethyt-m-toluamide insect repellents and reported out to the American association of poison control centers and finding out the toxicity of DEET over skin specially modern vector management

programs rely heavily on environmental manipulation, also referred to as source reduction or physical control Eldridge & Edman (2000) , and emphasis on non-chemical control methods is the preferred procedure for removal of all possible mosquito-breeding habitats from the property.

Schofield & White (1983) illustrated the importance of a good house design, which can eliminate breeding-sites for synanthropic arthropods such as fleas, bed bugs, ticks and container-breeding mosquitoes.

Rowland *et al*., (2004) worked on evaluation of synthetic repellents on mosquito nets in experimental huts against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes owing to the development of pyrethroid resistance in *Anopheles gambiae*, there is a need to develop chemical alternatives for use on mosquito nets.

To ensure that future mosquito outbreaks do not re-occur, educating the public on personal protective measures, elimination or destruction of mosquito larval habitats and protecting against day-biting mosquitoes, including the use of screening, protective clothing and repellents is an absolute must.

In a recent study by Hougaard & Dickson (1999) the mosquito magnet (American biophysics corporation North Kingstown), a mosquito collection device, reduced *Aedes sierrensis* ludlow, the western tree hole mosquito, populations when used in close proximity to breeding sources in around residential homes. However, only *Aedes Sierrensis* was caught using the mosquito magnet and other species of mosquitoes such as *Culex pipiens* (L.) were not captured by the trap during the study. Also, the study was of limited duration (2 months) and did not determine if

the mosquito magnet provided long-term control of *Aedes sierrensis*. The mosquito magnet has been shown to be an effective mosquito-sampling device for *Anopheline* mosquitoes such as *Anopheles sinensis* (Burkett *et al*., 2001).

In related work, (Burkett *et al*., 2002) reported that the mosquito magnet could significantly enhance current vector and disease surveillance efforts especially for the primary vector of Japanese encephalitis,*Culex tritaeniorhynchus* giles. Significantly greater numbers of mosquitoes were captured with mosquito traps using counterflow technology (e.g., mosquito magnet and counter flow geometry traps) when compared to standard light and carbon dioxide baited traps.

Godin *et al*., (2007) reviewed work from *in-vitro* permeation studies to clinical performance, presenting various experimental models used in dermal/transdermal research, including the use of excised human or animal skin, cultured skin equivalents and animal.

Barnard *et al*., (2001) studied on acute toxicity and persistence of three insect repellents, deet and two piperidines (AI3-35765 and AI3-37220), and evaluated against mosquito larvae of *Aedes albopictus* and *Anopheles albimanus Wiedemann* (Diptera:*Culicidae*) in the laboratory, and against natural populations of Ae. albopictus in the field and found a topical repellents (particularly AI3-37220) have good potential for development and use in the management of container-inhabiting mosquitoes because they deter oviposition and kill larvae.

Krzysztof *et al*., (2001) study on various *in-vitro* studies on penetration of terpenes from matrix-type transdermal systems through human skin. Polyurethane matrices containing up to 39% of the terpenes eucalyptol, L-limonene, D-limonene,

dipentene or terpinolene were produced. Release of the terpenes directly to the acceptor fluid, as well as through isolated human epidermis and dermis, was studied. In the presence of dermis the penetration profiles were very similar to the release profiles, indicating that dermis does not present a barrier for penetration of terpenes. For all terpenes the penetration was slower in the presence of epidermis. Release and penetration through the epidermis and dermis were fastest for dipenetene (mixture of D-limonene and L-limonene), being at least 3-4 times faster than for D-limonene and L-limonene. Large amounts of terpenes found in epidermis (approximately 1.5 mg/cm2) indicate that affinity of these compounds to the stratum corneum is very high.

Lakade *et al*., (2008) formulated and carried evaluation of sustained matrix tablet of anti-anginal drug, influence of combination of hydrophobic and hydrophillic matrix form, the objective of the present study was to develop hydrophilic polymer (HPMC) and hydrophobic polymer (Ethyl cellulose) based nicorandil matrix sustained release tablet which can release the drug up to time of 24 hrs in pre-determined rate. The formulation of nicorandil matrix tablet was prepared by the polymer combination in order to get required theoretical release profile. The influence of hydrophilic and hydrophobic polymer and granulation technique on nicorandil was studied. The formulated tablet was also characterized by physical and chemical parameters.

Dangi *et al*., (2012) developed a suitable matrix type transdermal patch of ondansetron hydrochloride using blends of two different type of polymeric combinations viz. PVP and HPMC K4 M and Eudragit L100 with

Povidone (PVP). The prepared formulations were subjected to various physiochemical evaluation test like moisture content loss, moisture absorption, flatness to study the stability of the formulations, *in-vitro* dissolution was performed to determine the amount of ondansetron hydrochloride present in the patches. Drug excipient interaction studies were carried out using Fourier transform infrared (FT-IR) spectroscopy technique. The *in-vitro* release of the drug from the formulations was studied using commercial semi permeable membrane. All the formulations were found to be suitable for formulating in terms of physiochemical characteristics and there was no notification in significant interaction between the drug and polymer used. *In-vitro* dissolution data showed that formulation of PVP: HPMC K4 M showed faster release of drug than the PVP: Eudragit L100 formulations during skin permeation studies. Skin irritation studies revealed that the batch containing PVP-Eudragit L100 has no erythema and oedema. Also reveal that PVP- eudragit L100 polymers are better suited than PVP-HPMC K4 M polymer for the development of ondansetron hydrochloride transdermal patches.

Kevin *et al*.,(2009) work, monolithic matrix transdermal systems containing tramadol HCl were prepared using various ratios of the polymer blends of hydroxy propyl methyl cellulose (HPMC) and Eudragit S 100 (ES) with triethyl citrate as a plasticizer. The concentration of HPMC and ES were used as independent variables, while percentage drug release was selected as dependent variable. Physical evaluation was performed such as moisture content, moisture uptake, tensile strength, flatness and folding

endurance concluded that medicated monolithic matrix transdermal ystems can be prepared from blends of HPMC and ES showed good mechanical performance. When high mechanical performance is required, higher amount of ES in the blends have to be used. *In-vitro* drug release profile indicates that the drug release is sustained with increasing the amount of ES in the blends and selection of a particular blend formulation can vary the diffusion of the drug significantly.

Shalin *et al*., (2011) review on sustained release drug delivery system. Now a days as very few drugs are coming out of research and development and already existing drugs are suffering the problem of resistance due to their irrational use specifically in case of drugs like antibiotics. Hence, change in the operation is a suitable and optimized way to make the some drug more effective by slight alteration in the drug delivery. Sustained release is also providing promising way to decrease the side effect of drug by preventing the fluctuation of the therapeutic concentration of the drug in the body.

Tyagi *et al*., (2010) studies on deltamethrin treated mosquito net. Study and characterize mosquito net for its material of construction and qualitative as well as quantitative determination of the mosquito repellent chemical deltamethrin present in it. Further assessment of the mode of incorporation of the deltamethrin in the fabric of the mosquito net was done, *i.e.* whether the deltamethrin was present as a coating on the surface of the mosquito net or it was incorporated in the bulk of the material of construction of the mosquito net.

Hazarika *et al*., (2012) carried out to evaluate the repellent activity of synthetic insecticides against vector mosquitoes, which suggest that synthetic insecticides have been highly effective in providing repelling the mosquitoes and other biting insect. The synthetic insecticides have been found to be consistent in activity even after a long time of use unlike the herbal based repellent which provide repellency initially but after some time the activity ceases .

Lt Col Bhatnagar & Col Mehta (2007) indicated that the synthetic repellents while applied along with the other repellents provide much more protection against mosquitoes as compared to applied singly.

Das *et al*., (2003) studied on repellent properties of three plant extracts-essential oil (steam distillate) of *Zanthoxylum limonella* (fruits), *Citrus aurantifolia* (leaf) and petroleum ether extract of Z. *limonella* (fruits) were evaluated as repellent against *Aedes* (S.) *albopictus* mosquitoes in mustard (Dhara) and coconut (Parachute) oil base under laboratory conditions. Three concentrations-10, 20 and 30% of the repellents were evaluated. Repellents in mustard oil afforded longer protection time against the bites of *Aedes* (S.) *albopictus* mosquitoes than those in coconut oil. At 30% concentration, 296-304 min protection time was achieved by the test repellents in mustard oil base while repellents in coconut oil exhibited 223.5-245 min protection time at the same concentration. Oil of *Z. Limonella* gave the highest protection time against the bites of *Aedes* (S.) *albopictus* mosquitoes at all theconcentrations than other herbal repellents tested both in mustard and coconut oil.

Shrivastava *et al*., (2011) studied on impact of deltamethrin on environment, use as an insecticide and its bacterial degradation . Worked over applying for a range of commercial crops and recreational uses, and by extension controls a variety of pests. The level of deltamethrin biodegradation in mixed cultures of benthic and plank tonic bacteria after 5, 10, and 15 days of incubation was higher than that in homogenous cultures. It was demonstrated that microorganisms from the *Sphingomonas paucimobilis* species and the *Moraxella* genus, among plank tonic bacteria, as well as *Burkholderia cepacia* and *Bacillus mycoides* species, among benthic bacteria, were the most effective in reducing the concentration of this insecticide.

Sarita *et al*., (2011) evaluate the effects of deltamethrin against field-collected adults of *Aedes aegypti* (*L*.) . Adults were selected with 0.025% deltamethrin for 40 successive generations. The selected adults were tested with 4% DDT and the emerging larvae were tested with various insecticides to study the cross-resistance spectrum. The knockdown and irritability studies were carried out in adult mosquitoes to investigate their behavioural response to deltamethrin. Suggesting the prolonged effective use of deltamethrin against *Aedes aegypti* as an adulticide.

3. AIMS & OBJECTIVES

3.1. Specific objectives:

To reach at the goal of the aim with following objectives have been set forth:

(i) To formulate deltamethrin-loaded sustained release mosquito repellent patches.

(ii) To evaluate the *in-vitro* physico-chemical characterization of deltamethrin loaded patches.

(iii) To access its mosquito repellency effectiveness against adult female *Aedes* (S.) *albopictus.*

3.2. Need of the study:

Despite tremendous advancement in the mosquito repellent preparations, external topical repellent preparations are preferred over other form of preparations, as they directly repel the mosquito from its bite. Commonly marketed repellent preparations vaporize without decomposition on heating at temperatures up to 400°C and produce varying repellent action on the mosquitoes, depending on the type of product and species of mosquito. As they produce smoke and as long as the compounds are vaporizing they give repellent action, later the repellent action is lost. There are hundreds of mosquito repellent companies in the market that are tricking people into purchasing non effective mosquito repellants. This is dangerous because diseases contracted from mosquitoes account for millions of deaths throughout the world. The problem is not with the repellent drugs, but the inefficient and poor delivery methods used sprays/lotions and pills are old fashioned and don't come close to the delivery capacity of patches. Therefore, number of synthetic repellents using different technologies has been explored to successful patch with improved performance, safety and efficiency in repelling action.

4. DELTAMETHRIN PROFILE

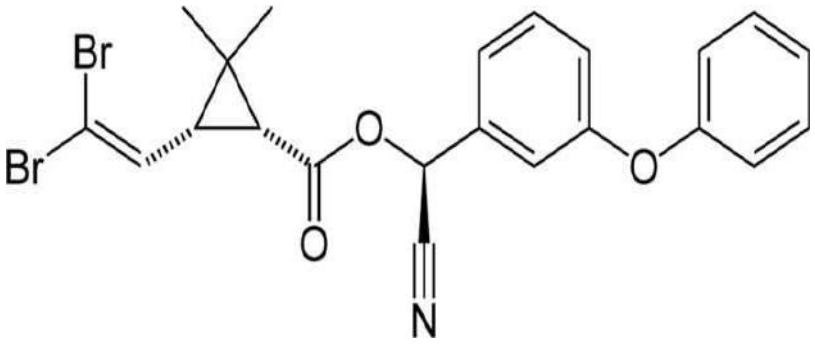
4.1. Common name: Deltamethrin

Physical and chemical properties: (Grelet 1990 & Lambert 1991)

Chemical name: (1R, 3R) [α-cyano (3-phenoxyphenyl) methyl] 3-(2, 2-dibromo-ethenyl)-2,2-dimethylcyclopropanecarboxylate (IUPAC)

Trade names: Butoflin ™ ; Butox ™; Decis ™; K-Othrin ™; K-Othrine ™ Dust; Striker ™ IEC insecticide

Chemical structure:



Empirical formula: $C_{22}H_{19}Br_2NO_3$

CAS registry number: 52918-63-5

Molecular weight: 505.2 gm/mole

Specific gravity: 1.053 g per mL at 20°C (Decis™) 0.5 g per mL at 20°C (Technical Grade of AI, TGAI)

Physical state: White to beige crystalline powder

Melting point: 98-101 °C

Solubility: Soluble in acetone, dimethylformamide, dioxane, ethyl acetate, and toluene (all 23-39%), relatively insoluble in water

Vapor pressure: 1.5 x 10^{-8} mmHg at 25°C (>90% AI) Octanol/Water

Partition coefficient: 2.7 x 10^{5} at 25°C

pH: 5.9 in a 1% aqueous dispersion

4.1.1. Description:

Pyrethroids insecticides are widely used insecticides in both agricultural and urban environments because of their high insecticidal effects, their relatively low mammalian toxicity, and their biodegradability (Motomura *et al*., 2001). They are widely used in agriculture to protect crops, in the household tom control pests, and in public health to control diseases caused by vectors or intermediate hosts (IPCS, 1990; Soderlund *et al*., 2002). Pyrethroids are synthetic analogs of pyrethrins, which are natural esters found in the pyrethrum extract from the flower heads of *Chrysanthemum cinerariaefolium*. They have been used extensively for over forty years and comprise 25% of the worldwide insecticide market (Shafer *et al*., 2005). That percentage share has increased substantially over the last few years in the United States (Lee *et al*., 2002), as a result of the United States environmental protection agency's (EPA's) restrictions on household and agricultural use of organophosphates. Pyrethroids were developed due to the instability that pyrethrins have to light and air. Pyrethroids have both greater stability and greater potency than their natural counterparts (Casida 1980). Pyrethroids popularity also stems from their insecticidal potency, slow development of pest resistance, and relatively low toxicity of most congeners in mammals.

Deltamethrin has very broad spectrum control (Tomlin 2006). It was first synthesized in 1974 and first marketed in 1977 (Vaysse *et al*., 1984). Deltamethrin is a synthetic pyrethroid insecticide which possesses an extremely high level of activity against a wide range of insects. It acts by both direct contact and ingestion (Worthing & Walker 1987). Technical grade deltamethrin contains more than 98% deltamethrin (FAO/WHO, 1981). It is stable to heat (6 months at 40 °C), light, and air, but unstable in alkaline media (FAO/WHO, 1981; Meister *et al*., 1983; Worthing & Walker 1983). Deltamethrin was first synthesized in 1974 and first marketed in 1977 (Vaysse *et al*., 1984). It is a pyrethroid insecticide that kills insects on contact and through digestion. It is used to control apple and pear suckers, plum fruit moth. Control of aphids, mealy bugs, scale insects, and whitefly on glasshouse cucumbers, tomatoes, peppers, potted plants, and ornamentals. It also controls numerous insect pests of field crops. Formulations include emulsifiable concentrates, wettable powders and granules. There are no known incompatibilities with other common insecticides and fungicides. Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which rapidly paralyze the insect nervous system giving a quick knockdown effect. Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Death of insects seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours. Deltamethrin poisoning occurs through cuticular penetration or oral uptake. The susceptibility of insects is dependent on a variety of factors and can vary, as with many insecticides, according to the environmental conditions. Flies are most susceptible to pyrethroid poisoning shortly before dawn. Many pyrethroids are not very active against cattle ticks, but some alpha cyano compounds (of which

deltamethrin is one) have higher activity than organophosphates or amidines, the former standard compounds for this purpose. Deltamethrin has very good residual activity for outdoor uses (field crops) and for indoor uses (mosquitoes, stable flies, horseflies, fleas, cockroaches, stored product insects). Deltamethrin has very broad spectrum control. It is considered the most powerful of the synthetic pyrethroids. It is up to three orders more active than some pyrethroids.

4.1.2. Pharmakokinetics:

4.1.2.1. Absorption (Soderlund *et al*., 2002; WHO 1999).

Deltamethrin is considered to be readily absorbed when administered orally. The carrier or solvent can affect the rate of absorption (National pesticide information center 2012).

- Pyrethroids are lipophilic. Absorption in the gastrointestinal tract and respiratory tract is higher compared to absorption through the skin.
- Rats absorbed 3.6% of the deltamethrin applied to their skin, which was then excreted within 24 hours. Since human skin is less permeable than rat skin, the absorption of deltamethrin through human skin is expected to be relatively weak.
- Deltamethrin was poorly absorbed from the gastrointestinal tract of lactating cows fed 10 mg/kg for three days.
- Deltamethrin was absorbed by rats after they were fed plant material containing bound residues of the chemical.

4.1.2.2. Distribution (Anadon A 1996; WHO 1999)

- Deltamethrin reached peak plasma concentrations in rats at 2.1 hours after a single oral dose. Deltamethrin distributed to nerve tissues and all regions of the brain tested (Gammon *et al*., 2012).

- There is little tendency for deltamethrin to accumulate in tissues.18 Studies with rats observed that orally administered deltamethrin was recovered in fat at slightly higher concentrations compared to other tissues.

- In rats, deltamethrin had a half-life in blood of 5.5 hours.

- One study found little accumulation in the major edible tissues when lactating cows were fed deltamethrin for three days at a rate of 10 mg/kg/day (National pesticide information center 2012).

4.1.2.3. Metabolism (WHO 1999; Roberts *et al*., 1999)

- Mammals generally metabolize pyrethroid through ester hydrolysis, oxidation, and conjugation. Ester cleavage is the main route of degradation in the body.

- Thiocyanate was the primary metabolite after rats were administered deltamethrin orally or intraperitoneally. Only the parent compound, deltamethrin, is considered to be toxicologically significant.

4.1.2.4. Excretion (Bradberry 2005; WHO 1999)

- In one study, excretion of deltamethrin fed to rats was almost complete in 48 hours. Approximately the same amount of the applied dose (36-59%) was found in the faeces and the urine.

- In other studies, the elimination half-life of orally-administered deltamethrin was 38.5 hours, and 33.0 hours when administered intravenously to rats.

- A study in lactating cows indicated that deltamethrin was excreted in milk in low amounts (0.42-1.60%) after exposure to a single oral dose18 . In another study, concentrations in the milk of cows peaked 7 days after dermal application of deltamethrin.

- One study found that Leghorn hen eggs contained low concentrations of deltamethrin residues after hens were fed 7.5 mg each day for three days. Residues in the eggs were detected within the first 24 hours after dosing. Peak residues were detected within 48 hours after the last dose.

- Deltamethrin and its metabolites were detected in the urine of workers within 12 hours of occupational exposure, and for up to 48 hours post-application human volunteers ingested a single dose of 3 mg deltamethrin and researchers tested urine, faeces, saliva, and blood samples. The highest levels in the blood were observed within one to two hours after the exposure. The elimination half-life ranged from 10.0-11.5 hours in plasma and 10.0-13.5 hours in urine. The majority of ingested deltamethrin (64-77%) was excreted in faeces and urine within four days of exposure.

4.1.3. Mode of action of deltamethrin:

It has been previously established that all pyrethroids have a common mechanism of action upon the voltage-gated membrane sodium channel (Chinn & Narahashi 1986). Their toxicology is dominated by acute pharmacological actions on excitability originating from this common mechanism (Ray *et al*., 2001). Deltamethrin

activates 'silent' sodium channels. The origin of these channels remains unknown. It is interesting to note however, that, in the central nervous system of arthropods and particularly of insects, cell bodies are generally inexcitable (Hoyle 1970; Pichon & Ashcroft 1985), which agrees with our findings in cultured neurones, but may become excitable and give fairly normal overshooting sodium spikes following colchicine treatment (Pitman 1975) or dissociation (Aldrich *et al*., 1988; Gundel *et al*., 1989). This indicates that, at least in these neurones, fast sodium channels may exist in the membrane in a non-functional configuration and may be transformed into functional channels under certain conditions. Insect neurones may prove to be very useful in the study of the expression of functional sodium channels during development and following various physical and/or chemical treatments. Deltamethrin effect on the parasite is mainly through contact and ingestion. As deltamethrin is extremely lipophilic, it easily penetrates the cuticles of insects and acarines.

Mechanism of action of pyrethroids in insects and invertebrates

Typical effects of deltamethrin are:

Hyperexcitation

Loss of coordination

Tremors, convulsions, tetanic spasms

Knock-down effect and dehydration and

Death

4.1.4. Toxicological effects: (WHO 1999)

4.1.4.1. Acute toxicity:

Deltamethrin produces typical type II motor symptoms in mammals. Type II symptoms include a writhing syndrome in rodents, as well as copious salivation. The acute oral LD (lethal dose) 50 in male rats ranged from 128 mg/kg to greater than 5,000 mg/kg depending on the carrier and conditions of the study; the LD50 for female rats was 52 mg/kg and other published values range from 31 to 139 mg/kg. Values ranging from 21 to 34 mg/kg were obtained for mice; while dogs had a reported LD50 of 300 mg/kg. The intravenous LD50 in rats and dogs was 2 to 2.6 mg/kg, and the dermal LD50 was greater than 2,940 mg/kg. The acute percutaneous LD50 for rats was reported to be greater than 2,000 mg/kg; greater than 10,000 mg/kg for quail; and greater than 4,640 mg/kg for ducks. The acute dermal LD50 for rabbits was greater than 2,000 mg/kg. No skin irritation and slight eye irritation were reported . Another study indicated skin irritation in rats and guinea pigs. The signs of poisoning produced in rats by deltamethrin are not the same as those produced by other pyrethroids. Blood pressure begins to drop promptly, but slowly; it tends to normalize about the time choreoathetosis (abnormal movements of the body of a combined choreic and athetoid pattern) begins but falls precipitously prior to death. The early signs, including choreoathetosis, are reversible, but rats that exhibit a tonic seizure and shock almost always die promptly. Acute exposure effects in humans include the following: ataxia, convulsions leading to muscle fibrillation and paralysis, dermatitis, edema, diarrhoea, dyspnea, headache, hepatic microsomal enzyme induction, irritability, peripheral vascular collapse, rhinorrhea, serum alkaline phosphatase elevation, tinnitus, tremors, vomiting and death due to respiratory failure. Allergic

reactions have included the following effects: anaphylaxis, bronchospasm, eosinophilia, fever, hypersensitivity pneumonia, pallor, pollinosis, sweating, sudden swelling of the face, eyelids, lips and mucous membranes, and tachycardia. Studies have shown many cases of dermal deltamethrin poisoning after agricultural use with inadequate handling precautions, and many cases of accidental or suicidal poisoning by the oral route at doses estimated to be 2-250 mg/kg.

4.1.4.2. Chronic toxicity:

In 2-year feeding trials, the reported NEL (no effect level) was 12 mg/kg diet for mice; and 2.1 mg/kg diet for rats. The dose without activity in rats over a 90-day period was 10 mg/kg/day. Suspected chronic exposure effects in humans include the following:choreoathetosis, hypotension, prenatal damage and shock. Workers exposed to deltamethrin during its manufacture over 7-8 years experienced transient cutaneous and mucous membrane irritation, which could be prevented by use of gloves and face masks. No other ill effects were seen.

4.1.4.3. Reproductive effects:

A reproductive 3-generation study in rats reported a reproductive NOEL (No observable effect level) to be greater than 2.5 mg/kg/day. Levels tested were 0, 0.1, 1.0 and 2.5 mg/kg/day. Oral administration of deltamethrin to mice on days 7 to 16 of gestation produced a dosage-related reduction of weight gain but no effect on the number of implants, fetal mortality, fetal weight or malformations.

Teratogenic effects: There were no reported teratogenic effects in mice, rats and rabbits. Deltamethrin has no teratogenic activity.

Mutagenic effects: There were no mutagenic effects in mice, rats and rabbits. Deltamethrin has no mutagenic activity.

Carcinogenic effects: No information was available.

4.1.4.4. Organ toxicity:

Deltamethrin is hydrolyzed by liver microsomal enzymes to 3-(2,2dibromovinyl) 2, 2-cyclopropane carboxylic acid 3phenoxybenzaldehyde.

4.1.5. Miscellaneous effects:

Analgesic effects of deltamethrin for thermic (hot plate test, 60°C) and mechanical stimuli were investigated in mice and rats, respectively. Deltamethrin prolonged the response-time to these tests. Although this action was not significant at 500 mg deltamethrin/kg body weight given orally, the reaction time was increased at 1000 and 1500 mg/kg given orally in aqueous suspension with 10% gum arabic (Chanh *et al*., 1981). In rats, treatment with deltamethrin increased mean arterial pressure and aortic output (Forshaw & Bradbury 1983). The cardiovascular effects of deltamethrin were due to both increased catecholamine release in peripheral vascular beds, and to a direct positive inotropic effect on the heart. (Krasnjih & Pavlova 1985) demonstrated induction of microsomal oxygenases in rats 20 hour after a single administration of 1/2 LD 50. Daily administration of $1/10^{th}$ LD 50 for 2 months reduced acetylcholinesterase activity in the serum, erythrocytes, liver, and cerebrum. It also led to some changes in the aspartate aminotransferase activity and the urea and protein contents of serum. When rats were dosed orally with a single dose of 1/2 LD 50 or 3 daily doses of 1/5 LD 50 deltamethrin, the activities of transferrin and ceruloplasmin in plasma, 20 hour after dosing, were unchanged. After the single dose,

microsomal monooxygenase activity was increased by 87%, and after the 3 doses, it was increased by 290% (Kagan *et al*., 1986).

4.1.6. Usage:

The primary use of deltamethrin (approximately 85% of the total production) is for crop protection (WHO 1990). Deltamethrin is also used to protect stored commodities such as cereals, grains, and coffee beans. Other uses include insect control for public health concerns, forestry, animal facilities, for animal ectoparasites, and as a wood preservative (WHO 1990) . While the initial California registration application is for the technical material (to be used in formulating products), anticipated California end-product uses include: treatment of cotton, residential and institutional establishments, non-food/feed areas of food/feed processing plants, granaries, and ornamental plants. Deltamethrin is a synthetic pyrethroid insecticide which possesses an extremely high level of activity against a wide range of insects (Worthing & Walker 1987), including *Lepidoptera*, *Hemiptera*, *Diptera* and *Coleoptera*. It acts by both direct contact and ingestion (Worthing & Walker 1987). Deltamethrin products are among the most popular and widely used insecticides in the world and have become very popular with pest control operators and individuals in the United States. This material is a member of one of the safest classes of pesticides: synthetic pyrethroids. This pesticide is highly toxic to aquatic life, particularly fish, and therefore must be used with extreme caution around water. Although generally considered safe to use around humans, it is still neurotoxin to humans. There are many uses for deltamethrin, ranging from agricultural uses to home pest control. Deltamethrin has been instrumental in preventing the spread of diseases carried by tick-infested prairie dogs, rodents and other burrowing anima. It is helpful in

eliminating and preventing a wide variety of household pests, especially spiders, fleas, ticks, carpenter bees, cockroaches and bed bugs. Deltamethrin is also one of the primary ingredients in ant chalk.

4.2. POLYMERS REVIEW:

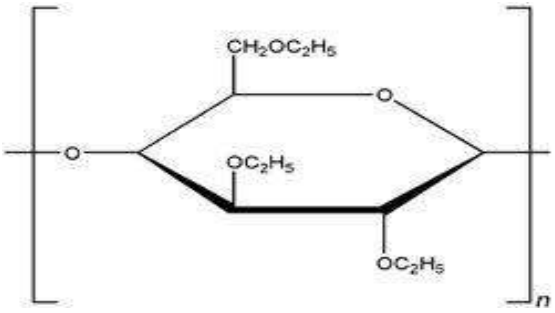
4.2.1. Ethyl cellulose:

Synonyms: Ethocel, Aquacoat

Chemical names and CAS registry number: Cellulose ethyl ether (9004-57-3)

Empirical formula: Ethyl cellulose is an ethyl ether of cellulose, a long chain polymer consisting of anhydro glucose units joined together by actual linkages (Raymond *et al*., 2009)

Structural formula:



Functional category: Coating agent; tablet binder; viscosity increasing agent.

4.2.1.1. Description: Ethyl cellulose is a tasteless, free flowing, white to light tan colour powder.

4.2.1.2. Typical properties:

Density (bulk): 0.4 g/cm3

Solubility: Practically insoluble in glycerine, propylene glycol and water. Freely soluble in chloroform, methyl acetate, and in mixtures of aromatic hydrocarbons and with ethanol (95%).

pH (2% w/w suspension): 5.0-7.5

4.2.1.3. Stability and storage conditions:

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated and to salt solutions although it is more sensitive to acidic materials than cellulose esters. The bulk materials should be stored in a dry place, in a well-closed container at a temperature between 7-32^{o}C.

4.2.1.4. Applications in pharmaceutical formulation or technology:

Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. The main use of ethyl cellulose in oral formulations is a hydrophobic coating agent for tablets and granules. Ethyl cellulose is also widely used in drug microcapsulation. In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions or gels, provided an appropriate solvent is used.

4.2.2. Polyvinylpyrrolidone (PVP):

Polyvinylpyrrolidone (PVP), also commonly called Polyvidone or Povidone, is a water-soluble polymer made from the monomer *N*-vinylpyrrolidone (Raymond CR *et al*., 2009)

Other names: PVP, Povidone, Polyvidone Poly[1-(2-oxo-1-pyrrolidinyl)ethylen] 1-Ethenyl-2-pyrrolidon homopolymer 1-2-pyrrolidinon-Polymere Copovidone PNVP

IUPAC name: Polyvinylpyrrolidone

Identifiers: CAS number 9003-39-8

4.2.2.1. Properties:

Molecular formula: $(C_6H_9NO)_n$

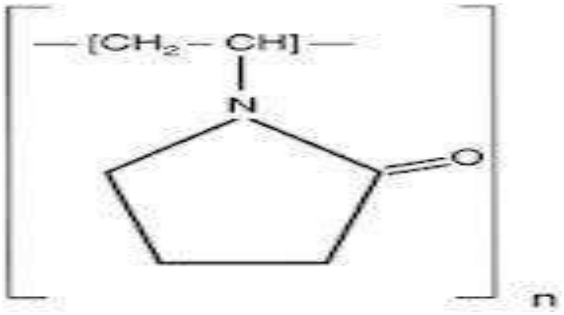
Molar mass: 2.500-2.5000.000 g/mol^{-1}

Appearance: white to light yellow, hygroscopic, amorphous powder

Density: 1.2 g/cm^3

Melting point: 150-180 °C (glass temperature)

Structural formula:



4.2.2.2. Applications in pharmaceutical formulation or technology:

Povidone possesses the following properties that make it ideal for numerous applications in drug manufacture:

• Solubility in all conventional solvents

• Adhesive and binding powers

• Film formation

• Affinity to hydrophilic and hydrophobic surfaces

• Ability to form complexes

• Thickening properties

4.3. PLASTICIZER REVIEW: (Raymond *et al*., 2009)

4.3.1. Dibutyl phthalate:

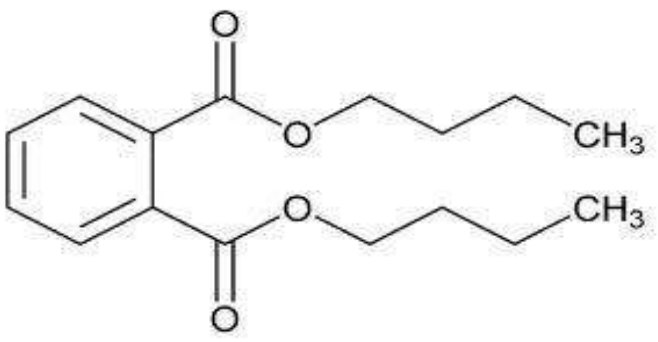
Synonyms: DBP; Di-n-Butyl Phthalate; n-Butyl phthalate; 1,2-Benzenedicarboxylic acid dibutyl ester; Phthalic acid dibutyl ester; o-benzenedicarboxylic acid, dibutyl ester; benzene-o-dicarboxylic acid di-n-butyl ester; dibutyl 1,2-benzenedicarboxylate; Benzenedicarboxylic acid, dibutylester; Dibutyl o-Phthalate

Substance name: Dibutyl phthalate

CASR number: 84-74-2

Molecular formula: $C_{16}H_{22}O_4$

Structural formula:



4.3.1.1. Description:

Dibutyl phthalate is a man-made chemical that is added to plastics and other chemicals. In plastics it helps keep them soft (a plasticizer). It is also used in elastomers, lacquers, explosives, printing inks, resin solvents, perfume oil solvents, paper coatings, adhesives, and nail polish. It is used as a solid rocket propellant

4.3.1.2. Physical properties: Dibutyl phthalate is a colourless, oily liquid with a weak odour.

Melting point: -35°C

Boiling point: 340°C

Specific gravity: 1.043

Vapour density: 9.6

4.3.1.3. Chemical properties: Dibutyl phthalate is soluble in most organic solvents, but only slightly soluble in water.

4.3.1.4. Applications in pharmaceutical formulations or technology:

DBT is used as a plasticizer in film coatings. It is also used as an insect repellent, primarily for the impregnation of clothing.

5. MATERIALS

5.1. Materials:

Deltamethrin was obtained as a gift sample from Defence Research Development and Establishment (DRDE), Gwalior, Madhya Pradesh, India. Polyvinylpyrrolidone (PVP K-30), Di-butyl-phthalate (DBT), and Ethyl cellulose (EC, ethoxy content 48-49.5 % w/w) were obtained from Himedia Laboratories Pvt. Ltd. Mumbai, India. Chloroform and Water (For HPLC) were obtained from Spectrochem Pvt. Ltd. Mumbai, India. Acetonitrile (For HPLC) was purchased from Merck Specialties Pvt. Ltd; Mumbai, India. Dimethyl sulfoxide (DMSO) was purchased from Loba Cheime Pvt. Ltd. Mumbai, India. All reagents and solvents used were of analytical grade and used as received without any further purification.

5.2. Insects: *Aedes* (S.) *albopictus*

The Tiger mosquito or forest day mosquito, *Aedes albopictus* (*Stegomyia albopicta*), from the mosquito (*Culicidae*) family, is characterized by its black and white striped legs, and small black and white striped body. It is native to the tropical and subtropical areas of Southeast Asia; however, in the past couple of decades this species has invaded many countries throughout the world through the transport of goods and increasing international travel (Scholte & Schaffner 2007). This mosquito has become a significant pest in many communities because it closely associates with humans (rather than living in wetlands), and typically flies and feeds in the daytime in addition to at dusk and dawn.

The insect is called a tiger mosquito because its striped appearance is similar to a tiger. *Aedes* (S.) *albopictus* is an epidemiologically important vector for

the transmission of many viral pathogens, including the West Nile virus, Yellow fever virus, St. Louis encephalitis (Randolf & Hardy 1998), dengue fever, and Chikungunya fever (Hochedez *et al*., 2006) as well as several filarial nematodes such as *Dirofilaria immitis* (Cancrini 2003).

A test was made using adult *Aedes* (S.) *albopictus* (an exotic species). *Aedes albopictus* is a known vector of dengue (Hawley 1988). It is persistent biters and common pests of humans, mammals, and birds. This species are known for contact with the WN virus transmission cycle in North America (Turell *et al*., 2001) and together support virus transmission in more than three dozen states in the United States (Darsie & Ward 1981; Moore & Mitchell 1997). The *in-vivo* evaluation of repellents against adult female *Aedes* (S.) *albopictus*, the Asian tiger mosquito. *Aedes* (S.) *albopictus* had access only to water 24 hour and had neither food nor water 24 hour before testing.

5.3. Pre-formulation study:

5.3.1 Literature survey of relevant topics from various books, journals, encyclopedias etc:

5.3.2 Physico-chemical characterization of deltamethrin and polymers

5.3.2.1 Physical properties study: The deltamethrin, EC and PVP were visually inspected for color, state and odor.

5.3.2.2 Solubility study: The solubility of deltamethrin, EC and PVP were studied on various organic and non-organic solvents.

5.3.2.3 Deltamethrin-polymer solubility study: Solubility in between deltamethrin and polymer were studied.

5.3.2.4. Melting point determination: The melting point of deltamethrin was studied on DSC analysis and further compared with official literature.

5.3.2.5. Compatibility studies:

(a) FT-IR study of deltamethrin:

The application of infrared spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixtures and as a tool in establishing of the structure. The infrared data is helpful to confirm the identity of the component and to detect the interaction of the components with the polymers. Infra red spectra of deltamethrin and polymers, alone and in physical mixtures were taken. Then it was investigated for any possible interaction between polymer and drug by FT-IR spectrophotometer (BRUKER, α Alpha- E, Germany). The samples for FT-IR analysis were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm^{-1} and resolution was 2 cm^{-1}.

(b) DSC study of deltamethrin-polymer blends:

The physicochemical compatibility between drug and polymers to be used in the formulation of deltamethrin-loaded sustained release mosquito repellent patches was also studied by using differential scanning calorimetry (DSC). The thermo grams obtained for pure deltamethrin, polymers and their physical mixtures were compared to ascertain any interactions. Sample were heated under conditions of DSC at temperature 50 to 250 ^{o}C at a rate of 10 ^{o}C / minute, Gas flow-Nitrogen at a rate of 20ml/minute (Perkin Elmer, Jade DSC, USA) and then thermo grams were obtained.

5.3.2.6. Development of HPLC method for estimation of Deltamethrin

The HPLC method developed in the DRDO laboratory was used in the experiment.

5.3.2.6.1. Prototype formulation development:

First blank patch were prepared using the prototype excipients found compatible in compatibility studies like ethyl cellulose, PVP and plasticizer. The prepared patches were evaluated for shape, smoothness, stickiness, clarity, homogeneity, flexibility and uniformity.

Then fixed amount drug is incorporated in the blank patch and the amount of deltamethrin is estimated by using validated HPLC procedure. The estimation of the deltamethrin was performed by using HPLC (Analytical technologies limited, Product model: UV 3000) and analytical column CHEMSIL ODS-column, 4.0×300 nm, 5 μm, by isocratic elution with mobile phase acetonitrile/water (80/20, v/v), flow rate 1.0 ml/min, UV detection at 245 nm, run time 7-10 minute.

Figure 1: High performance liquid chromatography (HPLC)

5.4. Formulation design:

5.4.1. *Preparation of Deltamethrin-loaded patches:*

From the results of prototype patch, formulation composition with various proportions of ethyl cellulose: PVP ratios were selected for formulation design and optimization.

Deltamethrin loaded mosquito repellent patches were prepared by solvent evaporation method using varying ratios of different blend of polymers. Di-butylphthalate is used as plasticizer at a fixed concentration of 20% w/w of dry weight of polymers. Initially, the polymers at a varied ratio were dissolved in chloroform and then deltamethrin and plasticizer were added on it. Then this mixture was molded into rings with defined surface area and controlled thickness over the backing membrane on a horizontal surface followed by solvent evaporation at an ambient temperature. The rate of evaporation was controlled by inverting the funnel. The patches were formed is then separated from the rings for further process.

5.4.1.1. Design and formulation of deltamethrin-loaded patches:

Table1: Composition of deltamethrin-loaded sustained release mosquito repellent patches

Formulation Code	EC:PVP K-30	Chloroform (ml)	Dibutyl phthalate (%w/w)	Deltamethrin (%w/w)
A1	1:2	3	25	25
A2	2:1	3	25	25
A3	3:0	3	25	25
A4	0:3	3	25	25
A5	1.5:1.5	3	25	25

5.5. Physicochemical characterization of Deltamethrin-loaded patches:

- ***Physical appearance:***

  All the deltamethrin-loaded mosquito repellent patches were visually inspected for shape, smoothness, stickiness, clarity, homogeneity, flexibility and uniformity.

- ***Uniformity of weight:***

  Weight variation is studied by individually weighing three patches from each formulation randomly and calculating their average weight. The

individual weight should not deviate significantly from the average weight and calculating mean along with standard deviation.

- ***Surface area:***

Surface area is studied on patches of each formulation by using millimeter scale and determines its mean and standard deviation values.

- ***Surface pH determination:***

The patches were allowed to keep in contact with chloroform for 2 hour at room temperature, and pH was determined by pH paper.

- ***Percent moisture uptake:***

Weighed patches are kept in a dessicator at room temperature for 48 hour. These are then taken out and exposed to 75.5% relative humidity using saturated solution of aluminium chloride in a dessicator until a constant weight is achieved. % moisture uptake is calculated as given below (Patel *et al*., 2012).

$$\% \text{ moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (1)$$

- ***Percent moisture content*:**

Three patches from each formulation were weighed and kept on desiccators containing fused calcium chloride at 37ºC until no change in weight of the individual patches was observed. This weight was noted as the

final weight. The percent content calculated using following formula (Patel *et al*., 2012; Satturwar *et al*., 2005; Dey *et al*., 2007).

$$\% \text{ Moisture content} = \frac{\text{Initial weigh} - \text{Final weight}}{\text{Final weight}} \times 100 \quad (2)$$

- ***Flatness:***

A patch should possess a smooth surface and should not constrict with time. This can be demonstrated in flatness study. For flatness determination, one patch is cut from the centre with (2x1cm) and applied on the skin. The length of patch is measured and variation in length is measured by determining percent constriction. Zero percent constriction is equivalent to 100% flatness (Kajal Ghosal *et al*., 2009).

$$\% \text{ constriction} = \frac{I_1 - I_2}{I_1} \times 100 \quad (3)$$

I_2 = Final length of each strip, I_1 = Initial length of each strip

- ***Measurement of thickness:***

The thickness of the patch is measured by digital micrometer (Mitotousu, Tokyo, Japan) at three different points of each patch. For each formulation, three patches were used. The average value for the thickness of single patch was determined along with its standard deviation values (Hui 2007).

- ***Scanning Electron Microscope (SEM) study:***

The external morphology of the blank patches and after drug loaded was studied by using an SEM (JEOL, JSM-6390 LV, England). The samples were mounted onto stubs using double sided adhesive tape and sputter coated with gold palladium. The coated patches were observed and photographs are taken at the required magnification at room temperature.

- ***Estimation of deltamethrin by high performance liquid chromatography (HPLC) method:***

The estimation of the deltamethrin was performed by using HPLC (Analytical technologies limited, Gujarat, India) with a UV/Visible detector and C_{18} column (CHOMASIL, particle size 5 µm, 250 mm×4.6 mm i.d) was used. Deltamethrin was separated by isocratic elution technique with a mixture of mobile phase containing acetonitrile:water (80:20% v/v), flow rate 1.0 ml/minute, and UV detection at 245 nm.

- ***Deltamethrin content:***

The patch of specified diameter was extracted with chloroform and kept it about 2 hours at room temperature in order to extract the deltamethrin completely from the polymeric matrix and centrifuged at 3000 rpm for 15 minute. Then fixed amount of resulting supernatant solution from above was analyzed for drug content using HPLC (Analytical technologies limited, Gujarat, India) at 245 nm.

- ***FT-IR spectrophotometry:***

The infrared data are helpful to confirm the identity of the component and to detect the interaction of the components with the polymers. Infrared spectra of deltamethrin and polymers, alone and in physical mixtures were taken. Then it was investigated for any possible interaction between polymer and drug by FT-IR spectrophotometer (BRUKER, α Alpha- E, Germany).

- ***Differential scanning calorimetric analysis:***

The physicochemical compatibility between the component and polymers used in the formulation of deltamethrin-loaded mosquito repellent patches was evaluated by differential scanning calorimetry (DSC) analysis. The DSC thermograms (Perkin Elmer, Jade DSC, USA) obtained for pure deltamethrin, pure polymers, their physical mixtures and formulated patches were compared to ascertain any interactions are there or not. Samples were heated at a temperature range between 50 to 250 ^{o}C at a heating rate of 10^{o}C/minute.

- ***Test animals:***

Healthy adult wistar strain albino rats (weighing 202-213g, 5-8 weeks of age, male) were obtained from Central Animal Resources, Defence Research Laboratory (DRL), Defence Research and Development Organization (DRDO), Tezpur, Assam, India. The animals were placed in polypropylene cages, with free access to standard laboratory diet (Pranav Agro Industries Limited, Sangli, Maharastra, India) and provided municipal

water ad libitum. They were hosed in an environmentally-controlled room with temperature of 22±3°C and 40-70% relative humidity with a 12 hour light/dark cycle. All of the animal experimental protocols were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Forest and Environment, Government of India.

- ***Inhalation toxicity:***

The component of the patches was extracted and dissolved in 5ml of 10% dimethyl sulfoxide (DMSO). Then this extracted solution was transfer to the sample holder of the nebulizing tube and run the machine till the solution was exhausted. After exposing, the animals were now studied by using a whole body plethysmometer (Whole body plethysmography, Data Sciences International, USA).

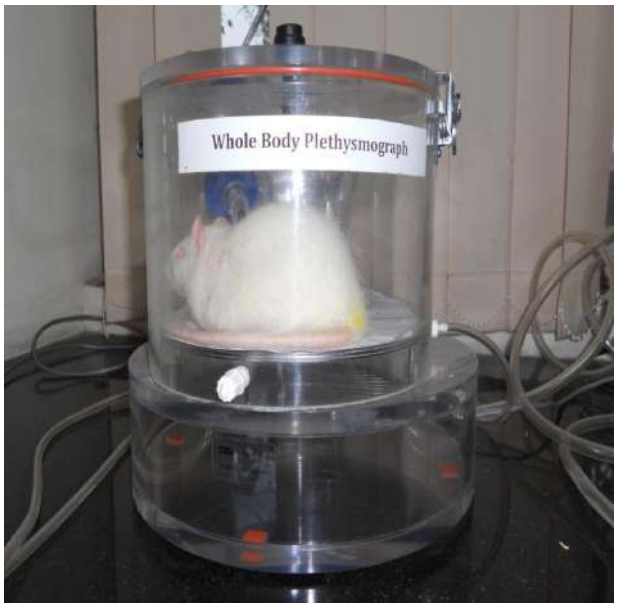


Figure 2: Whole body plethysmography

- ***Release study:***

The release study was carried out under accelerated conditions of higher temperature. The patches were kept in an oven at 40°C and withdrawn at different intervals of time from each batch and then extracted with chloroform and kept it about 2 hours at room temperature in order to extract the deltamethrin completely from the polymeric matrix and centrifuged at 3000 rpm for 15 minute. Then fixed amount of resulting supernatant solution from above was analyzed for deltamethrin content using HPLC (Analytical technologies limited, Gujarat, India) at 245 nm.

- ***Mathematical modelling of release kinetics***

To understand the mechanism of drug permeation kinetics from the prepared patches, the release data were fitted to various release kinetic equations:

Zero-order equation (cumulative percentage drug permeated *vs.* time):

$$Q_t = Q_0 + k_{0t} \quad \text{.....(4)}$$

Where, Q_t is the amount of drug release in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$), and k_0 is the zero order release rate (Wyatt 1999).

First-order equation (log cumulative percentage drug remaining to be permeated *vs.* time):

$$\ln Q_t = \ln Q_0 + k_1 t \quad \text{.....(5)}$$

Where, Q_t is the amount of drug released in time *t*, Q_0 is the initial amount of drug in the solution, and k_1 is the first order release rate constant.

Higuchi's model equations (Higuchi, 1961)*:*

$$Q = k_H t^{1/2} \quad \text{.....(6)}$$

Where, *Q* is the amount of drug release at time *t,* and k_H is the Higuchi diffusion rate constant.

Korsmeyer-peppas model (Korsmeyer *et al*., 1983):

$$Mt/M\alpha = Kt^n \quad \text{.....(7)}$$

Where, *Mt* is the amount of drug released at time *t, Mα* is the amount of drug released after infinite time, and *k* is a kinetic constant incorporating structural and geometric characteristics of the formulation and *n* is the diffusional exponent indicative of the drug release mechanism (**Table 2**).

Table 2: Value of 'n' and corresponding mechanism of drug release

Value of 'n'	Mechanism of drug release
n=0.5	Case-I (Fickian) diffusion or square root of time kinetics
0.5 < n < 1	Anomalous (non-Fickian) diffusion
n=1	Case-II transport/relaxation controlled release
n > 1	Super Case-II transport

- ***Test mosquitoes:***

  Laboratory-reared adult female *Aedes* (S.) *albopictus* mosquitoes were used under laboratory conditions. Initially, the larvae were reared in plastic pans and regularly fed with finely ground dog biscuit and yeast powder. Adult mosquitoes were maintained in cages and fed with 10% glucose solution.

- ***Repellency test procedure:***

  Both hands of human were employed for testing the repellent activity. Bare right hand was considered as control, whereas left hand loaded with patch was taken as test. The repellent activity was evaluated by inserting the hand into a customized test chamber (46 x 37 x 36 cm) under laboratory conditions. Hands were kept for one minute in the test chamber containing 100 unfed *A. albopictus* adult female mosquitoes. The number of mosquitoes landing and biting in test and control was recorded by two independent observers. The trials were conducted at 26±1°C and 80±5% RH in triplicates on three different days using three volunteers.

Figure 3: Mosquito chamber

- ***Data analysis:***

The percent repellency was calculated against control using **Equation 8**:

$$\text{Percent repellency (PR)} = C\text{-}N\times 100/C \quad (8)$$

Where, C= No of mosquitoes on control

N= No of mosquitoes on treated

The repellency index was indirectly calculated from percent repellency with **Equation 9**:

$$\text{Repellency index (RI)} = \frac{C\text{-}N\times 100}{C+N} \quad (9)$$

Where, C= No of mosquitoes on control

N= No of mosquitoes on treated

Mean and standard deviations for replicates were obtained at each time and analyzed using two-way analysis of variance (ANOVA) followed by Turkey Krammer test of multiple comparisons.

6. RESULTS

6.1. Pre-formulation study:

6.1.1. Physico-chemical characterization of Deltamethrin and polymers

6.1.1.1. Physical properties:

(a) Deltamethrin: Colourless crystalline powder; white or slightly beige powder.

(b) Ethyl cellulose: Ethyl cellulose is a tasteless, free flowing, white to light tan colour powder.

(c) PVP: White to light yellow, hygroscopic, amorphous powder

6.1.1.2. Solubility study:

(a) Deltamethrin:

Water Solubility: Almost insoluble in water.

Solubility in Other Solvents: Freely soluble in acetone, methylene chloride, chloroform but insoluble in water, methanol, ethanol, n- hexane.

(b) Ethyl cellulose: Practically insoluble in acetone and water. Freely soluble in chloroform, methyl acetate, ethanol and ethyl acetone.

(c) PVP: Freely soluble in acetone and chloroform but insoluble in ethyl acetone.

6.1.1.3. Deltamethrin-polymer solubility study: The deltamethrin-polymers are freely soluble in chloroform and methylene chloride.

6.1.1.4. Melting point determination: Deltamethrin melting point was found to be 107.85 degrees as per DSC analysis which matched the melting point value of the deltamethrin as reported in official literature.

6.2. Physicochemical characterization: The physicochemical characterization of deltamethrin-loaded patches is shown in **Table 3**.

Table 3: Physicochemical characterization of deltamethrin-loaded patches

Formulation code	A1	A2	A3	A4	A5
EC: PVP-K 30	1:2	2:1	3:0	0:3	1.5:1.5
Weight variation (gm)*	0.466±0.073	0.475±0.062	0.459±0.104	0.544±0.059	0.461±0.040
Thickness (mm)*	0.42±0.028	0.41±0.007	0.51±0.007	0.57±0.007	0.35±0.007
Surface area (cm^2)*	14.51±0.675	13.84±0.660	14.30±1.409	14.09±1.679	13.64±1.373
Moisture uptake (%)*	7.31±4.33	4.67±2.66	3.42±2.66	8.92±5.79	6.23±1.57
Moisture content (%)*	4.50±0.569	3.13±0.104	2.60±0.251	5.52±0.433	3.18±0.110
Deltamethrin content (%)*	50.35%	75.76%	77.60%	62.05%	66.41%

*All values expressed as mean ± SD (n=3)

6.3. Physical appearance: The physical appearance of deltamethrin-loaded patches is shown in **Table 4**.

Table 4: Physical appearance

Formulation code	Physical appearance
A1	Spericalsmooth,less sticky,clear,homogenous,uniform
A2	Sperical,smooth,sticky,clear,homogenous,uniform
A3	Sperical,smooth,nonsticky,clear,homogenous,uniform
A4	Sperical,smooth,sticky,clear,homogenous,uniform
A5	Sperical,smooth,sticky,clear,homogenous,uniform

6.4. Calibration curve of Pure Deltamethrin at 245 nm:

The calibration curve of pure deltamethrin at 245 nm was shown in **Figure 4.** The data of peak area (mv) is shown in **Table 5**. The calibration curve shows the linearity on a given concentration (µg/ml) as shown in **Table 5.** The correlation coefficient obtained was 0.996 and the equation of the regression line was y= 0.011x.

Table 5: Data of Concentration (µg/ml) vs. peak area (mv)

Concentration (µg/ml)	Peak area (mv)
6.25	384569
12.5	852638
25	1418531
50	2743216
100	5737382

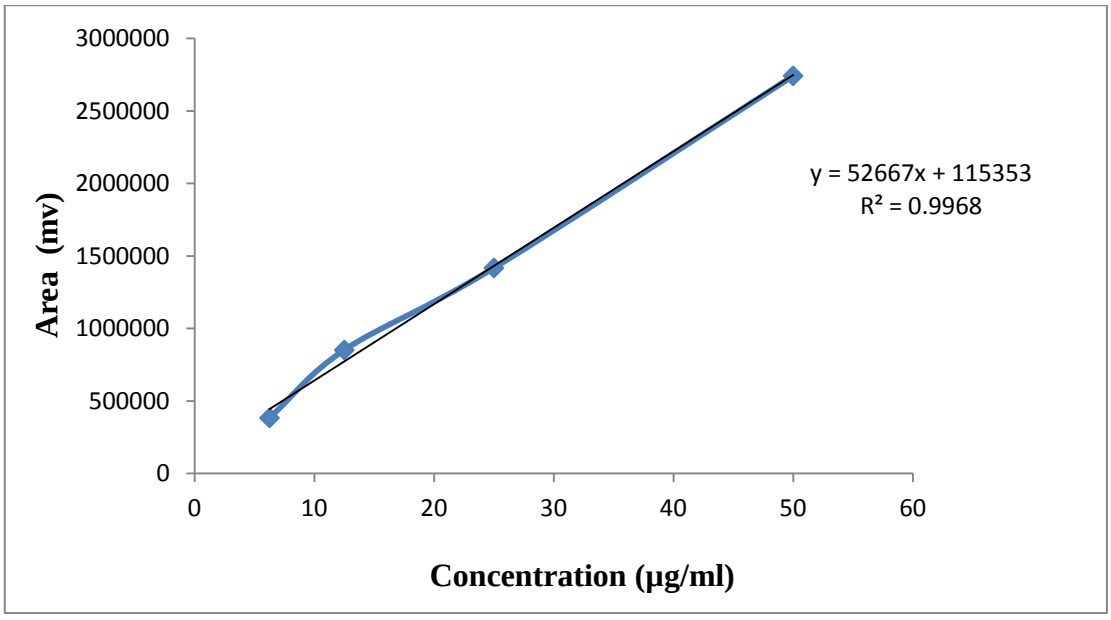


Figure 4: Calibration curve of pure deltamethrin at 245 nm

6.5. SEM analysis: The SEM analysis of deltamethrin-loaded patches and blank patch are shown in **Figure 5 & 6**.

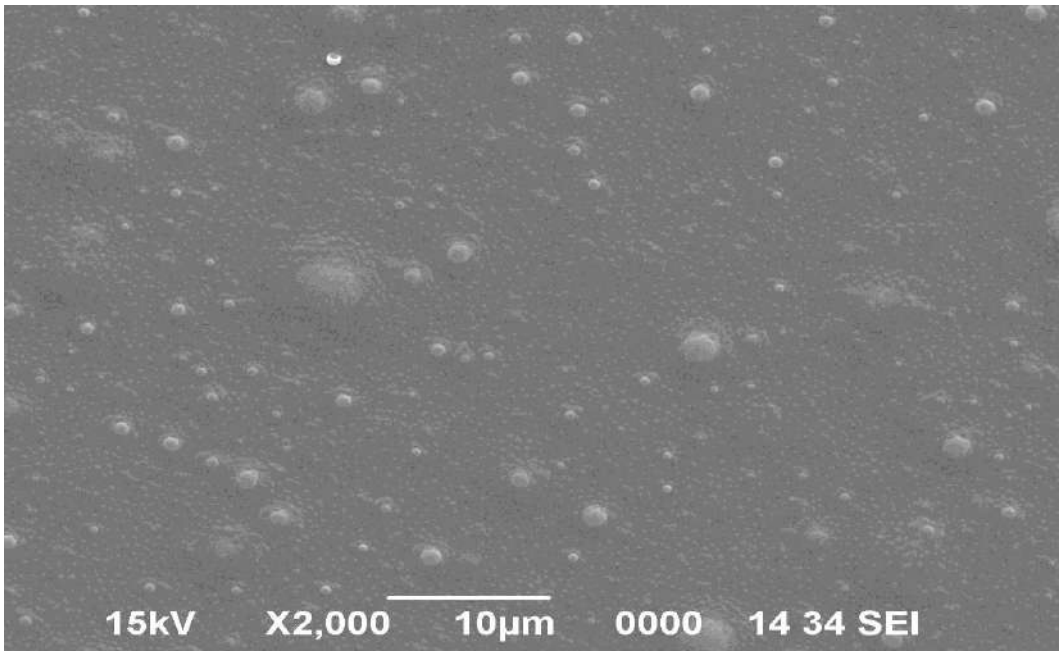


Figure 5: Scanning electron micrograph of deltamethrin-loaded patch

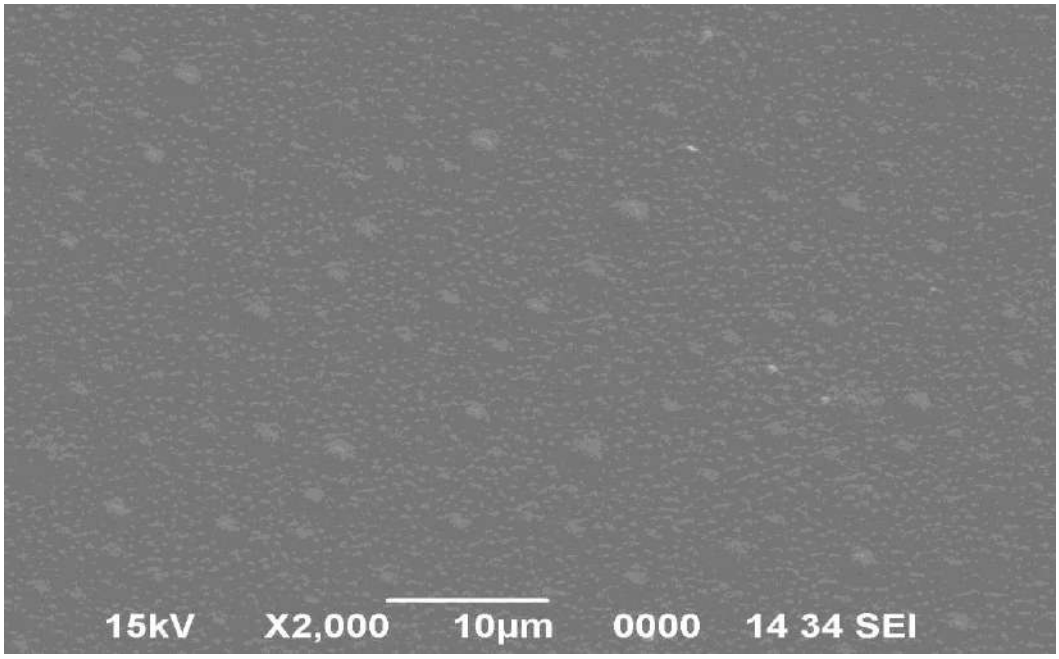


Figure 6: Scanning electron micrograph of of blank patch (A5)

6.6. FT-IR analysis: The FT-IR spectra of pure deltamethrin,ethyl cellulose, PVP and formulation A5 are shown in **figure 7, 8, 9 & 10**.

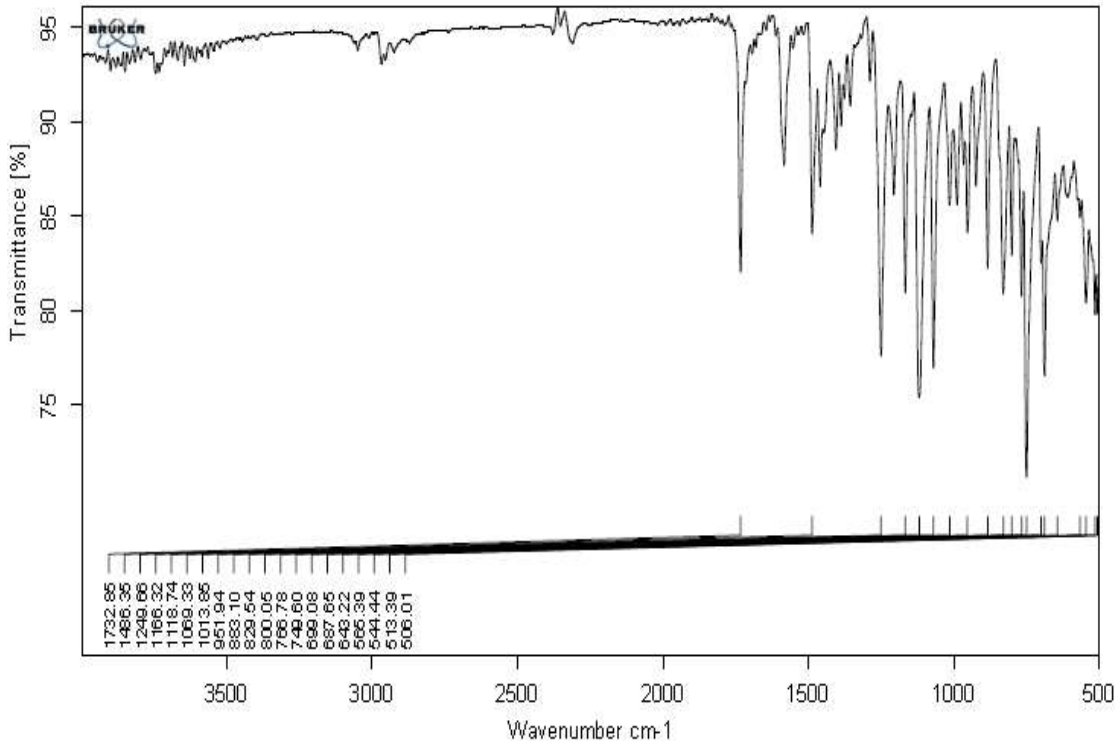


Figure 7: FT-IR spectra of pure deltamethrin

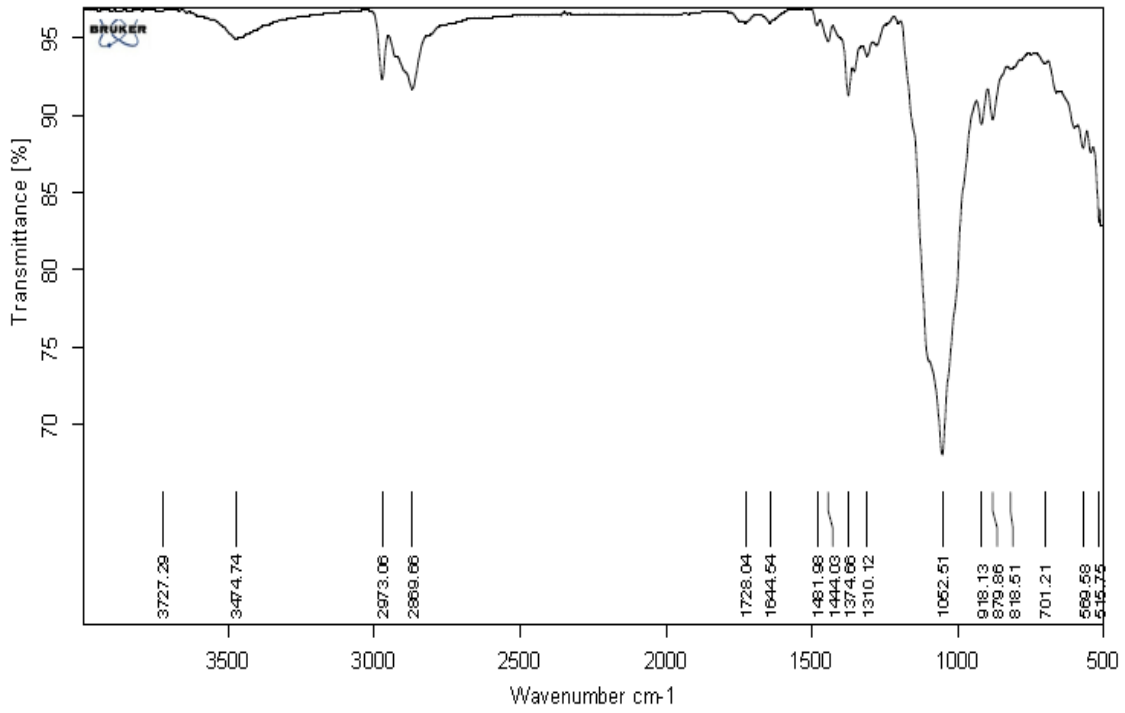


Figure 8: FT-IR spectra of ethyl cellulose

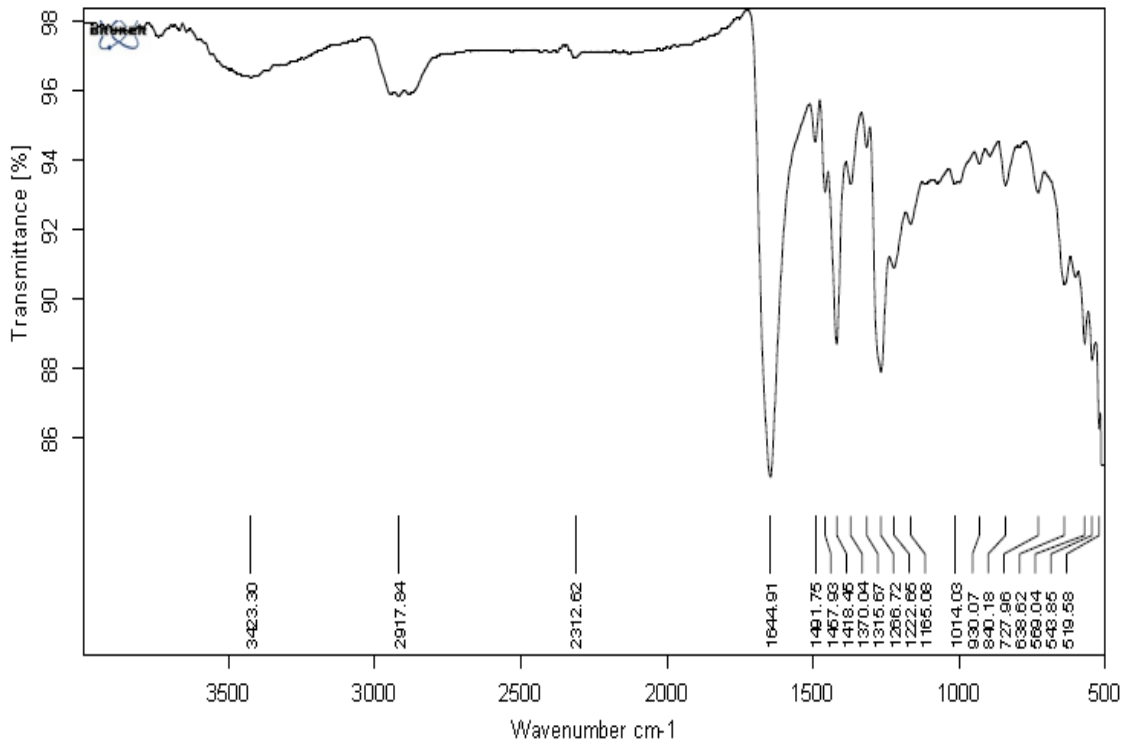


Figure 9: FT-IR spectra of Polyvinylpyrrolidone (PVP K-30)

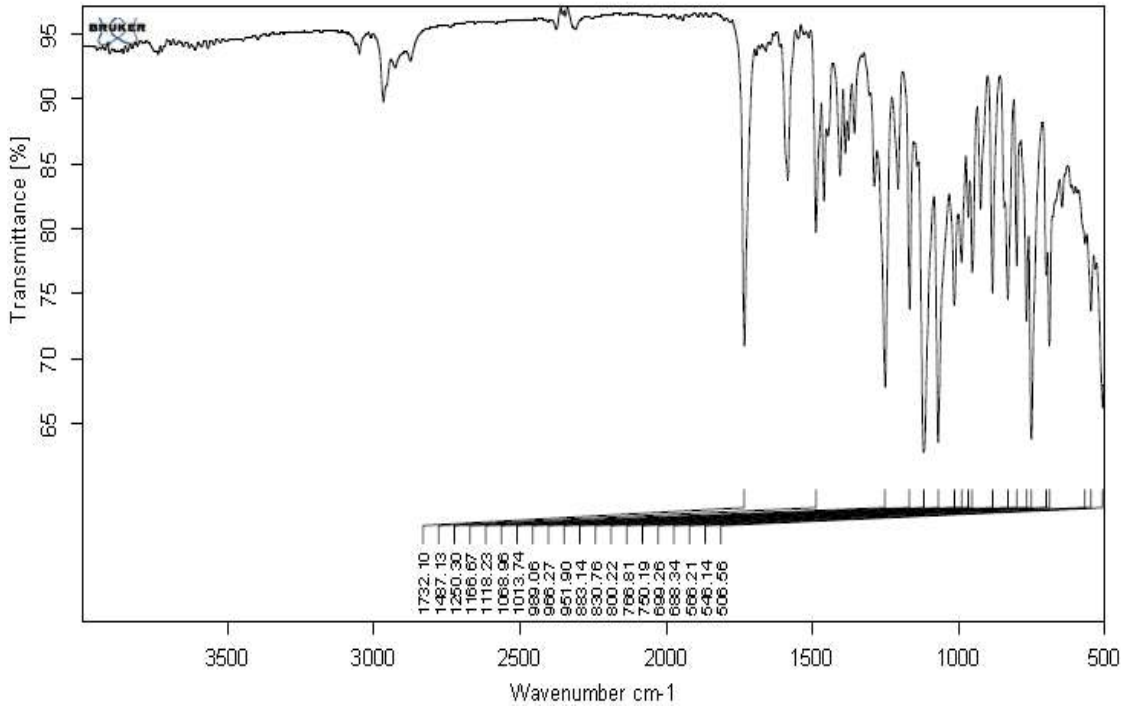


Figure 10: FT-IR spectra formulation A5

6.7. DSC analysis: The DSC thermogram of pure deltamethrin and deltamethrin-loaded patches are shown in **Figure 11** & **12**.

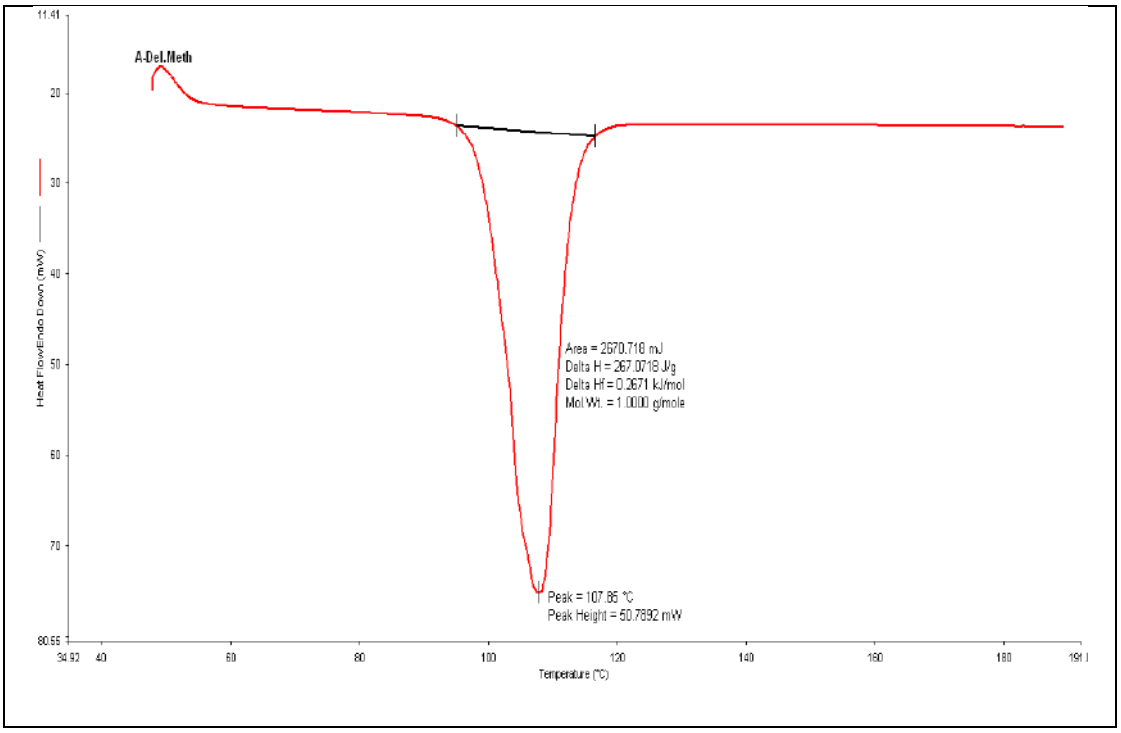


Figure 11: DSC thermogram of deltamethrin

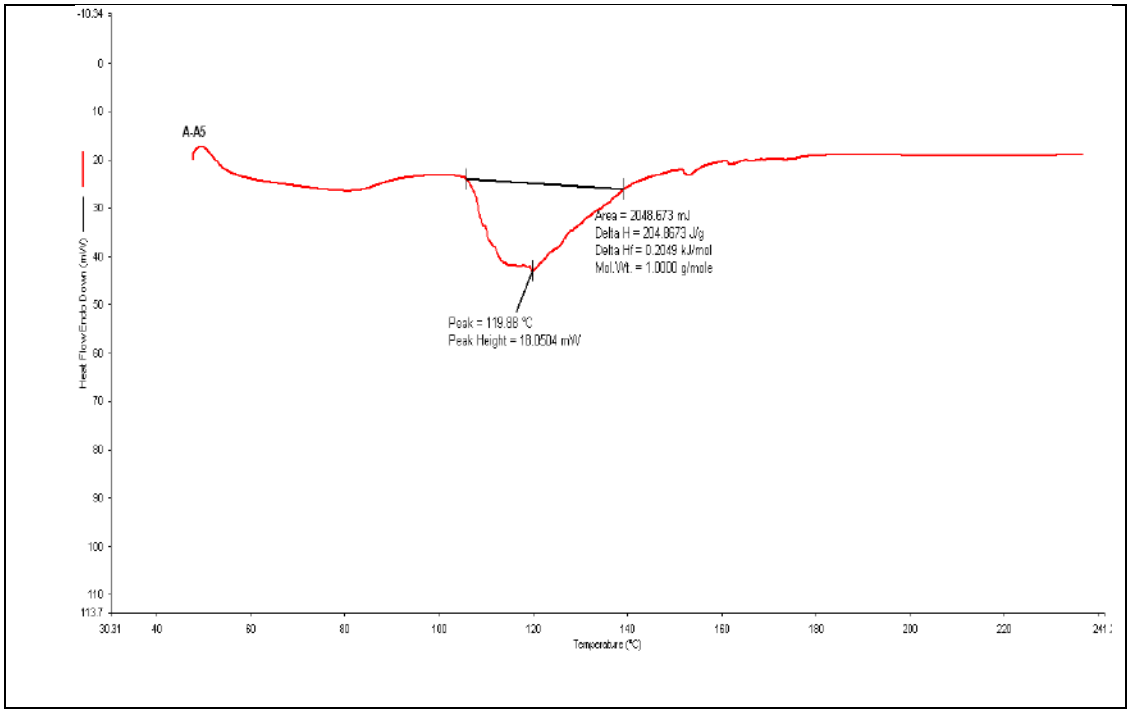


Figure 12: DSC thermogram of deltamethrin-loaded patch

6.8. Release kinetics: The release kinetics data of Zero-order model, First-order model, Higuchi model and Korsmeyer's model are shown in **Table 6, 7, 8 & 9** and the data for regression coefficient (R^2) is shown in **Table 10**.

Table 6: Data of Zero-order kinetic model

Time (hour)	% deltamethrin release
0	0
2	49.156167
4	67.491669
6	86.623988
8	97.713587

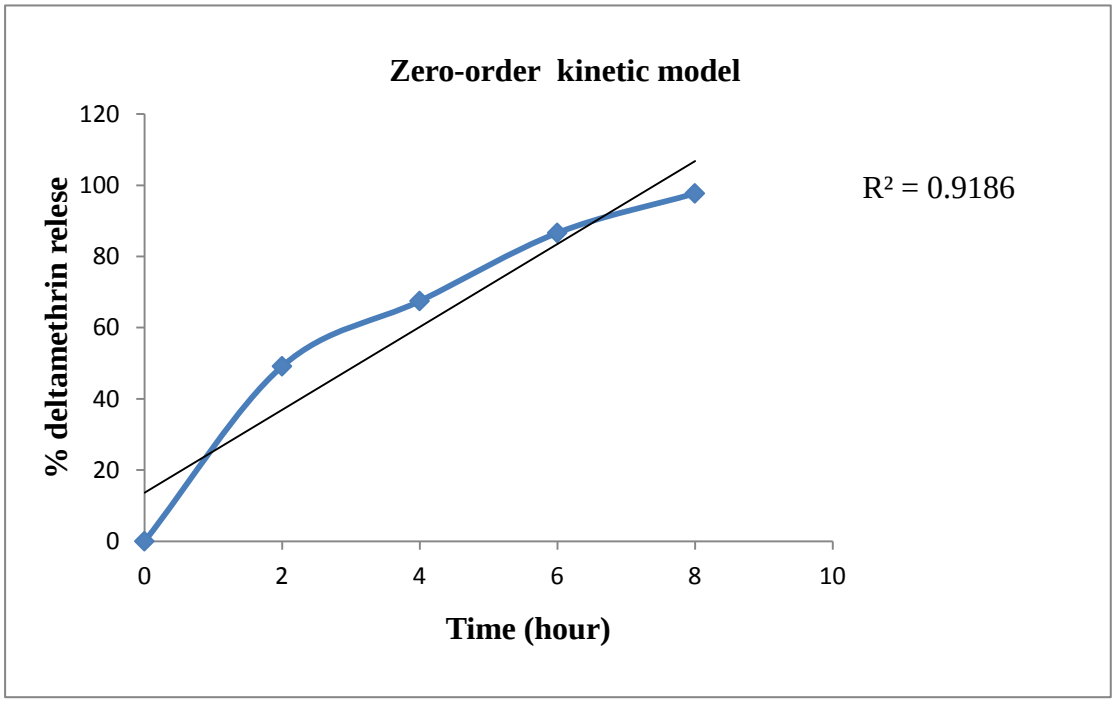


Figure 13: Zero order Plot

Table 7: Data of First-order kinetic model

Time (hour)	Log % deltamethrin release
0	0
2	3.895002319
4	4.212004169
6	4.46157678
8	4.582040614

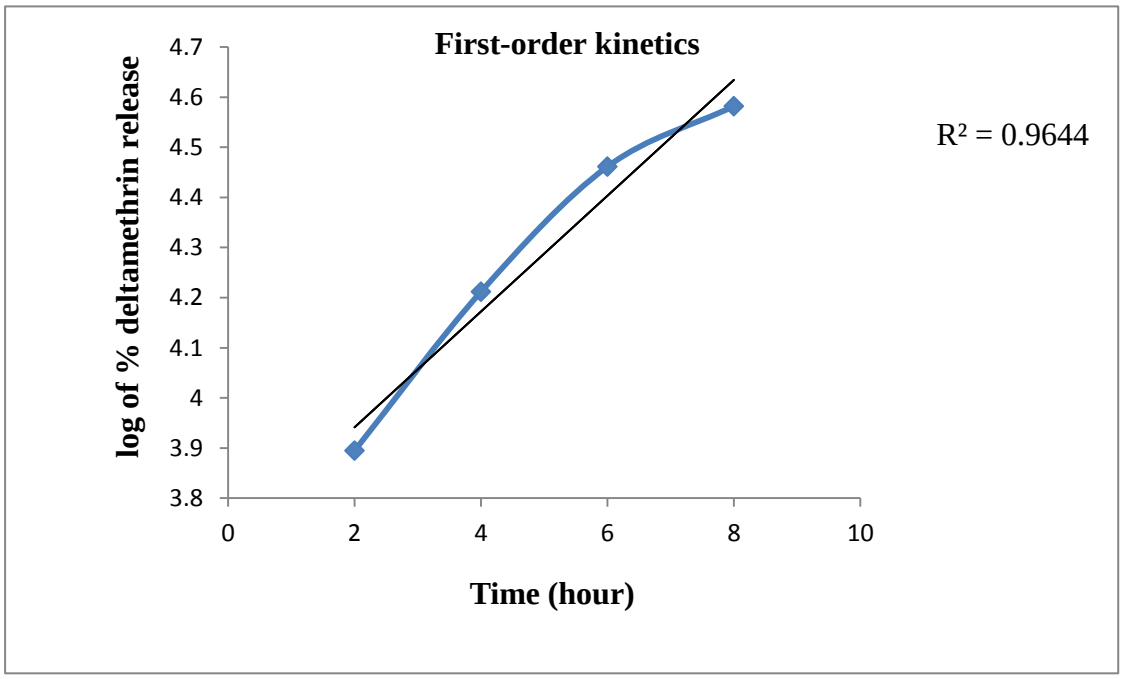


Figure 14: First order Plot

Table 8: Data of Higuchi model

Square root of time (hour)	% deltamethrin drug release
0	0
1.414214	49.156167
2	67.491669
2.44949	86.623988
2.828427	97.713587

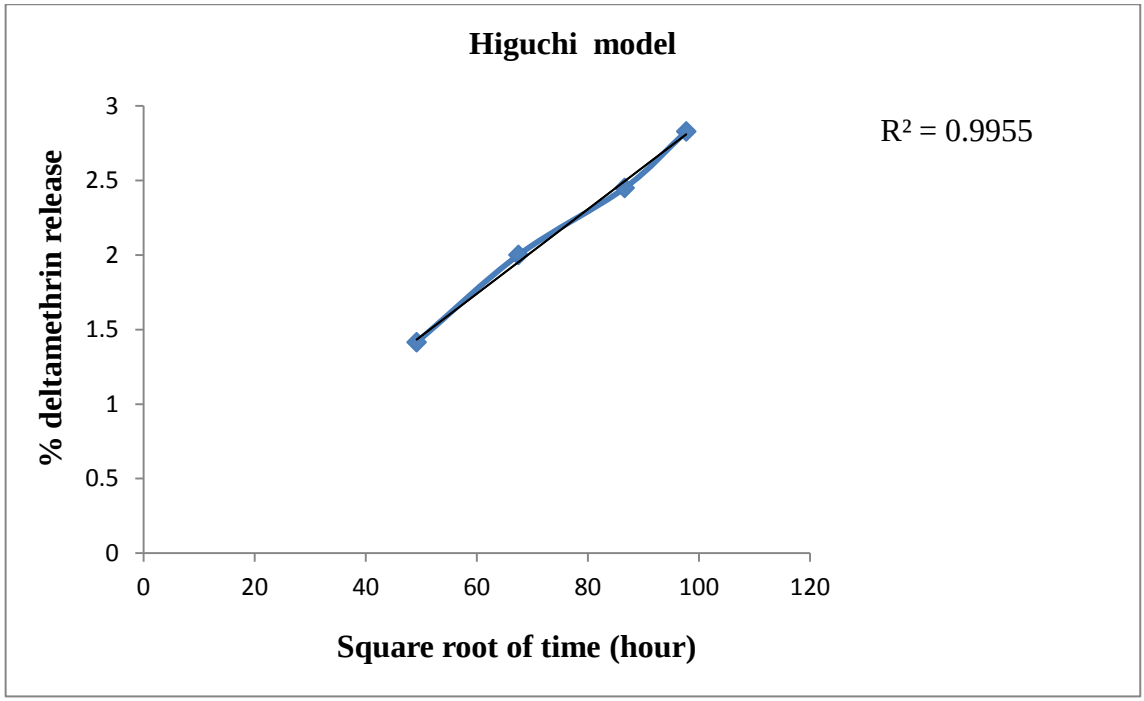


Figure 15 : Higuchi Plot

Table 9: Data of Korsmeyer's model

Log time (hour)	Log % deltamethrin release
0	0
0.693147	3.895002319
1.386294	4.212004169
1.791759	4.46157678
1.791759	4.582040614

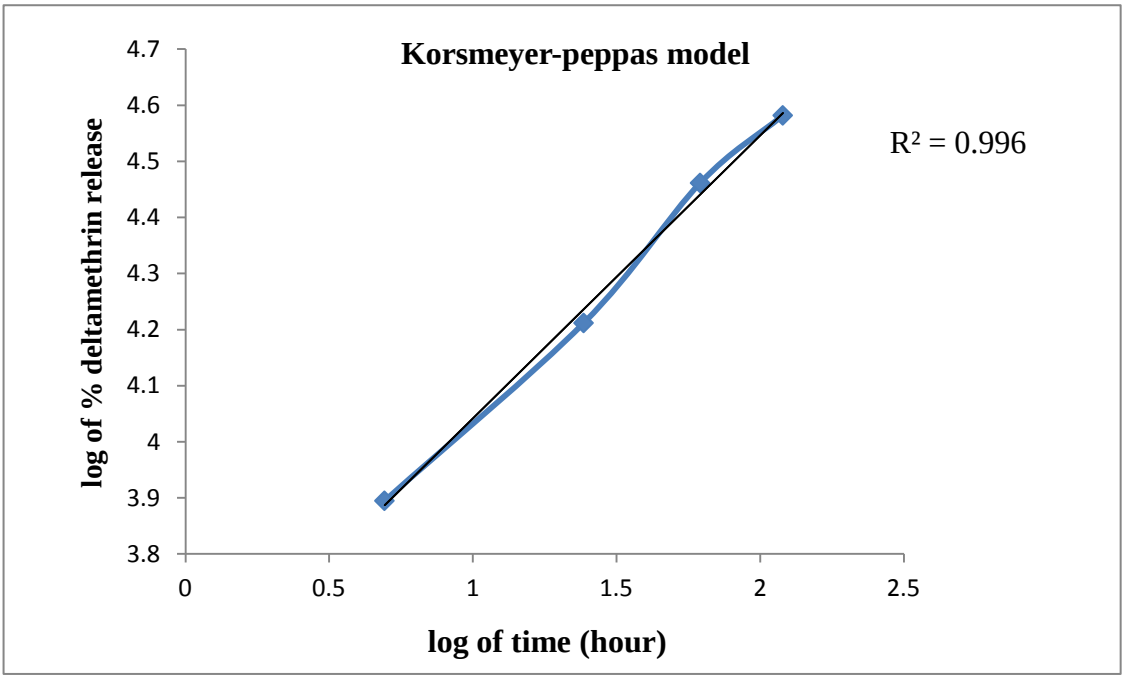


Figure 16: Korsmeyer-peppas Plot

Table 10: Data for regression coefficient (R^2)

Zero order Plot	First order Plot	Higuchi Plot	Korsmeyer-peppas Plot
0.918	0.964	0.995	0.996

6.9. Inhalation toxicity: The data for inhalation toxicity is shown in **Table 11**.

Table 11: Data for inhalation toxicity

Parameters	Penh*	BPM#	TV^
Control	0.54949	151.123	0.04906
Treated	0.75561	147.317	0.03777

*Enhance Pause #Breath per minute(ml/sec) ^Tidal volume(ml)

6.10. Repellency test results: The data of repellency test for landing is shown in **Table 12** and the data, figure for landing (Percent repellent) and landing repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory conditions of percent repellency are shown in **Table 13** & **Figure 17**.

Table 12: Repellency test data for landing

Time (minute)	**Control (Mean±SD)**	**Treated (Mean±SD)**	**Percent repellency (Mean±SD)**	**Repellency index (Mean±SD)**
30	6.66±3.78	1.66±0.57	70.6±17.8	72.2±17.61
60	6.33±2.31	3±1	51.8±10.5	54.8±10.3
90	6±2	2.33±0.57	59.7±8.67	62.0±8.85
120	5.33±3.21	2±1.73	65.7±9.75	67.7±8.22
150	2.66±0.57	1.33±0.57	50±16.6	51.3±16.1
180	3.33±0.57	1.33±0.57	61.1±9.62	62.4±9.04
240	5±1	2.33±1.15	55±18.02	57.3±16.1
300	5.33±2.08	2.33±1.15	57.9±8.36	60.2±6.35
360	4±1.73	1-66±0.57	56.6±5.77	58.3±6.35
420	4±3	1±1.73	85.7±24.7	86.7±3.02

*All values expressed as mean ± SD (n=3)

Table 13: Data for landing (Percent repellent)

TIME	MEAN
30	70.60606
60	51.85185
90	59.72222
120	65.74074
150	50
180	61.11111
240	55
300	57.93651
360	56.66667
420	85.71429

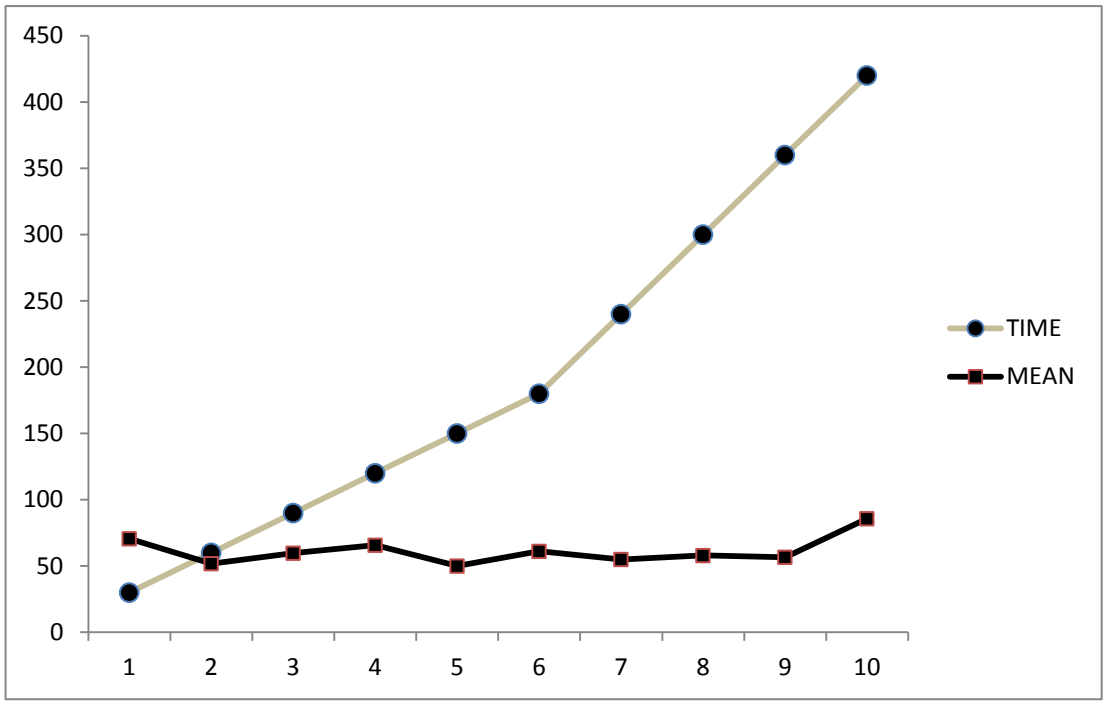


Figure 17: Landing repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory conditions of percent repellency.

6.10.1. The data, figure for landing (repellency index) and landing repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory conditions of repellency index are shown in **Table 14** & **Figure 18**.

Table 14: Data for landing (Repellency index)

TIME	MEAN
30	72.27273
60	54.85185
90	62.05556
120	67.74074
150	51.33333
180	62.44444
240	57.33333
300	60.26984
360	58.33333
420	86.71429

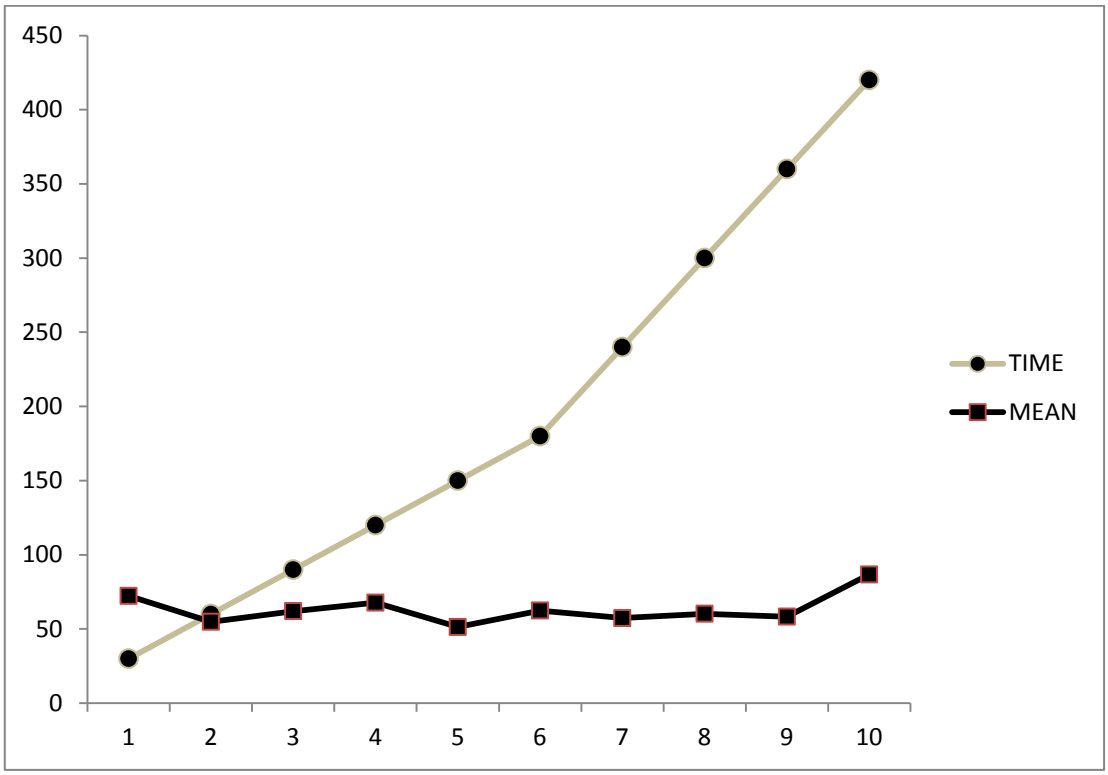


Figure 18: Landing repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory conditions of repellency index.

6.11. Repellency test results: The data of repellency test for biting is shown in **Table 15** and the data, figure for biting (Percent repellent) and biting repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory conditions of percent repellency are shown in **Table 16** & **Figure 19**.

Table 15: Repellency test data for biting

Time (minute)	Control (Mean±SD)	Treated (Mean±SD)	Percent repellency (Mean±SD)	Repellency index (Mean±SD)
30	1.66±0.57	0.33±0.57	83.3±28.87	77.7±38.49
60	1.66±0.57	0.66±0.57	66.5±28.8	55.5±38.49
90	1.33±0.57	1±0	16.6±28.8	11.11±19.24
120	1±0	0.33±0.57	66.6±57.7	66.6±57.7
150	1.33±0.57	0.33±0.57	83.32±8.8	77.7±38.4
180	1±0	0	100±0	100±0
240	1±0	0.33±0.57	66.6±57.7	66.6±57.7
300	1±0	0	100±0	100±0
360	1±0	0	100±100	100±0
420	1.33±0.57	0.33±0.57	100±50	77.7±38.4

*All values expressed as mean ± SD (n=3)

Table 16: Data for biting (Percent repellent)

TIME	MEAN
30	83.33333
60	66.66667
90	16.66667
120	66.66667
150	83.33333
180	100
240	66.66667
300	100
360	100
420	83.33333

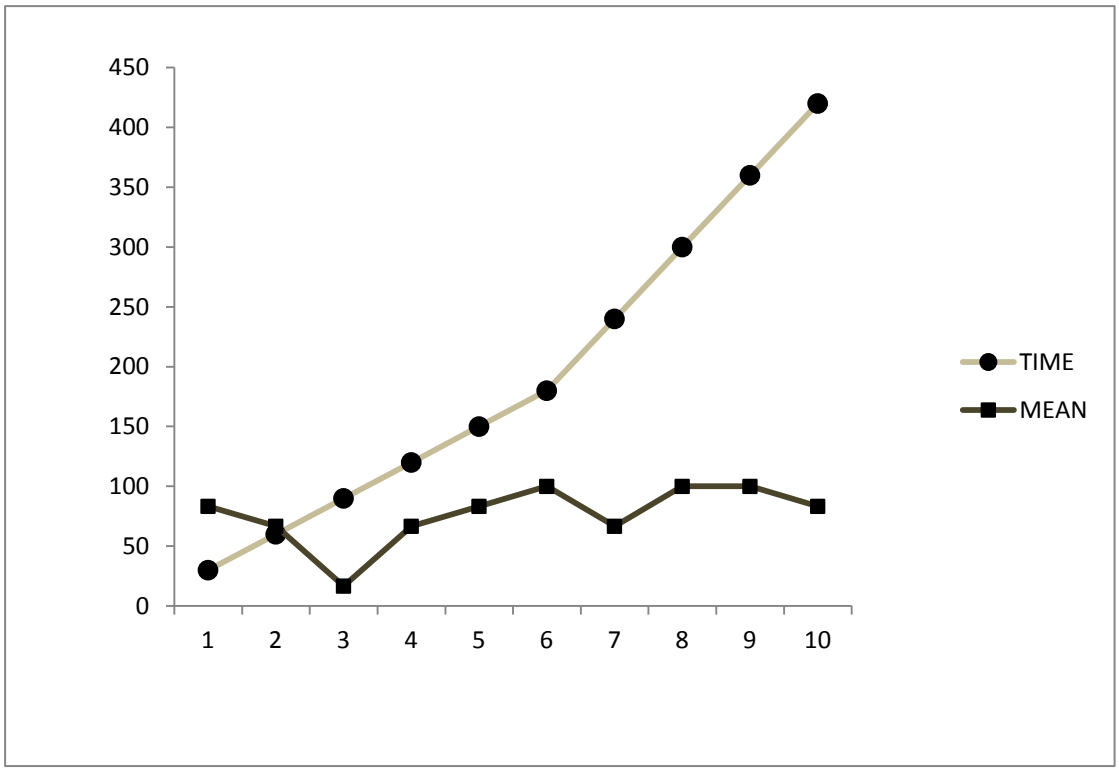


Figure 19: Biting repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory condition of percent repellency.

6.11.1. The data, figure for biting (repellency index) and biting repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory conditions of repellency index are shown in **Table 17** & **Figure 20.**

Table 17: Data for biting (Repellency index)

TIME	MEAN
30	77.77778
60	55.55556
90	11.11111
120	66.66667
150	77.77778
180	100
240	66.66667
300	100
360	100
420	77.77778

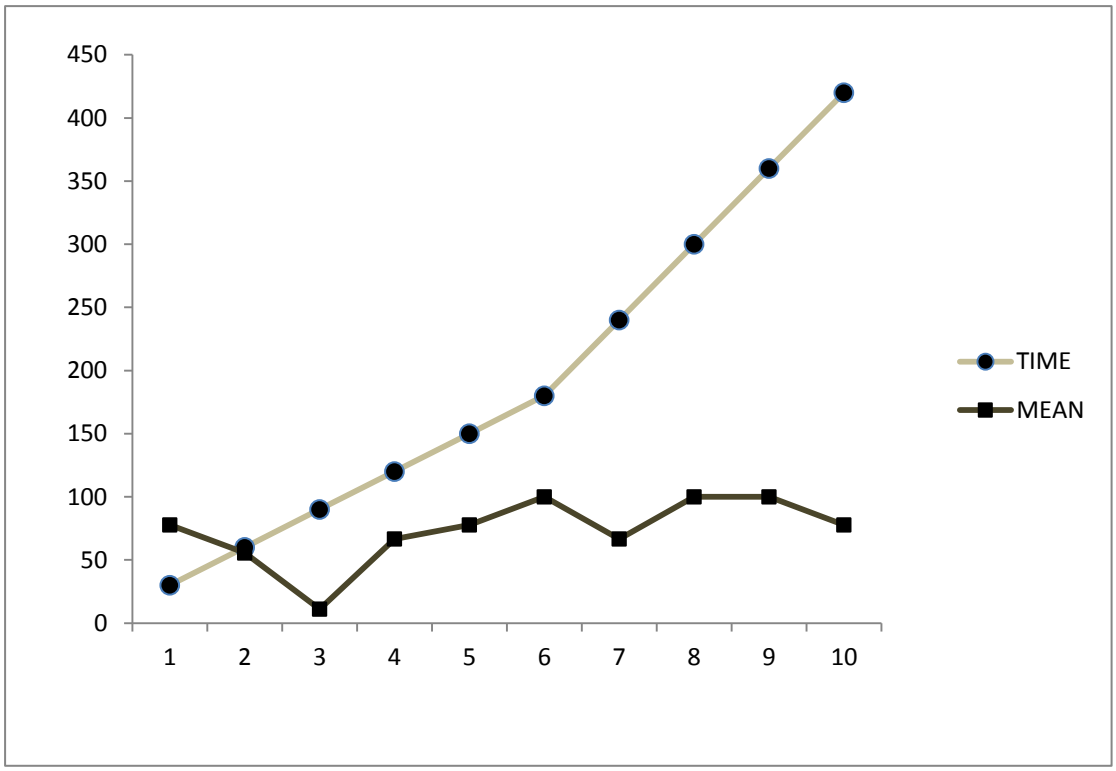


Figure 20: Biting repellent activity of deltamethrin-loaded mosquito repellent patches against *Aedes* (S.) *albopictus* under laboratory condition of repellency index.

7. DISCUSSION

Deltamethrin-loaded sustained release mosquito repellent patches were prepared by solvent evaporation method in a molded ring with defined surface area and controlled thickness over the backing membrane on a horizontal surface with an inverted funnel to control the rate of evaporation of the solvent. Different formulation (as shown in **Table 1**).

7.1. Physico-chemical evaluation of deltamethrin loaded patches

The physicochemical characteristics of the deltamethrin-loaded mosquito repellent patches along with different combination of EC and PVP were presented in **Table 3**. The weight variation of all the formulations varied in between 0.459 ± 0.104 to 0.544 ± 0.059. The surface area of all the formulation varied in between 13.64 ± 1.373 to 14.51± 0.675. The prepared patches were found to be uniform in thickness. The variation in the thickness of all the formulation was in the range between 0.35±0.007 to 0.57±0.007. The thickness uniformity and low weight variation indicates dosage uniformity in the patches. The values of the above physicochemical evaluation parameters are in appropriate ranges. The result may be because of constant polymer weights that are used while preparing the formulation. The results of moisture content (2.60±0.251 to 8.92±0.433) revealed that these properties were increased with the increase in hydrophilic polymer (PVP) content in the patches. Moisture uptake of these patches was found to vary from 3.42±2.66 to 16.44±2.66. The result values variation occurs due to the present of different ratios of polymers. Highest moisture uptake was found maximum in formulation A1 & A4 while

compared with A2 & A3 due to higher concentration of PVP K-30; as it is hydrophilic in nature, highly hygroscopic thereby increase in moisture uptake. In case of formulation A2 & A3 the moisture uptake is lower due to the nature of EC a water resistant. Low moisture uptake capacities protect the patches from microbial contamination and bulkiness during high humid conditions. There was found no difference in the length of the strips before and after cutting in longitudinal strips. This indicates zero constriction and 100% flatness. Hence these patches will maintain a smooth and uniform surface when they were placed onto the skin. The percent deltamethrin content was found to be from 50.35 to 77.60% of all the patches prepared, A3 showed promising results due to EC, due to less moisture uptake, thereby encapsulating deltamethrin into its core. All the prepared patches were spherical, smooth, less sticky, clear, homogenous, and uniform in nature. This is further confirmed by SEM studies. The pH of skin ranges from 5.8 to 6. The surface pH of all the patches (A1 to A5) was near 6 and hence, these patches should not cause any irritation in the skin.

7.2. SEM analysis

SEM photographs (**Figure 5 & 6**) demonstrated the homogeneous dispersion of the deltamethrin in the polymeric matrices.

7.3. FT-IR analysis

The deltamethrin-polymer interaction was studied by FT-IR analysis and is presented in spectra (**Figure 7 to 10**). The spectra (**Figure 7**) of deltamethrin has showed peaks at 1732.85 cm^{-1} due to carbonyl compound, peak at 1486.35 cm^{-1} showed ring stretch absorption C=C groups, peak at 1118.74.cm^{-1} is due to

aromatic C-O-C stretch , 1013.85 cm^{-1} due to aromatic ethers of C-O band groups, peak at 749.60 cm^{-1} showed out of plane bending due to =C-H group, and peak at 643.22 cm^{-1} due to strong stretch in aliphatic bromides. The spectra (**Figure 8**) of EC peak at 3474.74 cm^{-1} due to O-H group, peak at 2973.06 cm^{-1} showed symmetric stretch due to the presence of C-H group, peak at 1481.98 showed CH_2 bending and peak at 1374.66 cm^{-1} showed CH_3 bending. The spectra (**Figure 9**) showed peak at 3423.30 cm^{-1} due to amide N-H stretching , peak at 1644.92 cm^{-1} due to C=O group, peak at 1457.93 cm^{-1} due to methylene group bending absorption of PVP. Whereas in the spectra formulation A5 (**Figure 10**), the same characteristics peaks related to deltamethrin were noticed with slight variations. This ruled out the deltamethrin has no effect on polymeric interaction; hence the compound is stable in the formulation.

7.4. DSC Analysis

The DSC analysis of deltamethrin alone showed a sharp endothermic peak at 107.85 °C at corresponding to its melting point (**Figure 11**). The DSC analysis of deltamethrin loaded patch with polymers ethyl cellulose (EC):polyvinylpyrrolidone (PVP) showed a blunt endothermic peak at 119.88 °C with slight change in melting point of deltamethrin towards higher temperature (**Figure 12**). This change in temperature is might be due to the physico-chemical bonding of the drug with the polymer, which shifted the melting point of the drug towards higher temperatures.

7.5. Release study

The release data of best optimized formulation A5 was fitted into various release rate equations such as zero order, first order, Higuchi's square root time dependent diffusion and Korsmeyer- peppas exponential equations (**Figure 13**, **14**, **15** & **16**). The range of 'n' value for Korsemeyer-Peppa's equation -1 to 1. If the 'n' values of Korsemeyer-Peppa's equation is below 0.5, which indicates Fickian kinetics. If the 'n' value of Korsemeyer-Peppas equation is in between 0.5 to 1, this indicates non-Fickian kinetics. Here the formulation (A5) release kinetics fitted in Korsemeyer-Peppas equation and 'n' values showing 0.504. The kinetic studies revealed that the best optimized formulation A5 shows a Fickian diffusion controlled release mechanism governed by Higuchi kinetics as shown in (**Table 10**).

7.6. Inhalation toxicity

The animals were found to be non-toxic and there was no change in the inhalation pattern of their body. All the parameters of the animals were found within the normal range as shown in (**Table 12**).

7.7. Repellent test results:

Landing and biting repellent activity of deltamethrin-loaded mosquito repellent patches against *A. albopictus* performed under laboratory conditions has been presented in **Figures 17**, **18**, **19** & **20**. There was no difference in both percent repellency (df=29, F=2.248, p=0.0686) and repellency index (df=29, F=2.129, p=0.0824) for landing of mosquitoes at various time intervals.

Similarly, no difference could be observed for percent repellency (df=29, F=1.55, p=0.2046) and repellency index (df=29, F=2.07, p=0.0910) biting of *A. albopictus* mosquitoes.

Many studies have been carried out to evaluate the repellent activity of synthetic insecticides against vector mosquitoes, which suggest that synthetic insecticides have been highly effective in providing repelling the mosquitoes and other biting insects (Maia MF *et al*., 2012). The synthetic insecticides have been found to be consistent in activity even after a long time of use unlike the herbal based repellent which provide repellency initially but after some time the activity ceases (Maia MF & Hazarika *et al*., 2012). Other studies have indicated that the synthetic repellents while applied along with the other repellents provide much more protection against mosquitoes as compared to applied singly (Lt Col A Bhatnagar & Col VK Mehta 2007).

8. CONCLUSION

In this study, a deltamethrin-loaded mosquito repellent patch was found to be safe, and efficacious to skin. Patches developed with PVP and EC in combination are physico-chemically stable and showed good physical properties and evaluation parameters are in acceptable ranges. The preliminary *in-vitro* evaluations of various combinations were evaluated through this study. All the prepared patches formulated were stable at room temperature. The test on pH determination indicated that the prepared patches are safe for skin. FT-IR and DSC studies indicated compatibility between the deltamethrin and the excipients employed in the fabrication of patches. SEM study revealed the homogeneous dispersion of the deltamethrin in the polymeric matrices. The best optimized deltamethrin-loaded formulation i.e. A5 shows Fickian diffusion controlled release mechanism governed by Higuchi kinetics developed. There was no change in the inhalation pattern of the animals and found within the normal range. Landing repellent activity of deltamethrin-loaded mosquito repellent patches against *A. albopictus* performed under laboratory conditions found that there is no difference in both percent repellency and repellency index of mosquitoes at various time intervals. Similarly, no difference could be observed for percent repellency and repellency index biting of *A. Albopictus* mosquitoes. Based on the above observations, it can be reasonably concluded that PVP-EC polymers are better for the development of controlled release deltamethrin-loaded mosquito repellent patches and it can be used for prolong mosquito repellent application.

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