MAJOR PROJECT

MULTI-DECONTAMINATION COMPOSITE WIPE AGAINST CBR AGENTS

A Major Project Report submitted in partial fulfillment for the award of the degree of

MASTER OF TECHNOLOGY (M. Tech.)

In

POLYMER TECHNOLOGY

Submitted by

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[2K13/PTE/08]

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June 2015

DECLARATION

I, **Himanshu Shiwangi**, hereby certify that the work which is being submitted in this major project report entitled "**Multi-Decontamination composite wipe against CBR agents**" in the partial fulfillment for the award of the degree of Master of Technology (Polymer Technology) at **Delhi Technological University** is an authentic record of my own work carried out by me under the supervision of **Dr. Ram Singh** (Assistant Professor, Department of Applied Chemistry and Polymer Technology, DTU) and **Dr. Himanshu Ojha** (Scientist D, INMAS, DRDO, Delhi).

I, further declare that the project report has not been submitted to any other Institute/University for the award of any degree or diploma or any other purpose whatsoever. Also it has not been directly copied from any source without giving its proper reference.

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CERTIFICATE

This is to certify that the M.Tech major project entitled entitled "**Multi-Decontamination composite wipe against CBR agents**" submitted by **Himanshu Shiwangi**, Roll number 2K13/PTE/08, for the award of the degree of **"Master of Technology in Polymer Technology"** is a record of bonafide work carried out by him. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of the project report. He has done his work in collaboration with Dr. Himanshu Ojha (Scientist D, INMAS, DRDO, Delhi).

To the best of our knowledge and belief the content therein is his own original work and has not been submitted to any other university or institute for the award of any degree or diploma.

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HimanshuShiwangi

Contents

ABBREVIATIONS

S. No.	Symbol	Meaning
1.	IAEA	International Atomic Energy Agency
2.	INES	International Nuclear and Radiological Event Scale
3.	RED	Radiological Exposure Device
4.	RDD	Radiological Dispersal Device
5.	DTPA	Diethylene triamine pentaacetic acid
6.	EDTA	Ethylene diamine tetra acetic acid
7.	WBC	Whole body counter
8.	^{131}I	Radioactive isotope of Iodine
9.	^{127}I	Stable isotope of Iodine
10.	^{203}Tl and ^{205}Tl	Stable isotopes of Thallium
11.	HR-SF-ICP-MS	High resolution sector field inductively coupled plasma mass spectroscopy
12.	O/W	oil-in-water
13.	W/O	water-in-oil
14.	%	Percentage
15.	mg/L	Milligram/Litre
17.	g/mL	Microgram/Litre
18.	Gm	Gram
19.	h/day	Hour per day
20.	^{o}C	Degree Centigrade
22.	cm	Centimeter
23.	mm	Millimeter
24.	REEs	Rare-earth elements
25.	GFAAS	Graphite Furnace Atomic Absorption Spectroscopy

26.	ICP-AES	ICP Atomic Emission Spectroscopy
27.	PIT	Phase inversion temperature
29.	cm^2	Centimeter square
30.	h	Hour
31.	µl	Microlitre
32.	sec	Second
33.	ml	Millilitre
34.	DE	Decontamination Efficacy
35.	pH	Power of Hydrogen
36.	M	Molar
37.	HNO_3	Nitric Acid
38.	cps	Concentration per second
39	LLDPE	Linear Low Density Polyethylene

ABSTRACT

Elimination of harmful contaminants from humans and equipments comes under the category of decontamination of CBRN contaminants. The CBRN contaminants stand for Chemical, Biological, Radiological and Nuclear contaminants. The major objective is to minimize the burden of contaminations and to prepare the obtained minimized contaminated material for permanent disposal activities and protective storage. For decontamination chemical technique is very efficient and cost effective. Sometimes both humans and materials get contaminated with CBRN contaminants. Small amount of contaminants exposure cause change in cell structure. Due to higher amount sometimes death also occurs. Therefore, decontamination is very important for humans, at the same time it is very vital to minimize the waste and cost for the task required for personal decontamination.

Present work deals with the effectiveness of non woven composite wipes of different nano fibers and fibers along with a nanoemulsion for the decontamination of CBR contaminations. Decontamination in general is defined as the removal of hazardous material from areas where it is not wanted. Decontamination is applied to reduce the dose that worker may receive from a component or surface, to reduce the potential for airborne Chemical, biological, and radiological, (CBR) agents, or to reduce the disposal cost associated with the component or the material.

A nanofibrous web was developed which is based on Rayon fiber, Zinc titanate nanofiber and nano ZnO loaded polyester fibers. Because of its super-light weight, excellent resistance to heat radiation, and chemical and bacterial decontamination properties, it is used as a thin interlayer incorporating with highly porous fibrous materials so as to get high absorbency and entrapment properties. The results indicate that the decontamination of CBR contaminants can be effectively improved by adding appropriate nanofibers.

OBJECTIVES

1) To make multi decontamination composite Wipe for radiological, chemical and bacterial contaminants from CBR.

2) To carryout pre-formulation studies of the drug by spectroscopic techniques.

3) To evaluate nanoemulsion of *p-tert*-butylcalix [4] arene.

4) To characterize the prepared formulations by DLS & TEM.

5) To carry out *in vitro* & *ex vivo* decontamination efficacy evaluation using Nuclear medicine technique.

6) To carry out stability studies on optimized formulations as per ICH guidelines.

CHAPTER 1

INTRODUCTION

Nanoemulsions are heterogeneous dispersions of two immiscible liquids like oil-in-water (o/w) or water-in-oil (w/o) having a mean droplet size in the nano metric scale which has typical ranges from 20–200 nm, regardless of method of preparation [1].The term 'nanoemulsion' clearly indicates the nanoscale size range of the emulsion droplets and is distinctly different from the term 'microemulsions'. These nanoemulsions possess low viscosity, very high interfacial area and can have long-term colloidal stability. They can be prepared with a relatively small surfactant concentration of 3–10%. These interesting features of nanoemulsions have propelled scientists to explore applications of nanoemulsions in various fields [2,3].

Calixarene selectivity, affinity and extraction efficacy of uranium present in traces in biological media has been reprted in previous studies [4]. Nanoscale systems have already been developed for detoxification therapy and for actinides decorporation, but they have never been applied to radio-nuclides skin decontamination [5]. Cutaneous application of calixarene-containing nanoemulsion requires quick skin decontamination after contact with radionuclides. Therefore, the nanoemulsion has to display a superficial and efficient action with a least penetration into the skin. Nanoemulsion formulation provides a rapid penetration of active ingredients through skin due to the large surface area of droplets. Even sometimes it is found that nanoemulsion penetrate easily through rough skin. This property of nanoemulsion minimizes the additional utilization of special penetration enhancer which is responsible for incompatibility of formulation. Aurélie et al. prepared formulation displaying good chelating properties towards uranium [6]. In his paper, he had described the ability of this formulation to trap uranium and limit its transfer from the cutaneous contaminated site into the blood. Uranium percutaneous diffusion kinetics was assessed with Franz cells over 24 h through intact and excoriated pig ear skin biopsies, after or without application of the formulation. The results showed that prompt

application of the formulation allows 94% and 98% reduction of the amount of uranium diffused respectively through intact and excoriated skin.

Gadea et al. reported that chelating agents such as calcium or zinc diethylene triamine pentaaceticacid (DTPA) form compounds with specific radioisotopes poorly excreted by the kidneys, like rare earth sand actinides such as californium, plutonium, and americium, rendering them more easily excreted and enhancing elimination [7]. López et al. reported that chelating agents such as dyethilene-triamine-pentacetic acid (DTPA) as the zinc or calcium salts (Zn and Ca-DTPA) can expedite the removal of radioisotopes such as plutonium-239 or yttrium-90 [8]. Sodium bicarbonate is used to treat renal chemical toxicity of uranium and reduce the risk of acute tubular necrosis (which is generally a far greater hazard than its radiologic toxicity). Oral administration of insoluble Prusian blue is the countermeasure of choice for Cesium-137 (found in high concentrations for miles around Chernobyl following the accident) rubidium-82, or thallium-201. Oral calcium or aluminium phosphate solutions can block the absorption of strontium through competitive inhibition. Radioiodines are known from Chernobyl data to cause thyroid injury and to be carcinogenic, especially to the fetus and to children under 18 years of age. If taken within 4–6h of contamination, stable iodine in the form of nonradioactive potassium iodide (KI) saturates iodine binding sites within the thyroid and inhibits incorporation of radioiodines into the gland^{131}I and ^{137}Cs are the most significant for dose received by the exposed population in Chernobyl.

Kamboj et al. developed a superior formulation for skin decontamination of 99mTc-pertechnetate (a potential radiocontaminant), a topical gel formulation containing disodium edetatewasoptimized by using 2-factor, 3-level central composite design [9]. Validation of the optimization study with 13 confirmatory runs indicated a high degree of predictive ability of

response surface methodology (RSM). The optimized formulations were evaluated for drug content, *in vitro, in vivo, ex vivo* and skin irritation studies [9]. Liu et al. developed solid lipid nanoparticles (SLN) hydrogel for transdermal iontophoretic drug delivery [10]. The results indicated that SLN carbopol gel could be used as a vehicle for transdermal iontophoretic drug delivery under suitable electric conditions [10]. Lim et al. developed a novel approach for the use of hydrogel nanoparticles as effective carriers in transdermal delivery systems. Hydrogel nanoparticles, sized of 37 nm at dried state, were synthesized *via* a cross linking reaction between hyaluronic acid (HA) and polyethylene glycol [11]. The results suggested the importance of dispersion medium for the hydrogel nanoparticles to become effective drug carriers in topical/transdermal delivery systems [11].

In the document of U.S. Department of Health and Human ServicesFood and Drug AdministrationCenter for Drug Evaluation and Research (CDER) [12], the goals of internal decontamination are to reduce absorption and to enhance excretion of radioactive contaminants are given. Treatment is most effective if it is started as soon as possible after contamination. Radioactive contaminants may be internalized *via* inhalation, ingestion, or through wounds and skin. Treatment should be directed by knowledge of the specific radio-contaminant. Ideally, internal decontamination should begin during the first few hours if the treating physician suspects that radio-contaminants may have been internalized. After careful retrospective review of clinical data from human exposures resulting from nuclear detonations or nuclear reactor accidents, Prussian blue, potassium iodide (KI), and calcium-diethylene triamine pentacetate (Ca-DTPA) and zinc-diethylene triamine pentacetate (Zn-DTPA), when manufactured under conditions specified in an approved new drug application (NDA), were found safe and effective for the treatment of internal contamination with radioactive cesium, iodine, and plutonium,

americium, or curium, respectively. Currently, there are approved Prussian blue, KI, and Ca- and Zn-DTPA products in the United States [12].

Radioactive Contamination can arise from accidents involving nuclear reactors, industrial sources, or medical sources. The potential for these accidents has been present for many years. Recent events also have highlighted the potential for number of accidental radioactive contamination as a result of criminal or terrorist actions. Major radiation accident including Chernobyl nuclear reactor accident 1986, Goiania hospital irradiation source accident 1987 and Fukushima nuclear reactor accident 2011 resulted in contamination from individuals to many with Iodine-131, Strontium-90 and Cesium-137 [13].

External contamination

Contamination is the presence of a minor and unwanted constituent (contaminant) in a material, in a physical body, in the natural environment, at a workplace, etc. Contamination occurs by three main routes: inhalation, ingestion, and wound contamination [14]. A fourth and infrequent route is percutaneous absorption [15]. Radioactive contamination occurs when radioactive material absorbed from a contaminated Skin. External contamination occurs by the percutaneous absorption, which applies almost exclusively to radioactive isotopes. The target area for most contamination lies in the viable epidermis or stratum germinativum, where proliferative cells are located. The usual objective of dermatological therapy is to produce a designed therapeutic action at specific sites on the epidermal tissue. This requires diffusive penetration of the drug from the skin surface into the stratum corneum under the aegis of concentration gradient [21,22]. As long as these radioactive Contaminants remain in the body, they may pose significant health risks. The risks are largely long term in nature and depend not only on the type and concentration of the radioactive contaminant absorbed, but also on the

health status of the exposed individual. The only effective method of reducing these risks is removal of the radioactive contaminants from the Skin. However, early recognition of external contamination provides the greatest opportunity for radio contaminant removal. The uptake and retention of a radioactive contaminant is influenced by its portal of entry, chemistry, solubility, metabolism, and particle size [18-20].

Percutaneous absorption

The uptake of radio-nuclides through intact skin may cause unmanageable side effects especially for radio-nuclides with a long decay period. The critical site of radiation resulting from contamination is the stratum germinativum, where proliferative cells are located. The radiation dose reaching the basal cell layer of the epidermis involves predominantly beta and gamma radiation [21-23] (Figure 1).

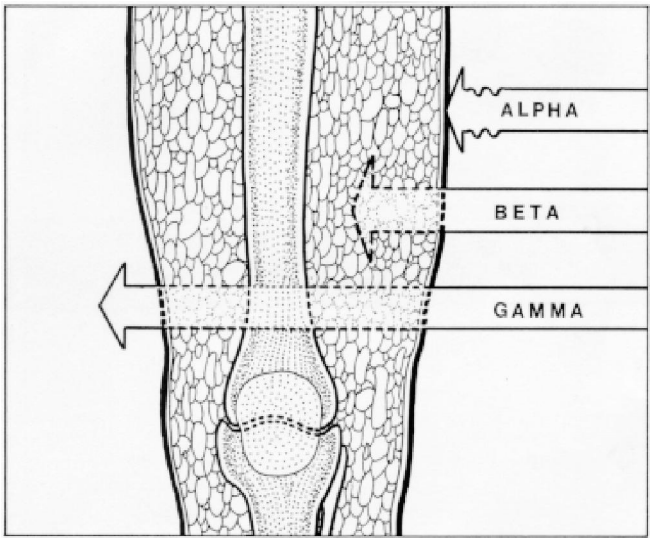


Figure 1. Radiation travelling through human tissue

The main pathway of a radioisotope from the skin to the systemic circulation is through hair follicles. W. Conrad Roentgen discovered X-ray in 1895, and further extensive working of

Curie family and Henri Becquerel results to radioactivity field [24-26]. Radioactive elements are those having an unstable atom, and its nucleus emits ionize radiation, i.e. alpha particles, beta particles, and gamma rays, in order to lose the extra energy retained inside [27,28]. This process of emitting radiation is accidental, and is known to be as Radiation Decay or Radioactivity.

The International Nuclear and Radiological Event Scale (INES) were commenced in 1990, by the International Atomic Energy Agency (IAEA), so that proper safety-significant information can be taken into action in case of nuclear emergencies. The scale is proposed to be logarithmic. There are total of seven nonzero levels on the INES scale, out of which three are incident-levels and four are accident-levels. There is a level zero as well. The level on the scale is determined by the highest of three scores: off-site effects, on-site effects, and defense in depth degradation. Each increasing level represents an accident approximately ten times more severe than the previous level (Figure 2).

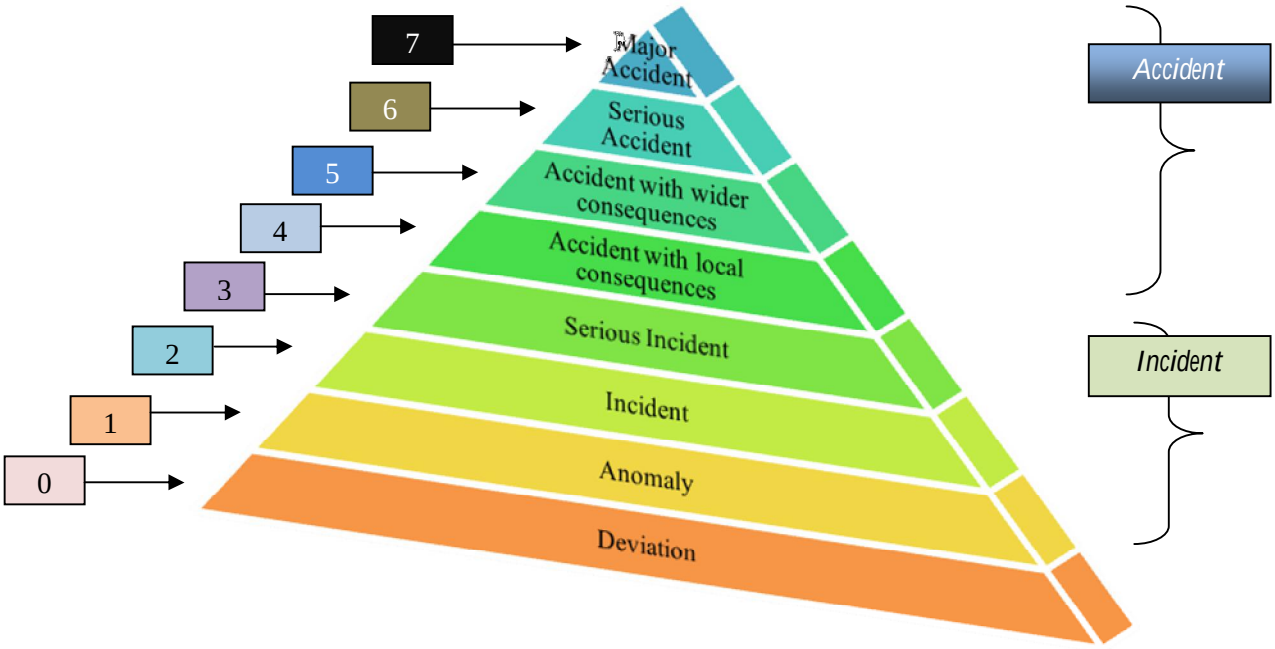


Figure 2. Levels in the Radioactive Emergencies

It has been proposed by the International agencies for radiation emergency management, that the radioactively affected area should be washed with soap and water as soon as it is feasible for the person in distress [29-31]. Either an acid soap or 25% solution of diethylene triamine pentaacetic acid (Ca-DTPA) can be used for the decontamination process of the radioactive contaminants, because it would help in removing some amount of harmful elements from skin thus reducing their intrusion inside the body [32-35]. Such simple methods help in removing the contaminants from the skin up to a certain level. There is a need of the broad spectrum of decontaminating agents which can remove most of the radioactive contaminants from the skin of the victims. Calixarene (Figure 3) are the new class of compound which is compatible to make the complex with most of the radioactive compounds. It is the product of the hydroxyalkylation of a phenol and an aldehyde, due to which a macrocycle or cyclic oligomer is formed [36,37]. Due to the presence of hydrophobic cavities, slighter small molecules or ions are held inside it, and thus calixarene and its derivatives are used to form complex with a wide range of cations, anions and neutral molecules.

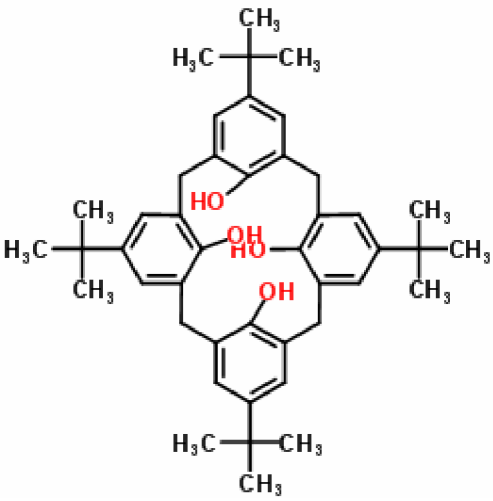


Figure 3. *p-tert*-butylcalix[4]arene

In the present work, decontamination efficacy of *p-tert*-butylcalix[4]arene based nanoemulsion was determined using Whole body counter (WBC) against ^{131}I and ^{201}Tl using Sprague-Dawley rat.

Iodine-131 (^{131}I), also known as radioiodine was discovered by Glenn Seaborg and John Livingood in 1938 at the University of California, Berkeley. It is a significant radioisotope of iodine, which has a half-life of about eight days, and is allied with applications like medical diagnostic and treatment procedures, natural gas production and nuclear energy [38]. It was the primary element of contamination in the Chernobyl disaster.

Thalllium (Tl) was discovered independently in 1861, by scientists, William Crookes and Claude-Auguste Lamy. It has 25 isotopes, with atomic masses ranging from 184 to 210 out of which only two, ^{203}Tl and ^{205}Tl are stable. The rest are radioactive in nature [39,40].

Whole Body Counter (WBC) is an important analytical tool for the quantitative analysis of decorporation measurements and routine control of contaminated persons [40-43].

Due to the safety concerns, ex-*vivo* evaluation of the complexation of *p-tert*-butylcalix[4]arene nanoemulsion with the stable isotopes of Iodine (^{127}I), Thalium (^{205}Tl) radio-nuclides were performed using high resolution sector field inductively coupled plasma mass spectroscopy (HR-SF-ICP-MS).

CHAPTER 2

EXPERIMENTAL

Materials and Equipments

Table 1. List of Materials used in the experimental work

S. No.	Name of Chemicals	Source
1	Para-tertbutylCalix[4]arene	Neelkanth Distributers,Vadodra, India
2	Paraffin oil	Sigma Aldrich, USA
3	Sorbitanmonooleate (Span® 80)	Sigma Aldrich, USA
4	Polyoxyethylene sorbitanmonooleate (Tween® 80)	Sigma Aldrich, USA
5	Chloroform	Rankem, India
6	Hydrochloric acid	Reachem lab, Chennai, India
7	Strontium Nitrate	Sigma Aldrich, USA
8	Dimethylsulfoxide	Sigma Aldrich, USA
9	Technetium-99m	Board of Radiation and Isotope Technology (BRIT) INMAS Delhi India.
10	Iodine-131	Board of Radiation and Isotope Technology (BRIT) INMAS Delhi India
11	Thallium-201	Nuclear Medicine Department of AIIMS, Delhi, India
12	Polyvinylpyrrolidon(PVP) polymer $[C_6H_9NO]_n$	Sigma Aldrich, Germany
13	Titanium Isopropoxide $[C12H_{28}O_4Ti]$	Sigma Aldrich, Germany
14	Zinc Acetate $[CH_3CO_2]_2Zn$	Sigma Aldrich, Germany
15	Acetic acid glacial $[CH_3CO_2H]$	Sigma Aldrich, Germany
16	Ethanol Solvent	INMAS Delhi India.
17	Acetone Solvent	INMAS Delhi India.
18	Polyester chips (PET)	CHEMCO group New Delhi.
19	LLDPE	CHEMCO group New Delhi.
20	Compatibilizer LLDPE-g-MA	CHEMCO group New Delhi.

Table 2. List of equipments used in the experimental work

S. No.	Name of Instruments	Name of Company
1	Needle-punching machine	3V International, NITRA
2	Whole Body Counter	Indigenously developed, WBC setup, INMAS, DRDO
3	High Resolution Sector Field Inductively Coupled Plasma Mass Spectroscopy (HR-SF-ICP-MS)	Thermo Scientific, Element XR^{TM}, USA
4	Differential Scanning Calorimetry	STARe Software (Mettler Toledo, 821e, Switzerland).
5	UV-Visible spectrophotometer	Cary100 Bio, Varian, Australia
	Magnetic stirrer	MLDX Remi, Mumbai, India
6	Electrospinning	EC-DIG IME Technologies, Netherlands
7	Melt spinning machine	LK-RBO6L, Guangdong, China
8	Homogenizer	TMP125 Omni International, Georgia
9	Dynamic light scattering (DLS)	ZS (La-900, HORIBA, U.K.).
10	Fluorescence spectroscopy equipment	Spectramax M_2 Germany.
11	Transmission electron microscopy (TEM)	Morgagni 268D, FEI, Holland.
12	Closed digestion vessel microwave system	Multiwave™ 3000, Perkin Elmer®, MA, USA
13	Weighing balance	Sartorius, Germany.
14	Digital balance	Shinko Sansui, Japan.
15	Digital pH meter	Orion 2-Star, Thermo Scientific
16	Hot air oven	Tempo, Mumbai
17	High purity water (Milipore)	SELECT®Ultra, Sigma-Aldrich, Germany

Experimental models

2-3 Months old adult male and female Sprague-Dawley rat weighing 180 ± 20 gm, in-bred in the experimental animal facility house of the Institute of Nuclear Medicine and Allied Sciences (INMAS) Delhi, were used for the experiment. Animals were kept under standard laboratory condition with photoperiod of 12 h/day and temperature of 25 ± 2^{o}C. Rats were housed individually in polyvinyl cage and fed standard animal food pellets (Golden Feeds, Delhi, India) and offered potable drinking water *ad libitum*. All the procedures were carried out in strict compliance with the Institutional Animal Ethics Committee (IAEC) INMAS.

Preparation of nanoemulsions

Phase inversion temperature (PIT) method was employed for the preparation of nanoemulsion [44,45]. *p-tert*-Butylcalix[4]arene was solubilised into the paraffin oil and non-ionic surfactants added with stirring (150 rpm) using a magnetic stirrer at 50^{o}C. Water was added slowly to the solution mixture and allowed to cool at room temperature under slight stirring at 150 rpm followed by homogenization at 1200 rpm for 2 min.

Preparation of Zinc Titanate Nanofiber [$ZnTiO_3$]

Two precursors of different Ti:Zn ratio with the help of Titanium isopropoxide and Zinc acetate for TiO_2-ZnO was prepared. 40% TiO_2 was prepared as 1st sample and 50% TiO_2 as 2nd sample, 50% sample was prepared to achieve perfect TiO_2-ZnO mixture. 40% TiO_2 sample was prepared, by taking 6 ml ethanol in a flask and adding 0.4 gm of Titanium isopropoxide and constantly stirring it, then 0.6 gm of Zinc acetate was added to it, Then 2 ml of Polyvinylpyrrolidone (PVP) polymer was added to it and as a result the 40% TiO_2 precursors was prepared. Similarly 50% sample was also prepared.

These precursors were kept for 6 hr with uniform stirring and then they were taken for electrospinning. For electrospinning, we used 3 mL syringe with internal diameter of 0.5 mm and the rate of pumping precursor from syringe was 1-2 mL/h with the 40-50% RH humidity parameter. Voltage applied was 10-15 KV. The fibers were collected on a foil of aluminium. The parameters were kept constant for both precursors to achieve a membrane of a uniform thickness. Then these nanofibers were annealed in a furnace with temperature (300-700^{o}C) and then they were cooled at 5^{o}C/min at controlled rate. It was carefully noted that these fibers were removed immediately from the foil and kept into a crucible which is further placed in the furnace. The fibre was subjected to heat treatment process immediately after electrospinning, due to the high moisture absorbency of PVP polymer. The diameter of nanofiber was ranging between 100-600 nm.

Preparation of Antibacterial fiber

Synthesis of Nano ZnO

In flask (A) 300 mL of ethanol was taken and 10 gm of Dehydrated Zinc acetate added to the flask and continuously stirred at 60 ^{o}C for 30 minutes. This will dissolve zinc acetate completely. Now in another flask (B) with 200 mL of ethanol, 12 gm of dehydrated oxalic acid was dissolved at 50 ^{o}C for 30 minutes.

Now, solution from flask (B) was slowly added to warm solution in flask (A) with continuous stirring. Thick gel of white colour was extracted and then it was calcined at 600 ^{o}C for 2 hrs. This finally gave ZnO nanoparticles.

Preparation of Master-batch

The master batch was prepared by compounding of LLDPE with (20% loading) of nano ZnO with (0.25%) compatibilizer in a twin screw extruder with 100 rpm and temperature range of 100-180 ^{o}C in different heating zones.

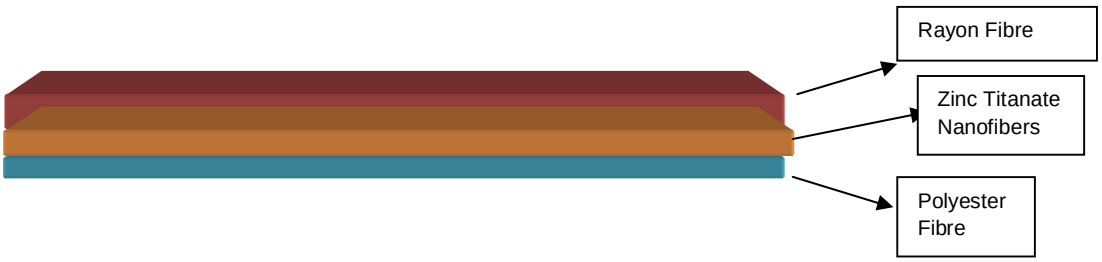
Melt spinning of fibres

Predried PET chips at 150 ^{o}C for 16 hours were used for melt spinning to remove moisture. Master batch was also dried for 4 hours at 60 ^{o}C. Melt spinning was done with 230 gm of PET chips and 20 gm of LLDPE loaded with 20% nano ZnO (PET/2% Nano ZnO). Temperature on different zones was 270, 285 and 290 ^{o}C (Above melting point of PET chips). For the prevention of oxidation in PET, Nitrogen gas in extruder zone was used. The filament coming out from the spinnerets were suddenly cooled by blowing cool air in 2 meter long quench duct. Then via passing through godet rolled it was collected in a draw roller. For spinning 107 m/min was the final.

Preparation of Composite Wipe

In order to manufacture the multilayered nonwoven Wipe, which consists of absorbent fabrics on top and Nano ZnO loaded polyester fibers with Zinc titanate nanofibers in middle. The multilayered were needle punched to form the flexible nonwoven Wipe. Needle punching technology use a series of barbed needles to interlock to give well unified fabrics [46,47]. It converts fiber into fabric without any process such as winding and weaving. Here fibers are first opened and then blended in the hopper feeder. Then they are separated into single fibers by a set of revolving rollers in a process referred to as carding. Now the fibers coming out of the carding

one by one were aligned in one direction as a partially coherent web. Multiple layers of carded webs are piled up to obtain necessary weight and are subsequently oriented in the cross direction by the cross lapper. Cross lapping provides the final nonwoven fabric with a good balance of strength in both directions. Then the cross lapped web is then subsequently needle punched using a series of needles to produce an interlocked fabric.



The needle punching technology gives intense flexibility to the fabric than other fabrics bonded by thermal or laminating process, due to the mechanical interlocking [48].

Characterization of nano-emulsion

Dynamic light scattering (DLS) measurements

The average globule size and polydispersity index (PDI) was determined by differential light scattering technique using Nanosizer/Zetasizer ZS (La-900, HORIBA, U.K.). The instrument utilizes a 4 mW He-Ne red laser at 633 nm. The light scattering is detected at 173^{o} by non-invasive backscatter technology using disposable polystyrene cuvettes (1 mL) with a measuring range from approximately 0.6 nm to 6 nm. Water was used to dilute the formulations to adjust the signal.

Transmission electron microscopy (TEM)

The surface morphology of nano-emulsion was observed using a transmission electron microscope (Morgagni 268D, FEI, Holland). Samples were mounted on copper gridding and dried under vacuum, were scanned at an accelerating voltage of 15 KV before observation.

Determination of pH

The pH of the nanoemulsions was determined using pH meter (Orion 2-Star, Thermo Scientific) calibrated to 7 ± 0.2.

Ex-vivo skin permeation studies using *p-tert*-butylcalix[4]arene nanoemulsion

Ex-vivo skin permeation studies were performed in a Franz diffusion cell with a diffusion area of 2.26 cm^2 and receptor volume of 22.5 mL. Abdominal rat skin was excised and washed with isotonic saline. The excised skin was then mounted between the donor and receiver chamber of the Franz diffusion cell with dermal side in contact with the receptor medium and the SC facing

towards the donor compartment. Donor compartment was filled with 2 mL of *p-tert*-butylcalix[4]arene loaded nanoemulsion. The temperature of the receiver compartment was at 32 ± 0.5 ^{o}C to simulate the skin temperature. At predetermined time interval of 15, 30, 45, 60 and 75 mins, 3 mL samples were collected from the receptor compartment and replaced with fresh receptor solution to maintain sink condition. All the collected samples were centrifuged (1500 g, 10 min) and analyzed for *p-tert*-butylcalix[4]arene content by UV-Vis absorption spectroscopy. Standard plot of *p-tert*-butylcalix[4]arene in chloroform was prepared by plotting absorbance values of *p-tert*-butylcalix[4]arene solutions against corresponding concentrations of standard solutions. Stock solution of *p-tert*-butylcalix[4]arene 20 µg/ml was prepared in chloroform and further diluted to concentration values 2 , 4, 6, 8, 10, 12, 14, 16, 18 µg/ml. Absorbance of the working solutions of *p-tert*-butylcalix[4]arene were further monitored using a UV-Vis spectrophotometer to obtain standard plot of *p-tert*-butylcalix[4]arene.

Visualization of skin penetration *in vivo*

The skin of the dorsal region was trimmed free of hairs by scissor (Figure 5). 2 mL solution of nanoemulsion formulation loaded with rhodamine 123 (5 mg/mL) was applied in a marked area of 1 cm^2 at the dorsal site of animals for 30 min and 5 h respectively. Thereafter, animals were euthanized by cervical dislocation. The excised rat skin was blotted dry in inert bloating paper to clean remaining formulation and frozen at −80 ^{o}C. Cryotome sections of 30 µm thickness were prepared and observed by normal and fluorescence light microscopy.

p-tert-Butylcalix[4]arene nanoemulsion skin interaction studies

Ex-vivo skin interaction studies were performed in a Franz diffusion cell with a diffusion area of 2.26 cm^2 and receptor volume of 22.5 mL [49]. Abdominal rat skin was excised and washed with isotonic saline solution, subcutaneous fatty tissue was removed. The SC was separated from a skin by digestion with 0.1% trypsin in a phosphate buffer solution (pH 7.2) at 37 ± 0.5 °C for 4 h. The isolated SC was rinsed with cold distilled water and dried by storage in desiccators over silica-gel [50]. The excised skin was then mounted between the donor and receptor compartment of the Franz diffusion cell with dermal side in contact with the receptor medium and the SC facing towards the donor compartment. Donor compartment was filled with 2 mL of drug loaded nanoemulsion/placebo nanoemulsion. Receptor compartment of Franz cell was filled with PBS (pH 7.4). The temperature of the receiver compartment was at 37 ± 0.5^oC to simulate the skin temperature. After 60 minutes skin was removed from cell and washed thrice with distilled water to remove the excess of nanoemulsion. Differential scanning calorimetry (DSC) thermograms of skin samples were recorded. SC was put into chloroform:methanol (2:1) for 48 hr for it delipidisation [51]. Untreated skin was taken as control and delipidised skin was taken as negative control. The DSC parameters were evaluated using STARe Software.

Decontamination studies of the developed *p-tert*-butylcalix[4]arene nanoemulsion

Contamination of the experimental model

Animals (*n = 4*) were divided into two groups

Group I - Decontaminated with placebo nanoemulsion formulation (with code, F1).

Group II - Decontaminated with nanoemulsion formulation containing 400 mg of *p-tert*-butylcalix[4]arene (with code, F2).

Hair of the thoraco-abdominal region (5×5 cm^2) of rat were removed 24 h before commencement of the experiment. Rat's hair was clipped-off closely to the skin using paired scissors and any cut through it was carefully avoided (Figure 5). After 24 h period, animals were examined for any damage and animals with absolute intact skin were selected for the experimentation [52]. ^{131}I (3.7 ± 0.185) and ^{201}Tl (3.7 ± 0.185 MBq) respectively were mixed in 0.1 mL saline and allowed to contaminate the exposed area of the experimental model (Rat) using 1 mL standard syringe plunger and air dried.

Figure 5. Hair removal of the experimental model

Abdominal skin of the Sprague-Dawley rat model was excised and washed with isotonic sodium chloride (NaCl) solution. The excised skin was then mounted between the donor and receiver chamber of the Franz diffusion cell with dermal side in contact with the receptor medium and the stratum corneum facing towards the donor compartment. Skin contamination was performed by depositing 500 µL of the individual standard solutions of the Thallium and Iodine (1000 mg/L iodide in water, prepared with high purity KI and water *Trace (*SELECT®Ultra, Sigma-Aldrich,

Germany) and 1000 mg/L Tl in 2% nitric acid, prepared with high purity $TlNO_3$, HNO_3 *Trace*SELECT® and water *Trace*SELECT®Ultra, Sigma-Aldrich, Germany).

Figure 6. Extracting the aliquots from Franz Diffusion Cell

Volume of the contaminated solution is kept to the minimum one which is required to cover uniformly skin surface and ensures the optimum transcutaneous diffusion conditions by avoiding lateral diffusion phenomenon. pH of the contaminated solution is adjusted to 5 by using 0.01 M acetate buffer solution. Contaminated solution kept up to 60 min along with the *p-tert*-butylcalix[4]arene nanoemulsion for the transcutaneous diffusion studies.

Sample analysis

The analysis of liquid in the receptor compartment of Franz diffusion cell for quantitative estimation of ^{205}Tl and ^{127}I were performed with the help of the HR-SF-ICP-MS (Thermo Scientific, Element XR^{TM}, USA). A multi elemental standard stock solution containing ^{205}Tl and ^{127}I (10 g/mL in 2% HNO_3, Sigma Aldrich, USA) was used to optimize signals before each ICP-MS measurement and to attain the best instrumental conditions. Aliquot was drawn after 15 minutes and diluted in 2% HNO_3 (HNO_3 *Trace* SELECT® and water *Trace* SELECT® Ultra, Sigma-Aldrich, Germany). Calibration curve of the standard stock solutions was plotted to calibrate the detector of HR-SF-ICP-MS. Statistical errors combination was made using the low of error propagation. In all analytical measurements standard deviations of ICP-MS measurements (< 2%) were negligible as compared to the standard deviations of the Thallium and Iodine percutaneous diffusion through the skin.

CHAPTER 3

RESULTS & DISCUSSION

Results

The *p-tert*-butylcalix[4]arene nanoemulsion with nonwoven Wipe has been synthesized as characterized by measurement of the size, shape, pH and zeta potential using DLS, TEM and pH-meter.

Compatibility of Dry nonwoven composite Wipe

Observation of chemical compatibility of the composite Wipe with four chemicals tested, in which the Wipe demonstrated no adverse effect with either Calixarene, Iodine, Technisium, Bleach in table X.

Material	Compatible with Calixarene	Compatible with Iodine	Compatible with Technisium	Compatible with Bleach
Nonwoven Dry Wipe (5 cm^2)	Yes	Yes	Yes	Yes

DLS measurements

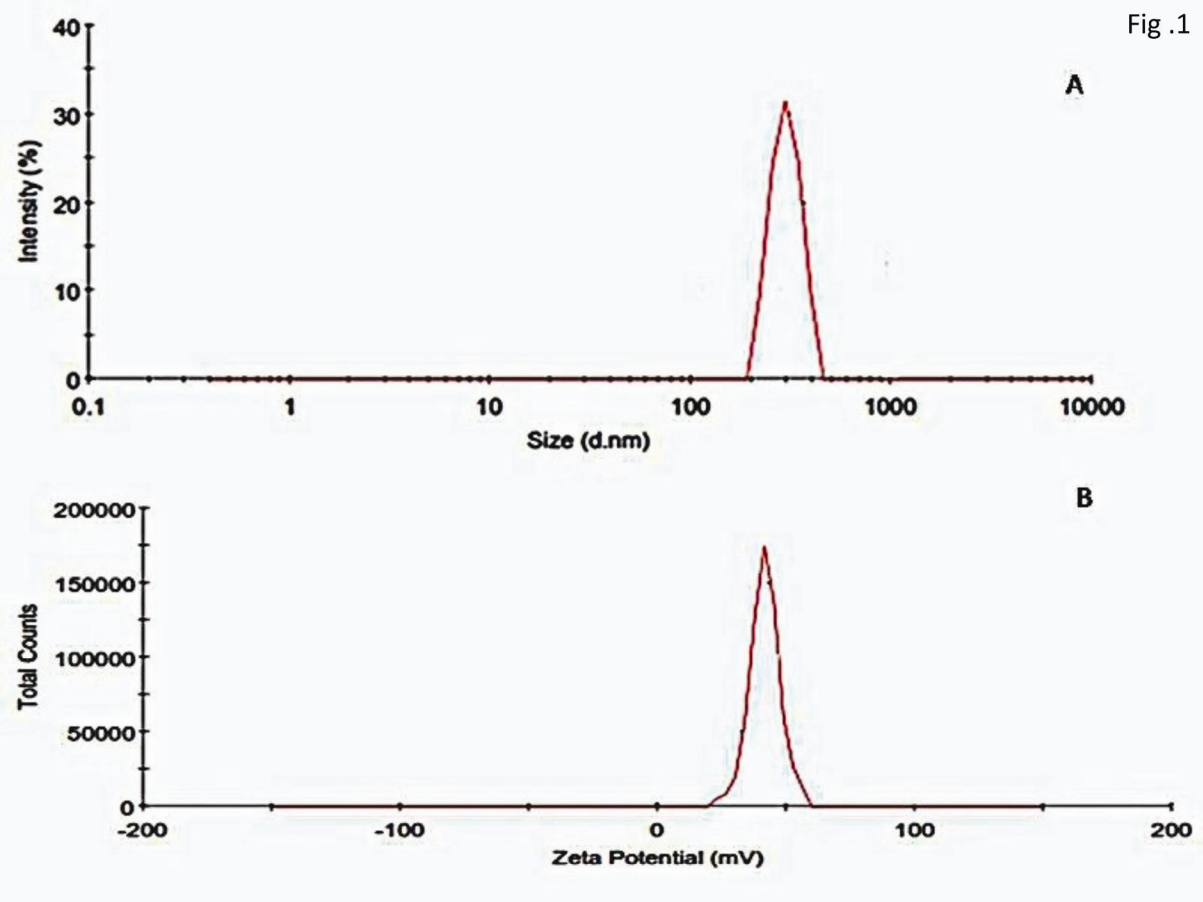
Droplet size and distribution

Fig. 1A shows the droplet size and size distribution of the formulation. The average size of the formulation was observed to be 500 nm. The difference in droplet sizes with varying ratios of surfactants was found to be significant ($p < 0.004$).

Zeta potential

The, zeta potential value suggesting degree of repulsion between neighbouring charged particles in dispersion for the formulation so prepared was found to be 40 mv (Fig.1B).

Fig .1

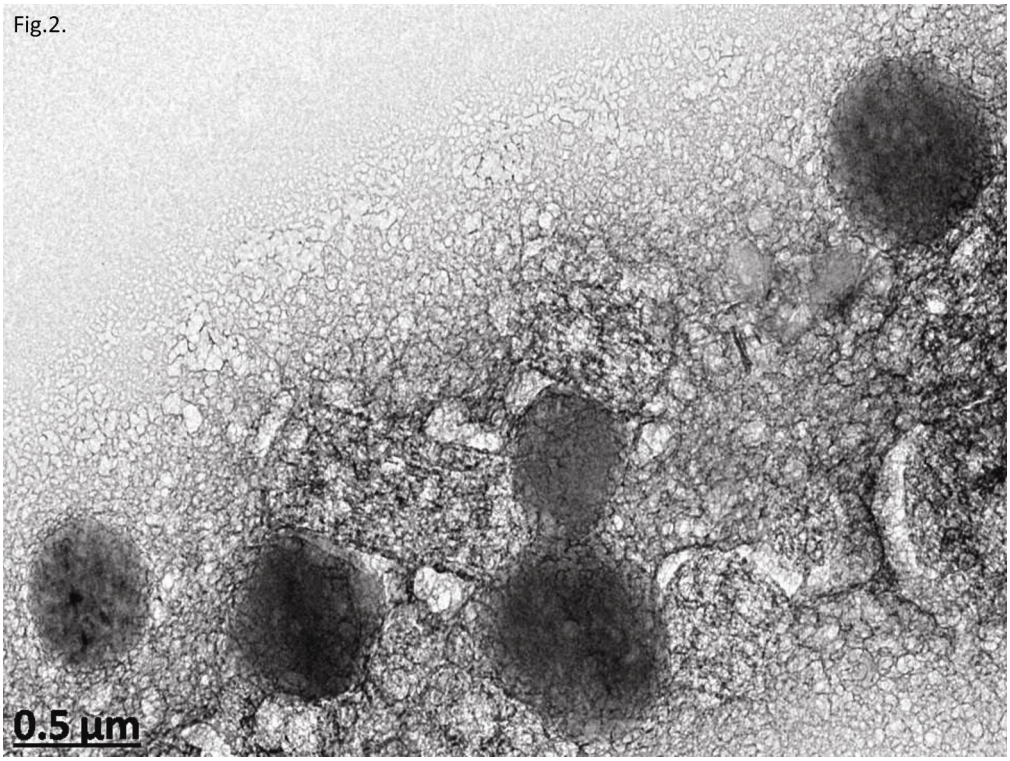


TEM measurements

The surface morphology as seen in figure 2, was spherical in nature with average size of 500 nm, confirming that the globules of the nanoemulsion are in the expected nano range.

Determination of pH

*p*H of optimized nanoemulsion was found to be 7.5 ± 0.2 which is favourable for topical application.



Ex-vivo skin complexation studies of the nanoemulsion with radionuclides

The individual skin samples contaminated with stable nuclides of thallium (^{205}Tl) and iodine (^{127}I) treated with placebo and *p-tert*-butylcalix[4]arene nanoemulsion and respective aliquots obtained from donor compartment of Franz diffusion cells were analyzed using HR-SF-ICPMS. The mass spectra of the digested skin samples that were not exposed to the ^{127}I and ^{205}Tl were also recorded around the mass of ^{127}I and ^{205}Tl. No significant peak at the mass of Iodine, i.e., 127 and the mass of Thallium, i.e., 205 were observed.

Concentrations of ^{127}I and ^{205}Tl were obtained using calibration plot (Figure 3) and concentration values as mean of at least 3 independent experiments, were presented in Table 2. Post 60 mins of contamination with 1000 µg/L of the standard stock solution, The concentration of the ^{127}I in the skin and aliquots treated with *p-tert*-butylcalix[4]arene were obtained as 0.164 ± 0.046 and 0.481 ±0.145 respectively, and the concentration values of ^{205}Tl were found to be

0.481 ±0.145 and 0.150 ± 0.081 in skin and aliquots respectively (Table 2). It was observed that the concentration values of the Iodine and Thallium was significantly higher in the skin samples treated with placebo nanoemulsion than those of obtained for skin samples treated with *p-tert*-butylcalix[4]arene treated nanoemulsion.

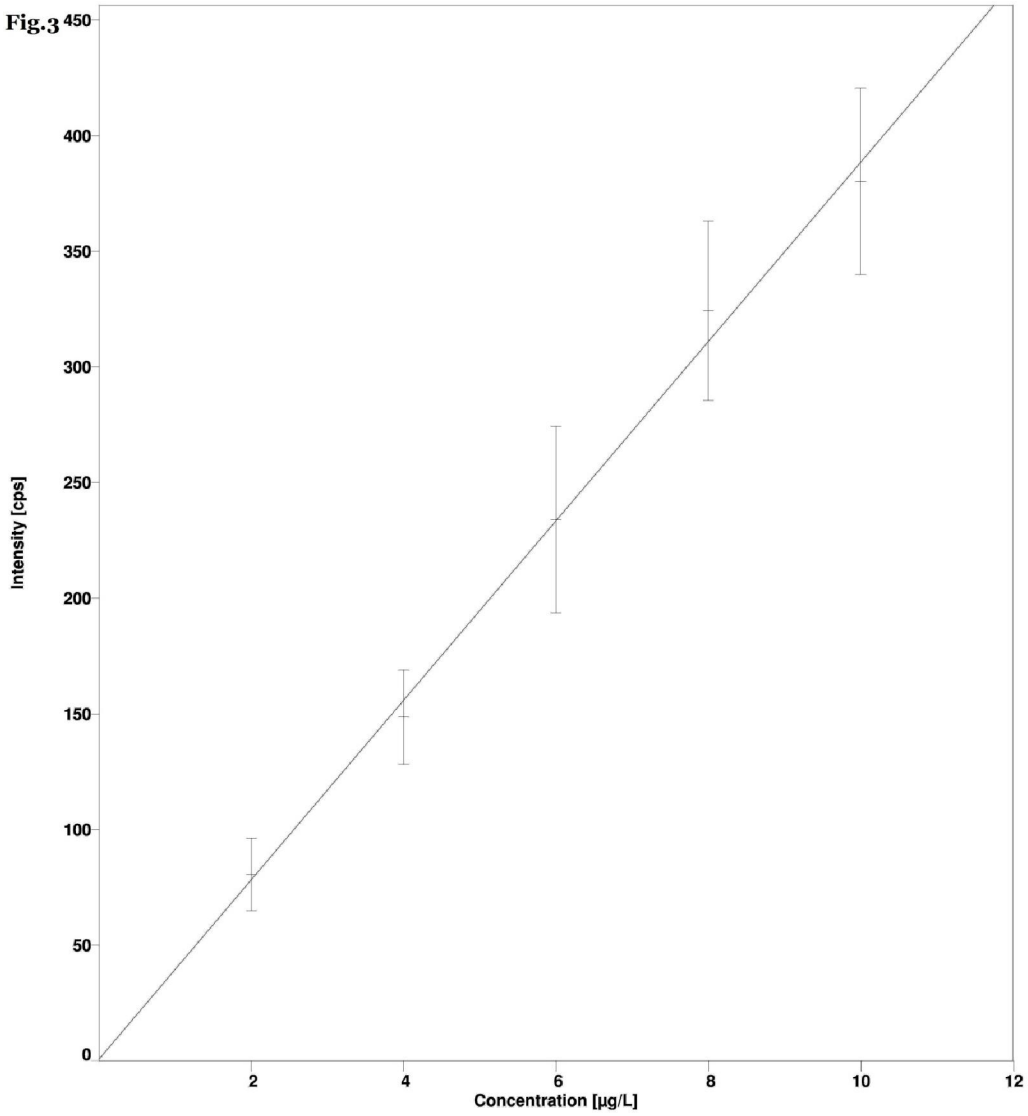
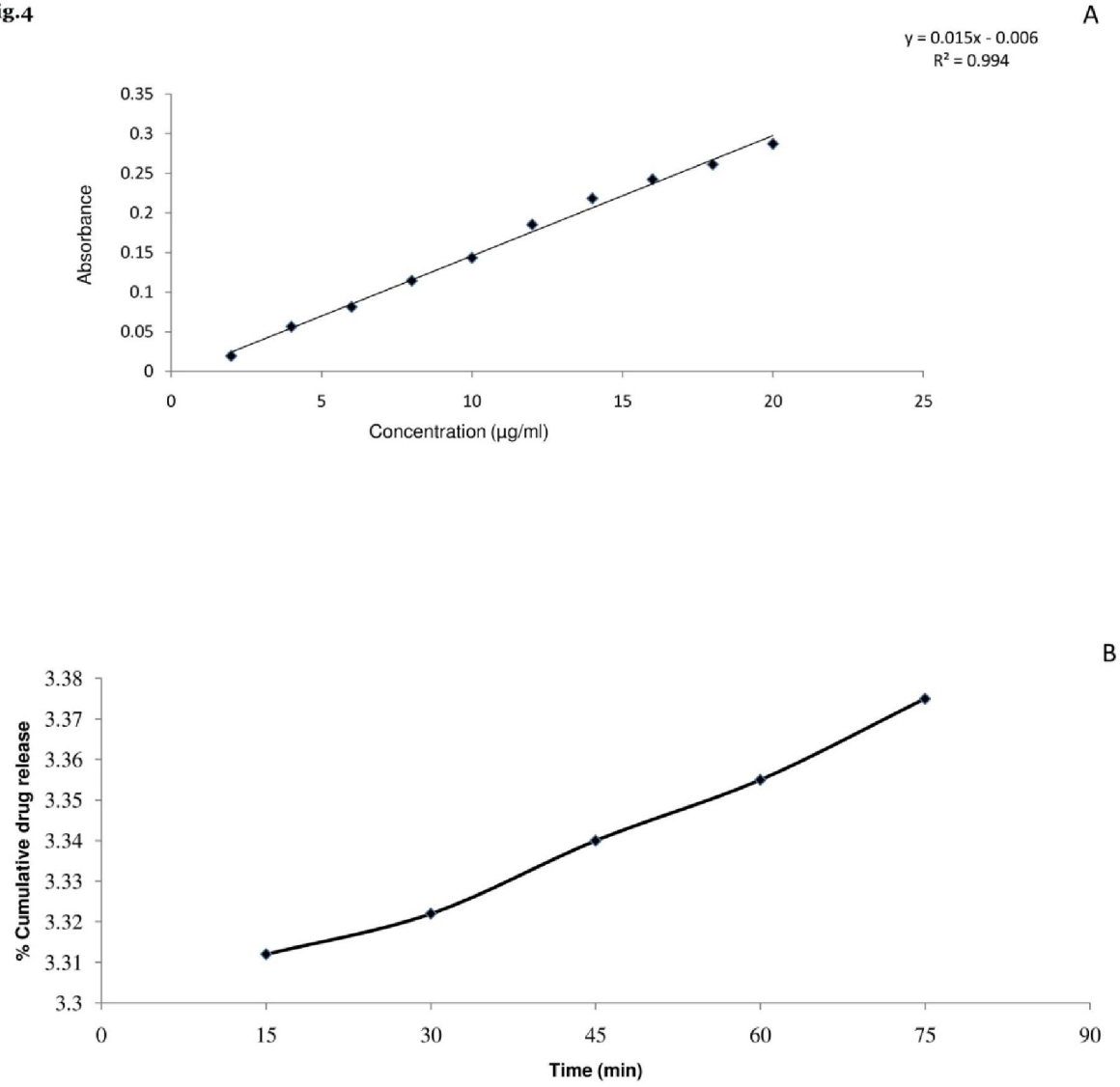


Figure 3

Ex-vivo skin permeation studies

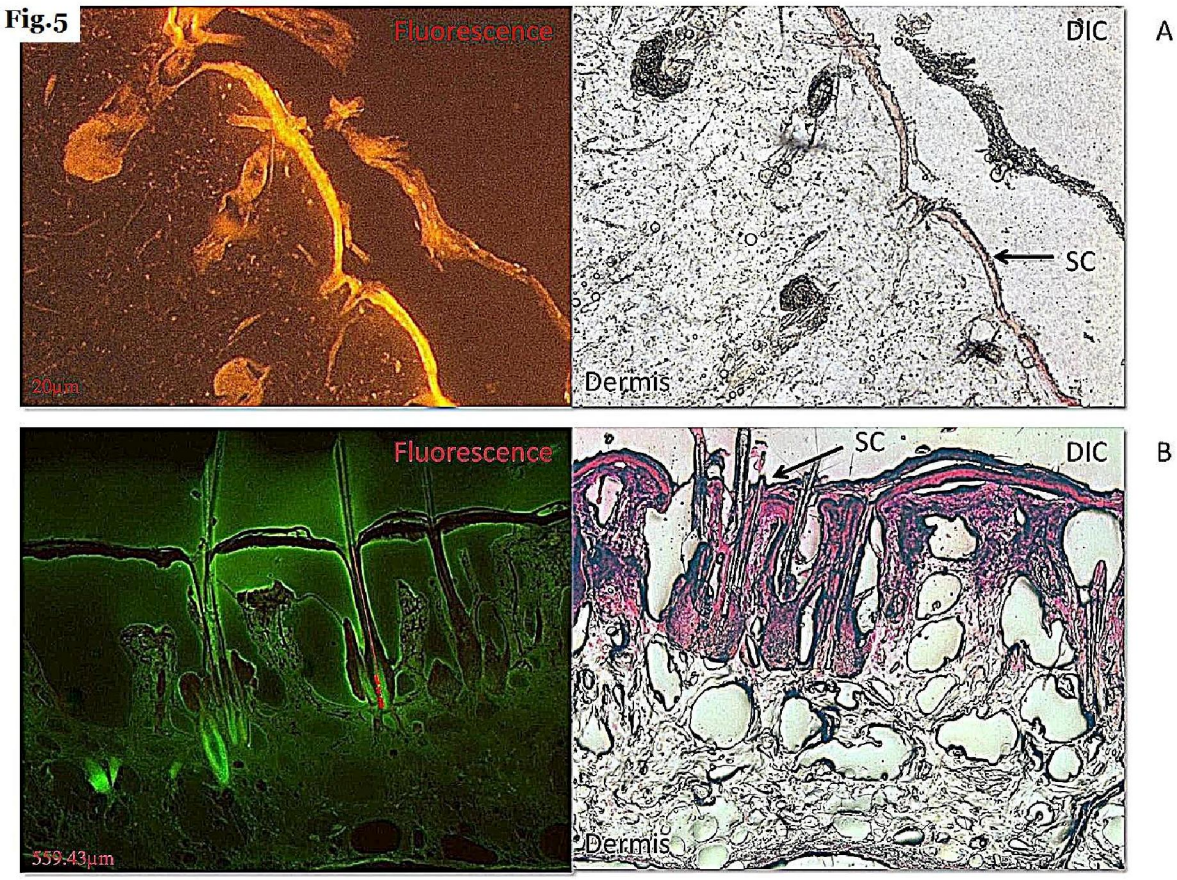
Fig.4



The skin permeation potential of nanoemulsion was characterized using *ex-vivo* model using UV-Vis absorption spectroscopy. Absorbance values of the working solutions *p-tert*-butylcalix[4]arene prepared in choloroform of concentration 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/mL were used to plot a standard plot (Figure 4A) to quantify the concentration of *p-tert*-butylcalix[4]arene in aliquots. The percentage cumulative drug release through rat skin in terms of concentration of *p-tert*-butylcalix[4]arene in aliquots were subsequently plotted against the

monitoring time. It was observed that maximum of 3.37% of *p-tert*-butylcalix[4]arene was released after 75 minutes of application of nanoemulsion (Figure 4B). Besides, it was observed that *p-tert*-butylcalix[4]arene release was found to increase linearly with time but very slowly.

Visualization of formulation skin penetration in vivo



Rat dorsal skin was treated with rhodamine 123 loaded nanoemulsion for ½ h and 5 h to accomplish an adequate penetration. According to the skin penetration data, typical native and the corresponding fluorescence images (50× magnification) of the formulation indicated localization of rhodamine 123 labeled nanoemulsion in the percutaneous region of rat skin (Figure 5A and Figure 5B). Therefore, the study suggested clearly the localized penetration apart than the direct penetration deep inside the skin, verifying the percutaneous localized permeation

across SC layer. Figure A shows the presence of the formulation at the epidermal region of the skin (20 µm) after the application, at a time interval of ½ h [53]. Figure B, derived from the margin of the treated area, showed the limited penetration that appeared to confined till the boundary of SC layer (559.43 µm) [54]. Nanoemulsion of *p-tert*-butylcalix[4]arene was effective in permeating rhodamine 123 up to 20 µm and 559.43 µm at a time interval of ½ h and 5 h respectively.

p-tert-butylcalix[4]arene nanoemulsion skin interaction studies

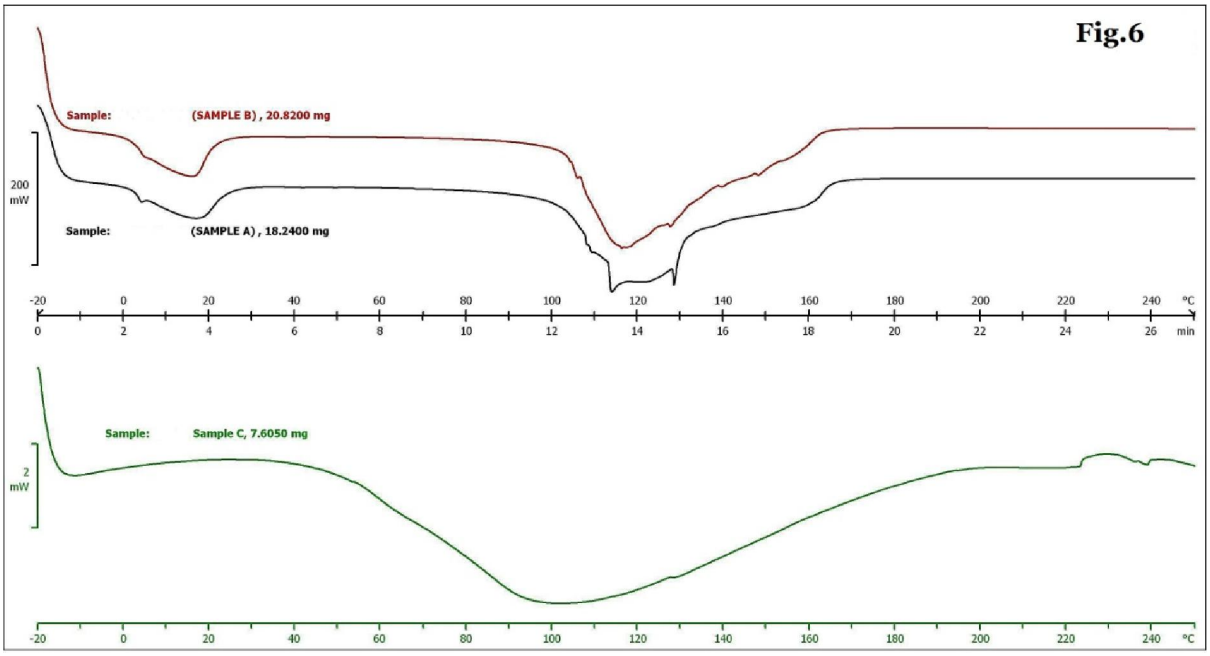


Figure 6 (A)–(C) shows the *DSC* thermogram of the control, *p-tert*-butylcalix[4]arene nanoemulsion, and delipidised (negative control) rat SC skin, respectively. A less commonly reported peak around 5°C –10 °C, attributed to superficial sebaceous lipids, was not observed in present experiments, which might be attributed to the low enthalpy of this peak [29]. Untreated rat skin (control) SC showed three endothermic peaks at temperatures 20°C (Labeled as, T1),

115°C (T2) and 130°C (T3) (Figure 6A). Similarly, the case of skin samples pretreated with *p-tert*-butylcalix[4]arene nanoemulsion, peaks are observed to appear at 20°C (T1) and 130°C (T3) but the peak around 115^{o}C (T2) was observed to show very low enthalpy and therefore appeared to be less significant in *DSC* thermogarm (Figure 6B). Figure 6C showed that for delipidised skin (negative control) all the three endothermic peaks in *DSC* thermogram were not found.

Ex-vivo Complexation studies of the nanoemulsion with radionuclides

The individual skin samples contaminated with (^{205}Tl) and (^{127}I) treated with placebo and *p-tert*-butylcalix[4]arene nanoemulsion and respective aliquots obtained from donor compartment of Franz diffusion cells were analyzed using HR-SF-ICPMS. In order to minimize the interference in HR-SF-ICPMS analysis, the mass spectra of the digested skin samples that were not exposed to the ^{127}I and ^{205}Tl were recorded around the mass of ^{127}I and ^{205}Tl (Figure A, B). No significant peak at the mass of Iodine i.e. 127 and the mass of Thallium i.e. 205 were observed.

^{127}I and ^{205}Tl concentrations were obtained using calibration plot and concentration values as mean of at least 3 independent experiments were presented in Table 3. Post 60 mins of contamination with 1000 µg/L of the standard stock solution. The concentration of the ^{127}I in the skin and aliquots treated with *p-tert*-butylcalix[4]arene were obtained as 0.164 ± 0.046 and 0.481 ±0.145 respectively and the concentration values of ^{205}Tl were found to be 0.481 ±0.145 and 0.150 ± 0.081 in skin and aliquots respectively (Table 3). It was observed that the concentration values of the Iodine and Thallium was significantly higher in the skin samples treated with placebo nanoemulsion than those of obtained for skin samples treated with *p-tert*-butylcalix[4]arene treated nanoemulsion.

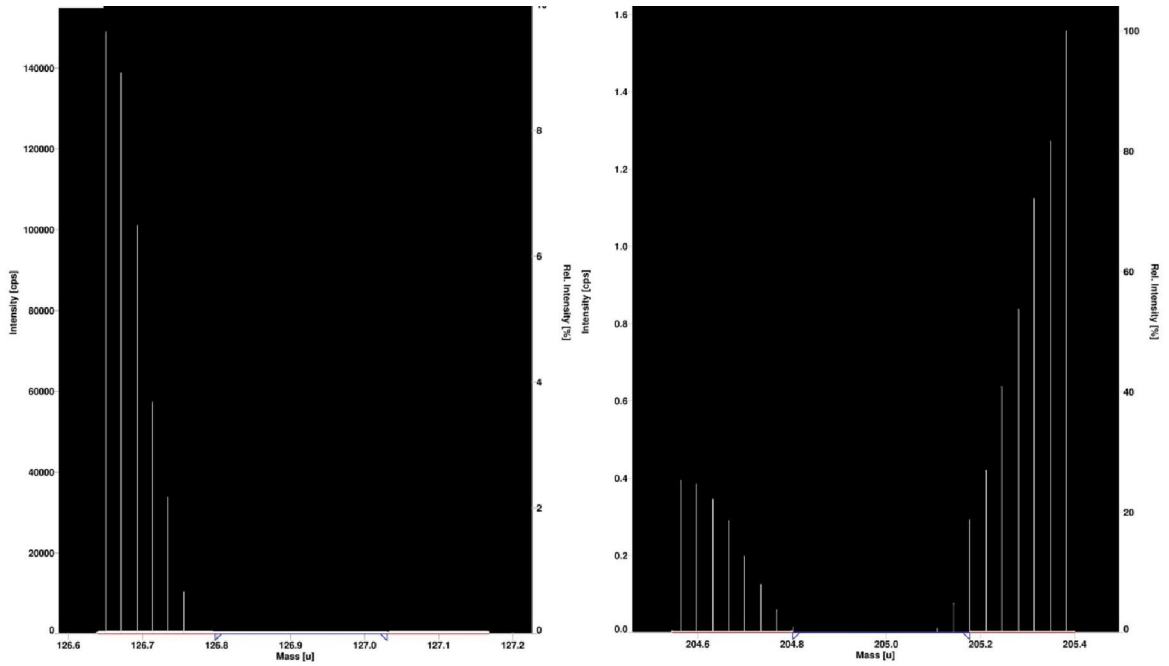


Figure 9. Mass spectra of the aliquot showing ^{127}I (A) and ^{205}Tl (B) concentration. X axis represents mass in atomic mass unit (a.m.u.), Y axis represents the signal intensity in counts per second (cps).

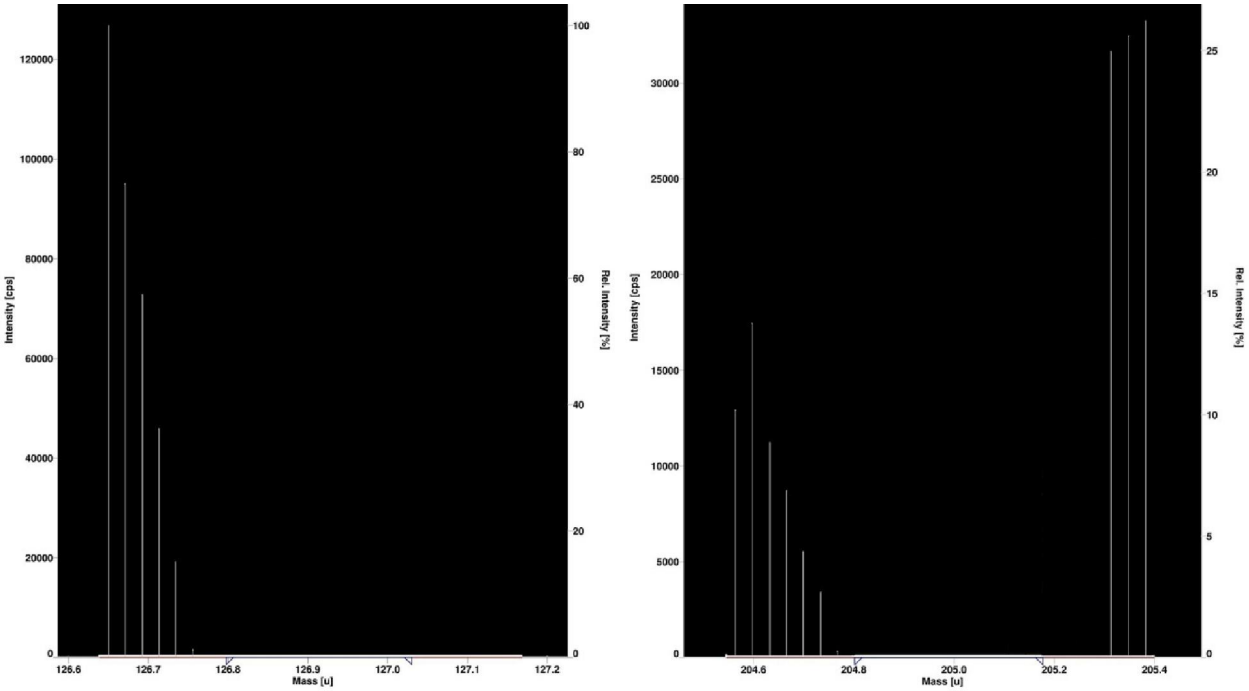


Figure10. Mass spectra of the individual skin samples revealed that there was no ^{127}I (A) and ^{205}Tl (B) retention by the skin. X axis represents mass in atomic mass unit (a.m.u.), Y axis represents the signal intensity in counts per second (cps).

Therefore, the data indicated that nanoemulsion was capable to make the complex with Iodine and Thallium and capable to remove them from contaminated skin.

Discussion

Decontamination of the radio-contaminated victim is an important task in the medical management of the nuclear and radiological emergency [30,55]. The prepared nanoemulsion consists of globules of size around 500 nm. This assured better stability against destabilization phenomena such as Ostwald ripening [31]. *p-tert*-Butylcalix[4]arene possess small size of hydrophobic cavity and binds efficiently with small metal ions and facilitate to encapsulate them or to bind them [32].

Main significant of choosing non-woven composite Wipe *via* needle punching technology was to achieve higher absorbency of the chemicals in bulk quantity and providing cost efficient fabric as compared to other products/wipes available in the market. The most important goal to achieve was to use fiber which provides high surface area along with the affinity of certain fibers towards the chemicals/contaminants and to retain it [48]. Zinc titanate nanofibers were fabricated *via* electrospinning where polyvinylpyrrolidone is used as a binder. These fibers are used because of their sorbency towards CW agents to disintegrate it, as its already proved. These nanofibers show good destructive reaction towards mustard and nerve agents, and product of reaction with fibers and stimulants are comparatively harmless [56]. Similarly Nano ZnO loaded polyester fiber is used for the antibacterial properties. The LLDPE-g-MA is used as a compatibilizer between ZnO and LLDPE. This fiber is very effective against *E. Coli* and *S. Aureus* bacteria [1].

The main reason for making this composite Wipe with nanoemulsion along with it, is to provide and cover all there major type of agents and to decontaminate it with one single product along with being cost effective. The goal of the complexation studies was to evaluate the ability of the *p-tert*-butylcalix[4]arene nanoemulsion to chelate ^{127}I and ^{205}Tl on the skin in rat model using Franz diffusion cell. The external face of the rat skin samples was put in contact with the bicarbonate-carbonate buffer as receptor fluid. All Franz diffusion cell experiments were performed under the occlusive condition to prevent the cross contamination with other metal ions, evaporation and concentration phenomena of solutions deposited in the Franz cell donor compartment during 60 min experiment. Skin decontamination potential of the *p-tert*-butylcalix[4]arene nanoemulsion was individually evaluated against ^{127}I and ^{205}Tl. It was find out after 60 mins there is significant decrease in the concentration of ^{127}I and ^{205}Tl in the aliquots of the receptor media of the Franz diffusion cells therefore, the monitoring time period was 60 mins. After the completion of monitoring time period it was observed that the digested skin sample contains the trace amount of the metal ions of ^{127}I and ^{205}Tl in skin samples treated with *p-tert*-butylcalix[4]arene nanoemulsion compared with those treated with placebo nanoemulsion. Moreover, mass spectra of the individual skin samples revealed that there was no ^{127}I and ^{205}Tl retention by the skin (Figure7A, B) and there is significant reduction of the ^{127}I and ^{205}Tl in the aliquot (Figure 8A, B) drawn at the end of the experiment. In case of the external contamination most of the radioactive contaminants remain superficial on the skin. The objective of the external decontamination is removal of radio-contaminant from the skin as soon as possible while preserving the integrity of the skin [33]. Since the skin is not the absolute barrier, the radio contaminants may pass through the skin. The medical management of the externally contaminated individuals requires particular attention in order to prevent the spread of the

contamination, limit internal intakes and reduce exposure to the radiation [34]. Thus, for the efficient decontamination the formulation has to be confined till the stratum germination of the epidermis maximum to the dermal papillae of the dermis. Skin permeation studies using UV-Vis spectroscopy showed that the trace amount of *p-tert*-butylcalix[4]arene present in the lower chamber of the Franz diffusion cell after 60 mins proved that the formulation could not penetrate skin. In order to clarify the preliminary percutaneous penetration mechanism, fluorescence microscopy performed to catch the direct impression of the process. Fluorescence emitted by rhodamine 123 labeled nanoemulsion provided direct evidences of percutaneous penetration. The fluorescence microscopy data confirmed that nanoemulsion was penetrated into skin to a distance of 20 µm and 559.43 µm after ½ h and 5 h respectively. Since the thickness of SC region extends maximally up to 15 mm, therefore, the data conformed the formulation was remained confined only up to the SC region of the skin [54]. As most of the radioactive compounds are remain localised on the percutaneous skin surface so there is the need of the localisation penetration in the percutaneous layer apart than the direct penetration deep inside the skin. Therefore, fluorescence microscopy results are consistent with those of *in-vitro* skin permeation study. This further ensures the formulation activity in the epidermis and dermal papillae of the skin. The nanoemulsion is capable to make the complex with the most of the radioactive ions in this region of the skin. In order to elucidate the possible changes caused by *p-tert*-butylcalix[4]arene nanoemulsion on the structure of the skin, the *DSC* study was performed out. Lamellar lipid structure provides the character to the SC layer of the skin. SC layer mainly consists of the corneocytes packed with keratin that are interconnected by a multilamellar lipid bilayer structure mainly composed of the cholestrol, cholestrol esters and sulphate, fatty acids and ceramide [36,37]. To further elucidate the mechanism of skin permeation of the *p-tert*-

butylcalix[4]arene nanoemulsion, its impact on the thermal properties of the SC intercellular lipids were studied by *DSC*. Commonly for untreated rat skin, *DSC* thermogram shows three endothermic peaks, observed around 20°C (T1), 115°C (T2) and 130°C (T3) correspond to lipid structure of skin. The association of these transitions with the intercellular lipids of the skin was well known in the literature [38,39]. When the skin is delipidised, due to loss of lipid structure all the three endothermic peaks get disappeared [57]. Similarly, in the present study when the skin delipidised with chloroform and methanol mixture in ratio 2:1 [51], it was observed that all the three endothermic peaks were not present. In the nanoemulsion treated skin sample DSC thermogram showed the lipid transitions at 20°C (T1), and 130°C (T3) but transition around 115°C (T2) were showed to possess very low enthalpy content thereby suggesting that all the three peaks are present. Therefore, *DSC* study confirmed the similarity of the lipid transitions in both of the skin samples treated with *p-tert*-butylcalix[4]arene nanoemulsion and untreated skin proves the compatibility of the nanoemulsion with that of the skin and further capable to preserve the integrity of the skin.

CHAPTER 4

CONCLUSIONS

- The results suggested that the *p-tert*-butylcalix[4]arene nanoemulsion is an efficient wide spectrum decontamination formulation against the radionuclides, and well capable to remove the radionuclide's from the SC layer of the skin, without compromising the integrity of the skin.
- The composite Wipe is very well compatible with the nanoemulsion and will equally help in decontamination of chemical and bacterial contaminants.
- Non woven fibers show great absorbency and stability.
- *Ex-vivo* complexation studies using HR-SF-ICP-MS confirmed the complexation of radionuclides with *p-tert*-butylcalix[4]arene by demonstrating the reduced metal content in aliquots obtained from receptor compartment of Franz diffusion cell.
- Skin penetration and permeation studies further confirmed limit of diffusion of formulation up to the SC region of skin. The skin interaction studies proved the skin compatibility of the formulation. Lower radio-active waste generation than the conventional decontamination method is the added advantage. Therefore, the formulation is effective and safe to use for skin decontamination purpose.

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