Trakia Journal of Sciences, Vol. 10, Suppl. 1, 65-72, 2012

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

OPTIMISATION AND EFFICACY EVALUATION OF THE TOPICAL GEL RADIATION DECONTAMINATION FORMULATION CONTAINING DISODIUM EDETATE AS CHELATING AGENT

S. Rana[1], Y. Karamalakova[2], A. W. Khan[3], S. Kotta[3], J. Ali[3], R. Chaudhary[1], A. Mittal[1], S. Sultana[4], S. H. Ansari[3], V. Gadjeva[2], R. K. Sharma[1*]

[1]Division of CBRN Defence, Institute of Nuclear Medicine and Allied Sciences, Delhi, India
[2]Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[3]Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi, India
[4]Department of Medical Elementology and Toxicology, Jamia Hamdard, Hamdard Nagar, New Delhi, India

ABSTRACT

Objective of the present study was to optimise radiation decontamination topical gel formulation and to evaluate its decontamination efficacy (DE) using pharmacoscinitigraphy technique. Disodium edetate was used as active pharmaceutical ingredient and sodium carboxymethyl cellulose (NaCMC) added to the formulation as polymer base. Different combinations of the active ingredient with polymer were used to ensure an optimal formulation with maximum DE and least occurrence of the dermal irritation / toxicity. Static counts were recorded before and after decontamination using Single Photon Emission Computed Tomography (SPECT). DE of the optimised disodium edetate topical gel was investigated and compared with formulation without active ingredient. Formulation could effectively remove ≥70% of the ^{99m}Tc radio-contaminant if applied 5-30 min after the contamination. Measured DE values were analyzed using one way ANOVA and students *t-test* were found to be statistically significant (p value <0.05). Visual inspection site of the application of formulation indicate that it is free from dermal irritancy and toxicity. Cumulative skin irritation of the tested optimised radiation decontamination topical gel was not observed.

Key words: Decontamination, topical gel, efficacy, toxicity, tissue equivalent

INTRODUCTION

Highly toxic radioactive materials (TRMs) are mainly used in Medicine, Agriculture, and Nuclear Energy production. Exposure / contamination by TRMs is an established risk amongst occupational workers. A consequence of the external contamination with radioactive materials, is the possible entry into systemic circulation followed by internal uptake of the TRMs by specific organs [1-2]. Three major routes of internalisation of the radioactive materials are through inhalation, ingestion and through intact / broken skin. Once TRMs reaches systemic circulations it tends to accumulate into their specific target organs, e.g., Thyroid for Iodine-131, Cesium-137 gets deposited in whole body, artery walls and adrenals, Stronium-90 in bone, and Cobalt-60 in liver, bone, kidney and gastrointestinal tract. The most frequently contaminated areas in human are the face, hands, hair headgears, shoes, gloves and outer clothing of the personnel / responders. The critical site of irradiation emanating from the radioactive contamination on the skin, is the stratum germinativum layer, where proliferative cells are located. The radiation dose reaching the basal cell layer of the epidermis is predominantly due to beta and gamma radiation [3-4]. The radio-nuclides decontamination / removal from the skin is an important task because any extra second of

***Correspondence to**: *Dr. Rakesh Kumar Sharma, Head, Division of CBRN Defence, Institute of Nuclear Medicine and Allied Sciences, Brig. S. K. Mazumdar Road, Delhi 110 054, India, Telephone No. +91-11-23968900, Fax No. +91-11- 23919509, E-mail: rksharmadrl@yahoo.com*

deposition not only results is exposure due to irradiation but will also facilitates their internalisation leading to systemic toxicities. Extent of the health hazards of contamination depends on physical and chemical properties of the radio-nuclides also [5-7]

A number of topical preparations / formulations are commercially available and have been evaluated for their relative efficiency against various radiological skin contaminants [8-18]. Decontamination of radioactive materials should be done rapidly and systematically. However, limitation of trained manpower restricts this process. During mass casualty events, self-decontamination formulations are advocated for effective decontamination [19-20]. Present study was planned to optimise radiation decontamination formulation and to evaluate their relative decontamination efficacy using nuclear medicine technique. ^{99m}Tc, the most commonly used radio-nuclide in diagnostic nuclear medicine with low gamma energy emission and short half life (6.020 h), was applied as the skin contaminant. Disodium salt of Ethylene Diamine Tetra Acetic acid (Na_2EDTA) is a powerful chelating agent that forms stable complexes with most metal ions. Due to its ability to sequester metal ions, it is widely used for chelating a number of heavy metals and radio-isotopes. A number of techniques were used for the detection of the EDTA in various samples [21-22]. Formulations containing disodium edetate offers high compatibility and lower toxicity. The potential physical and chemical interactions between active API / excipients interactions, such as incompatibility, acid base reaction, and physical changes may affect the stability and efficacy of the formulation [23]. The preparation should be such that the formulation runoff is minimised required wet contact time between the contaminant and the formulation would be maintained. The cost effective formulation should be devoid of toxicity and skin irritancy to the skin.

MATERIALS AND METHODS

1. Materials

^{99m}Tc sodium pertechnetate was obtained from Regional Centre for Radiopharmaceuticals, Board of Radiation and Isotope Technology (BRIT), Delhi, India. Symbia True point SPECT gamma camera, USA, was used for static counts and whole body imaging of the contaminated skin surfaces. Di-sodium edetate (Merck Ltd, Mumbai, India), sodium carboxymethyl cellulose (CDH Ltd., Mumbai, India), methyl paraben sodium, propyl paraben sodium (Titan Biotech Ltd., Rajasthan, India), triethalonamine (Fisher Scientific, Mumbai, India) and propylene glycol (CDH Ltd., Mumbai, India) were used. Other chemicals / reagents used were of analytical grade.

2. Methods

2.1. Experimental models

Decontamination efficacy studies of the topical gel were performed on 2–3 months old healthy male Sprague Dawley rats (weighing 200 ± 20 g) and human tissue equivalent models (Gammex RMI® USA). Animals were allowed to acclimatise for one week before start of the experiments in controlled environment at ambient temperature of 22±3 ^{0}C with relative humidity of 50 ±10%, (centrally air-conditioned with 100% fresh air replacement) and 12 h light/dark cycle. Experimental protocols were approved by Institutional Animal Ethics committees (IAEC) vide no. 111 INM/IEAC/2010/07/007.

2.2. Preparation of the Topical Gel

For the preparation of the gel, disodium edetate dissolved in water, methyl paraben sodium and propyl paraben sodium were added to it and stirred till it becomes clear. The solution was stirred further for 15 min. NaCMC was added to it with continuous stirring. Propylene glycol was added and the volume was made up with purified water. Prepared formulation was filled in lacquered plastic containers and stored at room temperature for further evaluation/use.

2.3 Physical Characterization of the Topical Gel

2.3.1 pH

To determine *pH,* 1.0 g of the gel was accurately weighed and dispersed in 100 ml purified water. The *pH* of the dispersion was measured using digital *pH* meter, which was calibrated before use with standard buffer solution at 4.0, 7.0 and 9.0. The measurements of *pH* were done in triplicate and average values were calculated [24].

2.3.2 Spreadability

To determine the spredability of the formulation, 0.5 g of topical gel was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 cm ×20 cm, over which a second glass plate was placed. A weight of

500g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading was observed [25].

2.3.3 Extrudability

To determine extrudability a closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 s was determined. The average extrusion pressure in grams was recorded.

2.3.4 Viscosity

The viscosity of the formulations was determined as such without dilution by R/S CPS Plus Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using spindle #C 50-1 having diameter of 50 mm and RHEO3000 software.

2.4 Contamination of the Experimental Model

24 h before commencement of the experiment hairs of the rats were clipped-off closely. Freshly prepared ^{99m}Tc (0.3±0.005 mCi) was used for skin contamination. Radio-isotopes were mixed appropriately in 0.1 ml of saline and applied over thoraco-abdominal area and / or tissue equivalent model (5 cm × 5 cm^2) patch using a syringe plunger. Contaminated surface was allowed to air dry. Lengths of contaminant exposure over the applied areas varied from 5 to 30 min. Animals were divided into 5 groups (n= 6).

Group I – Decontaminated with formulation (without API)
Group II – Decontaminated with formulation (0.25.0% API)
Group II – Decontaminated with formulation (0.5% API)
Group III – Decontaminated with formulation (1.0% API)
Group V – Decontaminated with formulation (1.25.0% API)

2.5. Decontamination protocol

Decontamination was performed by using cotton swabs (3 cm radius) applied on 1 g of the optimized topical gel. Procedure was performed in encircle movement from the periphery of the contaminated area toward the centre. Five consecutive decontamination attempts were performed. Each attempt was completed within 60 sec. Before and after each decontamination attempt, static counts (kilo counts per seconds) and images were taken from gamma camera. Acquisition time for recording static counts and images was selected at 180 sec. Camera parameters selected for the whole body imaging and static counts was at zoom level 2 with both detectors and 256 ×256 matrix sizes. Region-of-interest (ROI) software was used for count statistics.

2.6 Data analysis of decontamination efficacy

Radiation measurements were corrected for radioisotope decay so that decontamination data could be compared to initial contamination levels. Times of contamination and measurements were recorded to ensure that the measurements could be referred back to the initial contamination levels. Corrections were applied for background levels of radiation. Multiple readings were taken and the average was used as the background level and subtracted from all measurements.

Results, expressed as a percentage of residual contamination, were calculated as follows:

$$DF = \frac{C_0}{C_t}$$

Where DF denotes Decontamination Factor and

C_0 = Measurement of the initial contamination counts

C_t = Measurement of counts following decontamination attempt

Evaluation of the efficiency of a particular agent for decontamination was accomplished by determining the differential mean reduction of contamination from a radionuclide relative to the original contamination. Decontamination efficacy was expressed in terms of a percentage of the remaining contamination and was arrived at by using the following formula:

$$D = (1-1/DF) \times 100$$

Where D = decontamination efficiency and the other variables are the same as noted above.

2.7. Statistical analysis of the decontamination efficacy

Decontamination studies were performed in triplicates for each group. The mean for each data point was calculated from the three replicates and the error bars calculated from the standard deviations of the distributions.

Data are presented as mean ±standard deviation. Data were analyzed by one-way analysis of variance (ANOVA) with PASW statistics 18 software (USA). Results were found statistically significant and concluded at $p < 0.05$.

2.8 *In vitro* chelation efficacy

In vitro chelation efficiency (quantitative chelation) of the disodium in the gel was evaluated by using Instant Thin Layer Chromatography-Silica Gel (ITLC-SG). Na_2EDTA was radiolabelled with dry ^{99m}Tc (1 mCi) using Stannous chloride and ethanol present in the freshly prepared topical gel formulation and incubated in the formulation for 0.5 h. 20 µl of each sample was applied on the ITLC-SG strip and placed into beakers containing 100% acetone as mobile phase. After 15 min strips were removed from the beaker and cut into two parts (40:60 ratios). Static counts were measured using gamma well-type counter (Capintec, USA). Labelling efficiency of the EDTA was calculated by using the formula given below: [26]

$$\textit{In vitro}\ \text{chelaltion efficacy} = \frac{B}{B+T} \times 100$$

Where 'B' is the bottom of the ITLC strip and 'T' is the top of the strip.

2.8 Primary skin irritation/safety studies

Primary skin irritation test was conducted on Sprague Dawley rats in two groups (n = 6) to determine the potential of gel to produce irritation after a single topical application. Hairs were removed 24 h before applying the topical gel. 1 gm of the topical gel applied on cotton swab covered with 5 cm × 5 cm 4-ply gauz pad. The pad and entire trunk of each animal were then wrapped with semi adhesive plaster tape to avoid dislocation of the pad. Animals after dosing were caged individually. Control group of animals were treated same with normal saline. After 24 h cotton with gauz pads were removed. Following exposure, dermal irritation e.g., erythema and edema were evaluated for 2 weeks using method of Draize and co-workers [27]. Temperature at the site of the application of the topical gel was also measured with the infra red camera and was found with normal range.

RESULTS AND DISCUSSION

1. Characterization of topical gel

Pharmaceutical properties such as *pH*, spreadability, viscosity, extrudability of the formulations are very much critical to the successful development of the formulation. Optimized gel was found to be clear liquid, transparent and uniform in consistency. *pH* value of the optimized gel formulation ranged from 7.2 to 7.3 which are very close to skin *pH*. Spreadability of the formulation was 9–9.4 cm respectively. The extrudability of formulation was found to be 280-285 g which implies the ease of application of the topical gel. Viscosity was reported 330-348 pascals **(Table 1)**.

Table 1. *Characterization of the pharmaceutical parameters and stability studies the formulation (contains 1.0% API) at 25º C ± 2ºC and 60% ± 5% RH*

Formulations	Appearance	*pH*	Spreadability (cm)	Extrudability (g)	Viscosity (P)	Drug content (%)
Placebo	Transparent	7±0.2	9.0 ± 0.12	280 ± 0.11	330.53 ± 0.15	0
Na_2EDTA gel	Transparent	7±0.3	9.4 ± 0.1	285 ± 0.3	348.01 ± 0.04	99.04
After 3 months and 6 months						
Placebo	Transparent	70.1	9.0 ± 0.12	280 ± 0.11	330.53 ± 0.15	0
Na_2EDTA gel	Transparent	7±0.3	9.4 ± 0.1	285 ± 0.5	348.01 ± 0.05	98.08

2. Decontamination efficacy

^{99m}Tc was decontaminated in order to calculate the efficacy with respect to the different length of contaminant exposure. The results obtained were summated for each combination of contaminant and the mean count remaining after each decontamination attempt was calculated. The first two decontamination attempts were able to decontaminate 60 ±5% of the applied activity over 5-30 min while <5% of the applied activity was removed in the successive five attempts. **Fig. 1** shows the decontamination of the ^{99m}Tc with the formulation containing 1.0% of the disodium edetate at 5 to 30 min length of exposure was found to be most effective. DE of the formulations containing 0.25, 0.5 and 1.25% disodium edetate does not show significant increase in the efficacy (results not shown). Formulation with the 1.0% of the API was selected for further efficacy evaluation. Chelating agents forms complexes with the radioisotopes and facilitate to remove the contaminant. It represents that gel effectively forms stable complex with the ^{99m}Tc and also could prevent entry into systemic circulation through cutaneous absorption. Decontamination delayed long after radio-nuclide exposure was potentially effective to remove the contaminants from skin surface. **Fig. 2** presents the decontamination efficacy between 5 to 30 min lengths of exposure with ^{99m}Tc on human tissue equivalent model. Results clearly indicate that the decontamination attempt within 30 min of contamination could easily remove ≥70% of the applied activity while over that decontamination studies e.g., 1 h was found reducing of efficacy by ≥5% only. Various formulations for skin decontamination have been prepared for the removal of the radioactive contamination from skin. Intact skin layer is a barrier to small quantity of the radioactive nuclides but penetration will increase if outer layer of the skin is damaged as in the case of broken (wounded) skin [28-31].

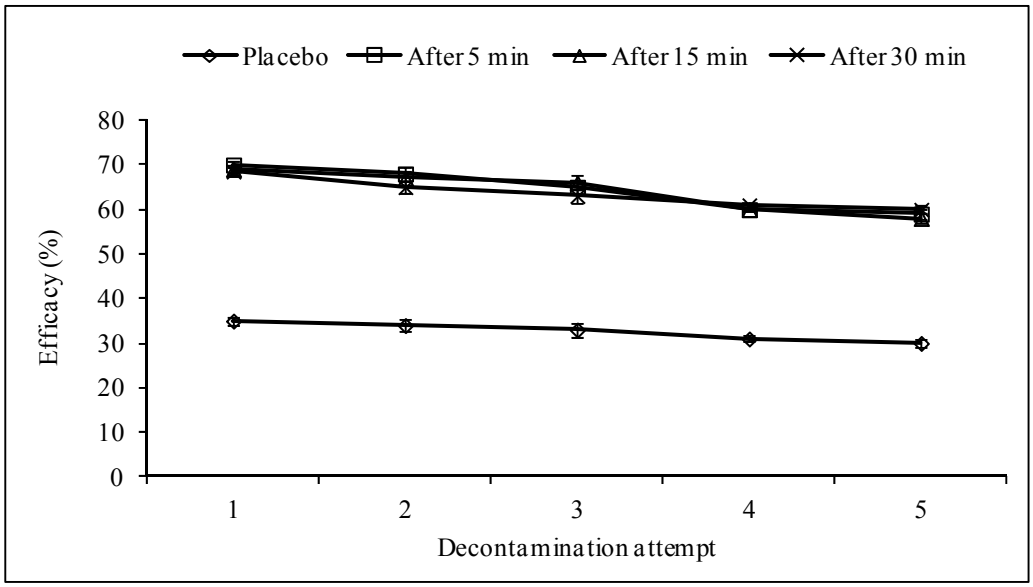

Figure 1. Decontamination efficacy of the topical gel with 1.0% of disodium edetate gel was evaluated on rat skin model using nuclear medicine technique. ^{99m}Tc was used as skin contaminant. Whole body imaging and static counts were recorded pre and post decontamination.

Rat skin decontamination efficacy was found more effective than the human tissue equivalent model. Radio-isotope possess tendency to tightly bind to the skin protein or may move toward the hair follicles that reduces the efficacy. **Fig. 1 and 2** demonstrate the quick reduction in percentage activity applied with the first and second decontamination attempt. This is presumably due mainly to the chelation reaction between loosely bound contaminant and the active ingradient of the gel. These common factors in the entire first and second attempt were apparent to a much smaller degree in successive attempts. Thus, it is in the third, fourth and fifth attempts that the truly

comparative values of the decontamination attempts were assessed. Further analysis by calculation of the standard deviations of the means, and using these figures to compare the significance of the results shown in **Fig. 1 and 2** is significantly better than 1 h length of the contaminant exposure (data not shown). **Fig. 3** representing scintigraph of the successive decontamination attempts. After each step of decontamination contaminant reduces could be clearly observed.

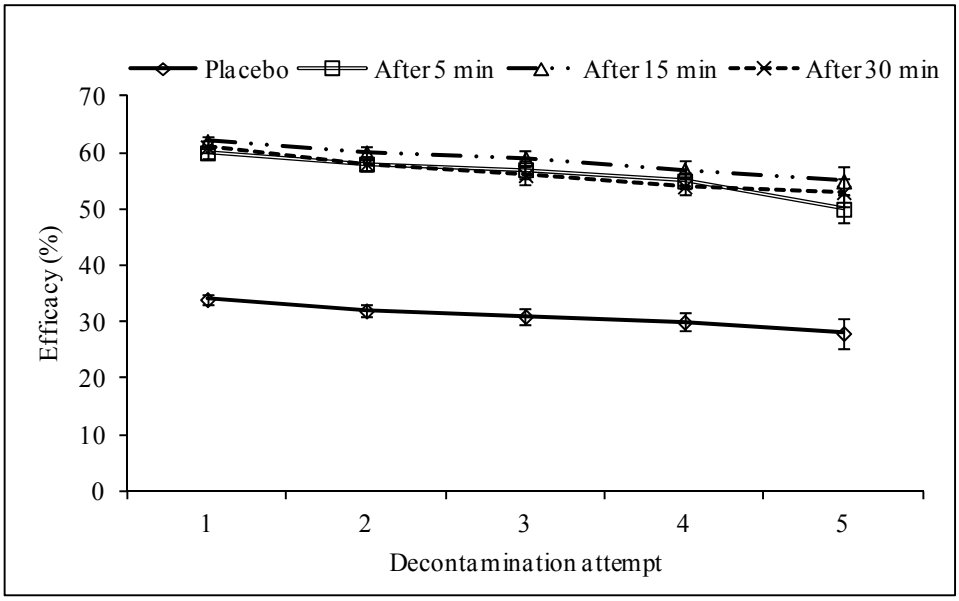

Figure 2. Decontamination efficacy evaluated on human tissue equivalent model applying optimised topical gel on cotton swabs. ^{99m}Tc was studied was applied as radiological skin contaminant. Decontamination factor was calculated after each pre and post wiping step to determine the effectiveness of the optimised topical gel.

In vitro chelation efficiency of the disodium edetate with ^{99m}Tc was found >95%. It indicates that disodium edetate gel could effectively chelate the radio-isotopes (>95%). Skin permeation study (*ex vivo* and *in vivo*) was performed using Franz cell chamber on rat skin / whole animal model and it was found that <5% of the EDTA permeated over 24 h.

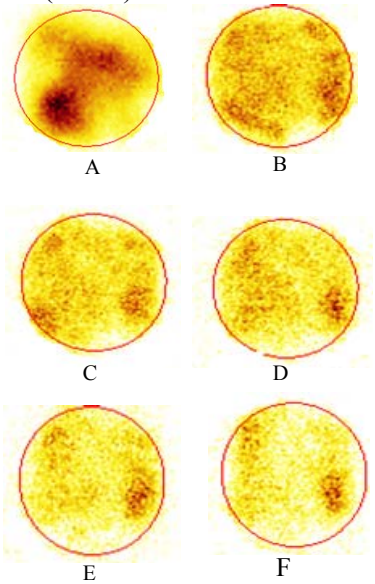

Figure 3. Scintigraph of the decontamination procedure representing successive attempts. After each step of decontamination procedure contamination level reduces gradually.
A - Contaminated skin/tissue equivalent surface
B - F Consecutive decontamination attempts

3. Skin toxicity

All animals survived, gained weight, appear active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. Skin patch where formulation was applied appeared normal i.e., without any erythema and edema.

CONCLUSION

The decontamination procedure with the use of optimised topical gel formulation removes most of the external contaminants. Water available for decontamination may itself be contaminated or scarce thereby limiting its availability. Therefore, the self usable skin decontamination topical gel formulation has been developed for self application after the release of the contaminant. Within 30 min of the contamination, application of the optimised gel formulation could effectively remove the radio-contaminant. No significant difference in the efficacy was found when decontaminated was done at 5 to 30 min. The optimised topical gel was found more efficacious for skin surface than the human tissue equivalent model. This work is a paradigm for the selection of the decontamination formulation based on chelation / complexion. Topical formulation was found to be safe, effective and non-irritant.

ACKNOWLEDGEMENT

The authors are grateful to Director, Institute of Nuclear Medicine and Allied Sciences (INMAS), Defence Research & Development Organization (DRDO), Delhi, India to provide laboratory facility. Thanks are also due to the Life Sciences Research Board (LSRB) for the project under which the formulation development and optimization work was carried out. SR would like to acknowledge the Council of Scientific & Industrial Research (CSIR), New Delhi, India for providing her the financial support. Authors are also grateful to the faculty of Nuclear Medicine for help in scintigraphic experimentation. Thanks are also due to the officials of Animal House Facility, for their help and support to providing animals and taking their proper care during the course of experiment.

REFERENCES

1. Yehezkelli, U., Dushnistky, T., Hourvitz, A., Radiation terrorism: the medical challenge. *Israeli Medical Association Journal,* 4:530-534, 2002.
2. William, G., Reifenrath, W.G., Hawkins, G.S., Kurtz, M.S., 1991.Percutaneous penetration and skin retention of topically applied compounds: An in vitro-in vivo study. *J Pharm Sci*, 80(6):526–532, 2002.
3. Nishiyama, H., Tunen, R.J. Van., Lukes, S.J., Feller, P.A., Survey of ^{99m}Tc contamination of laboratory personnel: hand decontamination. *Radiology,* 13(7):549-551, 1980.
4. Cox, R.D., Decontamination and management of hazardous materials exposure victims in the emergency department. *Ann Emerg Med*, 23(4):761-770, 1994.
5. Meineke, V., Van Beuningen D, Sohns T, Fliender, TM. Medical management principles for radiation accident. *Mil Med*, 168:219-222, 2003.
6. Wagner, R.H., Boles, M.A., Henkin, R.E.,Treatment of radiation and contamination. *Radiographics*, 14:387-396, 1994.
7. Schofield, G.B., Radioactive contamination of the skin. *J Soc Cosmet Chem.* 22:535-545, 1971.
8. Moore, P.H., Mettler, F.A., Skin decontamination of commonly used medical radionuclides. *J Nucl Med,* 21:475-476, 1980.
9. Rana , S., Bhatt, S., Dutta, M., Khan, A.W., Ali, J., Sultana, S., Kotta, S., Ansari, S.H., Sharma, R.K., Radio-decontamination efficacy and safety studies on optimized decontamination lotion formulation. *Int J Pharm,* 434(1-2):43-48, 2012.
10. Gregory, J., An investigation into the removal of radioactive contamination from the hand. *Brit J Industr Med*, 10:32-40, 1953.
11. Kamboj, S., Sharma, D., Bala, S., Ansari, S.H,, Ali, J., Kumar, P., Nishad, D., Rana, S., Sharma, R.K., Optimization of disodium edetate topical gel using central composite design and evaluation for external radioactive decontamination. *Elixir Pharm,* 42:6144-6150, 2012.
12. Levitin, H.W., Siegelson, H.J., Dickinson, S., Haraguchi, H., Nocera, A., Turineck, D., Decontamination of mass casualties: re-evaluating existing dogma. *Prehosp Disaster Med,* 18(3):200–207, 2003.
13. Petitot, F., Frelon, S., Moreels, A.M., Claraz, M., Delissen, O., Tourlonias, E., Dhieux, B., Maubert, C., Paquet, F., Incorporation and distribution of uranium

in rats after a contamination on intact or wounded skin. *Health Physics*, 92(5): 464-474, 2007.
14. Kumar, v., Goel, R., Chawla, R., Silambarasan, M., Sharma. R.K., Chemical, Biological, Nuclear and Radiological Decontamination: Recent trend and future perspectives. *J Pharm Bioall Sci* 2(3): 220-238, 2010.
15. Khan, A.W., Kotta, S., Ansari, S.H., Sharma, R.K., Kumar, V., Rana, S., Ali, J., Chemical, biological, radiological, and nuclear threats-Decontamination technologies and recent patents: A review. *J. Renewable Sustainable Energy,* 4, 012704 (2012); http://dx.doi.org/10.1063/1.3688029
16. Felton, J.S., Rozas, C.J., Decontamination of human skin experimentally soiled by radioactive materials. *Arch Environ Health,* 1:87-95, 1960.
17. Schulte, J.H., The problem of radioactive contamination of skin. *Archives of Environmental Health,* 13:96-101,1966.
18. Rana, S., Dutta, M., Soni, N.L., Chopra, M.K., Kumar, V., Goel, R., Bhatnagar, A., Sultana, S., Sharma R.K., Decontamination of Human and Rabbit Skin Experimentally Contaminated with 99mTc Radionuclide Using the Active Components of "Shudhika"-a Skin Decontamination Kit. DOI:10.1017/S1049023X1200057X
19. Harrison, W.D., The fate of radioiodine applied to human skin. *Health Phys*, 9:993-1000, 1963.
20. Henson, P.W., A note on some aspects of skin contamination by certain radionuclides in common use. *Brit J Radiol,* 1:87-93, 1972.
21. Narola, .B, Singh, A.S., Mitra, M., Santhakumar, P.R., Chandrashekhar, T.G., A Validated Reverse Phase HPLC Method for the Determinationof Disodium EDTA in MeropenemDrug Substance with UV-Detection using Precolumn Derivatization Technique. *Analytical Chemistry Insights*, 6:7–14, 2011.
22. Katata, L., Nagaraju, V., Crouch, A.M., Determination of ethylenediamine tetraacetic acid, ethylenediaminedisuccinic acid and iminodisuccinic acid in cosmetic products by capillary electrophoresis and high performance liquid chromatography. *Anal Chim Acta,* 579, 388:177–184, 2006.
23. Bharate, S.S., Bharate, S.B., Bajaj, A.N., Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredient: a comprehensive review. *J Excipients and Food Chem*, 1(3), 3-26, 2010.
24. Mohanambal E., Arun K., Abdul H. S. A., Formulation and Evaluation of *pH*-triggered *in situ* Gelling System of Levofloxacin. *Ind J Pharm Edu Res,* 45(1): 58-64, 2011.
25. Soares, L.A.L., Ortega, G.G., Petrovick, P.R., Schmidt, P.C., Optimization of tablets containing a high dose of spray-dried plant extract: A technical note. *AAPS Pharm Sci Tech. 6E,* 367-71, 2005.
26. Bonacucina, G., Martelli, S., Palmeiri, G.F., Rheological, mucoadhesive and release properties of carbopol gels in hydrophilic cosolvents. *Int J Pharm* 282:115–130, 2004.
27. Draize, J.H., Woodward, G., Calvery, H.O., Methods for the study of irritation and toxicity of substaces applied topically to the skin and mucous membranes. *J Pharmacol Ex Ther,* 82:377-390, 1944.
28. Bolzinger, M.A., Bolot, C., Galy, G., Chabanel, A., Pelletier, J., Bariancon, S., 356 Skin contaminations by radiopharmaceuticals and decontamination strategies. *Int J Pharm*, (1–2): 44–49, 2010.
29. Bauerova, K., Kassai, Z., Koprda, V., Harangozo, M., Contribution to the penetration of radionuclides across the skin. Concentration dependence of strontium through the skin in vitro. *J Appl Toxico,* 21(3):241-243, 2001.
30. Koprda, V., Harangozo, M., Kassai, Z.,Transfer of radionuclides across skin barrier of animal skin models in vitro. *J Radioanaly Nucl Chem*, 246(3):509-509, 2000.
31. Merrick, M.V., Simpson, J.D,, Liddell, S., Skin decontamination- a comparison of four methods. *British Journal of Radiol,* 55:317-318, 1982.