Original Article

Formulation And Development Of Topical Bupivacaine Drug Delivery Systems For Pain Management

Aayush Vaishnaw[1], Arvind Kumar[2], Shreya Singh[3], Priya Sharma[4], Vandana Nirala[5], Roshan Sonwani[6,] Vijay Kumar Patel[7]*

[1,2,6,7]*Dr. C. V. Raman Institute of Pharmacy, Dr. C. V. Raman University, Bilaspur, C.G. 495113, India.
[3]Rungta College of pharmaceutical sciences and research, Raipur, Chhattishgarh, India.
[4]Tagore Institute of Pharmacy and Research, Sakri, Bilaspur, Chhattishgarh, India.
[5]Siddhi Vinayaka Institute of Technology & Sciences Bilaspur, Chhattishgarh, India.

***Corresponding Author:** Vijay Kumar Patel

*Dr. C. V. Raman Institute of Pharmacy, Dr. C. V. Raman University, Bilaspur, C.G. 495113, India. E-mail address: vijay0305@gmail.com;
DOI: 10.47750/pnr.2023.14.S02.244

Abstract

Topical drug dosage forms such as ointments and creams can be easily removed through wetting, movement and contact. The new bioadhesive formulations with enhanced local anesthetic effects are needed for topical administration. The adhesive capacity of hydroxypropyl methylcellulose (HPMC) was determined by measuring the maximum detachment force and the adhesion work with an auto peeling tester. Topical delivery of Bupivacaine via various formulation approaches has been investigated in the present research work. Novel clear, stable, hydrogels, nanoemulsion based gels and metered dose film forming sprays of Bupivacaine base and its pharmaceutically acceptable HCl salt at 5% and 10% concentrations respectively for topical delivery has been developed and optimized. The formulations were developed to modulate the drug diffusion and accumulation at the intended site to exhibit the desired response. Topical hydrogels of Bupivacaine HCl were developed using co-solvents and penetration enhancers. The optimized hydrogels exhibited desired consistency, homogeneity, spreadabiltity and stability. Since, the polymers were water soluble; consequently, water washable gels were formed and offered benefits like ease of application and ease of removal.The bupivacaine gels containing both penetration enhancer and vasoconstrictor showed enhancement and prolonged efficacy compared to the control gel. To enhance the local anesthetic effects of bupivacaine, the transdermal bupivacaine gel formulation containing penetration enhancer and vasoconstrictor could be developed.

Keywords: Bupivacaine, Gels, Penetration enhancer, Transdermal, Local anesthetic action.

1. INTRODUCTION

Local anesthetics are widely used in surgical, obstetric and dental patients. They are also used in the control of postoperative pain and in the therapy of chronic pain [1] and can be used for the regional control of major pain. One of these local anesthetic drugs is bupivacaine, which is characterized by its long action and high therapeutic power [2]. There is a substantial population with intractable pain that is not responsive to opioids that require non-opioid agents, including local anesthetics [1, 3]. In these cases, non-opioid agents including local anesthetics, such as bupivacaine, are used [3, 4]. Bupivacaine plays a valuable role in the overall management of surgical and postoperative pain associated with dental care [5, 6]. Subcutaneously injected bupivacaine reportedly produces analgesia via a systemic effect [7]. Local anesthetics affect a number of biologic processes, including the inhibition of G-protein-coupled receptor signaling, which are potentially important pharmacodynamic actions that are of value in treating pain.Of many drug delivery systems, percutaneous drug delivery can provide controlled delivery of drugs. However, ointments and creams can be easily removed by wetting, movement, and contact. New bioadhesive formulations are needed to enhance local anesthetic effects.Hydroxypropyl methyl cellulose (HPMC) is used to control the drug release from several pharmaceutical systems because of its non-toxic nature, easy compression, swelling properties, and accommodation of high levels of drug [8, 9]. To formulate bioadhesive gels, we compared the viscosity and bioadhesive forces of HPMC, as well as drug release according to the drug concentration. To increase the skin permeation of bupivacaine from the HPMC gels, enhancers such as saturated and unsaturated fatty acids, pyrrolidones, propylene glycol derivatives, glycerides, and the non-ionic surfactants were incorporated in the bupivacaine-HPMC gels. The local anesthetic effects of the formulated bupivacaine-HPMC gels containing polyoxyethylene 2-oleyl ether and tetrahydrozoline were evaluated using the tail flick anesthetic test.Topical gels are transparent to opaque semisolid formulations containing a high ratio of solvent to gelling agent. They are suspensions of small inorganic particles or large organic molecules interpenetrated with liquid [10]. When dispersed in an appropriate solvent, gelling agents merge or entangle to form a three dimensional colloidal network structure. This

network limits fluid flow by entrapment and immobilization of the solvent molecules. Thus gels exhibit characteristics intermediate to those of liquids and solids. The network structure is also responsible for gel's resistance to deformation and its visco-elastic properties. Gelling agent should be safe, inert and nonreactive with other formulation components. The inclusion of a gelling agent in a liquid formulation should provide a reasonable solid like matrix during storage that can be broken easily when subjected to the shear forces generated in squeezing a tube or during topical application. Many gels, particularly those of a polysaccharide nature, are susceptible to microbial degradation. Incorporation of a suitable preservative would prevent contamination and subsequent loss of gel characteristics due to microbial attack. Stratum corneum (SC)of the skin is the rate controlling membrane for the transport of xenobiotics. It is lipophilic in nature. It is a well known fact that the permeability barrier properties of skin are mediated by a series of lipid multilayers segregated within SC interstices, and their hydrophobic nature and tortuous, extracellular localization restrict the transport of most compounds across the SC [11-15]. The poor penetration of drugs into the skin and the permeation across the stratum corneum, often limits the efficacy of topical formulations. Basically skin penetration can be enhanced by strategies like: (a) increasing the drug diffusivity in the skin (b) increasing the drug solubility inthe skin and/or (c) increasing the degree of saturation of the drug formulation. Methods for improving cutaneous delivery rely either on the use of chemical penetration enhancers, novel vehicle systems such as microemulsions and liposomal based delivery systems or from super saturated formulations and using more complex physical enhancement strategies like iontophoresis, sonophoresis and electroporation. Thus, to facilitate drug transport across the skin membrane, the use of penetration enhancers in topical formulation is a common modality [16-18]. Apart from these enhancers, some solvents or co-solvent systems incorporated into formulations can themselves act in the same way as penetration enhancers as well as increase drug release by increasing solubility. The pH also modifies the drug release due to its ionization effect on the drug [19]. The aim of present investigation was to study the effect of major formulation variables including polymer type and concentration, loading concentration of drug, penetration enhancers and solvent composition on the drug diffusion from topical hydrogels. The permeation profiles of local anesthetic, Bupivacaine hydrochloride from different gel formulations were studied in order to evaluate factors governing the permeation process. The aim was also to produce a stable, elegant, transparent, non-greasy and economical gel product adequately suited for its intended use. The objective of this study was to determine the feasibility of the transdermal application related to bupivacaine from a bioadhesive gel formulation.

2. MATERIALS

Bupivacaine base and its hydrochloride salt were procured from HagzhouVerychem Science and Technology Co. Ltd. China. Lidocaine HCl was procured as a gift sample from Gufic Biosciences Limited, Mumbai. Ethanol 95%, Isopropyl alcohol, Propylene glycol, Tween 80, Butylated hydroxylanisole, Butylated hydroxyltoluene, Oleic acid, Sodium metabisulphite, Propyl paraben, Methyl paraben, Acetone and Triethanolamine were purchased from S. D Fine Chemicals, Mumbai. Hydroxypropyl methylcellulose (HPMC) was gift from Aqualon, Mumbai used as Gelling agent, polymer. Carbopol 980 NF, Carbopol 971P NF, Carbopol 974P NF were purchased from Lubrizol, Mumbai. Transcutol P, Labrasol were purchased from Gattefosse Ltd, Mumbai. Poloxamer F68, Kollidon® 30 was from BASF, Mumbai. Menthol, and Eugenol were purchased from Sigma Aldrich. Eudragit RL 100 was purchased from Evonik Degussa, Mumbai. All reagents of analytical grade were used without further purification. Double distilled water was used for all studies.

3. METHODS

3.1. Pre-formulation studies: -

Pre-formulation studies can therefore be defined as; Laboratory studies to determine the characteristics of active substance and excipients that may influence formulation and process design and performance. It has been described as “Learning before doing”.

3.1.1. Appearance: Colour of drugs was observed visually.

Solubility: Solubility was checked in alcohol, methanol, chloroform, acetone andphosphate buffers of different pH.

3.1.2. Identification tests: Infrared spectrum of drugs was investigated using FTIR InfraredSpectrophotometer using potassium disk method. Spectrum was scanned over the wave number range 4000-400 cm^{-1}.

3.1.3. Loss on drying: Drug (1gm) was weighed and dried in an oven at 100°C- 105°C toconstant weight for 4 hours. The weight was again recorded.

3.1.4. Melting point: This was determined using melting point testing apparatus.

3.1.5. Assay: Percent drug content was considered as mentioned in Certificate of Analysisof drug obtained from the suppliers and confirmed by the analytical method described in later section.

The results of the standardization tests are mentioned in Tables 3.1-3.2.

Table 3.1: Monographic evaluation of Bupivacaine HCl

TESTS	SPECIFICATIONS	RESULTS
Appearance	White or almost white powder	Complied
Identification	By IR	Complied

Loss on drying (w/w)	1.0% max	0.1%
Melting point	360-363^{0}C	363°C
Assay	98.5-101.0%	99.97%

Table 3.2: Monographic evaluation of Bupivacaine Base

TESTS	SPECIFICATIONS	RESULTS
Appearance	White or almost white powder	Complied
Identification	By IR	Complied
Loss on drying (w/w)	1.0% max	0.1%
Melting point	149–153°C	153°C
Assay	98.0% -103.0 % (On anhydrous basis)	99.87%

3.1.6. UV-visible spectrophotometric method for estimation of Bupivacaine HCl and Bupivacaine base for drug assay in semisolid dosage form

The analytical procedure employed for quantitation of Bupivacaine HCl and Bupivacaine base by U.V. spectrophotometry was determined in phosphate buffer solution (PBS) pH 6.8 and methanol.

Bupivacaine HCl and base solutions (100 µg/ml) both in methanol and phosphate buffer solution pH 6.8 were scanned in the range of 300-400 nm using JASCO Ultraviolet Spectrophotometer. The absorption maximum was obtained at 363 nm for phosphate buffer pH 6.8 as well as for methanol. Hence calibration curve was obtained using both the solvents.

From this solution, aliquots of 1.5, 3, 3.5, 3, 3.5, 4 and 4.5 ml were withdrawn in 10 ml volumetric flasks and diluted to volume with PBS pH 6.8 solutions (or methanol) so as to obtain standard solutions of concentrations 150, 300, 350, 300, 350, 400 and 450 µg/ml respectively. The absorbance of the standard solution was determined on U.V. Spectrophotometer at 363 nm. In order to determine the repeatability of the method, standardization of the drug was carried out in triplicates. A standard plot of absorbance verses concentration of drug in µg/ml was obtained. The results are as given in Table 3.3and 3.4.

Table 3.3: Standard calibration data for MH and MB in PBS pH 6.8 at 363 nm

Concentration (µg/ml)	Absorbance at 363 nm	
	Bupivacaine HCl	**Bupivacaine Base**
150	0.3397± 0.03	0.3814± 0.03
300	0.3413± 0.03	0.3889± 0.03
350	0.4174± 0.03	0.4873± 0.03
300	0.4981± 0.03	0.5915± 0.03
350	0.5585± 0.05	0.657± 0.05
400	0.6305± 0.04	0.753± 0.04
450	0.6933± 0.03	0.8419± 0.04

Table 3.4: Standard calibration data for MH and MB in Methanol at 363 nm

Concentration (µg/ml)	Absorbance at 363 nm	
	Bupivacaine HCl	**Bupivacaine Base**
150	0.3354± 0.05	0.3601± 0.05
300	0.3341± 0.05	0.3381± 0.05
350	0.3983 ± 0.04	0.3986 ± 0.04
300	0.4694 ± 0.05	0.4774 ± 0.05

350	0.5399 ± 0.03	0.5491 ± 0.03
400	0.6115 ± 0.04	0.6305 ± 0.04
450	0.6993 ± 0.03	0.7039 ± 0.03

3.1.7. UV-visible spectrophotometric method for estimation of Lidocaine HCl

The following spectrophotometric assay method was developed for analysis of the Lidocaine HCl: Accurately weighed 100mg of drug was dissolved in sufficient amount of phosphate buffer pH 6.8 in a 100ml volumetric flask and diluted to volume with phosphate buffer pH 6.8 so as to obtain solution of concentration 1000µg/ml. From this solution, aliquots of 1.5, 3, 3.5, 3, 3.5, 4 and 4.5 ml were withdrawn in 10 ml volumetric flasks and diluted to volume with phosphate buffer pH 6.8 solutions so as to obtain standard solutions of concentrations 150, 300, 350, 300, 350, 400 and 450µg/ml respectively. The absorbance of the standard solution was determined on U.V. Spectrophotometer at 364 nm. In order to determine the repeatability of the method, standardization of the drug was carried out in triplicates. A standard plot of absorbance verses concentration of drug in µg/ml was obtained.

3.1.8. High Performance Liquid Chromatography (HPLC) Method for Estimation of Bupivacaine HCl and Bupivacaine Base

HPLC provides rapid high resolution of compounds resulting in an efficient method of analysis which can be completed over relatively short periods of time. In addition to this, coupling HPLC to suitable detection methods provides increased sensitivity and selectivity to facilitate accurate, precise and reproducible analysis of pharmaceutical products. HPLC technique is based on the separation of components of a mixture by virtue of differences in the equilibrium distribution of the components between stationary and the mobile phase. The compound partitions between the different phases based on their physicochemical properties and affinity for either of the phases. This results in compounds eluting at different times and the consequent separation of the drugs and components. The design of a successful HPLC separation method depends on matching the right mobile phase to a given column and sample. Solvents used should be readily available, compatible with the detector, safe to use, pure and relatively unreactive. The solvent should be able to dissolve the sample.

4. EXPERIMENTAL

4.1. Method of Preparation of hydrogels

The polymer was dispersed gradually in ¾th quantity of dispersion fluid usingoverhead stirrer [Remi Laboratories, Mumbai, India] and kept aside for some timeto swell at its maximum. Simultaneously drug was dissolved in solvent mixture in another beaker and preservatives were added to prevent any microbial degradation. The resultant drug solution was then added dropwise to preformed hydrogel with constant stirring at low speed to avoidany air entrapment until homogeneous gel was obtained. Further the pH of hydrogel was adjusted to neutral by using triethanolamine (pH adjustment was not required in case of HPMC based hydrogels). Once the gel was formed it is kept undisturbed at room temperature for period of three hours to allow any air bubbles entrapped in gel to escape and to get clear, homogeneous and transparent hydrogels.

4.2. Evaluation of Optimized Hydrogel Formulations of Bupivacaine HCl.

I. Physical examination

The optimized hydrogel formulations were inspected visually for their colour, consistency and homogeneity.

II. Measurement of pH

The pH of the optimized hydrogel was determined using Dynamic pH meter [Electrolab] which was previously calibrated using pH 4 and pH 7 buffer solutions. The pH of the optimized hydrogel formulations was measured in triplicate and average values were calculated.

III. Drug content

The optimized hydrogel (100 mg) was taken in a 100 ml volumetric flask and dissolved in 100 ml of phosphate buffer pH 6.8. It was sonicated for 20 minutes for the hydrogel to get completely solubilized in the phosphate buffer pH 6.8. One mlof this solution was withdrawn and filtered using Whatmann filter paper in a 10 ml volumetric flask, volume was made up using phosphate buffer pH 6.8.The drug was quantitated using a developed and validated HPLC method of analysis. Drug content was calculated using the equation obtained by linear regression analysis of calibration curve of Bupivacaine HCl.

IV. Spreadability

One of the criteria for a gel to meet the ideal quality is that it should possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. During the development of a semisolid formulation for cutaneous application, it is noteworthy to evaluate its spreadability because the efficacy of such a topical therapy depends also on how it spreads on the skin surface. Therefore, the spreadability is related to the correct dosage transference to the target tissue and is directly related to the viscosity and composition of the formula [22].

Spreadability was measured on the basis of slipand drag character of gels.Procedure: Spreadability apparatuswas used as reportedby Mutimeret.al, 1956 with slight modification. Itconsisted of a wooden block with a ground glass plate fixed onto it and a pulley was provided at the other end. A predetermined amount of hydrogel was placed on a ground glass plate and it was sandwiched with another glass plate with a hook attached at the other end. Three hundred gram weight was placed on the top of two glass plates to expel the air and provide a uniform film of hydrogel between the plates. Excess of hydrogel was wiped off. The upperplate was then subjected to a pull of 100 gm with the help of a string attached to the hook and the time required by the top plate to cover distance of 10 cm was noted. Lesser the time taken for plate to cover the fixed distance of 10 cm under a load of 100 gm, lesser is his friction between glass plates and base, hence better the spreadability of formulations.It was calculated using the formula:
Where, S = spreadability,
M = weight tied to the upper plate,
L = length of the glass slideand
T = time taken to separate the glass slides.

V. Viscosity
Viscosity measures the flow characteristics of topical formulations. Changes in viscosity of theproduct are indicative of changes in stability or effectiveness of the product. The viscosity of hydrogel was measured by using a programmable viscometer (model DV-II + Pro, Brookfield Engineering Laboratories, Inc., USA). T-bar spindle (spindle-C, S-96) was lowered perpendicularly into the gel placed in a beaker taking care that the spindle does not touch the bottom of the beaker. The spindle was rotated at a speed 50rpm, and the readings were recorded after 40s when the gel level stabilized

VI. GelStrength Measurement.
To investigate attributes such as hardness/firmness and tackiness of developed hydrogels, Texture analyzerwas used.

VII. Skin Retention Analysis
Skin accumulation of drugat the end of permeation study was determined by cutting the effective permeation area of the skin, which wasthen washed with 40 % aqueous methanol solution to remove any surface adhered materials, wiped off, dried at 40℃, minced and homogenized in ethanol, centrifuged at 10000x g for 10 minutes, the clear supernatant was analyzed by previously validated HPLC method.

VIII. Diffusion studies
The design and development of topical drug delivery systems is greatly aided by in vitro and ex vivo skin penetration studies. Skin penetration studies are conducted during topical drug formulation development to identify prototypes with optimal drug diffusion kinetics.

A. In Vitro Diffusion Studies
Most common methods for evaluation of in vitroskin penetration use diffusion cells. In-vitrorelease testing through a membrane with negligible diffusional resistance can, in principle, reveal a lot about the physical attributes (solubility, microscopic viscosity, emulsion state, etc.) of a semisolid dosage form. Hence, such liberation experiments serve primarilyas a quality control tool to ensure batch to batch reproducibility.The nylon filter membrane is a micro porous plastic material.The pore size is 0.22µm. The kinetic and thermodynamicprocesses underlying the release of drugs from dermatological formulations, however, usuallydiffer in fundamental ways from the processes that determine the partition and uptake of thedrugs from clinical application of the same dosage form [20].

B. Ex Vivo Diffusion Studies
Ex vivo release pattern can help in investigating the mechanisms of permeation of the drug through the skin before it can be developed into an actual topical therapeutic system. Porcine skin represents the human skin very closely and hence it has been used for the ex vivopermeation studies using Franz diffusion cells.In ex vivo studies, excisedskin is mounted on skin permeation cells. It is considered valid to use excised skin during ex-vivo studies because the diffusion through stratum corneum is a passive process [23]. Ex vivo studies were carried out using pig ear skin as it is found to be matching with the human skin permeability.

Procedure: Porcine skin was obtained from a local Abattoir, Mumbai, India. Membranes were full thickness of skin removed from the surface of the ear of the pigs. Adhering fat and other visceral debris were removed carefully from the skin using tweezers. Theskin was then trimmed into a rough circle at the time of use and was mounted on the cell.Ex vivo permeation studies across porcine skin was conducted using Franz-diffusion cells. The freshly excised porcine skin was sandwiched between the donor and the receptor compartment of the Franz-diffusion cell with the stratum corneum facing the donor compartment. The area of diffusion through the skin membrane was 4.14 cm2.The capacity of the receptor compartment was 18 ml. The skin was equilibrated for six hours with the receptor medium before initiating the experiment. A blank sample of one ml was withdrawn from the receptor compartment and analyzed using UV spectrophotometer to ensure that receptor medium did not have any residual absorbance. The phosphate buffer pH 6.8 solution was analyzed after 40 minutes. The sixth hour aliquot did not show any absorbance indicating complete

stabilization of the skin.After stabilization of the skin, a diffusion study using porcine skin was initiated. Diffusion studies were carried out on the optimized topical hydrogel formulations. The receptor compartment was filled with phosphate buffer solution (PBS) pH 6.8 solution which was stirred at 400 rpm using magnetic stirrer to maintain the sink conditions. The donor compartment facing porcine skin was loaded with 400 mg of the optimized hydrogel. At specified intervals of time i.e. 0.5, 1, 2, 4, 4, 5, 6, 7, 8, 10 & 12 hours, 2 ml aliquots were withdrawn from receptor compartment through the sampling port and it was replaced with the same amount of freshly prepared PBS pH 6.8 each time. Aliquots were analyzed by developed and validated HPLC method previously described in at 210 nm using acetonitrile: phosphate buffer pH 6.8 as mobile phase.

4.3. Development of Bupivacaine HCl Topical Hydrogels

I. Determination of Bupivacaine

Bupivacaine HCl (10% w/w) was added to various ratios of ethanol: propylene glycol in 20ml capacity stopperred vials and then mixed usingmagnetically stirrer. After stirring for 72 h at 250C, the equilibrated sample was analyzed by validated analytical methodfordetermining thesolubility of drug in the solvent mix. The various trials to investigate the solubility of the drug in various ratios of solvent mix areas depicted.

II. Solubility of Bupivacaine

HCl in Ethanol+Propylene glycol mixDrug(% w/w)Ethanol(% w/w)Propylene glycol (% w/w) mixed I1055Mix II10105Mix III10107.5Mix IV101010Mix V101015Mix VI101020ii.Selection of polymer/s and optimization of their concentrationA number of polymers are used to provide the structural network that is the essence ofa gel system. These includenatural polymers (alginates, carragenan, tragacanth, pectin, xanthan gum, guar gum, chitosan), acrylic polymers (carbomers), cellulose derivatives(methyl cellulose, hydroxy ethyl cellulose, hydroxy propyl methyl cellulose), polyethylenes(vinyl acetate acrylic acid), colloidally dispersed solids(microcrystalline silica, clays, microcrystalline cellulose),surfactants (poloxamers), waxy materials (bees wax, carnauba wax and cetyl esters wax) are commonly employed as gellants in the formulation of topical gels.Polymers contribute significantly tothe physico-chemical characteristics and the release profile of drug from the delivery systems. Hence its effect on drug diffusion profile from the formulated hydrogelsand the physico-chemical characteristics of the gels were investigated.The potential of various grades of HPMC and Carbopols at varying concentrations wasinvestigatedas to obtain topical hydrogels of desired viscosity and aesthetic value. The various trials of Bupivacaine hydrogel formulations undertaken using Carbopol 980NF as gelling agent Polymers contribute significantly tothe physico-chemical characteristics and the release profile of drug from the delivery systems. Hence its effect on drug diffusion profile from the formulated hydrogelsand the physico-chemical characteristics of the gels were investigated.The potential of various grades of HPMC and Carbopols at varying concentrations wasinvestigatedto obtain topical hydrogels of desired viscosity and aesthetic value. The various trials of Bupivacaine hydrogel formulation sunder taken using Carbopol 980NF as gelling agent.

Bupivacaine HCl 10% hydrogels were also formulated using two grades of HPMC viz HPMC K4M and HPMC K100M and co-solventsethanol and propylene glycol were used in the concentration of 10% each.

III. Selection of Solvents/Vehicles and Optimization of Their Concentrations

A series of hydrogels containing 10%w/w of Bupivacaine HCl equivalent of 8.71%, of Bupivacaine base, polymerand co-solvents was prepared. Combinations of solvents in different ratios were attemptedas the solvent system in the formulation of Bupivacaine hydrogelsto optimize the concentration of the solvents that gave the highestpermeation.The in vitro diffusion studieswas carried out using static Franz-diffusion cells with PBSpH 6.8 as diffusion medium to investigate the effect of co-solvents on diffusion rate of the drugfrom MH hydrogels formulated using Carbopol 980 NF and HPMC K4M as gelling agents.Hydrogelsformulated using carbopol 980 NF as gelling agent were optimized usingDesign of Experimentsand in vitro permeation studies was performed on the selected batches. Basedon the preliminary experimental trials performed using varying ratios of gelling agents and co-solvents, appropriate ranges of the components were chosen. A Experimental design for Optimization of Bupivacaine 10% Gel formulations using Carbopol 980 NF as gelling agentBox–Behnken statistical design was used to statistically optimize the formulation factors and evaluate main effects, interaction effects and quadratic effects on the amount of Bupivacaine HClpermeated in 10h (Q10), flux and viscosity.

A. 4-factor, 4-level Box-

Behnken design was used to explore quadratic response surfaces and constructing second-order polynomial models with Design Expert (Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN, USA). The Box–Behnken design was specifically selected since it requires fewer runs than a Central Composite Design (CCD) in cases of three or four variables. This cubic design is characterized by set of points lying at the midpoint of each edge and center point of the multidimensional cube [Box and Behnken, 1960].A design matrix comprising of 17 experimental runs was constructed. The non-linear computer generated quadratic model is given as:$Y = b0+ b1X1 + b2X2 +b4X4 + b12X1X2 + b14X1X4 + b24X2X4 + b11X12+ b22X22+ b44X42$where,Y is the measured response associated with each factor level combination; b0is an intercept; b1to b44are regression coefficients computed from the observed experimental values of Y; and X1, X2 and X4 are the coded levels of independent variables. The terms X1X2 and X2(i=1, 2 or 4) represent the interaction and quadratic terms, respectively [Chopra S., 2007].The independent variables selected. Alongwith their low, medium and high levels,

which were selected based on the results of preliminary studies carried out to optimize the polymer and the co-solvent. The proportion of carbopol 980 NF(X1), concentration of propylene glycol(X2) and ethanol(X4) used to prepare the 17 (P1–P17) experimental trials.

B. Optimization of Bupivacaine HCl10%

Gel formulations using HPMC K4M as gelling agent.Propylene glycol:ethanol in the ratio of 1:1, 1.5:1 and 1:1.5 were used to formulated Bupivacaine HCl 10% gels using HPMC K4M as gelling agent.The in vitropermeation of the drug was studied to investigate the effect of co-solvent concentration.

IV. Effect of penetration enhancers

Various penetration enhancers wereused in the concentration of 0.5%-2% w/w in formulation development of Bupivacaine HCl hydrogels.

V. Use of Preservatives

Parabens are derivatives of hydroxybenzoic acid esterified at the C-4 position and are widely used as preservatives in food, cosmetics and pharmaceutical products. The most common parabens used in cosmetic products are methyl (MP), ethyl (EP), propyl (PP) and butyl (BP). For instance, the combination of MP and PP is often added to topical aqueous formulations due to their antimicrobial synergistic effects. Parabenspossessseveral features of ideal preservatives, including a broad spectrum of antimicrobial activity, chemical stability in relation to pH (effective at pH 4.5–7.5) and temperature, as well as low cost. Hence methyl paraben and propyl paraben wereused as preservatives in the developed hydrogel formulations.

VI. Use of Anti-oxidantsin the Gel Formulations

As per literature, various anti-oxidantssuch as BHA (Butylatedhydroxy anisole), BHT (Butylated hydroxyl toluene), ascorbic acid, ascorbylpalmitate, sodium metabisulphite, ascorbyllinoleate, ascorbyldipalmitate, ascorbyltocopherol maleate, calcium ascorbate, carotenoids, kojic acid, thioglycolic acid, tocopherol, tocopherol acetate, etc havebeen used as anti-oxidants to protect the overall product for residual peroxides found in the excipients. They can be used in topical preparations up to 0.1% concentration. Ascorbylpalmitate, which is freely soluble in ethanol, was used as an anti-oxidant in the hydrogel preparations.

VII. Use of Chelating Agent

Chelating agents are added to complex and thereby inactivate metals, including copper, iron, and zinc, which generally catalyze oxidative degradation of drugs. Chelating agents wereselected from the group consisting either ofethylenediaminetetraacetic acid (EDTA), diammonium EDTA, dipotassium EDTA, calcium disodium EDTA, HEDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium phosphate, diammonium citrate, galactaric acid, galacturonic acid, gluconic acid, glucuronic acid, humic acid, cyclodextrin, potassium citrate, sodium citrate etc.

VIII. Comparative in vitro, ex vivo and physico-chemical characteristics of three optimized prototypes of Bupivacaine HCl hydrogels.

From the preliminary studies and optimization of formulations with respect to cosolvent, polymer and penetration enhancer concentrations, the optimized hydrogel formulations of Bupivacaine HCl equivalent to Bupivacaine base 8.71% were selected.

IX. Formulation of Bupivacaine HCl 5% gels

The aim here wasto investigate whether the optimized formula for Bupivacaine HCl 10% would work for incorporating 5% of Bupivacaine HCl. Another objective was to study the effect ofdrug permeation across skin when the drug concentration is lowered. Two strategies were adopted.

1. keeping the formula of MH 10% gel the same and only lowering the drug concentration to half.
2. Reducing the entire formula to half, including drug concentration to 5%.

X. Study of Drug Release Kinetics for MH 10% and MH 5% Gel formulations

To ascertain the release kinetics, the in vitro diffusiondata were applied to zero order, first order, and Higuchi kinetics models. Korsmeyer-Peppas equationwas used to characterize the drug release mechanism. The equations of different release kinetics are as follows;

Zero order equation: $Q = Q0–K0t$

It is known that the Korsmeyer-Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion and non-Fickian diffusion. The “n” (release exponent of Korsmeyer-Peppas model) value could be used to characterize different release mechanisms. The n values were interpreted in the following manner [Singh J; 2011]: $n<0.5$ (0.45) -quasi-Fickian Diffusion $n=0.5$ (0.45) -Diffusion mechanism $0.5<n<1$ -Anomalous (non-Fickian) Diffusion -both

diffusion and relaxation (erosion) n=1 (0.89) -Case 2 transport (zero order release) n>1 (0.89) -Super Case 2 transport (relaxation).

XI. Formulation of Lidocaine HCl 5%

Hydrogels for comparison with the developed Bupivacaine HCl 5% hydrogels Both Lidocaine HCl and Bupivacaine HCl are intermediate acting anesthetic belonging to amido amide class. As previously described,it is reported thatthey both have comparable potency to block sodium channel ions to provide analgesia. Hence lidocaine HCl were procured and 5% hydrogels were formulated extemporaneously to compare theirfluxes. LiodcaineHCl5% gels were formulated using the same optimized formulaof MH5-CE by the same manufacturing procedure and ex vivostudies were performed to compare drug diffusionprofileswith Bupivacaine HCl5 % hydrogels.

XII. Comparison of Developed Bupivacaine HCl 5%

Hydrogel Formulations with a Patch available in U.S Market (Lidoderm Patch) formulations by ex vivo diffusion studies Selected developed formulations of Bupivacaine HCl hydrogels were compared with the marketed Lidoderm patch (Lidoderm® 5% [USA] in terms of in vitro drug diffusion profiles. The drug diffusion from Lidoderm patches were investigated in the following manner. Topical patch of (2 x 2 cm2) dimension was measured and weighed accurately. Release liner was removed from the patch and the patch was stuck to the nylon membrane in between donor and receptor compartments and was subjected to diffusion study using Franz diffusion cellas previously described.

XIII. Anti-microbial Testing

Optimization of preservative concentration in semi-solid formulations is a central partof formulation development. The minimum acceptable limit of preservatives in a drug product must be demonstrated as microbiologically effective by performing a microbialchallenge assay as specified in USP Chapter <51>and described herein. Preservatives in the Product: Methylparaben: 0.18% and Propyl paraben: 0.02% the representative challenging microorganisms for antimicrobial effectiveness testing used was:

a. E.coli: Escherichia coli ATCC 8749
b. S.aureus: Staphylococcus aureus ATCC 6548
c. P.aeruginosa: Pseudomonas aeruginosa ATCC 9027

XIV. Stability Testing

The purpose of stability testing is to provide evidence on how the quality of a drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and air. It enables the establishment of recommended packaging and storage conditions and the shelf life of the preparation. The stability studies were performed on the optimized formulations to assess their ability to keep their chemical and physical characteristics. The formulations were subjected to stress conditions and their physical and chemical attributes were recorded. Six selected prototype formulations were placed under stability testing.

i. Bupivacaine HCl 10%and 5%, Carbopol 980 gel formulated using Eugenol 0.5% as penetration enhancer.
ii. Bupivacaine HCl 10%and 5%, HPMC K4M gel formulated using Eugenol 0.5% as penetration enhancer
iii. Bupivacaine HCl 10%and 5%, HPMC K4M gel formulated using Carvone 0.5% as penetration enhancer.

XV. Container Closure

Compatibility Based on literature search on packaging system considered for dispensing pharmaceutical gels, Aluminum collapsible tubes lacquered internally and Lamitube (Provided as Gift sample from EsselPropack, Mumbai) were used. Lamitubesprovides more resistant to air and moisture. Lamitubes –ABL, Aluminum Barrier Laminates were used. The multilayer tubes (lamitube) made from laminates with aluminium foil barrier combine the excellent barrier advantages of traditional metal tubes & the attractive visual and tactile feel of the plastic tubes. The lamitube body and the shoulder provide excellent barrier properties against permeation of gases like oxygen.

To confirm the package suitability, the gel formulations were dispensed both in aluminum collapsible tubes and Lamitubes. Stress testing at 40°C ± 2°C/75% RH ± 5% RH for 6months included testing of attributes of the drug product that are susceptible to change during storage andare likely to influence quality, safety, and/or efficacy. These included the pH, consistency, homogeneity and drug content. Validated analytical procedures were applied for determination of the above attributes.

5. RESULTS AND DISCUSSION

5.1. UV-visible spectrophotometric method for estimation of Bupivacaine HCl, Bupivacaine base and Lidocaine HCl for drug assay in semisolid dosage forms

In the present work, U.V spectrophotometric method for the quantitation of Bupivacaine HCl and base and Lidocaine in topical dosage form was developed for routine analysis. The method was developed by using phosphate buffer solution pH 6.8 and methanol as solvents as the drug showed good solubility in both the solvent systems. In proposed method, absorption maxima was obtained at 263 nm for Bupivacaine base & HCl and 264 nm for lidocaine and the calibration curve obeyed Beer-Lambertz law in the concentration range of 100-450 µg/ml with correlation coefficient (r^2) of 0.9999

and 0.9998 in phosphate buffer pH 6.8 and methanol respectively. The developed method was validated according to ICH guidelines for validation of analytical procedures. Limit of detection was found to be 20 µg/ml and limit of quantification was 60 µg/ml for Bupivacaine HCl and base. The low values of percentage relative standard deviation showed that the developed method was precise. All statistical data proved validity of proposed method, which can be applied for assay of Bupivacaine. Although the proposed method was found to be linear, precise and accurate, it is not very sensitive for the quantification of Bupivacaine from *in vitro*/*ex vivo* diffusion media. Hence a more sensitive HPLC method was developed for the analysis of Bupivacaine HCl and base from topical formulations.

5.2. Analytical Method Development and Validation for Quantification of Bupivacaine from *In vitro* diffusion media

The initial mobile phases tried were based on published data on Bupivacaine. However a well resolved peak of drug could not be obtained. The developed mobile phase used for the quantitation of Bupivacaine from the diffusion media consisted on acetonitrile: phosphate buffer solution pH 6.8 (6:4 v/v). The validation was carried out to demonstrate the suitability of the developed method for quantitation of Bupivacaine from the *in vitro* diffusion media i.e., the method should be sensitive enough to detect low concentrations of the active and should be repeatable and linear. The analytical method was validated for linearity and precision.

Linearity: The linear regression coefficients for the constructed calibration curves ofBupivacaine demonstrated linearity with r^2 value greater than 0.999.

Precision: Intra-day and Inter-day precision of Bupivacaine analyzed at three differentconcentrations showed % RSD values < 2. This indicated that the developed method for quantification of drug from the *in vitro* diffusion media was precise.

Thus an analytical method for *in vitro* diffusion studies was developed and found to be selective, sensitive, linear and precise.

5.3. Analytical Method for extraction of drug from Porcine Ear Skin.

Bupivacaine could be easily extracted from the porcine ear skin using tissue homogenizer for homogenizing the skin sample and methanol as solvent for extraction since the drug has excellent solubility in methanol. The suitability of the method was further verified by performing the detailed validation of the method as per ICH guidelines.

Specificity*:* The control skin sample HPLC spectra were compared with HPLC spectrafrom Bupivacaine spiked skin sample. The retention time of the drug was recorded at 5.8 min. In the chromatogram of the skin extracts, skin components did not interfere with the peak of interest. Since no interference between the drug and skin matrix components was observed in the HPLC spectra, the method was proved to be selective and specific.

Linearity*:* Calibration curve constructed for Bupivacaine by plotting the graph ofconcentration versus Bupivacaine area was found to be linear in the range of 2.0 to 10µg/ml. The analytical method showed a regression coefficient greater than 0.999 on all the three days. Thus the linear regression analysis demonstrated acceptability of the method for quantitative analysis of Bupivacaine in the skin samples.

Intra-day and Inter-day Precision: The observed lower values of relative standard deviation, lower % RSD values <2, at both, intra-day and inter-day analysis indicated the method to be precise. It showed the acceptability of the method with adequate intra-day and inter-day precision.

Repeatability: SD, % RSD and SE displayed low variance for three separate days for Bupivacaine. This demonstrates the method to be repeatable for the analysis of Bupivacaine from the skin homogenate.

Recovery*:* The recovery was calculated from the Bupivacaine concentration with the nonskin sample and compared with the spiked skin homogenate. The mean recovery of Bupivacaine was 96.34%, 98.45% and 97.59% at concentrations of 2.0, 8.0 and 10.0 µg/mL respectively.. The average recovery over the entire analytical range was 97.46%. From the recovery rates, it can be concluded that the extraction procedure provided a reliable quantitative determination of the drug in skin extracts.

5.4. Analytical Method for extraction of drug from Rat Skin.

The linear regression coefficients and calibration curves in the range of 0.1 to 0.5 µg/mL of Bupivacaine showed that the extraction method from epidermis and dermis of rat skin homogenate is linear with R^2 value greater than 0.998 on three separate days. The chromatogram showing analysis of blank and drug in rat skin homogenate are shown in Figs 2.26 and 2.27 and the standard calibration curve of MB in the presence of rat skin matrix.

5.5. Analytical Method for determination of drug content from Tape Strips.

The 3M Micropore tapes were investigated with the aim to extract the drug efficiently and selectively and to eliminate the interference of tape constituents such as adhesives and polymer with the peak of interest. When Chloroform and isopropyl alcohol was used as extraction media, interference with the active peak was observed. However, when methanol was used as extraction media, no interference due to tape strip constituents and skin components was observed at the Rt of the active, Bupivacaine.

Specificity: The method developed here proved to be selective since the retention times for other compounds present in the skin or in the adhesive tape, analyzed under the same chromatographic conditions, was not similar to those obtained for Bupivacaine showing no interference between them and the tape matrix constituents or the skin components, thereby validating the specificity of the technique.

Intra-day and Inter-day Precision: The observed lower values of relative standard deviation, lower % RSD values <2, at both, intra-day and inter-day analysis indicated the method to be precise. It showed the acceptability of the method with adequate intra-day and inter-day precision.

Repeatability: lower % RSD values for Bupivacaine thus confirming that the method could be repeatable and reproducible and the precision was found to be acceptable for analytical purposes.

Linearity*:* Chromatograms recorded over a concentration range of 0.5 to 5.0 µg/mLyielded a linear relationship between the peak area and the concentrations. The method demonstrated the acceptable linearity with regression coefficient greater than 0.996. The typical calibration curves obtained for Bupivacaine.

Recovery*:* The mean recovery for Bupivacaine from the tape strips was found to be98.34% at a concentration of 0.5 µg/mL, 97.47% at a concentration of 1.5 µg/mL and 96.45% at a concentration of 2.0 µg/mL. Therefore, the average % recovery over the entire analytical range was 97.42. High recovery of the drug from the tissue matrix is a desirable outcome of sample preparation and is therefore important characteristic of extraction procedure. From the recovery rates, it could be concluded that the extraction procedure provides a reliable quantitative determination of drug in tape extracts.

5.6. Analytical Method for extraction of drug from Plasma.

Bupivacaine was extracted from the plasma matrix using liquid–liquid extraction using dichloromethane as an organic solvent under alkaline conditions.

Linearity: Chromatograms recorded over a concentration range of 0.05 to 0.5 µg/mL yielded a linear relationship between the peak area and concentration. The method demonstrated acceptable linearity with regression coefficient greater than 0.998.

Recovery: The recovery study of Bupivacaine from the plasma samples was studied at three concentrations of 0.08 µg/mL, 0.12 µg/mL and 0.22 µg/mL and the mean recovery was found to be 99.45%, 98.86% and 100.03% respectively. From the recovery data of Bupivacaine, it could be concluded that this extraction procedure was efficient and gave good recoveries.

The LOD and LOQ of Bupivacaine in rat plasma matrix were found to be 20 and 60 ng/mL with mean % accuracy of 99.67%. Hence, the analytical method developed and validated for the quantitation of Bupivacaine in rat plasma matrix was sensitive, precise and accurate. This developed and validated HPLC method will be useful for estimation of drug from topical formulations.

5.6.1. Standard preparation

About 10 mg of Bupivacaine reference standard was accurately weighed and dissolved in the mobile phase to make 100 ml solution. A 10-ml aliquot of the resulting solution was further diluted to 100 ml with mobile phase.

5.6.2. Test preparation

About 500-mg, accurately weighed, portion of the Bupivacaine gel was dispersed by sonication for 10 min in 50 ml of mobile phase and diluted to 100 ml with the mobile phase. The solution was filtered using a 0.45-µm filter membrane discarding the first 15 ml. Samples of the Bupivacaine gel and placebo gel were stressed under the following conditions, and test preparations were prepared using the stressed samples.

5.6.3. Thermal degradation

Bupivacaine gel was weighed and placed in a sealed glass tube. The glass tube was placed into a heated tube furnace (Lindberg/Blue) at 150◦C for 4 hrs. The glass tube was then allowed to cool to room temperature. Then the sample was analyzed by HPLC.

5.6.4. Photo-degradation

Bupivacaine gel samples were spread as thin layers on the inside walls of two quartz UV cells. The cells were exposed uncovered, sample side up, to UV (254 and 365 nm) (output: 300mW/cm^2 at 6") and white light under ambient conditions for 48 hrs. After 48 hrs, the light source was removed and sample was analyzed.

5.6.5. Oxidative degradation

Hydrogen peroxide (3%, 3mL) was added to diluted Bupivacaine gel sample. This sample was then analyzed after 4 h to estimate degradation of Bupivacaine.

5.6.6. Acid degradation

Hydrochloric acid (5N, 3mL) was added to gel sample and heated at 60◦C for 4 h. After 4 h, the solution was brought to ambient temperature and to this solution, 6mL of acetonitrile was added and drug was extracted.

5.6.7. Base degradation

NaOH (5N, 3mL) was added to gel sample and heated at 60◦C for 4 h. After 4 h this solution was brought to ambient temperature and to this solution, 6mL of acetonitrile was added and drug was extracted.

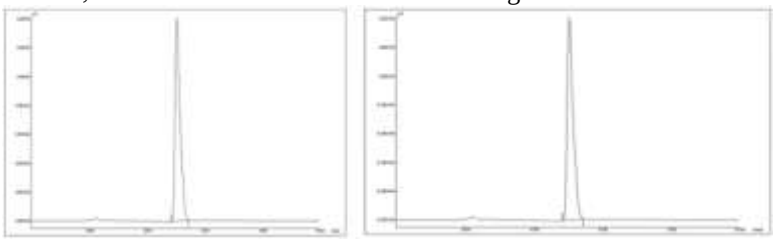


Fig 5.1: Chromatogram of Reference Bupivacaine

The stability and degradation profiles of Bupivacaine in gel samples were evaluated with the long-term objective of developing stable topical formulations of this drug. Chromatogram of Bupivacaine extracted in the gel sample at 210 nm showed no interferences between the drug and excipient peaks. The stability profile of Bupivacaine gel samples at high temperature and light conditions was monitored by high performance liquid chromatography (HPLC). Forced degradation of Bupivacaine gel sample was performed at extreme heat, light, acidic (pH<2.0) and alkaline (pH>10.0) conditions and by addition of hydrogen peroxide (oxidizing agent). Bupivacaine was very stable to acid and base treatment, no degradation product was seen. Also, it was found stable to oxidation. There were no significant changes in the chromatograms obtained with Bupivacaine gels subjected to oxidative, heat, light, acid or base stress, when compared to the chromatograms obtained with the reference sample. The physical appearance of the gel, however, changed when it was subjected to heat stress—the gel appeared to lose its viscosity.
The developed HPLC procedure separated the excipients and other peaks from the Bupivacaine peak and will be utilized for the analysis of Bupivacaine in compounded Bupivacaine topical formulations.

5.7. Skin Irritation Potential

The skin irritation testing was carried out to evaluate the primary skin irritation potential of the developed Bupivacaine topical formulations.

A. Mortality/Morbidity

There were no deaths or evidence of impending death during the in-life period.

B. Clinical Observations

Topical application of three Bupivacaine topical formulations (Test code for the active formulation: FMH 10, FMB 5 and FMDTS 5) for a period of 24 hr with occlusion did not produce any significant change in the primary irritation index (PII) as determined by visual examination of the treated skin at 1, 24, 48 or 72 hours after removal of the gauze. The PII scores obtained from the area of skin treated with the formulations containing the drug (right flank) were not materially different from the PII scores obtained from the area of skin treated with their corresponding vehicle formulations (left flank) (Formulation Code: VE-FMH-10, VE-FMB-5 and VE-FMDTS-5 for the three active formulations respectively). All PII scores recorded at 1, 24, 48 and 72 hr after a 24 hr application of the three active formulations of Bupivacaine and its corresponding vehicle formulations were observed to be <2. Therefore, the formulations of Bupivacaine (Test code for the active formulation: FMH 10, FMB 5 and FMDTS 5) and its vehicle formulations (Test code for the vehicle formulations: VE-FMH-10, VE-FMB-5 and VE-FMDTS-5) may beconsidered nonirritating. None of the above formulations was observed to produce any observable adverse local or systemic effects.

C. Body Weight

None of the treated animals in the present study showed any significant change in the mean body weight of the animals receiving the treatment of various formulations tested in the present study (Table 5.1).

D. Histopathology

Histopathological evaluation of necropsy samples of skin where Bupivacaine topical (Test code for the active formulation: FMH 10, FMB 5 and FMDTS 5) or vehicle control gels (Test code for the vehicle formulations: VE-FMH-10, VE-FMB-5 and VE-FMDTS- were applied showed no significant gross pathological changes or toxic effects, and the microscopic examination was unremarkable (Figure 5.2).

FMH 10 FMB 5 FMDTS 5

Fig 5.2: Representative pictures of rabbit skin 24 h post treatment with developed formulations.

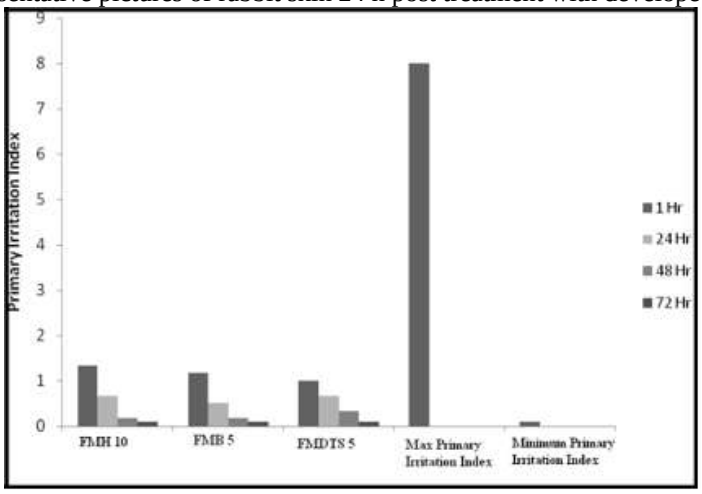


Fig 5.3: Effect of topically administered Bupivacaine formulations as assessed in terms of the Drug Irritation Index.

Data Tables:

Table 5.1: Body Weight

Body Weight (Kg) at Time (Hr) Post-Dose						
	Group I		Group II		Group III	
Animal Number	1 Hr	72 Hr	1 Hr	72 Hr	1 Hr	72 Hr
1	2.34	2.22	3.41	3.48	2.63	2.63
2	3.45	3.54	2.14	2.11	2.33	2.33
3	2.98	2.83	2.58	2.71	3.25	3.25
4	3.56	3.69	2.95	2.92	2.86	2.86
5	3.23	3.20	3.45	3.61	3.21	3.21
6	3.67	3.61	3.12	3.19	2.49	2.49
Mean ± SD	3.20 ± 0.49	3.18 ± 0.56	2.94 ± 0.51	3.00 ± 0.55	2.79 ± 0.38	2.79 ± 0.38

Modified Draize Scale Score

GROUP I: Table 5.2
Treatment(s) Name/ Code: FMH 10
Dose, Route and Site of Application: 500 mg gel/ 4 cm^2, topical, Right flank.

Animal Number	1 hr			24 hr			48 hr			72 hr		
	ER	ED	PII	ER	ED	PII	ER	ED	PII	ER	ED	PII
1	1	1	2	1	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	1	0	0	0	0	0	0	0	0	0
4	2	1	3	1	1	2	1	0	1	0	0	0
5	1	1	2	1	0	1	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0.83	0.50	1.33	0.50	0.17	0.67	0.17	0.00	0.17	0.00	0.00	0.00

Treatment(s)/ Code: Vehicle Gel of FMH 10
Dose, Route and Site of Application: 500 mg gel/ 4 cm^2, topical, Left flank.

Animal Number	1 hr			24 hr			48 hr			72 hr		
	ER	ED	PII	ER	ED	PII	ER	ED	PII	ER	ED	PII
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	1	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0.17	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

GROUP II: Table 5.3.
Treatment(s) Name/ Code: FMB 5
Dose, Route and Site of Application: 500 mg gel/ 4 cm^2, topical, Right flank.

Animal Number	1 hr			24 hr			48 hr			72 hr		
	ER	ED	PII	ER	ED	PII	ER	ED	PII	ER	ED	PII
1	1	0	1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	1	0	0	0	0	0	0	0	0	0
4	1	1	2	1	0	1	0	0	0	0	0	0
5	2	1	3	2	0	2	1	0	1	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0.83	0.33	1.17	0.50	0.00	0.50	0.17	0.00	0.17	0.00	0.00	0.00

Treatment(s)/ Code: Vehicle Gel of FMB 5
Dose, Route and Site of Application: 500 mg gel/ 4 cm^2, topical, Left flank.

Animal Number	1 hr			24 hr			48 hr			72 hr		
	ER	ED	PII	ER	ED	PII	ER	ED	PII	ER	ED	PII
1	1	0	1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0.17	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

GROUP III: Table 5.4.
Treatment(s) Name/ Code: FMDTS 5
Dose, Route and Site of Application: 500 mg/ 4 cm^2, topical, Right flank. x

Animal Number	1 hr			24 hr			48 hr			72 hr		
	ER	ED	PII	ER	ED	PII	ER	ED	PII	ER	ED	PII
1	2	1	3	1	1	2	1	0	1	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	2	0	2	1	0	1	1	0	1	0	0	0
5	1	0	1	1	0	1	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0.83	0.17	1.00	0.50	0.17	0.67	0.33	0.00	0.33	0.00	0.00	0.00

Treatment(s)/ Code: Vehicle of FMDTS 5
Dose, Route and Site of Application: 500 mg gel/ 6 cm^2, topical, Left flank.

Animal Number	1 hr			24 hr			48 hr			72 hr		
	ER	ED	PII	ER	ED	PII	ER	ED	PII	ER	ED	PII
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

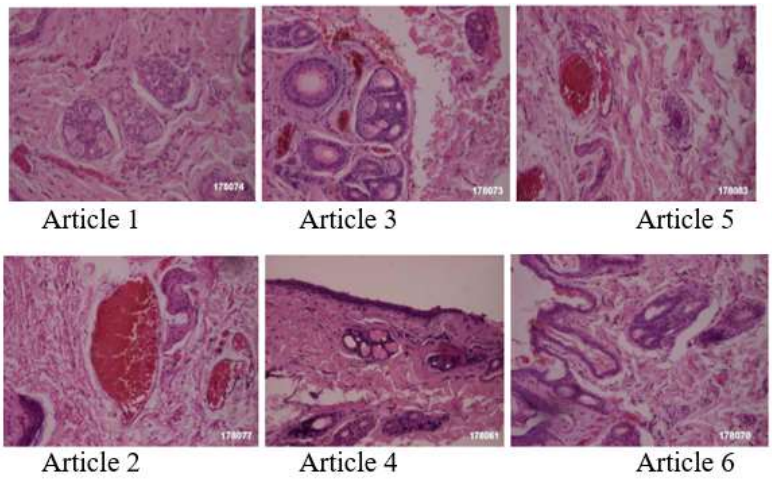


Fig 5.4: Representative micrographs demonstrating the effect of topically administered Bupivacaine formulations on the skin at the end of 24 h

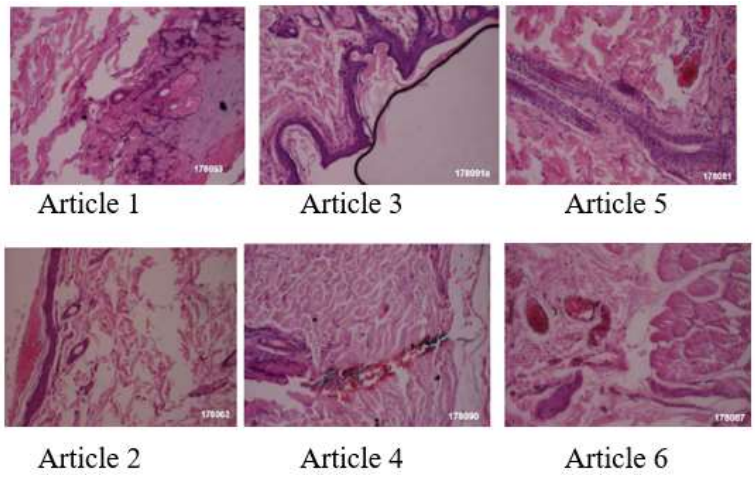


Fig. 5.5: Representative micrographs demonstrating the effect of topically administered Bupivacaine formulations on the skin at the end of 72 h

Results of the Skin Irritation studies showed that:

1. 24-hour topical application of vehicle gel formulations (Test code for the vehicle formulations: VE-FMH-10, VE-FMB-5 and VE-FMDTS-5) does not exert any significant irritant effect on rabbit skin over a 72-hour observation period post application.
2. 24-hour topical application of Bupivacaine topical formulations (Test code for the active formulation: FMH 10, FMB 5 and FMDTS 5) does not exert any significant irritant effect on rabbit skin over a 72-hour observation period post-application.
3. 24-hour topical application of Bupivacaine topical formulations (Test code for the active formulation: FMH 10, FMB 5 and FMDTS 5) does not produce any observable toxic signs or symptoms, or death over a 72-hour observation period post-application.
4. 24-hour topical application of vehicle gel formulations (Test code for the vehicle formulations: VE-FMH-10, VE-FMB-5 and VE-FMDTS-5) does not produce any significant histopathologic changes in rabbit skin.
5. 24-hour topical application of Bupivacaine topical formulations (Test code for the active formulation: FMH 10, FMB 5 and FMDTS 5) does not produce any significant histopathologic changes in rabbit skin.
6. Therefore, the Bupivacaine formulations may be considered safe at least for single dose topical administration without producing any acute irritant effect on skin.

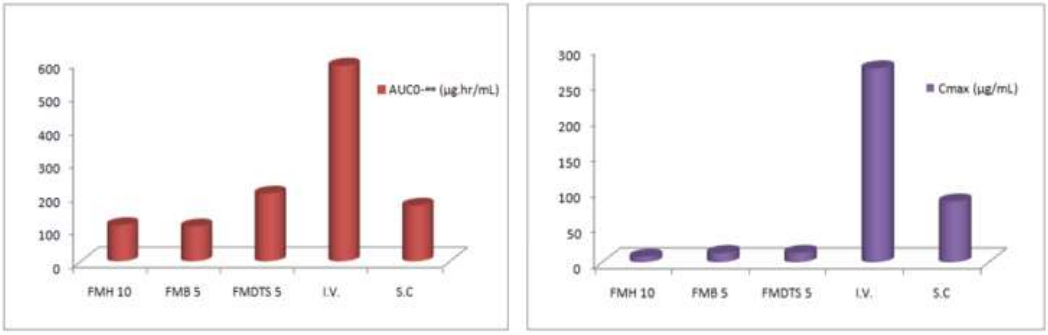
5.8. Pharmacokinetic Studies

Pharmacokinetic studies of topical Bupivacaine formulations in Bupivacaine provided information on the extent, rate and duration of Bupivacaine absorption from the formulations through the skin.

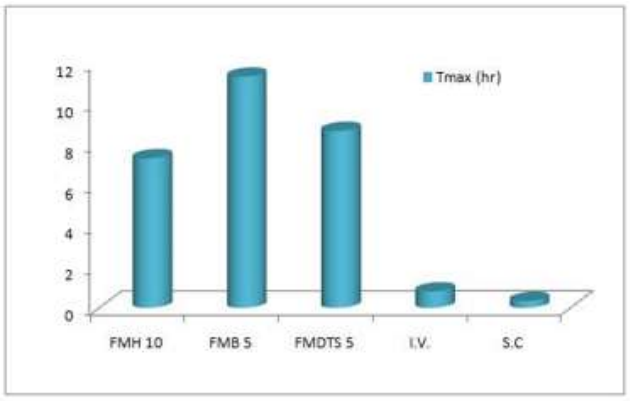
A non-compartmental model was applied. The plasma concentrations of Bupivacaine from various test formulations at different time intervals were subjected to pharmacokinetic analysis. Pharmacokinetic parameters like C_{max}, t_{max}, AUC were calculated from the conc. Vs time plot (Table 4.17). Trapezoidal rule was employed to calculate AUC. The C_{max} of the test formulations was 7.53 µg/mL, 12.83 µg/mL and 13.06 µg/mL for FMH 10, FMB 5 and FMDTS 5 Bupivacaine topical formulations respectively. For FMDTS 5, T_{max} to reach C_{max} was 13.05 h and was the highest amongst other formulations tested. The $AUC_{0-\infty}$ (µg.hr/mL) values was in the following order, FMDTS 5> FMB 5> FMH 10. For Bupivacaine administered intravenously and subcutaneously, C_{max} of 272 µg/mL at T_{max} 0.8 h and C_{max} of 85.2 µg/mL at T_{max} 0.33 h respectively were observed in rabbits.

Table 5.5: Pharmacokinetic Parameters Calculated for Test Formulations and I.V and S.C Bupivacaine formulations

PK Parameters	FMH 10	FMB 5	FMDTS 5	I.V.	S.C
$AUC_{0-\infty}$ (µg.hr/mL)	108.67	104.67	203.33	588	167.2
C_{max} (µg/mL)	7.53	12.83	13.06	272	85.2
T_{max} (hr)	7.33	11.33	8.67	0.8	0.33



(a) **(b)**



(c)

Fig 5.6: Comparative Pharmacokinetic Profiles a) $AUC_{0-\infty}$, µg.hr/mL b) C_{max} ,µg/mL and c) T_{max}, hr of Test formulations vs I.V & S.C Bupivacaine

6. SUMMARY & CONCLUSION

Preformulation Studies

Standardization of drugs and excipients: Bupivacaine base and itspharmaceutically acceptable hydrochloride salt were used for the purpose of research work and were standardized as per monographic specifications and Certificate of Analysis. Table illustrates various tests and observations and specifications for the both forms of Bupivacaine. The drugs passed the tests for identity, purity and the results were found to comply with the pharmacopoeial limits and were used for further incorporation in the formulation of topical drug delivery systems.

Analytical Method Development and Validation: For estimation of drug informulations, tape strips, various layers of skin and plasma; simple, accurate, precise and less time consuming assay methods were developed and validated as described in Chapter 2.5.The UV spectra for both salt and the base form are depicted For HPLC analysis of drug; the mobile phase consisted of phosphate buffer solution pH 6.8: acetonitrile (60: 40 v/v). The retention times for Bupivacaine base and Bupivacaine HCl were 5.6 and 5.4 mins respectively.

All the developed analytical methods were validated for linearity and range, precision, accuracy and percent recovery as per the ICH guidelines. The assay methods developed and validated for Bupivacaine were observed to be sensitive, specific, and accurate with high precision and % recovery.

Formulation Development of Topical Delivery Systems of Bupivacaine salt andbase Novel Topical Bupivacaine hydrogels formulated using permeation enhancers

Topical gels are transparent to opaque semisolids semi-solid formulations containing a high ratio of solvent to gelling agent. Initially, preliminary trials were conducted to select and optimize the concentration of gelling agent, solvent, and penetration enhancer for formulation development of Bupivacaine HCl hydrogels. Bupivacaine HCl at two different concentration levels, 5% and 10% w/w was used to investigate the drug releaseprofiles. Three prototype hydrogel formulations were optimized for Bupivacaine HCl 10% strength. Bupivacaine HCl 5% hydrogels were formulated based on the optimized formulae of 10% hydrogel formulation. Once the preliminary studies were completed, further formulation optimization was carried out using statistical Design of Experiments method by considering the concentration of gellant, Carbopol 980 NF, co-solvents propylene glycol and ethanol as independent variables and % drug permeated at the end of 10 hrs, viscosity (Pa.s) and flux (µg/cm^2/hr) as the dependent variables. Box Behnken Design of Experiment (using Design Expert® software, version 8.1.) was used. Various batches (17 in number) were formulated using low, medium and high values of the independent variables and their responses obtained on carrying out the *in vitro* diffusion studies.

The relative impermeability of stratum corneum offers major resistance to percutaneous absorption of most of the drugs. Therefore, attempts were made to reduce this barrier resistance reversibly by using penetration enhancers along with co-solvents like ethanol and propylene glycol. Various penetration enhancers incorporated in the hydrogel formulations included isopropyl myristate, transcutol P, menthol, eugenol, carvone, oleic acid, eucalyptol, geraniol, limonene, labrasol, Brij-35. Amongst these, eugenol and carvone showed maximum enhancement of the rate of drug diffusion across the barrier membrane, thus, it was incorporated in the optimized formulations. The *in vitro* % drug diffused were 85.85% and 87.56% from eugenol and carvone based hydrogels respectively formulated using HPMC K4M as gallant.

In vitro permeation studies were carried out to select the optimumformulation. Physico-chemical characteristics such as gel viscosity, spreadability, pH and drug content were assessed.

Nanoemulsion based gels of Bupivacaine for topical delivery

Nanoemulsions are optically transparent nanometric sized emulsions with particle sizes between 100 and 500 nm, composed of the oil, surfactant, co-surfactant and water. However, the application of the nanoemulsion to the skin is inconvenient due to low viscosity. To increase their viscosity and to make them more suitable for the topical application, gelling agents have been incorporated to form nanoemulsion gels.

Stable nanoemulsion based hydrogel formulations for topical delivery of Bupivacaine were formulated. Solubility of Bupivacaine base was evaluated in various oils, surfactants and co-surfactants to identify the components of the nanoemulsion. Nanoemulsions were formed by spontaneous emulsification technique. The pseudo-ternary phase diagrams for nanoemulsion regions were constructed using oleic acid as oil, a blend of a high HLB surfactant, caprylo caproyl macrogol-8-glyceride (Labrasol) and low HLB cosurfactant, purified diethylene glycol monethyl ether, Transcutol P (1:1 ratio) and water as hydrophilic phase. Phase diagrams were constructed to obtain the optimum concentration ranges of oil, surfactant and co-surfactant as in Fig 3.2.3. Once the preliminary studies were completed, further formulation optimization was carried out considering the concentration of oil, S_{mix} (surfactant-cosurfactant mix) and water as independent variables and % drug permeated at the end of 12 hrs and flux (μg/cm^2/hr) as the dependent variables. Box Behnken Design of Experiment (using Design Expert software, version 8.1.) was used. Responses of the 17 different batches formulated using factorial design.

Pharmacokinetic Studies

Pharmacokinetics of the developed topical formulations of Bupivacaine was studies in Chinchilla rabbits. Various pharmacokinetic parameters like C_{max}, T_{max} and AUC_{0-t} were determined and reported. The low systemic absorption rate indicated that Bupivacaine might have accumulated significantly in the layers of the skin which was desired to achieve anti-neuropathic response. Plasma Bupivacaine concentrations remained well below systemically toxic concentrations, and no obvious clinical side effects were observed in any rabbits used in the study.

Irritation Studies

The potential of the developed Bupivacaine topical formulations to produce ocular irritation (reversible changes in the eye) or corrosion (irreversible tissue damage) whenapplied to the eye of rabbits was assessed (Section 4.5). Also its potential to produce dermal irritation and corrosion when applied to the skin of rabbits/rats was investigated (Section 4.6). Primary Irritation Index (PII) of the formulations was calculated. Ocular application of the Bupivacaine topical formulations and their vehicle controls did not produce any significant change in the ocular lesion score in terms of the corneal opacity, iritic, conjunctival hyperemia and chemosis over 72 hours and may be considered "not irritating" to the rabbit eye. Topical application of all the three optimized Bupivacaine formulations for 24h did not exert any significant irritant effect on rabbit skin over a 72-hour observation period post-application and did not produce any significant histopathologic changes in rabbit skin. Therefore, the developed and optimized topical formulations of Bupivacaine were considered safe for single dose topical administration without producing any acute irritant effect on skin.

7. CONCLUSION

Topical delivery of Bupivacaine via various formulation approaches has been investigated in the present research work. Novel clear, stable, hydrogels, nanoemulsion based gels and metered dose film forming sprays of Bupivacaine base and its pharmaceutically acceptable HCl salt at 5% and 10% concentrations respectively for topical delivery has been developed and optimized. The formulations were developed to modulate the drug diffusion and accumulation at the intended site to exhibit the desired response.

Topical hydrogels of Bupivacaine HCl were developed using co-solvents and penetration enhancers. The optimized hydrogels exhibited desired consistency, homogeneity, spreadabiltity and stability. Since, the polymers were water soluble; consequently, water washable gels were formed and offered benefits like ease of application and ease of removal. Nanoemulsion system composed of oleic acid as oil, Smix of labrasol: Transcutol P and distilled water have been proposed for topical delivery of Bupivacaine. A topically applied nanoemulsion is expected to penetrate the stratum corneum and exist intact in the horney layer. Once it enters into the stratum corneum, nanoemulsions may simultaneously alter both the lipid and the polar pathways. Greater drug penetration Novel metered dose topical spray formulations of Bupivacaine base were developed using different polymers. Solution spray formulations were developed and filled in containers with metered dose spray pumps to provide propellant free delivery. The metered dose topical spray of Bupivacaine base will prove as an alternative to conventional topical delivery for relief of neuropathic pain.

8. REFERENCE

1. Dahm P, Lundborg C, Janson M, Olegard C, Nitescu P. Comparison of 0.5% intrathecal bupivacaine with 0.5% intrathecal ropivacaine in the treatment of refractory cancer and noncancer pain conditions: results from a prospective, crossover, double-blind, randomized study. Reg. Anesth. Pain Med. 2000;25:480–487.
2. Ritchie JM, Greene NM. Aneste´sicos locales. In: Goodman LS, Gilman AG, editors. Las Bases Farmacolo´gicas de la Terape´utica. 7th ed. Madrid: Editorial Me´dicaPanamericana; 1989. pp. 300–317.
3. Tsai YC, Wang LK, Chen BS, Chen HP. Home-based patient controlled epidermal analgesia with bupivacaine for patients with intractable herpetic neuralgia. J. Formos. Med. Assoc. 2000;99:659–662.
4. Moore PA. Long-acting local anaesthetics: a review of clinical efficacy in dentistry. Compend. Contin. Educ. Dent. XI. 1990:22–30.
5. Saxen MA, Newton CW. Anesthesia for endodontic practice. Anesthesia in Dentistry. 1999;43:247–261.

6. Moore DC. Lipid rescue from bupivacaine cardiac arrest: a result of failure to ventilate and maintain cardiac perfusion. Anesthesiology. 2007;106:636–637.
7. Duarte AM, Pospisilova E, Reilly E. Reduction of postincisional allodynia by subcutaneous bupivacaine. Anesthesiology. 2005;103:113–125.
8. Klech CM, Gels, Jellies. In: Encyclopedia of Pharmaceutical Technology. Swarbrick J, Boylan JC, editors. Vol. 6. New York: Marcel Dekker Inc.; 1999. pp. 415–439.
9. Nairn JG. Solutions, emulsions, suspensions and extracts. In: Gennaro AR, editor. Remington: The Science and Practice of Pharmacy. Philadelphia: Williams and Wilkins Inc.; 2000. pp. 745–7.
10. Tur KM, Ch`ng HS. Evaluation of possible mechanism of bioadhesion. Int. J. Pharm. 1998;160:61–74.
11. Blanco-Fuente H, Anguiano-Iga S, Otero-Espinar FJ, Blanco-Mendez J. In-vitro bioadhesion of carbopol hydrogels. Int. J. Pharm. 1996;42:169–174.
12. Shin SC, Kim HJ, Oh IJ, Cho CW, Yang KH. Development of tretinoin gels for enhanced transdermal delivery. Eur. J. Pharm. Biopharm. 2005;60:67–71.
13. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J. Pharmacol. Exp. Therp. 1941;72:74–79.
14. Cho CW, Choi JS, Shin SC. Enhanced local anesthetic efficacy of bioadhesive ropivacaine gels. Biomol. Therap. 2011;19:357–363.
15. Choi JS, Shin SC. Enhanced bioavability of ambroxol by transdermal administration of the EVA matrix containing penetration enhancer in rats. Biomol. Therap. 2010;18:106–110.
16. Tanojo H, Bouwstra JA, Junginger HE, Bodde HE. In-vitro human skin barrier modulation by fatty acid: skin permeation and thermal analysis studies. Pharm. Res. 1997;14:42–49.
17. Oh SY, Jeong SY, Park TG, Lee JH. Enhanced transdermal delivery of AZT (Zidovudine) using iontophoresis and penetration enhancer. J. Control. Rel. 1998;51:161–168.
18. Green PG, Guy RH, Hadgraft J. In-vitro and in-vivo enhancement of skin permeation with oleic-acid and lauric acids. Int. J. Pharm. 1988;37:251–255.
19. Sonwani R, Kumar A, AVaishnaw A, Kashyap P, Patel P, Tamboli R, Jain DK et al "Development And Formulation Of Microemulsion For Topical Drug Delivery Of Fluconazole". Journal of Pharmaceutical Negative Results 2022 (13) 2257-2265. https://doi.org/10.47750/pnr.2022.13.S05.354.
20. Ammar H. O., Ghorab M., et.al., Evaluation of chemical penetration enhancers for transdermal delivery of aspirin; Asian Journal of Pharmaceutical Sciences, 2007, (3): pp. 96-105.
21. Alison M. C., Harriet M. L. Lidocaine Patch 5% Drugs 2000; 59 (2): 245-249.
22. Arellano A., Santoyo S., et.al., Influence of propylene glycol and isopropyl myristate on the in vitro percutaneous penetration of diclofenac sodium from carbopol gels; European Journal of Pharmaceutical Sciences, 1998 (7), 129-135
23. Ahmed M. El Gendy, H. W. Jun, and Alaa A. Kassem., In Vitro Release Studies of Flurbiprofen from Different Topical Formulations Drug development and industrial pharmacy, 2002 (28), 823–831.