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Formulation and development of bioadhesive transdermal gel of ropivacaine loaded nanoparticles for enhancement of anesthetic effect: Preclinical study in animal model

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Abstract: A transdermal drug delivery system (TDDS) is one of the most attractive approaches and is popular due to high patient compliance, low risk and ease of applicability. To formulate a bioadhesive gel with Ropivacaine-loaded nanoparticles for enhancement of the local anaesthesia. The ionotropic gelation method was used to formulate nanoparticles and characterized for particle size, zeta potential, PDI, drug loading and surface morphology. The optimized nanoparticulate formulation was further used in the development of bioadhesive gel and characterized for clarity, pH, bioadhesive strength, drug content, viscosity, *ex-vivo* skin permeation and *in vivo* Tail Flick test on a rat model. Among nanoparticle formulations, NP4 formulation was found to be the ideal formulation based on Physico chemical parameters. The F6 bioadhesive gel was considered optimised amongst all the formulations. The F6 gel showed an excellent skin permeation profile over 14 hr as compared to other formulations. This formulation showed maximum anesthetic effect compared to other formulations as observed in the tail flick test. F6 formulation containing RPV nanoparticles showed 3.32 folds increase in anesthetic activity as compared to the control gel. Bioadhesive transdermal gel containing RPV nanoparticles would be a potential alternative strategy for improving the anesthetic effect

Keywords: Ropivacaine, gel, anaesthesia, bio adhesion, nanoparticles, permeation.

INTRODUCTION

Transdermal drug delivery system (TDDS) is one of the most attractive approaches but it is a quite potential alternative approach to the parenteral and invasive route of administration. TDDS is popular due to high patient compliance, low risk, ease of applicability and lowest rejection rate in patients (Vega-Vásquez *et al*., 2020). The skin care sector, including cosmetics, as well as the pharmaceutical business, may be able to use TDDS. This strategy can avoid local drug concentration build-up and non-targeted medication delivery because it primarily requires local administration (Mali *et al.,* 2015). The phrase "drug delivery system" (DDS) comprises various methods through which the release and distribution of the drug in various cells, tissues and organ systems take place so that they can exert their effects as effectively as possible (Vargason *et al.,* 2021). An ideal DDS enhances the therapeutic activity of the active compound with minimising adverse and side effects (Shankar *et al*., 2022). Various route of administration is explored in the medical and pharmaceutical field including oral, parenteral, nasal, vaginal and buccal but stands out among them as an appealing strategy (Li *et al.,* 2019). The difficulty is that only a little portion of the medication may be absorbed via the skin tissue. To overcome this problem, novel TDDS methods have been developed extensively and have gained popularity as administrative strategies. A competitive edge over current medication delivery methods may also be offered by such development in terms of the delivered dose, cost-effectiveness and therapeutic efficacy (Li *et al.,* 2019).

The total amount of active ingredient absorbed in topical applications varies significantly depending on a variety of variables, including the size of the application region, the frequency and strength of administration and the viscosity or thickness of the applied vehicle. Application place, age and skin condition are other variables that affect medication absorption. An active substance can more easily penetrate the non-keratinized dermis (Li *et al.,* 2019).

Topical gels are three-dimensional polymeric matrices of natural or synthetic gum with a significant amount of physical or chemical cross-linking that have a liquid phase that is contained inside a semisolid solution (Kolimi *et al.,* 2022). Topical gels are an excellent contender for a wide range of applications because of their behaviour in the middle of solid and liquid components. Topical gels have received a lot of attention recently since they are a topic that appeals to scientists and professionals (Kolimi *et al.,* 2023). The majority of topical gels are made using organic polymers, such as carbomers, which provide an attractive, transparent appearance. Some bases that include a trace of oily compounds have an emollient

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effect on dry skin. It is more significantly, composed of bases of non-volatile oleaginous materials, including hydrocarbon bases, which create a skin barrier that is occlusive to stop the escape of skin moisture that enters the environment (Kaur *et al.,* 2016). Gels are becoming more and more common since they are more stable and can release drugs under regulated conditions than other semisolid preparations like creams, ointments, pastes and so forth. The drug's bioavailability may be improved by the gel formulation's improved absorption properties (Kolimi *et al.,* 2022). The potential for the gel formulation's therapeutic usage in patients may be revealed by a thorough examination of the stability properties over an extended period. The polymer has a higher potential for application as a topical medication delivery dosage form since it is water-soluble and forms a water-washable gel. A topical gel is a safe and effective therapy option for the management of skin problems, according to clinical data (Kaur *et al.,* 2016).

Nanoparticulate drug delivery technology is one of the most promising new drug delivery methods because it has the potential to successfully formulate and improve the therapeutic effectiveness of many different medications (Khairnar *et al.,* 2022). It had created a fantastic foundation for effective medication targeting and therapy. Because of their tiny size, they can pass through numerous biological barriers and collect any bodily cell to provide efficient medication administration. The biodistribution of medications with high therapeutic potency is improved by nanoparticles, enhancing therapeutic effectiveness and decreasing nonspecific toxicity (Budha *et al.,* 2021). In addition to all of these benefits, polymeric nanoparticles can result in prolonged release profiles of the pharmaceuticals they are encapsulating and high loading capacities of therapeutic and imaging agents due to their high surface-area-to-volume ratios (Khairnar *et al.,* 2022).

Amongst different agents used to generate anaesthesia Ropivacaine (RPV), an amide type of local anesthetic is an option agent for pediatric anaesthesia. The medication has been shown to have an action similar to that of Bupivacaine but has shown decreased risk ratios of toxicity to the cardiovascular as well as the central nervous system when taken by i.v. infusion. Healthy volunteers showed less sensitivity to the impacts than did sufferers (Chen *et al.,* 2015). When compared to Bupivacaine, the toxicity was cut by 25%. Due to the clear separation of the motor and sensory blocks that RPV generated in comparison to Bupivacaine during clinical trials and its decreased lipid solubility, the medication is ideally suited for postoperative analgesia (Cederthoim 1997).

Ropivacaine (RPV) is an active amide-type local anesthetic agent with claimed therapeutic and chemical characteristics that are comparable to those of bupivacaine (Yu *et al.,* 2021). In comparison to bupivacaine, RPV is less hazardous to the cardiovascular and neurological systems following intravenous administration. When compared to bupivacaine, RPV demonstrated relatively few central nervous system symptoms in healthy human volunteers and was determined to be at least 25% less hazardous (Martini *et al.,* 2002). Due to its somewhat reduced lipid solubility in clinical testing, RPV demonstrated a clear separation of the motor and sensory blocks and also allowed for a quicker recovery from the motor blockade than bupivacaine. The aforementioned benefits make RPV an excellent choice for postoperative analgesia. The present investigation is an attempt to develop a novel formulation of transdermal nanoparticulate gel of the anesthetic drug ropivacaine to enhance its anesthetic effect.

MATERIALS AND METHODS

Materials

Ropivacaine (RPV) was obtained from Baoji Guokang Bio-Technology Co., Ltd. China and Chitosan (CS) was obtained from Sigma Aldrich, USA. Tripoly phosphate (TPP) and acetic acid and Folic acid were obtained from Shanghai Chemical Co. (Shanghai, China). Carbopol 934, methylparaben and triethanolamine were purchased from Sigma Aldrich USA.

Manufacturing of RPV-NP

The RPV-chitosan nanoparticles were created using the ionotropic gelation process, which involved dissolving chitosan in an acetic acid solution and stirring continued at room temperature. Chitosan was made to dissolve in a surfactant (Tween-80; 1 percent v/v) while the drug in the organic phase of the acetone solution was introduced dropwise to the aqueous phase using various quantities to create an o/w emulsion while being stirred. Under stirring, TPP was introduced dropwise at various concentrations to an o/w emulsion as a cross-linking agent. To allow the organic solvent to completely evaporate, it was held overnight. The production of nanoparticles was the result of the interaction between the negative TPP group and the positive amino group of the CS. Using a cooling centrifuge, nanoparticles were separated by centrifugation at 20,000 rpm for 15 min at -800 C. The supernatant was then used to assess free RPV by HPLC (Dong *et al.,* 2020). The formula composition of nanoparticles is presented in table 1.

Table 1: CS-RPV nanoparticle formulation compositions

Formulations	NP1	NP2	NP3	NP4
RPV (mg)	50	50	50	50
Acetone (ml)	1	1	1	1
Chitosan (mg)	25	50	75	100
3% Acetic acid (ml)	15	15	15	15
Tween 80 (ml)	0.2	0.4	0.6	0.8
TPP (mg)	0.5	1	1.5	2
Purified water	q.s.	q.s.	q.s.	q.s.

Preparation of nanoparticulate gel

Carbopol 934 as per batch quantity was dissolved in double distilled water (100ml) with methylparaben at a concentration of 0.5 % w/v and the mixture was agitated until it formed a clear, translucent gel. The pH was then adjusted to 7 and triethanolamine was gradually added. At room temperature, it was then left to stand for 24 hr. Finally, by slowly swirling, drug-loaded nanoparticles were mixed into a gel. The formula composition of nanoparticulate gel is presented in table 2.

Table 2: Formulation of nanoparticulate bioadhesive transdermal gel

Ingredient	F1	F2	F3	F4	F5	F6
RPV-NP (% w/w)	1	1	1	1	1	1
Carbopol 934 (%w/w)	0.5	1	1.5	2	2.5	3
Methylparaben (mg)	0.35	0.35	0.35	0.35	0.35	0.35
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Water (ml)	q.s. to prepare 100 ml					

Evaluation of Nanoparticles

Particle Size, zeta potential and particle size distribution (PDI)

All these parameters were determined by Zetasizer (Malvern/DTS 4.1). The nanoparticulate suspension was diluted with double distilled water and sonicated for 20 min to get a properly dispersed nanoparticulate suspension. This suspension was loaded in a particle size analyser at room temperature and samples are analysed (Gomathi *et al.,* 2017).

RPV loading efficiency

The RPV concentration in the supernatant solution was determined after centrifugation and samples were analyzed by the HPLC method. The loading efficiency was determined by using the formula presented below (Gomathi *et al.,* 2017).

% DLE = Target Loading - Unloaded NLX / Target Loading X 100

Scanning electron microscopy (SEM)

The surface morphology study was performed using SEM. This technique provides information about surface morphology, size and shape (Gomathi *et al.,* 2017).

Evaluation of transdermal gel

Physical appearance

All the formulations were checked for their visual physical appearance and observation of any foreign particle, aggregate, etc. This can be done against a dark and white background (Zaki *et al*., 2022).

pH of the formulation

The digital pH meter was previously calibrated with buffer and then the electrode was dipped into a sample of gel formulation and pH was determined (Zaki *et al*., 2022).

Drug content

About 100mg of nanoparticulate gel was weighed and dissolved in ethanol. The solution (5 ml) was diluted with ethanol up to 25ml. Then the total drug content of the formulation was estimated by performing UV visible spectrophotometry (Zaki *et al*., 2022).

Viscosity

The viscosity was determined at room temperature using a Brooke field digital viscometer (DV-II + Pro) with spindle no. 6 at 5 rpm. Values were recorded (Zaki *et al*., 2022).

Bioadhesive strength

The bioadhesive strength of the gel formulation was determined by calculating the minimum force of detachment required from the biological membrane. This membrane was fixed to a small glass slide using adhesive tape and it was attached to the bottom of the beaker (100 ml) from the outside and then kept in a 500ml glass beaker. Phosphate buffer pH 6.8 was poured into the beaker and the level was adjusted just above the biological membrane. Accurately weighed gel (1 gm) was applied on the lower side of the biological membrane followed by placing preload of 100 gm on a glass slide for 10 min to ensure proper adhesion between gel and biological membrane. The weight required to detach the test sample from the biological membrane was determined and an average value was calculated.

Ex-vivo skin permeation of RPV

The RPV permeation was studied using a Franz diffusion cell fitted with a rat skin membrane obtained from the local slaughterhouse. The membrane was properly maintained in normal saline solution at room temperature and placed between two compartments of the diffusion cell. Gels equivalent to 50mg of RPV were applied uniformly on the skin. pH 6.8 buffer as release media was filled in the receptor compartment with a constant temperature of 37± 0.5^{o}C at 30 rpm. The sampling (0.5 ml) was done at predetermined time intervals of up to 1 hr and for each withdrawal, an equal volume of buffer was added to maintain the sink. Withdrawn samples were analyzed using HPLC at 280 nm wavelength and drug diffused through the membrane was calculated.

In vivo tail flick test

The study utilized about 24 healthy Wister rats (7-9-week-old; 280-320 gm weight) divided into three groups each containing eight rats. Animal experiments were carried out according to the guidelines of the institutional animal ethical committee approved by the Department of Anesthesiology, Jiangsu Taizhou People's Hospital, Taizhou City, 225300, China. In this test, a total of 3 groups were prepared, group 1 (control group), group 2 (F5) and group 3 (F6). The rat was attached to the tail-flick test device. (Orchid Scientific's Tail flick Analgesia

meter was used in this study. An apparatus had an arrangement of having the single control switch for light and timer activated simultaneously when the tail flicked. The time it took from turning on the light to witnessing the tail flicker was recorded. Between 50 seconds of cutting was maintained to avoid injury due to temperature. Application of 50 mg of the drug was done and the aesthetic test was started. This was repeated every 5 min until the duration time was reduced to the desired value. The linear trapezoidal rule was used to calculate the rat tail-flick curve. All of the groups' efficacy factors were compared. By using the equation below efficacy factor (EfF) was determined (Kondamudi *et al.,* 2016) by considering Area Under Effective Concentration (AUEC).

EfF=(AUEC of bupivacaine patch containing enhancer) / (AUEC of the control patch)

The basis for the tail-flick test of the rat is, the latency of the removal of the tail from the heat source was evaluated using a rat tail exposed to a source of light or radiant heat. The tail-flick technique is used to assess nociception, analgesic efficacy and tolerance development.

STATISTICAL ANALYSIS

The statistical evaluation was done by application of Design expert software version 2.0 and statistically significant parameters were evaluated

RESULTS

Physicochemical characterisation of NP's

The ionotropic gelation technique was the most appropriate method for RPV-CS-NP. The drug loading efficiency was found between 10.25 to 20.18% with particle sizes ranging from 172-350 nm. The PDI values showed that the formulated nanoparticles had uniform size distribution within nanoparticulate suspension (See table 3). Also, the zeta potential values (19.25 to 23.90 mV) of the suspension confirmed the physical stability of the nanoparticles.

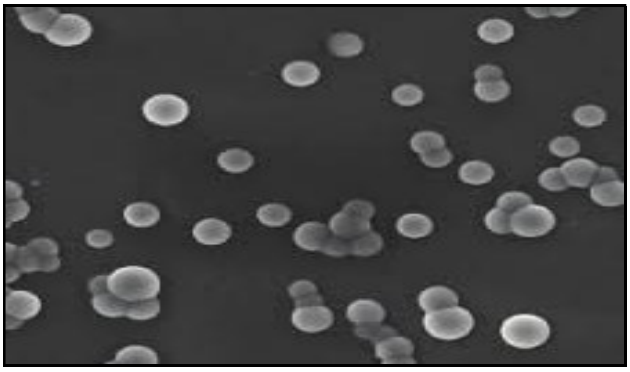

Fig. 1: Scanning electron microscopy of nanoparticles.

Table 3: Characterisation of RPV-CS-NP

Code	DL (%)	PSD (nm)	Zeta potential(mV)	PDI
NP1	10.25±0.11	350±0.54	19.25±0.16	0.29±0.016
NP2	12.47±0.21	192±0.65	20.70±0.22	0.32±0.022
NP3	17.51±0.09	270±0.72	21.72±0.10	0.22±0.075
NP4	20.18±0.24	172±0.47	23.90±0.21	0.24±0.045

Surface morphology

It was found that the nanoparticles (NP4) were found to be spherical, smooth and without a crack in nature (fig. 1). From all these observations NP4 formulation was considered as optimized nanoparticles and it was utilised in the manufacturing of transdermal gel.

Physico-chemical evaluation of transdermal gel

The various Physico-chemical properties of the gels are presented in table 4.

Table 4: Physicochemical properties of RPV-CS-NP loaded gel.

Formulation	Visual Appearance	Clarity	pH	Content (%)	Viscosity (cPoise)	Bioadhesive strength (dyne/cm^2)
F1	Hazy	Nonclear	7.1±0.2	89.20±0.2	8050±1.75	1790.51±2.45
F2			7.5±0.17	91.11±0.4	10025±1.20	2025.12±2.90
F3			7.0±0.22	93.50±0.6	11450±2.11	2250.50±3.24
F4			7.2±0.27	97.89±0.3	12790±1.71	2471.41±2.26
F5			7.5±0.24	98.57±0.7	14145±3.11	2535.65±5.51
F6			7.4±0.32	99.89±0.5	16920±2.51	2742.72±4.11

The formulation containing RPV-CS-NP gels was found to be hazy and nonclear. The clarity and transparency of the formulation were lost due to the presence of RPV-loaded nanoparticles otherwise the gelling system formed with Carbopol 934 polymer is clear and transparent. pH of all the formulations was found in the 7.0 to 7.5 range which is within the neutral pH range and is very well compatible with the skin without causing any irritation. A direct relationship was observed between drug content and concentration of Carbopol 934 polymer. The maximum drug content was observed in the F6 formulation due to higher polymeric concentration as compared to other formulations. The viscosity of the gel formulations was found between 8050 to 16920 cps. Which is feasible for topical drug delivery. The viscosity and bioadhesive strengths (1790.51 to2742.72 dyne/cm^2) of the formulations were found to be linear with polymer concentrations.

Ex vivo permeation study

The comparative drug permeation through a mucosal membrane is presented in fig. 2. The F6 formulation showed an excellent skin permeation profile over 14 hr as compared to other formulations.

In vivo tail flick test

Table 5 showed the AUEC0-120 min of rat tell flick test for the formulated gels. Control formulation showed the AUEC 950.45±120.5 (sec/min) for which efficacy is 1.

Formulation F5 showed 100% drug permeation at 12hr while F6 formulation was found to be more sustained up to 14hr. So, it was concluded that the drug permeation of F6 was maximum than other formulations.

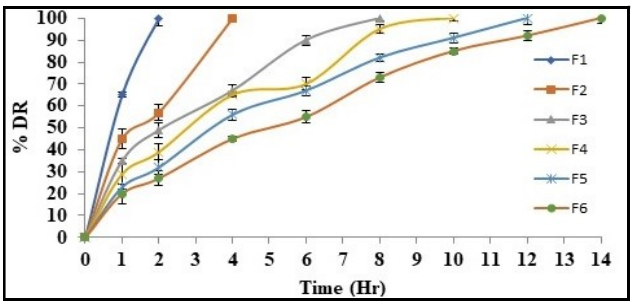

Fig. 2: Comparative *ex vivo* permeation study through rat skin membrane in pH 6.8 phosphate buffer.

Table 5: The comparison of AUEC for Bupivacaine patches containing enhancer.

Formulation	AUEC (sec/min)	Efficacy factor
Control	950.45±1.205	1
F5	1590.21±1.766	1.67
F6	2237.11±1.1235	2.35

A graphical representation of % cumulative drug permeation has been shown in fig. 3.

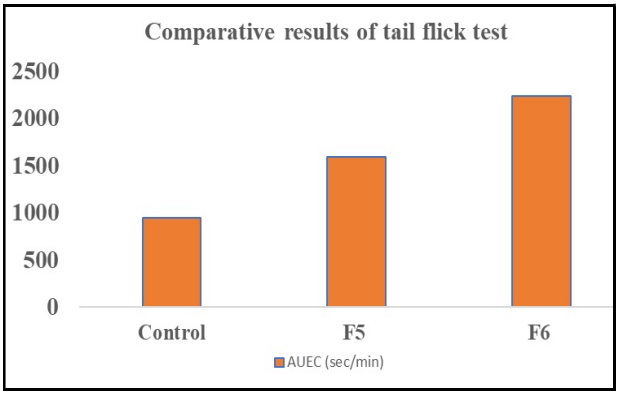

Fig. 3: Comparison of AUEC for RPV bioadhesive gel.

Formulation F5 showed AUEC 1590.21±176.6 (sec/min) while formulation F6 showed the highest AUEC i.e., 2237.11±112.35 (sec/min) than the other formulation. From the results, it was observed that bioadhesive gel containing nanoparticles showed the anesthetic effect at 25 min and control gel showed it for 16min. The bioadhesive gel formulation containing RPV nanoparticles showed 3.32 folds increase in anesthetic activity as compared to the control gel (fig. 3). From the study, it was confirmed that gel formulation containing nanoparticles showed a more prolonged anesthetic effect.

DISCUSSION

The present research work deals with the development of bioadhesive transdermal gel containing nanoparticulate RPV to enhance the anesthetic effect. The nanoparticles were prepared by using chitosan polymer due to their excellent biocompatible potential in drug delivery systems. The nanoparticles were formed due to the interaction between CS (+ve charged) and TPP (-ve charged) (Bangun *et al.,* 2018). The loading efficiency was directly associated with the concentration of CS used in the formulation. Increasing the CS concentration helped in the maximum loading of the RPV. The transdermal gel was developed by using Carbopol 934 polymer. This polymer is widely used in various pharmaceutical formulation developments including gel, cream and ointment due to its excellent compatibility with the biological system (Sabalingam *et al.,* 2022). The formulations developed with this polymer can be applied in cavities including the nose, eye, vagina, etc. Apart from all these applications, it is also used in various topical as well as transdermal formulations. This observation clearly showed that the active ingredient in the form of nanoparticles was uniformly distributed at higher concentrations of polymers resulting in the achievement of maximum drug content. This permeation profile was also found to be dependent on the polymeric concentration. The higher polymeric concentration played an excellent role in the retardation of drug permeation through the skin membrane (Kurakula *et al.,* 2021). In drug permeation studies, we have studied and observed RPV bioadhesive gel formulation containing nanoparticles. Therefore, later we tested the anesthetic effects of the formulated gels using a rat tail-flick analgesio meter.

Bioadhesive transdermal gel of ropivacaine loaded nanoparticles has enhanced the anesthetic effect by improving the delivery of ropivacaine, a local anesthetic, through the skin and into the underlying tissues. Ropivacaine loaded nanoparticles increased the surface area available for drug absorption and improve drug retention on the skin, which resulted in a prolonged and sustained release of the drug.

The bioadhesive properties of the gel allow it to adhere to the skin, increasing the residence time of the drug and allowing for a more efficient transfer of the drug through the skin barrier. The nanoparticles used in the gel can also help to protect the drug from degradation and increase its stability, further enhancing the effectiveness of the anesthetic.

Additionally, the use of nanoparticles can allow for a more targeted delivery of the drug to specific areas, reducing the risk of systemic toxicity and improving the therapeutic efficacy of the drug. This targeted delivery can also result in a lower dose of the drug being required, reducing the risk of adverse effects.

Overall, the combination of bioadhesive transdermal gel and ropivacaine loaded nanoparticles can improve the

anesthetic effect by improving drug delivery and increasing drug retention, leading to a more prolonged and sustained release of the drug with potentially lower doses and fewer side effects.

CONCLUSION

In contrast to the traditional drug delivery system, the recently developed bio-adhesive transdermal gel containing RPV nanoparticles would be a potential alternative strategy for improving anesthetic effects. To prove the safety and effectiveness of the formulation, other clinical applications must be investigated.

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