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DEVELOPMENT OF IN VITRO RELEASE TEST FOR CAPSAICIN TOPICAL GEL FORMULATIONS BY USING FRANZ DIFFUSION CELL

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ABSTRACT

In vitro release test (IVRT) was developed for evaluation of release profile of Capsaicin from Capsaicin topical gel formulations. The method was developed using a Vertical Franz diffusion cell, commercially available synthetic membranes, Hydro alcoholic receptor medium and quantification by HPLC with UV detection. Good release profile of Capsaicin was observed with 0.2µm Teflon membrane, Ethanol: Water (70:30, %v/v) as receptor medium, 32°C as compartment temperature and 8 hrs study time. The aliquots of the samples at different time intervals were analyzed by HPLC with UV detection at 280 nm. Cumulative release and flux value of Capsaicin at the end of the study were found about > 15 µg/cm^2 and 2.0 µg/cm^2/hr respectively. Linearity was established for Capsaicin in the concentration range of 0.25-6.25 µg/ml with correlation co-efficient of 0.9998. Method showed discriminative power and sensitivity in the terms of quantification at lower concentration. Method can be utilized in pharmaceutical and Cosmetic industries for monitoring of batch to batch uniformity with respect to change in process and formulation composition and for comparative IVRT study of Generic formulations to built confidence prior to Clinical study.

KEYWORDS: Capsaicin, IVRT, Franz Diffusion Cell, HPLC

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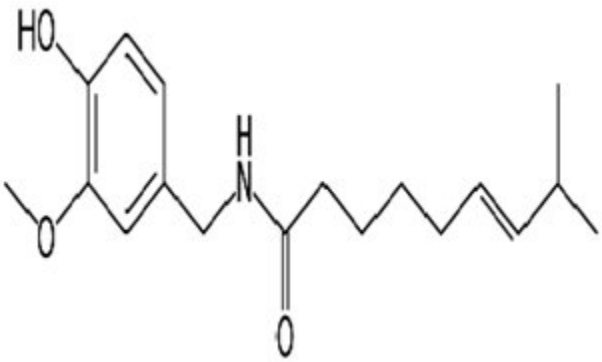
INTRODUCTION

Capsaicin is the active component of Chilli peppers, they are generally known as ripen fruits of various species of genus capsicum. Generally their taste is representing a hot sense consisting of capsaicinoids as the major group of organic compounds closely related to the family of alkaloids, and they are known to be biosynthesized and accumulated in the placenta of Capsicum fruits[1,2]. Burning and painful sensations associated with capsaicin result from its chemical interaction with sensory neurons. Capsaicin, as a member of the vanilloid family, binds to a receptor called the Vanilloid receptor subtype 1[3]. Capsaicin is used in topical ointments as well as in transdermal patches to relive pain of peripheral neuropathy. It may be used as cream for the temporary relief of minor aches and pains of muscles and joints associated with arthritis. It may be used in concentration range of between 0.025% and 0.075%[4]. Structure and molecule details of Capsaicin are given in figure 1.



IUPAC Name: 8-Methyl-N-Vanillyl-trans-6-nonenamide

Molecular Wt: $C_{18}H_{27}NO_3$

CAS No: 404-86-4

Partition co-efficient: 3.04

Figure 1
Structure and molecule details of Capsaicin

The use of Franz diffusion cell to assess skin permeability has evolved into a major research methodology, providing key insights into the relationship between skin, drug and formulations[5,6]. The key parameter for any drug product is its efficacy as demonstrated in controlled clinical trials. The time and expense associated with such trials make them unsuitable as routine quality control methods. Therefore in vitro release test (IVRT) by Franz diffusion cell are often used to assure that product quality and performance are maintained over time and in the presence of change. An in vitro release rate can reflect the combined effect of several physical and chemical parameters, including solubility and particle size of the active ingredient and rheological properties of the dosage forms[7]. Franz diffusion cells are normally used with excised human or animal skin. However, when biological skin is not readily available, synthetic membranes employed in drug diffusion study by Franz cell have two functions: simulation of the skin and quality control[8-10]. Synthetic membranes for quality control should have a minimum diffusion resistance to drugs and only act as a support to separate the formulation from the receptor medium[11,12]. The Food and Drug administration (FDA) has suggested that simple, porous synthetic membranes are suitable for assessing topical formulations performance as they act as a support yet are not rate-limiting barriers. The purpose of this study was to develop an in vitro release test (IVRT) for Capsaicin topical gel formulations by using Franz diffusion cell and application of the developed method in pharmaceutical and cosmetic industries. This study was performed with different synthetic membranes and hydro alcoholic medium as receptor medium to establish best suitable

conditions for the diffusion of Capsaicin from formulations to receptor medium through synthetic membrane.

MATERIALS AND METHODS

Instruments and Reagents

Vertical Franz diffusion cell with auto sampler (Make: Logan), Alliance HPLC system with UV detection and Empower software (Make: Waters), XS205 dual range balance (Make: Mettler Toledo), Bandelin sonorex sonicator, volumetric flasks, pipettes and beakers were used for this study. Capsaicin standard (Potency: 98%), Ethanol HPLC grade(Make: Merck), Methanol HPLC grade (Make: RFCL), Acetonitrile HPLC grade (Make: RFCL), Water HPLC grade and Glacial acetic acid HPLC grade were used for this study.

In vitro release test parameters

The study was performed with Franz cells consisted of six vertical cells and a water bath used to maintain the temperature at 32 ± 1°C. Approximately 200 to 300 mg of Capsaicin topical gel samples were applied on 0.2µm Teflon membrane (Synthetic membrane) of the donor chamber and completely occluded by covering dosage form with parafilm**.** The receiver chambers were filled with Ethanol: Water (70:30, %v/v) as receptor medium and stirred at 600 rpm with magnetic stirrer. A receptor medium was selected because of the low aqueous solubility of Capsaicin. All membranes were pre-wetted in the receptor medium for 15 minutes before use. Each study was run for 8 hrs and samples were withdrawn at 0.5, 1, 2, 4, 6 & 8 hrs intervals from each cell and replenished with fresh receptor medium. Quantification of Capsaicin in samples was performed by HPLC method which is described below. The cumulative amount of drug release and Flux were calculated and plotted graph of cumulative drug release Vs the square root of time for each formulation. Gel samples with different composition and rheological properties were studied by this method and evaluated difference in drug release with respect to change in formulation composition and change in physical attributes of the formulation.

Quantification method (HPLC)

Quantification of Capsaicin in the IVRT samples was performed by RP-HPLC with UV detection at 280 nm. Separation was achieved on a Grace Alltima C18 (25cm × 4.6 mm, 5 µm) column maintained at 40°C. Mobile phase as mixture of 0.1% v/v Glacial acetic acid, Methanol and Acetonitrile (40:35:25, % v/v) was used at flow rate of 1.0 ml/minute with isocratic mode for Capsaicin peak elution. Samples were injected with 50 µl of injection load and run time of 22 minutes. Linearity curve for Capsaicin in the receptor medium was made in the concentration range of 0.25– 6.25 µg/ml and injected into HPLC, plotted linearity curve of Concentration vs Area for Capsaicin. All samples of IVRT study were analyzed by HPLC within established linearity range.

RESULTS AND DISCUSSION

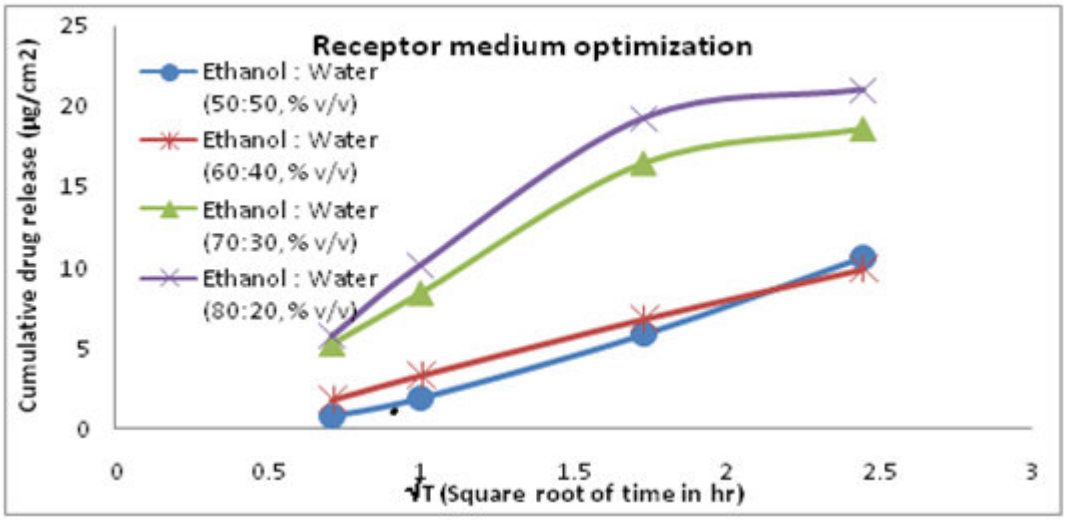
Receptor medium, Membrane, other Franz diffusion cell apparatus parameters and HPLC method for quantification are key parameters for IVRT method development and were optimized as mentioned in a), b), c) and d).

a)Receptor medium selection

The most important factor for the selection of receptor medium is the solubility of the drug in the medium. The receptor medium should provide a diffusional sink for the active ingredient released from the semisolid formulation. Generally it is similar to the physiological condition of the skin, it is also imperative to ensure that the release of the drug can be measured without bias[13,14]. Appropriate receptor medium such as aqueous buffer for water soluble drugs or hydro-alcoholic medium for sparingly water soluble drugs or another medium with proper justification can be used. Capsaicin is insoluble in water and soluble in alcohol. Therefore solubility of capsaicin in different compositions of water and alcohol (hydro-alcoholic medium) were tried and found that medium containing ≥ 50% alcohol in water

is good enough to achieve sink condition. Hence IVRT experiments were performed with different compositions of Ethanol and water such as 50:50, 60:40, 70:30 & 80:20 % v/v as receptor medium, 0.2µm Teflon membrane as barrier, about 200 mg of sample application and time period of 8 hours. Then observed release profile of Capsaicin from gel formulation by using different receptor mediums. The relationship of Q (Cumulative amount release) vs √T (Square root of time) is derived from Higuchi model[15]. Cumulative release of Capsaicin vs √T with respect to different receptor mediums are shown in figure 2.

Figure 2
Effect of different receptor medium on release of Capsaicin (n=3)



The results are demonstrated that capsaicin release was increase with increase in Ethanol concentration in receptor medium. Good release of Capsaicin such as 18.63 µg and 20.98 µg were found with Ethanol and Water composition of 70:30 and 80:20, % v/v respectively. But air bubble formation was found in receptor chamber just below the membrane with Ethanol: Water (80:20, % v/v) as receptor medium and variability of release within sets of cells was observed while with Ethanol: Water (70:30, % v/v), no such observation was found. Hence Ethanol: Water (70:30, % v/v) was selected as receptor medium for IVRT study.

b)Membrane selection

The membrane of choice allow the drug to diffuse into the receptor medium as it is released from the sample and should not contain any leachables that can cause interference to the quantification of drug. Three different synthetic membranes 0.2µm Nylon, 0.2µm Teflon and 0.2µm polysulphone were tried with Ethanol: Water (70:30, % v/v) as receptor medium for In vitro release study of Capsaicin from topical gel formulations. Before starting in vitro release study, membrane binding study and pretreatment of membranes with medium were performed. Membrane-binding studies were performed to determine whether Capsaicin is bind to specific membrane or not[16]. Capsaicin standard solution of 4.5µg/ml concentration was prepared and filtered through each of above membranes in triplicates and analyzed all filtered solution against unfiltered solution by HPLC. The results of membrane-binding study are summarized in table 1 and enabled that recovery of capsaicin was found > 94% with all three membranes, while within three membranes, Recovery of Capsaicin was found >98% by using Teflon membrane compared to Nylon and Polysulphone membranes. Pretreatment of membranes were performed by soaking in

receptor medium for 15 minutes to avoid any variability in release profile. Then IVRT of capsaicin topical gel formulation was performed with each of membrane in triplicates cells by using Ethanol: Water (70:30, % v/v) as receptor medium. Each study was performed for 8 hrs and determined cumulative drug release for each membrane. The study results enabled that 0.2µm Teflon was showing good release about 20.98 µg/cm^2 compared to 0.2µm Nylon and 0.2µm Polysulphone membranes showing result of 17.62 µg/cm^2 and 15.89 µg/cm^2 respectively (figure 3). Hence 0.2µm Teflon membrane was finalized for this study.

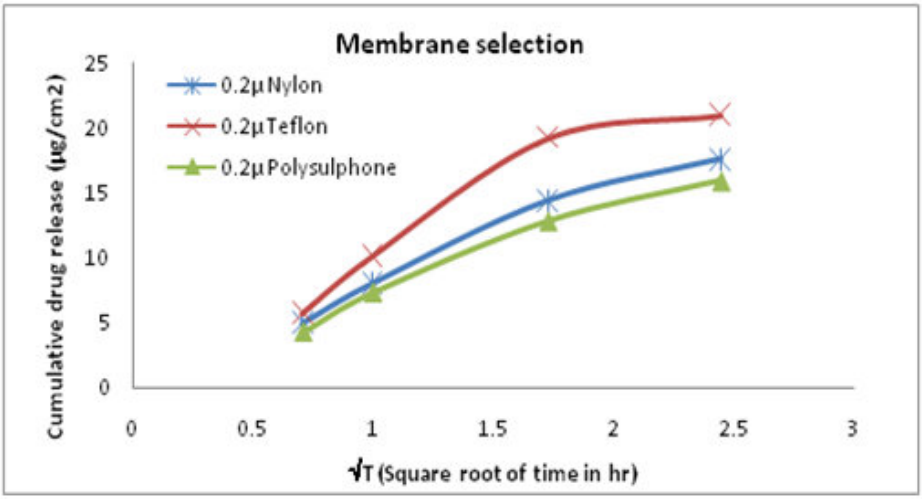
Table 1

Membrane binding study of Capsaicin with different synthetic membranes (n=3)

Membrane	Membrane binding (% Recovery)	% RSD
Nylon	96.3 ± 1.14	1.18
Teflon	98.7 ± 0.71	0.72
Polysulphone	94.1 ± 0.95	1.01

Figure 3

Effect of different synthetic membranes on release of Capsaicin (n=3)



c) Other Equipment parameters & Drug release calculation

Study temperature to be selected based on application of formulations on target organ. Basic application of Capsaicin gel formulations on skin, hence 32°C temperature was used throughout experiments. Gel sample quantity applied (200 mg) on membrane was selected based on amount required for uniform spreading and covering of membrane area. Generally six Franz diffusion cells are used for a test as in dissolution testing to nullify individual dosage form variability. Sampling intervals of this study were finalized as 0.5, 1, 2, 4, 6 & 8 hrs based on sufficient release of Capsaicin. Sampling was performed with auto sampler and there was a complete replacement of sample with fresh receptor medium. And therefore no limitation for collection of small volume of sample at each interval and complete replacement of sample with fresh receptor medium resulted in a easy to achieve sink condition for drugs having poor solubility.The cumulative amount (Q) of Capsaicin released per surface area of membrane is determined by following equation.

$$Q = [C_n V + \sum_{i=1}^{n-1} Ci S] / A$$

Where,
Q = Cumulative amount of Capsaicin released per surface area of membrane (µg/cm^2)
C_n= Concentration of Capsaicin (µg/ml) determined at nth sampling interval.
V = Volume of individual Franz diffusion cell, 11 ml
$\sum_{i=1}^{n-1} Ci$ = Sum of concentration of Capsaicin(µg/ml) determined at sampling intervals 1 through n-1
S = sampling volume, 11 ml
A = Surface area of sample well, 1.766 cm^2

d) HPLC method for quantification

A sensitive analytical method was developed in order to quantify low level of Capsaicin in receptor medium. Good separation of Capsaicin in the presence of Gel matrix was achieved by using Grace Alltima C18 (25cm × 4.6 mm, 5µm) as stationary phase and mixture of 0.1% v/v Glacial acetic acid, Methanol and Acetonitrile (40:35:25, % v/v) as mobile phase. Mobile phase was run at flow rate of 1.0 ml/minute in isocratic mode with run time of 22 minutes to achieve separation (figure 4). Capsaicin was detected at 220 nm wavelength in order to enhance sensitivity. Other parameters such as 50µl as injection volume and column temperature of 35°C were finalized during development. Linearity of the method was established in the concentration range of 0.25 – 6.25 µg/ml. Correlation co-efficient of the linearity plot and linearity equation were found to be 0.9998 and y = 28479x – 438.9 respectively (figure 5).

Figure 4
Representative chromatogram of standard

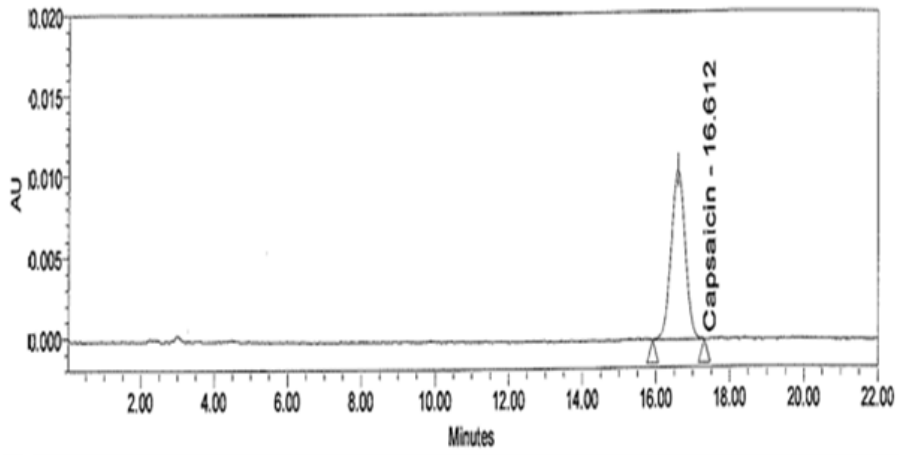
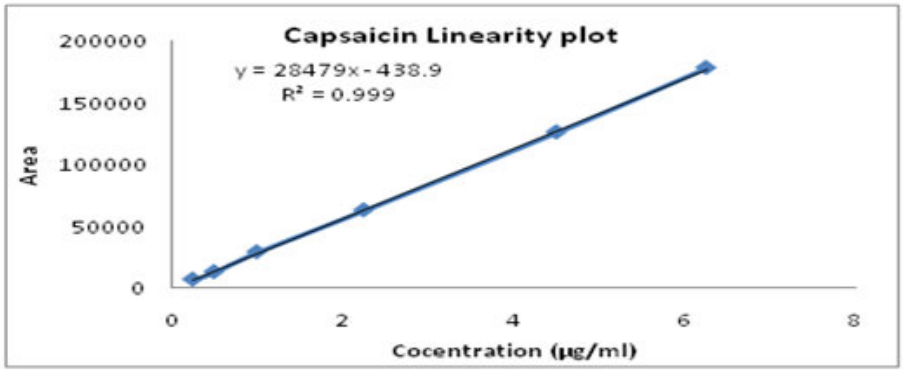


Figure 5
Linearity plot of Capsaicin



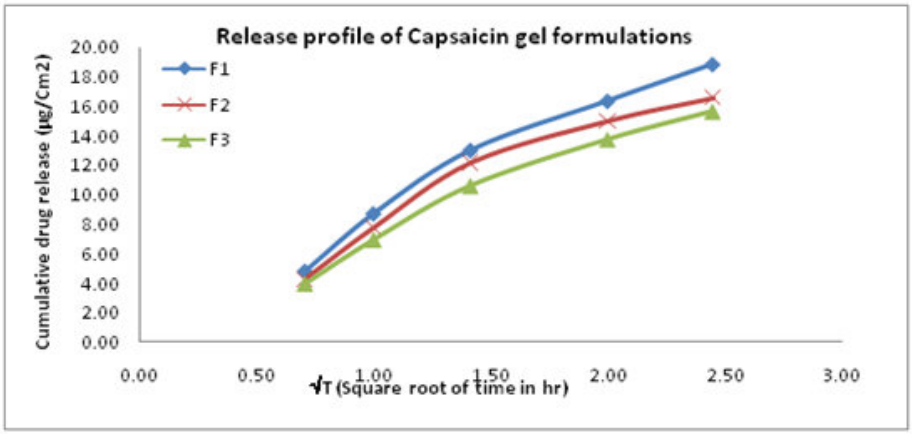
e) Application of IVRT for release monitoring of different Capsaicin gel formulations.

Different formulations of Capsaicin gel F1 (Capsaicin with 7% Propylene glycol in gel composition), F2 (Capsaicin with 28% Propylene glycol in gel composition) and F3 (Capsaicin with 30% Propylene glycol in gel composition) were studied by developed method and compared release profile of Capsaicin (figure 6). The results of Flux, Cumulative drug release, viscosity of the formulations are shown in table 2. Results revealed that formulation F1 is showing good release of Capsaicin compared to F2 & F3. Results indicated that release rate of Capsaicin is affected by change in propylene glycol concentration in formulation composition and change in physical attribute (Viscosity) of the formulations.

Table 2
Flux and Cumulative release of Capsaicin formulations

Formulations	Capsaicin (% w/w)	Viscosity in cP (By CAP 2000+, Spindle 1, 400rpm)	Flux (µg/cm²/hr) (n=6)	Cumulative drug release (µg/cm²) (n = 6)
F1	0.025	160.8	2.356 ± 0.21	18.85 ± 1.66
F2	0.025	287.8	2.069 ± 0.09	16.55 ± 0.72
F3	0.025	339.4	1.960 ± 0.13	15.68 ± 1.07

Figure 6
Comparative release profile of three capsaicin gel formulations



CONCLUSION

The developed IVRT method propose the use of synthetic membranes for in vitro release study, which mimic the skin requirements at early product development stage. This method is sensitive in terms of quantification of Capsaicin in concentration range of 0.25- 6.25 µg/ml having linearity correlation co-efficient of 0.9998. This method was utilized to evaluate release profile of Capsaicin from different topical gel formulations. This method showed discriminative power with respect to change in composition of formulation and physical attributes of the formulations. This method provides a useful tool to assess the drug product quality and sameness as required by SUPAC-SS. This method can be utilized in pharmaceutical and cosmetic industries for monitoring of batch to batch uniformity with respect to change in process and formulation composition and for comparative IVRT study of Generic formulations to built confidence prior to Clinical study.

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