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Research Article

An Overview on Automated In Vitro Release Testing (Ivrt) For Topical Formulation

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ABSTRACT
In vitro release testing (IVRT) is used to check the release characteristics of any semi-solid topical drug formulations. A various diffusion system such as Franz diffusion cell, vertical diffusion cell system, horizontal diffusion cell system, static diffusion cell, side by side diffusion cell, immersion cell, flow through diffusion cell, are being used as a manual release system, but suffer from numerous manual errors. Therefore, valid and standard diffusion system is desirable for determination of release characteristics of topical formulations. To overcome these errors, recently, the manual system has been replaced by automated in-line diffusion cell system. This automated IVRT offers many advantages over manual system that include continuous flow of receptor solution, automated sampling, required less time, also safeguarding the hazards of the environment by limiting physical contacts, thereby overcome all the problems which are associated in manual diffusion system. An automated IVRT diffusion system is an advanced technique that is being used for development and evaluation of topical formulations. In this review, we are demonstrating the advanced IVRT diffusion system along with its various validation parameters and factors associated with sample analysis. Furthermore, the potential advantages and future prospective of it are also discussed.

Keywords: Diffusion, In Vitro Release Testing (IVRT), In-Line Cell (ILC), Validation and application.

1. INTRODUCTION

The FDA released the guideline for industry related to scale-up and post approval changes: recommended chemistry, manufacturing, and controls (CMC) test to support each level of change (1).]. In May 1997 SUPAC-SS regulatory guidelines were released for In Vitro Release Testing for Non-Sterile Semi-Solid Dosage Forms. According to the SUPAC-SS, development and evaluation of topical products are done. If there are some changes in their composition, its manufacturing site and equipment, source of excipient and changes in batch size (2, 3). In vitro release testing (IVRT) is used as a tool for the development and evaluation of semi-solid dosage form such as creams, ointment and gels. So, there is a need of simple, reliable and reproducible method for the determination of release characteristics of topical semi-solid dosage form by using a diffusion cell. IVRT method is used to check and validate the repeatability and reproducibility (i.e. accuracy and precision) (4, 5). An in vitro release rate can reflect the combined effect of several physical and chemical parameters, including solubility and particle size of the active pharmaceutical ingredient (API) and rheological properties of the dosage form (6). Manual IVRT methods such as Franz diffusion cell, vertical diffusion cell system, horizontal diffusion cell system, static diffusion cell, side by side diffusion cell, immersion cell, flow through diffusion cell are being used for the in vitro release and permeation performance of topical formulations. It is also helpful to understand the physicochemical characteristics and performance of the topical formulations (4) (7).

A proper IVRT method needs to mimic skin permeation kinetics, including donor, membrane and a receptor medium that are analyzed for drug concentration (8). The most common IVRT method employs an open chamber design like the Franz diffusion cell system and can be used with a synthetic membrane or human cadaver skin, applied formulation and receptor solution. The membrane separates the donor compartment containing the test formulation from the receptor compartment that is filled with selected receptor medium.

Diffusion of the drug from the semisolid product across the membrane is monitored by assay of sequentially collected samples of the receptor medium. At pre-determined time points an aliquot of medium is removed from the receptor compartment for drug content analysis, usually by high-performance liquid chromatography (HPLC).

The receptor compartment is filled with fresh receptor medium after each sampling (6). But these manual systems have various problems like manual errors by the addition of receptor solution, manual sample collection, maintain sink condition by manually adding receptor solution as discusses in **Table 1**.

In recent years, the diffusion cell design has been modified and the automated system elevated in order to ease of operation, make user friendly, and provides a fast and reproducible technology. Kumar P et al (1993), compared the Franz diffusion cell and Enhancer Cell and found the Enhancer cell has certain advantages when compared to the Franz cell such as reduced investment, loading the cells and set up of the equipment and sampling techniques, especially with automation(9). Cordoba-Diaz M et al (2000), performed in vitro permeation studies by using an automated in-line flow-through diffusion system and provided good reproducibility, the continuous through the receptor chamber helps to maintain sink conditions throughout the experiment (10). Shiow-Fern Ng et al (2010), performed in vitro studies by using Franz diffusion cell and found a robust and reproducible method, frequent sampling rates and maintained sink condition (11). Alves AC et al (2015), was evaluated permeation of transdermal nanoparticles using a Franz diffusion cell with computer-controlled sampling. This system provides conventional techniques by real time analysis of collecting samples and the operator is able to stop sampling whenever required (12). Kanfer I et al (2017), compared insertion cell and vertical diffusion cell (VDC) and found that insertion cell is easier to use and

readily adaptable for use with the compendial flow-through apparatus. Easy to use and remove the air bubbles at the membrane-liquid interface (8). Upadhyay Y et al (2019), Comparison IVRT of Diclofenac Topical Formulations Using an In-Line Cell Automated Diffusion System and found that this diffusion systems provides automated sampling, time-saving, and accurate discriminate results also determine a fast and accurate technique for topical formulations (23)

Automated IVRT (ILC 07), is similar to a flow type manual diffusion system that contain a very small volume of receptor solution. The main advantage of this automated system over a manual system is that, it eliminates the errors related to the actual amount of sampling and deviation in sampling time throughout the experiments. It is based on the continuous flow principle, the continuous flow through the receptor chamber helps to maintain sink conditions throughout the experiment (10). It provides a fast, automated, suitable, and reproducible technique for IVRT and overcome all the problems which are associated with the manual system because of the automated and accurate sampling and constantly flow of receptor solution (23).

2. DEMONSTRATION OF AUTOMATED IVRT (ILC 07)

It is also known as In-Line Cell 07 (ILC 07) automated diffusion system because this equipment incorporated seven in-line flow-through diffusion cells. This instrument is with small volume of receptor solution and similar to a flow type Franz diffusion cell. The basic principle is continuous flow and measure the flux over time.

2.1. Components and Principle of ILC 07

The automated ILC consists of donor and receptor chambers in which the membrane of interest is placed in the horizontal plane and clamped by adjustable lock. A peristaltic pump with multiple channels flows the selected receptor solution from a reservoir bottle and send the receptor solution to the cells through a distribution manifold. ILC system has a fraction collector and a heating circulator bath to warm the cells at a desired temperature (i.e., 32 ± 1.0 ºC) and time point of sample collection is decided and release samples are collected in scintillation vials (21) (22). All components of the ILC are as shown in **Figure.1**.

The basic principle of this system is a continuous flow of receptor solution and maintains the sink conditions throughout the experiment. Due to the continuous flow of receptor solution there is no need of refilling and sampling time is also elapse (10).

		Drug release from semisolid dosage forms follows the Higuchi **Equation (1)**, as shown
	below,	
		$Q = 2C0 \bullet (Dt/n)1/2$ ---------- **Equation (1)**
	Where,	

Q = amount of drug released per unit area of application, C0 = initial concentration of drug,
D = diffusion coefficient of drug, t = time.

This equation is applicable when the drug is solubilised in a formation matrix and the amount of drug released is less than 30%. The amount of drug released is proportional to the square root of time, therefore, a plot of average cumulative release versus time should yield a straight line, the slope of which is used to calculate flux (amount released/cm2/h). The release of the drug is studied during a period of 4–6 hours, which is the typical duration of application for a topical product.

2.2. ILC Automated Diffusion System Assembly Setup Procedure

The assembly setup procedure of automated diffusion system ILC) is as follows. It starts with turning on the heating circulator bath and the temperature of water bath to be set at 45 degree C.

Before starting the experiment, Purging is required to remove the bubbles. The temperature is measured with the help of an infrared thermometer and after this, dose is applied. Membrane of interest is placed inside the cell chamber and selected receptor solution is to be filled in donor chamber And temperature of the membrane surface is maintained at 35 ±2 °C. After getting a desired temperature the application dose is placed into the cell and donor chamber place over it. The receptor solution which is filled in the reservoir is continuously pump using a peristaltic pump at a defined volume to time ratio to the ILC. The sample is collected at the pre decided interval period using scintillation vials and the samples will be analyzed (10)(21)(22).

Predose sample is collected during membrane equilibration and before formulation application to ensure that there is no interference with retention time (RT) of Analyte due to receptor solution, membrane and assembly setup. Desired topical formulation is applied onto the membrane surface of each cell with the help of a dosing syringe and spread the formulation onto the membrane. A parafilm is used to occluded the donor chamber of each cell. The spring clamp is placed over the donor chamber and is screwed. Release samples are collected in scintillation vials over the set sampling time points including predose sample and after this, time points are defined. All collected samples can be diluted using receptor solution (If required collected sample can be diluted to obtain the desire concentration in the calibration curve range). Collected samples will be analyzed and will give a release concentration (23).

3. FACTORS AFFECTING THE ANALYSIS OF SAMPLES IN IVRT

There are various factors that affects the analysis of samples:

3.1 Temperature

The temperature of an in vitro system should be controlled to maintain a target temperature and minimize variations in experimental conditions (24). Increasing and decreasing in the temperature will affect the analysis of the samples. The maximum tolerable skin surface temperature was approximately 42–43°C in vivo. The temperature difference between skin surface and TDS surface increased with increasing temperature, or with increasing TDS thermal resistance in vivo and in vitro (25). With an increase in temperature, the lag time decreased (20).

3.2 Volume of Samples

Volume of the collected sample affects the analysis of samples, if the volume of all samples which are collected from different-different cells are not of same volume then there will be variation in the result and flux rate also.

3.3 Sampling Time

To plot actual linearity, A minimum of six sampling time points is required to plot actual linearity. If the samples were not collected on proper time than the total amount of compound to cross the membrane in a given time period and how much compound crosses a membrane at short time intervals, the total amount of compound that crosses the membrane over a longer sampling period, or the total amount of compound found within the membrane after a given exposure period will not be analysed. Flux rate also will not be calculated (16).

3.4 Flow Rate of Receptor Solution

The volume of receptor solution pumped through the receptor compartment of the cell is an important parameter. Analysis of samples can be varied by changing the volume and flow rate through the receptor compartment (24).

In order to maintain sink conditions, the volume pumped through the cell in a given time should be significantly greater than the volume of the receptor compartment (20).

3.5 Amount of Applied Dose

The amount of applied dose is an important factor that affects the analysis of samples with an increase in the concentration of drug dose also increase of flux (26).

3.6 Occluded Donor Chamber

An air exposure can affect the release rate if the donor chamber is not occluded during the experiment (15).

4. VALIDATION PARAMETERS FOR ILC

The validation of ILC is done by considering following parameters as follows:

a. Volumes of donor and receptor chambers for each cell.
b. Temperature control in the different chambers.
c. Pumping system / flow rates and variability of sampling volume
d. Evaporation of receptor fluid from the collected samples.

4.1 Volumes of Donor and Receptor Chambers for Each Cell

All volumes will be measured by gravimetric methods by filling the chambers with Milli-Q water and assuming a density of 1 g/ml. All the determinations will be made in triplicate foreach cell. The volume of both donor and receptor compartment must be homogenous for each cell.

4.2 Temperature Control in the Different Chambers

The whole equipment will be assembled and a laboratory film (Parafilm) will be placed instead of a diffusion membrane in each cell. The system will be filled with Milli-Q water and a flow rate of receptor fluid of about 4 ml/h will be selected 0.5 ml of water will be placed in each donor compartment and the temperature will be measured at 30 and 60 min in each chamber using a calibrated thermometer. The experiment will be carried out programming two temperatures 35 and 37°C. No significant differences between the obtained values in donor and receptor chambers at 30 and 60 min.

4.3 Pumping System / Flow Rates and Variability of Sampling

Flow rate of peristaltic pump is calibrated by taking three readings of dispensing Milli Q water from each tubing till defined time. E.g. 0.1 ml flow of Milli Q water from each tubing till 10 minutes will dispense 1ml of total volume. If not pump needs to be calibrated by feeding the obtained volume in the pump and further pump will adjust itself according to the feed value (i.e. it increases / decreases its rotation speed). A good linear relation will be found the flow rates of medium into the receptor chambers and variability of samples.

4.4 Evaporation of Receptor Fluid from the Collected Samples

Weight the empty scintillation vial and weight the vial after sampling by using a balance.Empty vial weight / after sampling vial weight × 100 (10).

5. ADVANTAGES OF AUTOMATED DIFFUSION SYSTEM OVER MANUAL DIFFUSION SYSTEM

ILC system defines the suitability of simulation drug release and analyze the quality and reproducibility of the topical formulations (22). It is a conventional technique, due to the real time rugged and robust automated sampling (20). Replacement of laborious and tedious manual procedures, aiming precise control of time event due to automated sampling and unattended operation over at least 24 h (12). The continuous flow through the receptor chamber helps to maintain sink conditions and the elimination of saturation throughout the course of an experiment and replenishing is not required because of the continuously flow receptor solution. This kind of cell is more suitable for simulation of in vivo conditions, in comparison the classical static Franz-type cells (10). Manually sampling from the diffusion cell, collecting sample aliquots, and replacing receptor medium can be a time-consuming task for the analyst (21). The collection of the samples is flexible and reproducible and the volume and flow rate through the receptor compartment of the cell cannot be varied as compare to manual system. ILC is less time consuming as compared to manual system due to the automatic fraction collector(23). In manual system during sampling, gas is often generated between the donor and receptor and interferes with the formulation diffusion. It does not occur in ILC system (27). ILC is suitable for those drugs which has a lower solubility in the receptor compartment (17). ILC have a large-capacity donor chamber to allow appropriate loading of the applied compound and a low volume receiving chamber that ensures rapid removal of penetrant at relatively low pumping rates (26) .

6. FUTURE PROSPECTIVES OF ILC

It employed diverse aims towards quality control of pharmaceutical topical preparations by in vitro analysis such as permeation, release and dissolution test. It will be a more conventional technique - Due to the real time monitoring will provide reliable kinetic information that predicts the outcomes by allowing more complete permeation profiles. Real time monitoring will afford considerable flexibility and kinetic profile will observed by using adapted

sampling frequency. Replenishing is not required in ILCs after collection of each time interval and maintain sink conditions as the receptor solution flowing continuously through donor cells. Due to the automatic sampling there is no need of any person for sample collection and less time consuming. Automated dissolution systems are popular throughout R&D and QC laboratories worldwide.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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Table.1: Stages of development of diffusion cells from manual to automatic system

Type of Diffusion System	Features	Limitations	Published/ Manufactured by	References
Franz diffusion cell	Simple and low cost less technical issues.	Poor reproducibility	Permegear Riegelsville, PA, USA	(13, 14)
Vertical diffusion cell (VDC)	Robust and reproducible nature and sensitivity to both chemical and physical changes to the formulation	Occurrence of air bubbles at the membrane-liquid interface that affects the precision, accuracy, and reproducibility of the result	(Hanson, Logan, PermeGear	(15) (8)
Insertion cell	Easy to use and remove the air bubbles at the membrane-liquid interface.	More technical problems		(8)
Side by side diffusion cells/ Valia-Chien cell	Easily customized with different volumes, amberization and additional porting, suited for iontophoresis.	Suspended water for bath temperature control. It is adequate for rapidly diffusing compound, Its broader applicability is unknown	Permegear	(16, 17)
Static vertical diffusion cell	Inexpensive, Evaluate and validate system with simple design	Temperature control is less, manual sampling errors and inability to agitate the solutions		(18)
Flow through diffusion cell	Automatic sampling make it convenient and maintains the sink conditions by continuous flow and used for less soluble receptor solution drugs	Expensive and technical problems occurs	Crown glass	(19)
Kelder-cells	Automatic sampling, sink conditions maintain by continuous flow of receptor solution, and unattended operation over at least 24 h.	NA	Plexiglas	(20)

Enhancercell	Reduced investment, loading the cells and set up of the equipment and sampling techniques, especially with automation.	NA	Van K el Industries, Inc., (Edison, NJ)	(9)
In line cell07	Time-saving, automated sampling, accurate discriminative results provide a fast, automated, convenient, and reproducible technique.	NA	Permegear, Riegelsville, PA	(23)

NA=Not Applicable.

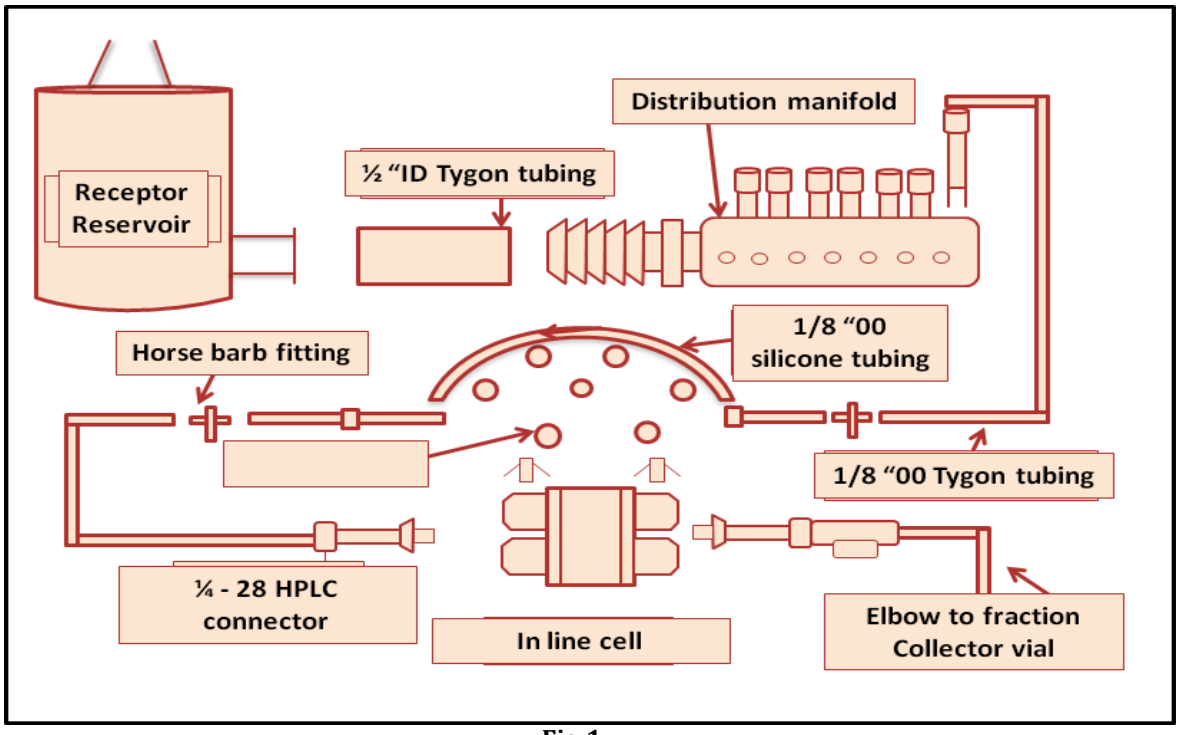

Fig:1