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COMPARATIVE IN-VITRO DISSOLUTION STUDY OF VARIOUS MARKETED BRANDS OF ALPRAZOLAM TABLETS

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Abstract:

Alprazolam is a benzodiazepine anxiolytic commonly prescribed as a sleeping aid and for the treatment of anxiety disorders. The current study was undertaken with the aim of analyzing quality of commercially available brands of alprazolam tablets available in market. To assess the quality, locally available 0.25 mg alprazolam tablet of seven different manufacturers were selected and certain physico-chemical parameters like weight variation, hardness, friability, disintegration time and dissolution profile etc. were evaluated using in-vitroanalytical methods. All the tablet brands met the requirements of IP as they showed acceptable weight variation and friability. Brands were slightly different in hardness, disintegration time and dissolution profile from each other. The hardness of all the brands was found to bein the range of 1.50±0.18 to 4.21±0.11 kg-ft. In water medium the disintegration time of all brands were found to be 0.57±0.45 to 2.22±0.23 min. Five out of seven brands showed better dissolution profile as they released more than 90% drug in 30 min. The study revealed that most of the marketed alprazolam tablets met the BP standards for physico-chemical properties which are the indicators of drug quality. It can be concluded that drug products should always comply standard quality parameters that are the prerequisites for getting satisfactory clinical effects.

Key words: Alprazolam, anxiolytic, anxiety, disintegration, dissolution

INTRODUCTION

Alprazolam is a triazolobenzadiazepine that is a benzadiazepine with a triazolo ring attached to its structure. The chemical name of alprazolam is 8-chloro-1-methyl-6-phenyl-4H-s-triazolo [4,3][1,4] benzodiazepine. Alprazolam is a white crystalline which is soluble in methanol or ethanol but having no appreciable solubility in water at physiological pH. It is a short-acting in the benzodiazepine class used to treat anxiety disorders and as an adjunctive treatment for depression. Anxiety or tension associated with the normal stress of everyday life usually does not require treatment with medicines. Alprazolam was invented by Pfizer and marketed under the Xanax1. Alprazolam is a benzodiazepine which affects chemicals in the brain that may become unbalanced and cause anxiety and is most commonly used to relieve anxiety, nervousness and tension associated with anxiety disorders. It is also used to treat panic disorders. Clinically all benzodiazepines cause a dose related central nervous system depressant activity varying from mild impairment of task performance to hypnosis.

- **PHARMACODYNAMICS OF ALPRAZOLAM**

CNS agents of the 1,4 benzodiazepine class presumably exert their effects by binding at stereo-specific receptors at several sites within the central nervous system. Their exact mechanism of action is unknown. Clinically, all benzodiazepines cause a dose-related central nervous system depressant activity varying from mild impairment of task performance to hypnosis.

- **PHARMACOKINETICS OF ALPRAZOLAM**

➢ **ABSORPTION**: oral administration, alprazolam is readily absorbed. The peak plasma concentration is reached about 1.5 to 2 hours after administration of alprazolam orally tablets given with water. When taken with water. Plasma levels are proportional to the dose given over the dose range of 0.5 to 3.0 mg, peak levels of 8.0 to 37 mg/ml are observed. The elimination half-life of alprazolam is approximately 12.5 hours (range 7.9 -19.2 hours) after administration of alprazolam tablets in healthy adults.

Food decreased the mean C_{max} by about 25% and increased the mean T_{max} by 2 hours from 2.2 hours to 4.4 hours after the ingestion of a high-fat meal. Food did not affect the extent of absorption (AUC) or the elimination half-life.

➢ **DISTRIBUTION:** *In vitro* alprazolam is bound (80 percent) to human serum protein. Serum albumin accounts for the majority of the binding.

- **METABOLISM**: Alprazolam is extensively metabolized in humans, primarily by cytochrome P450 3A4 (CYP3A4) to two major metabolites in the plasma: 4-hydroxyalprazolam and α-hydroxy alprazolam. The plasma concentrations of 4-hydroxyalprazolam and α-hydroxyalprazolam relative to unchanged alprazolam concentration were always less than 4%. The reported relative potencies in benzodiazepine receptor binding experiments and in animal models of induced seizure inhibition are 0.20 and 0.66, respectively, for 4-hydroxyalprazolam and α-hydroxy alprazolam. Such low concentrations and the lesser potencies of 4-hydroxy alprazolam and α-hydroxyalprazolam.
- **ELIMINATION**: Alprazolam and its metabolites are excreted primarily in the urine.

- **DISSOLUTION TESTING**

The definition of dissolution is deceptively simple. It is the process in which a solid substance goes into solution. For dosage forms containing an active solid ingredient, the rate of dissolution may be critical to absorption. Obviously, in most instances, dissolution of the active solid material is affected by a variety of factors such as the media in which the drug is dissolving, the temperature of the media, and the affinity for the solid particles to dissolve in the media. There are numerous other factors, such as excipients, coatings, and pH, which have an effect on the rate of dissolution. While the most rapid absorption is from a solution, most dosage forms are solids, either tablets or capsules. One must also consider dissolution from suspensions and suppositories. Several chapters in this text cover various dosage forms as the theme for the discussion on dissolution. The theory is the same regardless of the dosage form design, but obviously, the rate of dissolution and the limitations are different for each individual dosage form. Any process of drug release and subsequent absorption into the blood stream must consider dissolution of the solid. Wetting of the material, be it hydrophilic or hydrophobic, is the first critical step and precedes deaggregation. This process may also be considered disintegration. The drug then dissolves into the dissolution media, be it in vitro or in vivo. As a rule, suspensions dissolve faster than capsules since some deaggregation has already occurred. Tablets usually have the slowest dissolution rate, either by design to allow a sustained, controlled release or by the nature of the wetting process. The earliest obvious reference to dissolution (1897) was by Noyes and Whitney, where they stated that the dissolution rate is governed by the rate of diffusion of a saturated thin layer forming instantly around the dissolving material. The work of Noyes and Whitney concentrated on physico-chemical aspects and not bioavailability. In 1951, Edwards showed that aspirin tablets would have poor analgesic activity due to poor dissolution. Theoretical models of dissolution

continued to be developed in the early 1900s by Brunner, when he adapted Fick's Law of diffusion. In the 1930s the cube root law, which describes a linear relationship between dissolution rate and cube root of time, came into favor. By the 1950s, dissolution was further studied and began to be recognized as a factor in bioequivalence, although it was not until the 1960s DISSOLUTION that dissolution and absorption rates would be correlated. Beginning in the 1960s a multitude of research papers reported a correlation between dissolution and bioavailability. During this time, many researchers believed and hoped that dissolution would directly relate to, and predict, bioavailability. However, even forty years later, we realize that although we can often correlate in vivo–in vitro activity with dissolution, it does not predict biologic or therapeutic activity. There are a myriad of factors that have an effect on dissolution such as agitation rate, vessel shape, wobble of the equipment, temperature, and others. The most one can expect is an equivalence test on different products, assuming all variables are held equal or, as in most cases, the slight variations in the tests cancel one another. Today dissolution is readily identified as a quality control issue and used to prove batch-to-batch relationships and equivalence. For many drugs, similar dissolution profiles are generally accepted as producing bio-equivalent lots. It is generally accepted that the last 30 years have seen the science of dissolution become mature, and it is recognized that there are limits to what dissolution testing can scientifically prove. It is universally accepted as a quality control tool. We now understand the factors that have an effect on and control the rate of dissolution. Solubility, particle size, and crystalline states are all intrinsic factors that have an effect on the rate of dissolution. Diluents, excipients, binders, granulating agents, and lubricants all play a role in dissolution as well. Obviously, the dosage form itself is critical. All of these factors will be addressed in this text. Rapid dissolution is not always the goal in formulation. If one desires a controlled- or sustained-release dosage form, the factors that affect the dissolution rate may be manipulated to obtain the desired effect. The pharmaceutical formulator can use methods of controlling dissolution to readily obtain a desired release profile. While the remainder of the book is divided into chapters by dosage form, many factors remain the same regardless of the dosage form while some are specific to the individual dosage form and dosage form design.

➢ DISSOLUTION APPARATUS OVERVIEW

Over the past forty years, two basic techniques have evolved for in vitro dissolutiontesting, the stirred beaker method and the flow-through procedure.The stirred beaker system places the test specimen and a fixed volume of fluidin a vessel, and stirring provides mechanical

(hydrodynamic) agitation. This systemwas adopted as the official dissolution method in *USP XVIII* in 1970 and describedas the rotating basket method, USP Apparatus 1.The rotating paddle method was adopted as an official dissolution method bythe USP several years later and became USP Apparatus 2. The origin of officialequipment developed from a number of baskets and stirring devices is shown. Some of the needs for the flow-through type apparatus included a change of pHor any other change in the dissolution medium. Difficulties had also arisen for anumber of sparingly soluble drugs, which were difficult to investigate with a limitedvolume of media. The flow-through system was first adopted by the DeutscherArzneimittelcodex (German Pharmaceutical Codex, DAC) in 1981.Two flow-through apparatus were eventually added to the *USP* in 1990 to overcomesome of the experimental difficulties from the use of the single vessel methodology.They became known as USP Apparatus 3, Reciprocating Cylinder, andUSP Apparatus 4, Flow-Through Cell.The most common dissolution apparatus used throughout the world are the basketand the paddle. These methods are simple and robust and are generally flexibleenough to allow dissolution testing for a wide variety of drug products. For thisreason, Apparatus 1 and 2 should be used for dissolution method developmentunless shown to be unsatisfactory. Other in vitro dissolution apparatus such as thereciprocating cylinder and the flow-through cell system described in the *USP* maybe considered, if needed.More drug release equipment, USP Apparatus 5 and 6, deal with transdermalsystems. USP Apparatus 7 (Alza-type) was developed for the analysis of transdermal systems as well as a variety of drug release systems such as osmotic pumpsand implants. Because of the diversity of delivery systems and the evolving natureof understanding in the area of drug release, different experimental modificationsmay be needed to obtain a suitable in vivo correlation with in vitro release data.Alternatives or modifications to established methodology should be considered onthe basis of proven superiority for a particular product. If the release of the activedrug substance from an individual drug product cannot be accommodated by oneof the major compendia apparatus, appropriate modifications have to be developed.However, unnecessary proliferation of alternative dissolution apparatus should notbe encouraged due to the reproducibility problems that plagued early dissolutionequipment; this will also hinder regulatory acceptance.

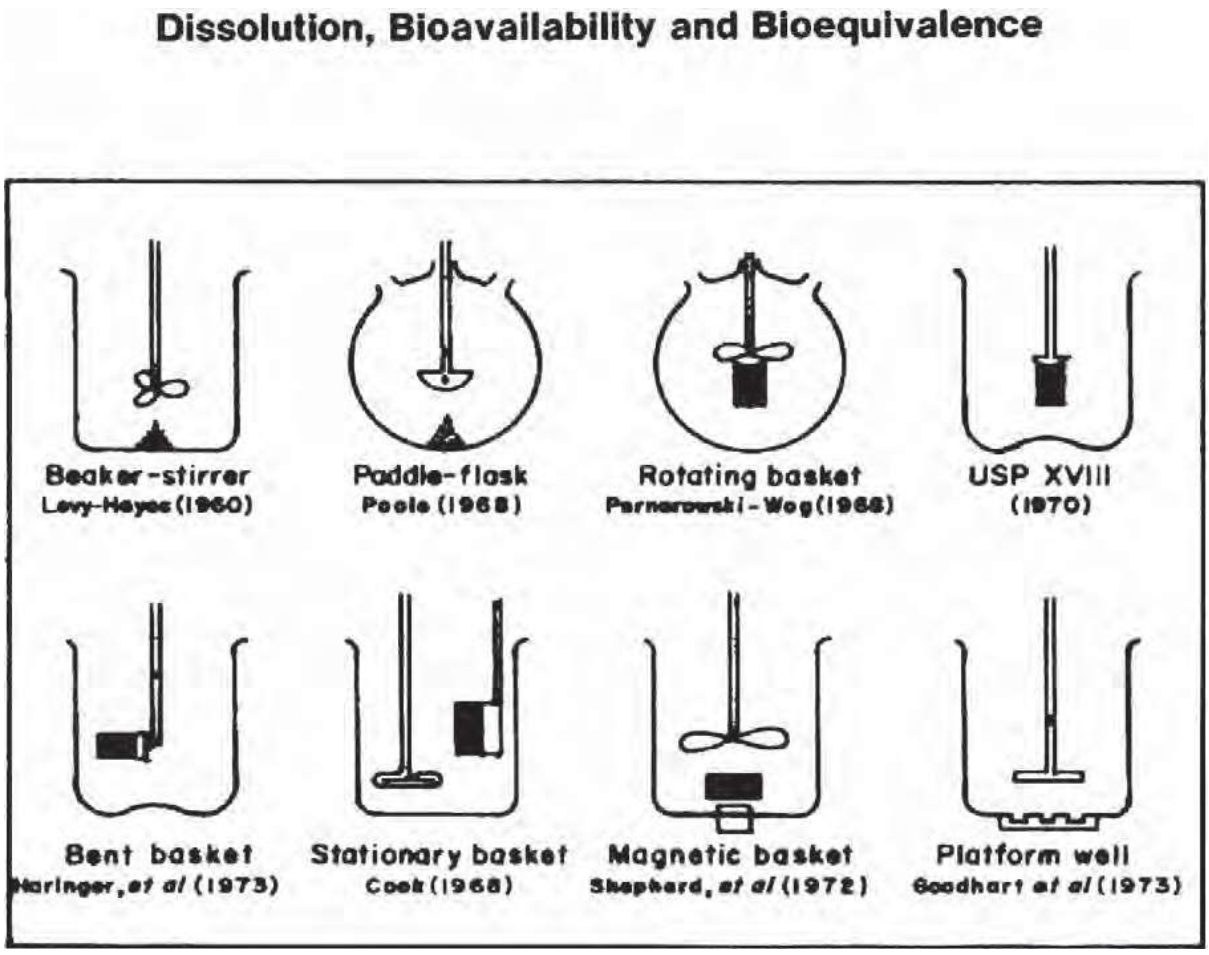


Figure. *Different designs of dissolution vessels and stirrers that have been utilized in major nonofficial methods.*

A. USP APPARATUS 1 AND 2—ROTATING BASKET AND PADDLE

USP Apparatus 1 is called the rotating basket apparatus and USP Apparatus 2 is called the rotating paddle apparatus.

- **ROTATING BASKET**

A general description of the rotating basket apparatus consists of shaft and basket component fabricated from 316 stainless steel. Unless otherwise specified in a test method or official monograph, a 40-mesh basket is used. The basket shaft assembly containing the product is lowered into a 1000-mL vessel and rotated at a specific speed within media, which is maintained at a specific temperature. The rotating basket method is routinely used for capsule formulations at an agitation speed of 50–100 rpm. Rates outside a range of 50–150 rpm are generally unacceptable because of irreproducibility associated with the hydrodynamics below 50 rpm and turbulence above 150 rpm. High turbulence in the vessel leads to a loss of discriminatory power associated with the dissolution method. The vessel used for Apparatus

1 and 2 is typically a 1000-mL hemi spherical shaped vessel made of glass or suitably inert material. Vessel volume should be between 500 and 1000 mL with 900 mL used historically. One-liter vessels are tubing-based with dimensions of 98–106 mm i.d. and 160–210 mm in height. Larger vessels have been developed over the years to allow more volume for poorly soluble compounds. The USP 2-L vessel has dimensions of 98–106 mm i.d. and 280–300 mm in height. The USP 4-L vessel has dimensions of 145–155 mm i.d. and 280–300 mm in height. The official basket used for Apparatus 1 is a 40-mesh design, meaning there are 40 openings per linear inch. Openings are equal in both directions producing a standard square weave. *USP* specifies that 40-mesh (40 40) screen be manufactured with wire having a 0.010 diameter. Dissolution baskets are fragile and require proper handling and care. Attachment to or removal from the basket shaft requires holding the upper rim. When not in use, baskets should be stored in protective cases. Baskets should be carefully inspected for damage or excessive wear since defective or misshaped baskets will affect test results. The standard 40-mesh basket with a 0.01 wire size results in a 0.381-mm square aperture. For comparison, the *Japanese Pharmacopeia* specifies 0.011 wire diameter resulting in 0.425-mm square hole resulting in a 36-mesh basket. The baskets are not interchangeable and could result in a 24% difference in *USP, EP,* and *JP* baskets when testing the USP disintegrating calibrator, Prednisone. Current Physical Parameters and Tolerances: Wobble 1 mm basket lower rim Dimensions per USP Height 25 2 mm Centering 2 mm center line Speed 4% of set speed Vessel Temp. 37 0.5 C Time points 2% of specified time In addition to the current physical parameters, some pharmaceutical laboratories have adopted more stringent parameters gleaned from a Parma Subcommittee on Dissolution Calibration proposal to the *USP* (*3*) to maintain a higher degree of control over the rotating basket apparatus: Optional Parameters and Tolerances: Shaft wobble 0.5 mm total run out Basket wobble 1.0 mm total run out Basket exam No defects at time of use Shaft verticality Vertical using bubble level Speed 2% set speed Vibration 0.2-mil displacement Typical products tested with the rotating basket are capsules, tablets, floaters, modified-release products, beads, and suppositories. Suppository testing may be performed with a slotted Palmieri basket with or without glass beads. Several allowable variations of the standard 40-mesh basket exist including a basket with a gold coating 2.5- m thick (0.0001-inch). Larger vessels accommodating up to two and four liters are now allowable variations in the *USP*. Such vessels are advantageous for poorly soluble drugs. Some non-official variations of the rotating basket include Teflon baskets, o-ring baskets, 10-mesh through 2300-mesh (5-micron), three-fin, mini, and bolus baskets for veterinary products. Examples of these baskets are shown in Figure 4. Occasionally a small

volume apparatus may be required for low-dose, high potency products. Such a variation consists of a mini-basket apparatus based on USP Apparatus 1 with 100- or 200-mL vessels. Small volume apparatus has a typical operational minimum volume of 30-mL. Shown in Figure 5 are a mini basket and an official rotating basket.

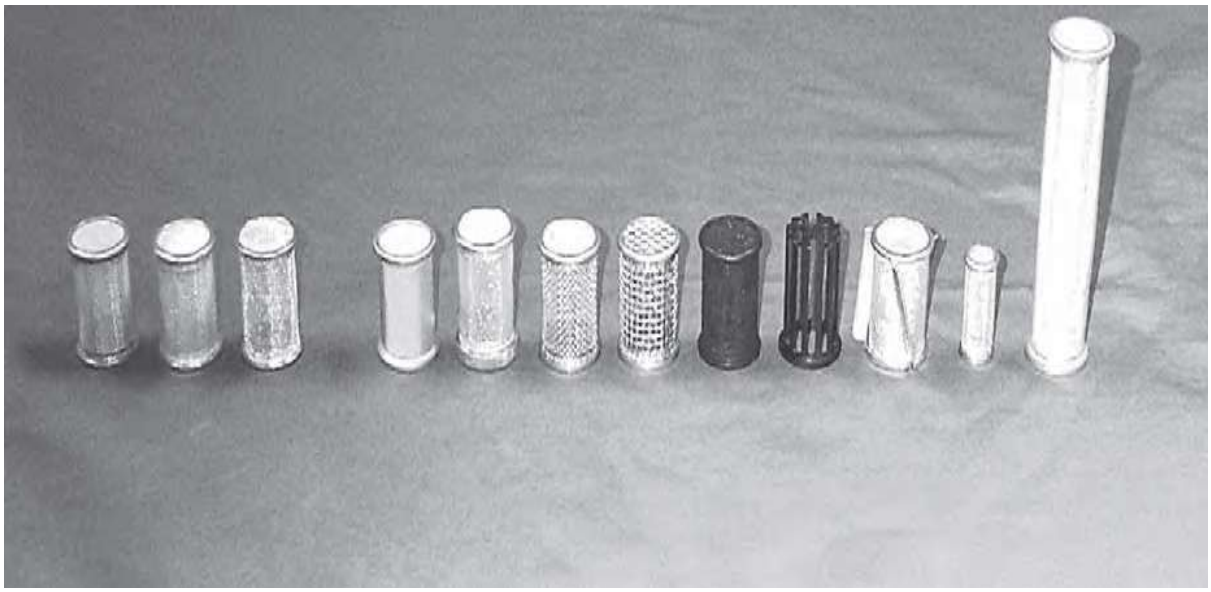


Figure. Examples of non-official variations of the rotating basket.

Figure. Mini basket and official rotating basket.

B. GENERAL APPARATUS 2 DESCRIPTION ROTATING PADDLE

USP Apparatus 2, the rotating paddle method, followed the development of the rotating basket method with better stirring characteristics. The paddle blade is fixed to the bottom of the shaft and rotates at a height of 25 mm from the inner bottom of the vessel. The paddle consists of a metallic or suitably inert, rigid blade and shaft composing a single entity. The paddle blade and shaft may be coated with a suitable inert material.

The paddle is lowered into a 1000-mL vessel and rotated at a specific speed within media, which is maintained at a specific temperature. The rotating paddle method is routinely used at an agitation speed of 25 to 75 rpm. Rates outside a range of 25 to 75 rpm are generally unacceptable because of irreproducibility of the hydrodynamic effects below 25 rpm and turbulence above 100 rpm. High turbulence in the vessel leads to a loss of discriminatory power associated with the method. Agitation rates around 25 rpm but less that 50 rpm are acceptable for suspensions. For solid dosage forms with excessive coning, rotational speeds around 75 rpm may be necessary to improve the data. As with any variance from normal operating parameters, atypical conditions must be supported with data from normally accepted conditions for justification of USP Apparatus 2. When dissolution profiles exhibit inappropriately dissolving drug substance during method development, adjustments outside the normal rotational speed may be warranted. Any time a method references *USP* General, the dosage unit must be allowed to settle to the bottom of the vessel before rotation of the paddle begins. To aid the dosage unit settling to the bottom of the vessel, a small, loose piece of stainless steel wire consisting of a few turns may be attached to a dosage unit that would otherwise float.

Figure. Examples of Apparatus 2 paddles.

Since the construction of the sinker has such an impact on the hydrodynamics in the bottom of the vessel, individuals have sought to standardize the USP design. A unique standardization utilizing cork borers was presented in the *PharmacopeialForum* as a stimuli article. The guidance suggested a method to construct sinkers by hand with the use of a cork borer which would minimize the variability resulting from different interpretations of the construction of a USP sinker. In addition to sinking floating dosage forms, sinkers may assist in keeping a dosage form from sticking to the vessel inappropriately as in the case with some film-coated tablets. Sinkers must be adequately described in laboratory standard operating procedures to eliminate hydrodynamic variation associated with different sinker devices. Sinkers of other descriptions may be used if properly validated. Many different sinkers have evolved, some of which are based on the wire helix design.

Current Physical Parameters and Tolerances:

Wobble	Not specified in USP
Dimensions	per USP
Height	25 ± 2 mm
Centering	±2 mm center line
Speed	± 4% of set speed
Vessel Temp.	37 ± 0.5 C
Time points	± 2% of specified time

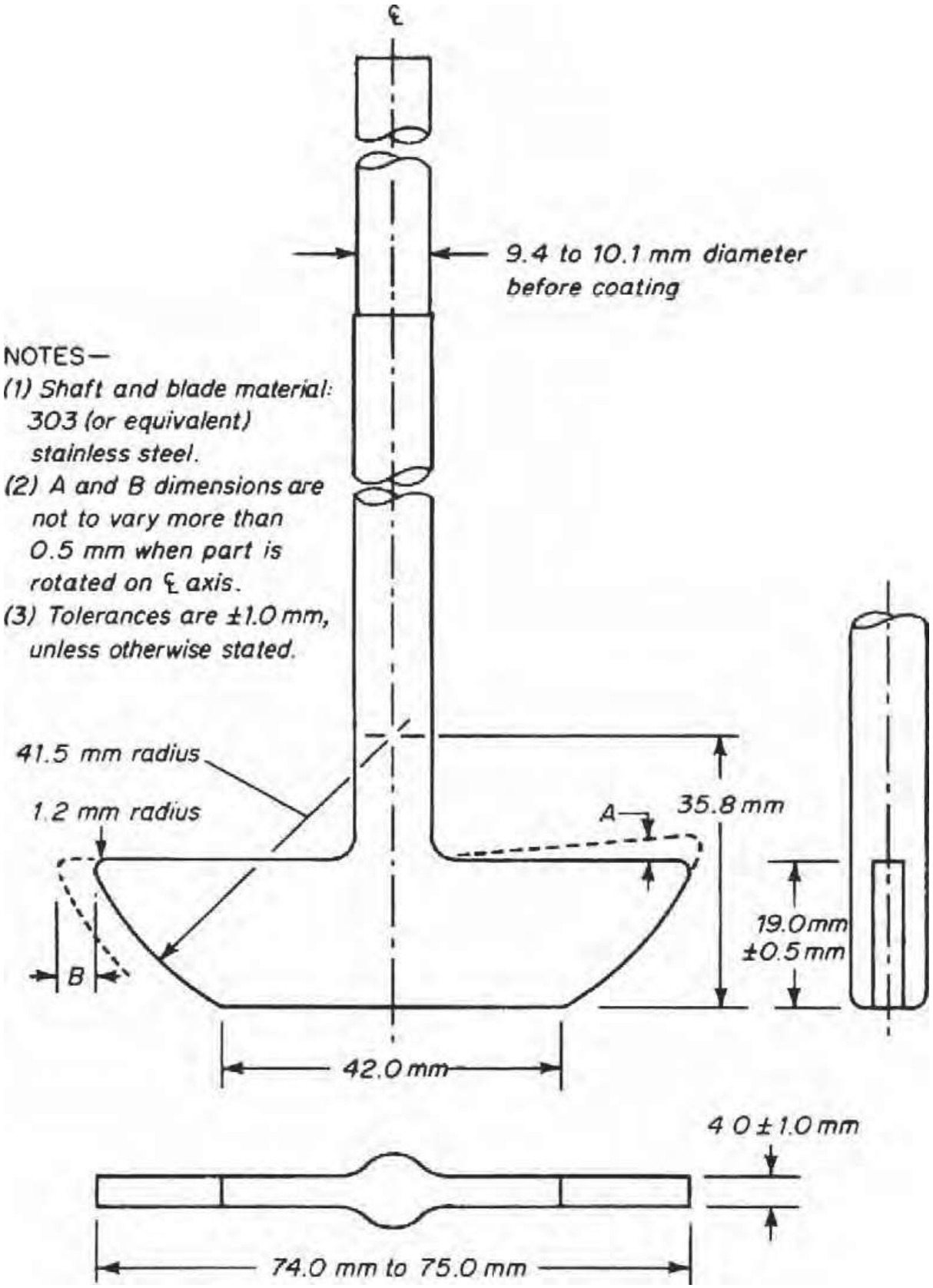


Figure. USP Apparatus 2 paddle specifications.

As mentioned in the previous section on physical parameters for basket, some laboratories have adopted more stringent parameters for the paddle apparatus to maintain a higher degree of control.

OPTIONAL PARAMETERS AND TOLERANCES:

Shaft wobble	≥ 0.5 mm total runout
Paddle exam	No defects at time of use
Shaft verticality	Vertical using bubble level
Speed	±2 RPM of set speed
Vibration	≤ 0.2-mil displacement

Typical products tested by the paddle method are tablets, capsules (with sinkers), hydrogel tablets, suspensions, powders, microparticles, and transdermals (paddleover- disk method).

- **CALIBRATION OF USP APPARATUS 1 AND 2**

In the early 1970s, scientists began to evaluate significant apparatus-to-apparatus differences in dissolution results. Monograph specifications could not be enforced due to variability in results from lab to lab and apparatus to apparatus. In 1978, the USP established and issued the first official dissolution calibrator tablets and reference standards. The primary purpose was to control vibration since most other parameters could be controlled by mechanical measurements. Since that time, dissolution apparatus used under current Good Manufacturing Practices (cGMPs) should be challenged with an apparatus suitability test as outlined in the *USP* Dissolution. At the time of this printing, the suitability test must be conducted with USP dissolution calibrators of the disintegrating and non-disintegrating type, Prednisone and Salicylic Acid tablets, respectively. The word calibration is somewhat of a misnomer since the ‘‘calibrator’’ tablets do not actually calibrate anything, as a weight would be used to calibrate an analytical balance. At this time there is no predefined period of calibration; however, our current Good Manufacturing Practices as outlined in 21 CFR Parts 210 and 211 require calibration of analytical equipment according to prescribed schedules. These prescribed schedules are generally established by various organizations to limit the liability originating from test results obtained on apparatus that may fall out of calibration as a result of age, environmental factors, or relocation. In the event of obtaining an outside-of-target dissolution result or a suspect result during calibration or routine analysis, a detailed review

of the equipment, method, materials, and analyst documentation should take place. The investigation should be thoroughly documented and include all observations and explanations for the aberrant result by showing a cause-and-effect relationship, corrective action, and eventually the retest. One change at a time should be made prior to retesting to isolate the cause of the aberrant result. Recheck all physical parameters after any adjustments are made and perform retesting on a set of six tablets.

A laboratory review checklist consisting of the following areas should be implemented to perform a thorough evaluation: check calculations, reread samples that were non-conforming, examine spectrophotometer and any automatic sampling equipment, review sampling technique, review standard preparation, review media preparation. Documentation of the investigation should include a description of the failure with a full data summary, the laboratory review checklist, description of the findings, corrective actions, additional physical adjustment of the apparatus, specific reasons for the run to be invalidated such as a crack in the vessel, and the retest(s).

- **ABERRANT DISSOLUTION DATA INVESTIGATION**

The most common sources of error in dissolution testing for the rotating basket and paddle methods are deaeration, paddles and baskets conformance, condition of vessels, vibration and environmental issues, sampling technique, and filtering issues.

- **DEAERATION**

While numerous deaeration techniques have been utilized, some are better than others. The USP recommends heating media to approximately 41 C followed by vacuum filtration through a 0.45- m filter under vigorous stirring. After filtration, continue to draw the vacuum for five additional minutes. In theory, the best way to remove dissolved gases is through boiling, but this is a waste of energy and time resources. However, heating media to 41 C and applying a vacuum can achieve boiling at a lower temperature. The filter is simply used to provide a pressure gradient. Once the media passes through the filter, air is immediately and efficiently stripped out. Media is then measured and gently poured into the dissolution vessel and allowed to equilibrate to 37 C in the vessel. Alternate deaeration techniques have been used such as helium sparging or vacuum ultra-sonication. Critical parameters for helium-sparing methods include gas flow rate, type of diffuser, and time per volume. The efficiency of alternate deaeration techniques must be demonstrated and documented through validation.

Historically, media has been measured and gently added to the vessel with the aid of a cylinder or other calibrated ‘‘to deliver’’ device. Media should be delivered with an accuracy of 1%, which currently rules out most ‘‘class A’’ graduated cylinders because they are calibrated in 10-mL increments and are not capable of measuring the 9-mL tolerance for the typical 900-mL media volume. Alternately, media could be measured in a ‘‘to deliver’’ class A volumetric flask if it has been calibrated at the intended measuring temperature. An alternate media-measuring technique employed by many automated systems is gravimetric measurement.

Dissolution media at a controlled elevated temperature may also be weighed by correcting for pre-determined density. This is how many automated delivery systems measure and transfer media to the vessel.

- **VIBRATION**

For dissolution equipment to operate correctly, the area must be maintained free from excessive vibration from sources such as centrifuges, vacuum pumps, fume hoods, shakers, ultrasonic cleaners, and unstable bench top and construction. All such sources of external vibration must be eliminated. Internal sources of vibration may be caused by tension or dirt on drive belt, worn parts and bearings, and turbulence in the water bath. Make sure the deflector shield is in place in the water bath.

Preferably use a vibration meter during calibration periods to obtain a baseline measurement. If suspect or aberrant dissolution results are obtained on the apparatus, current vibration measurements may be compared to the level obtained during calibration to see if this could have contributed to the suspect data. Vibration measurements should be part of the routine physical calibration of the apparatus to detect vibration from unwanted sources prior to obtaining data.

- **SAMPLING AND FILTERING**

The filter is essential to stop the dissolution test by removing undissolved drug product as well as particulate matter and turbidity from the sample. Filters must be tested for drug adsorptivity to show that they do not bind drug substance. Filters should also be evaluated for efficiency to demonstrate that drug substance did not pass through the filter and continue to dissolve. Separate, clean, dry filters and glassware must be used when sampling each vessel. Generally, the first several milliliters should be discarded prior to sample collection for analysis but the specific amount discarded must be determined through validation. Sample

aliquots must be filtered immediately after the sample is drawn, otherwise the dissolution process continues.

C. BASKETS AND PADDLES

Basket and paddle stirring elements must be checked for USP conformance. Routine physical observations of baskets and shafts should be conducted to ensure integrity of the stirring element. Physical observation of basket and shafts should include that they are straight and roll evenly on a flat surface. Any Teflon coating must not be chipped or peeling, which adds to the turbulence in the vessel. Check surfaces for corrosion or discoloration due to prolonged exposure to hydrochloric acid. Stainless steel, while resistant to rust and corrosion, will be attacked by chloride ions, which will cause pitting in the surfaces and a reduction in the wire diameter used in the baskets. A basket will maintain specifications of 40-mesh unless it is misshapen, but the basket micron rating will change over its lifetime due to corrosion. A non-lustrous pewter appearance is an indication that the basket integrity is failing. Particles may fall out of the basket too early causing lower results if the wire diameter is significantly reduced due to corrosion. Gold coating up to 2.5- m thick is an allowable variation for baskets to inhibit corrosion associated with a stainless steel basket. The basket surface must be smooth, not wrinkled or misshapen, and must not have a frayed appearance; the basket should be replaced, if necessary.

USP basket clips must be tight since loose clips impart excessive wobble. Non-

USP baskets such as o-ring attachments without clips must be validated to show that there is no change in test results. USP Prednisone calibrator tablets run in o-ring baskets have exhibited up to a 10% suppressed result over the USP clip type basket.

Regarding air bubbles, several observations should be made when starting a dissolution run. Bubbles occasionally form underneath the disk and sometimes hold a tablet in the upper portion of the basket and do not allow one side of the dosage unit to contact the media for several minutes. If this is not noticed, a non-disintegrating dosage form similar to the salicylic acid calibrator tablet will produce results on the low side of the expected range. Bubbles that form under a basket will alter the performance of the basket and cause failures since dissolution media will not circulate through the basket properly. Bubbles forming in the mesh will also change the characteristics of the basket by blocking the openings and virtually changing the mesh of the basket. The latter condition is usually a result of poor media deaeration.

- **VESSELS**

Dissolution vessels should be serialized or numbered and maintained in their original positions. This reduces the opportunity for a defective vessel to be moved from one apparatus to another, causing random failures. The inner surface of vessels should be routinely checked for irregularities, scratches, cracks, pits, and unevenness or surface aberrations. Vessels should be acquired from a reputable manufacturer since the inner surface must have a defect-free hemispheric bottom. Vessels manufactured with poor quality control will exhibit shallow, protruding, or asymmetrical bottoms, which will greatly affect the dissolution results due to increased turbulence.

Vessels need to be checked for cleanliness. Scum, film, or sticky residues build up over time and can greatly affect dissolution rates. Vessels must be scrupulously clean. Tablets are to be dropped into non-rotating medium and allowed to settle to the bottom of the vessel prior to starting the rotation of paddles. Observe the dosage unit after introduction and record unusual observations such as sticking, floating, bubbles, and irregular shape of the cone.

Figure. Cone formation (12a) and Peak vessel configuration (12b).

- **NON-COMPENDIAL VARIATIONS**

Several unofficial modifications have developed to provide advantages over traditional dissolution apparatus. These have been occasionally implemented to improve the hydrodynamics of the dissolution test but only when the modification has proven to be superior to traditional dissolution apparatus.

- **PEAK VESSEL**

The peak vessel reduces the inherent inconsistencies in the hydrodynamics of standard hemispherical dissolution vessels. An inverted peak is incorporated into the bottom of the vessel, displacing the unstirred zone, preventing cone formation.

- **SMALL-VOLUME DISSOLUTION**

To maintain quantitative levels of analyte during the dissolution test, a reduction in vessel volume accompanied by an alteration in apparatus design may be required. For oral dosage units containing concentrations of analyte at the microgram or nanogram level resulting from highly potent, low dose compounds, small-volume dissolution apparatus may be required and are generally quantitation limited. The justification for the small-volume dissolution apparatus is primarily due to the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

- **MEGA PADDLE**

Another device that was introduced to overcome some of the hydrodynamic anomalies associated with the dissolution test is the mega paddle. The mega paddle was introduced as a stimuli article in the *Pharmacopeial Forum*.

FIGURE. MEGA PADDLE.

Its primary purpose was to improve the mixing characteristics within the vessel, eradicate coning, and produce better stirring from a low energy system that will not cause particle shear. The mega paddle may also be useful for 2-L and 4-L vessels where greater fluid movement is required. This modified paddle has not been as widely accepted as the peak vessel for improving hydrodynamics within the vessel.

- **STATIONARY BASKET**

The stationary basket assembly is used with traditional rotating paddle apparatus. This modification suspends the dosage unit contained in a basket device just above the rotating paddle. Several variations of the stationary basket have evolved. One system utilizes a basket held in place by a disk with clips similar to the rotating basket apparatus with the exception

that a 0.25-inch shaft mounts the stationary basket to the evaporation cover. In addition to a standard USP 40-mesh basket, 10- and 20-mesh baskets have been used. Another variation was introduced in the *USP26 First Supplement* for a Felodipine monograph. This design utilizes a quadrangular basket of stainless steel wire gauze, which is suspended 1 mm above the rotating paddle.

➢ QUALIFICATION OF NON-COMPENDIAL EQUIPMENT

While no USP specifications or calibration procedure are available for small and large volume vessels, peak vessels, mega paddles, or stationary baskets, the pertinent physical characteristics should be measured. Detailed specifications of the modified equipment must be documented and reliable sources for the equipment should be available. Apparatus should be calibrated with one-liter vessels to indicate that the apparatus is suitable under standard conditions. Physical parameters of height, centering, speed, wobble, and temperature need to be measured against current USP criteria and documented.

Non-compendial dissolution apparatus are only to be used in extraordinary conditions with demonstrated and documented superiority over conventional ‘‘official’’ dissolution apparatus. Non-compendial dissolution apparatus should also demonstrate discrimination of variation from batch to batch, utilize sufficient volume to be analytically quantifiable, be rugged (transferable), precisely and reproducibly manufactured, and commercially available. Additional calibration regimens should be implemented whenever practical.

D. USP APPARATUS 3 AND 4—RECIPROCATING CYLINDER AND FLOW-THROUGH CELL

These apparatus are found in *USP* Physical Test Chapter 724 Drug Release. USP Apparatus 3 is called the reciprocating cylinder apparatus, and USP Apparatus 4 is called the flow-through cell.

➢ RECIPROCATING CYLINDER GENERAL APPARATUS 3 DESCRIPTION

A presentation at the 1980 Federation International Pharmaceutique (F.I.P.) drew attention to acute problems associated with USP Apparatus 1 and 2 dissolution results. The conference inspired the concept for the USP Apparatus 3. The USP Apparatus 3, also known as the Bio-Dis, is an excellent apparatus for developing controlled-release products because it can quickly and easily expose products to mechanical and physiochemical conditions which may influence the release of the products in the GI tract. The Bio-Dis Extended Release Tester was designed to test the dissolution rates of extended-release products or any dosage form

requiring release profiling at multiple pH levels. The capability for product transfer from one pH to another makes it an excellent candidate for delayed-release products.

USP Apparatus 3 has seven inner tubes, which mechanically traverse six rows of corresponding, media-filled outer tubes. Six of the tubes are for testing drug product while the seventh row is maintained for blank media or standard solutions. The reciprocating cylinder apparatus consists of sets of cylindrical, flat-bottom glass vessels with corresponding sets of reciprocating cylinders. A motor and drive assembly reciprocate the cylinders vertically inside the vessels. The cylinders are allowed to move from row to row to expose the un-dissolved drug product to various pH levels.

When the reciprocating cylinder test begins, the inner tubes descend slowly into the first row of the vessels, then the reciprocating motion starts. After the programmed time for this row expires, the inner tubes rise above the vessels, drain for the programmed time, and automatically move to the next row. The process is repeated for each row.

The reciprocating cylinders are glass tubes fitted with top and bottom caps containing screens designed to contain the product under evaluation. As the cylinder reciprocates vertically the drug product is constantly exposed to media contained in the vessel. The 300-mL outer tubes remain in contact with the water bath to maintain the medium temperature at 37 C.

The seventh position of the reciprocating cylinder apparatus may be modified for each row to contain a blank and standard at the specified pH, to be sampled for automated analysis.

CURRENT PHYSICAL PARAMETERS AND TOLERANCES:

Temperature	37 ± 0.5 C
Dip rate (DPM)	±5% of set speed
Stroke Distance	10.0 ± 0.1cm
Bottom screen	per method
Top screen	per method (optional)
Time points	± 2% of specified time

- **USP 3 CALIBRATION REQUIREMENTS**

The reciprocating cylinder apparatus does have a calibration program as outlined in the *USP* utilizing Chlorpheniramine Maleate tablets as the single-unit calibrator.

- **FLOW-THROUGH CELL GENERAL APPARATUS DESCRIPTION**

The flow-through cell was primarily developed for poorly soluble solid dosage forms. Limitations of volume and pH change associated with traditional rotating paddle and basket apparatus prompted the development of the flow-through cell.

The flow-through cell is made up of three transparent parts, which fit into each other. The lower part consists of two adjacent chambers connected to an overflow device.

The dissolution media passes into the lower part of the chamber then flows upward to an exit in the upper chamber, which leads to a filter assembly. The middle part of the cell has a cavity and holder designed to contain the dosage unit. Typical flow rates from 4 mL/min up to 16 mL/min are traditionally used.

With a flow-through system, the specimen is placed in a small column, which is continuously flushed with a stream of fluid, simultaneously providing the medium and the mechanical agitation for dissolution of the drug substance.

USP Apparatus 4 can be run as an open or closed system. The open system provides a large volume of multiple solvents as needed. An open system provides media from one or multiple pH sources and collects separate discrete samples as they elute from the flow-through cell.

The closed systems circulate the media through the cell from a reservoir. A closed system with a small media reservoir could reduce volume to below 100 ml for low-dose compounds.

Products that may be tested in Apparatus 4 include extended-release dosage forms, beads, suppositories, powders, and implants.

CURRENT PHYSICAL PARAMETERS AND TOLERANCES:

Temperature	37 ± 0.5 C
Pump rate	240–960 mL/hr
Std flow rates	4, 8, 16 mL/min
Flow rate accuracy	± 5%
Pulsation	120 10 pulses/min
Cell Diameters	12 and 22.6 mm

E. USP APPARATUS 5, 6, AND 7—PADDLE OVER DISK, ROTATING CYLINDER AND RECIPROCATING HOLDER APPARATUS

These apparatus were originally designed and are primarily used for the analysis of transdermal delivery systems. However, the reciprocating holder apparatus has been utilized for a number of extended-release products other than transdermal delivery devices. These apparatus are fully described in the *USP* (*6*) as follows: paddle over disk as USP Apparatus 5, the rotating cylinder as USP Apparatus 6, and the reciprocating holder as USP Apparatus 7.

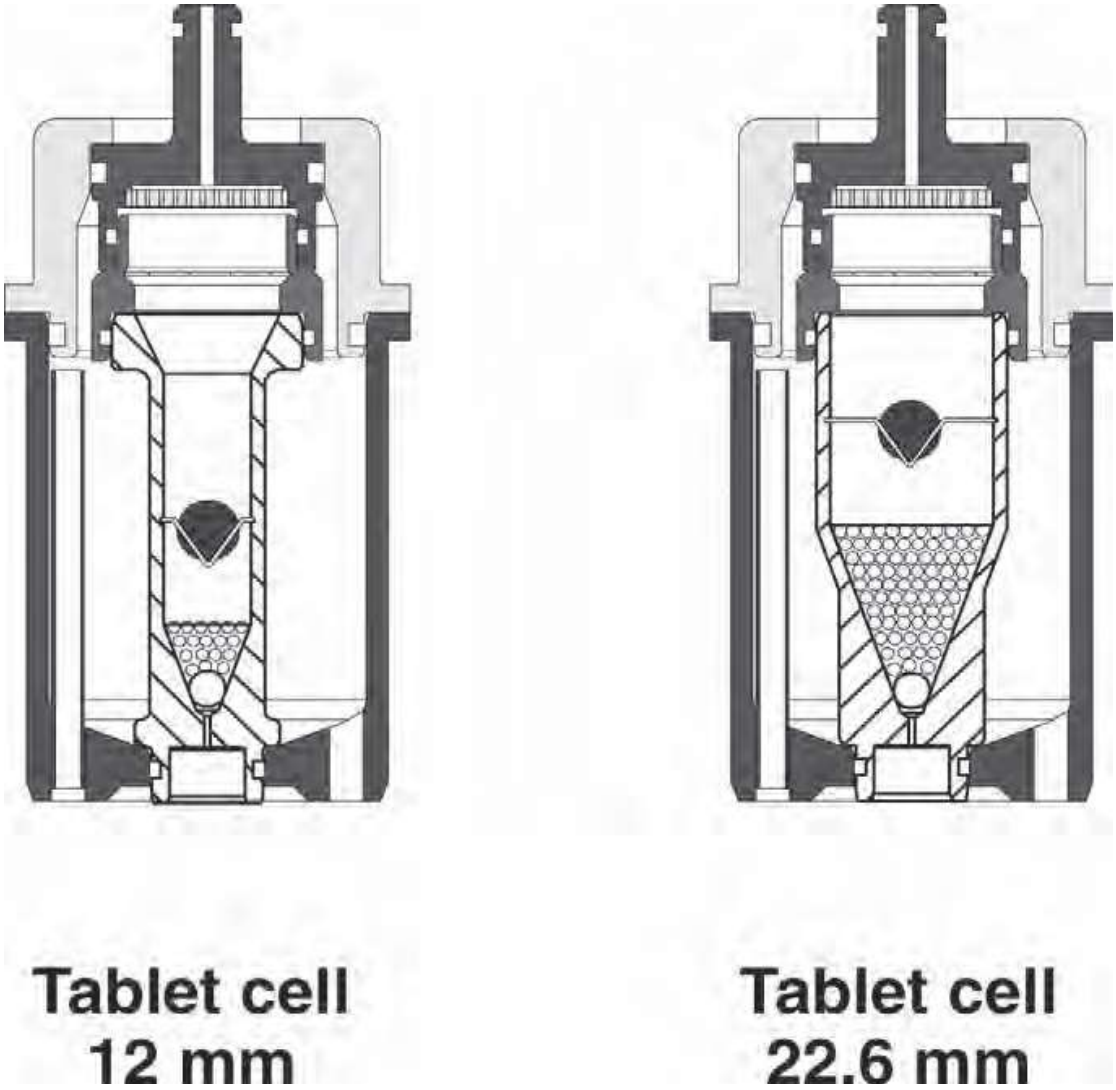


FIGURE. USP APPARATUS 4: FLOW-THROUGH CELLS.

The analysis of transdermal delivery systems occurs typically at 32 C, similar to the temperature of the skin. Before the 1980s, the skin was seldom regarded as a suitable route for administration of drugs to systemic circulation. However, the transdermal route offered several potential advantages for the systemic delivery of drugs. Drugs with narrow

therapeutic indices can be good candidates for transdermal route because of the absence of the peak-and-valley feature associated with the in vivo absorbance of conventional oral dosage forms. Transdermal systems provide controlled blood levels of potent drugs. Lastly, because of its noninvasive delivery, a transdermal patch may be removed easily if toxicity or side effects Constant surface method in the form of the modified Woods apparatus, which compresses the active pharmaceutical ingredient into a disk of known surface area (0.5cm2). The Woods apparatus is found under *USP* General Chapter 1088 Intrinsic Dissolution (*7*). The dissolution rate obtained by this method is termed the *intrinsicdissolution rate*, and is characteristic of each solid compound in a given solvent under the fixed experimental conditions. The modified Woods apparatus consists of a punch and die which contains the compressed pellet of bulk drug substance.

The die is attached to the holder, which is inserted into the dissolution apparatus capable of holding the device. The test begins by lowering the intrinsic device containing the drug substance into the dissolution vessel and rotating.

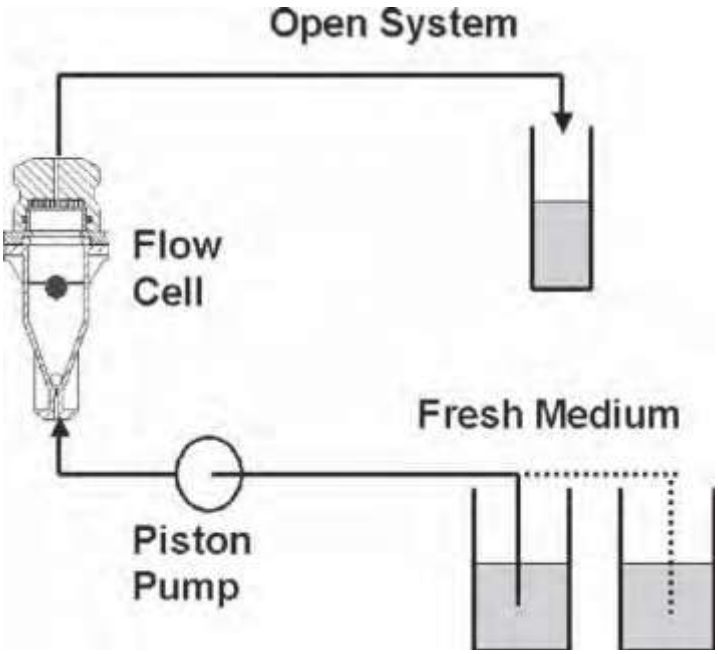


FIGURE. USP APPARATUS 4 OPEN SYSTEM.

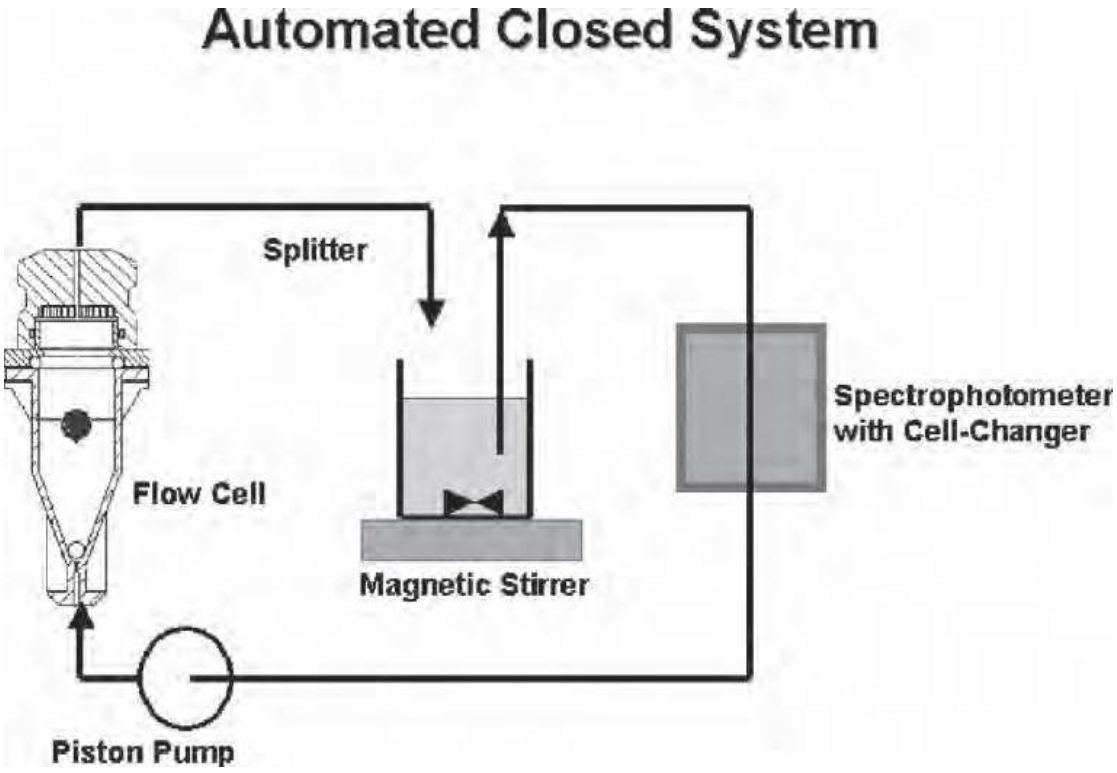


FIGURE. USP APPARATUS 4 CLOSED SYSTEM

Samples are obtained to a point where at least 10% of the drug contained in the device has dissolved. The intrinsic dissolution may be calculated by plotting the cumulative amount of the drug substance dissolved per the constant surface area against time until 10% has dissolved. The cumulative amount of drug substance dissolved for a specific area is obtained by dividing the amount dissolved at each time point by 0.5 cm2. Performing a linear regression on the data points up to the point that 10% has dissolved will yield the intrinsic dissolution rate from the slope of the regression line. The value obtained is based on the Noyes-Whitney equation and is generally expressed as milligrams dissolved per minute per centimeter squared (mg/min/cm2). Intrinsic rates greater than 1.0 mg/min/cm2 have negligible problems with dissolution rate limitations, but rates less than 0.1 mg/min/cm2 suggest problems with dissolution rate.

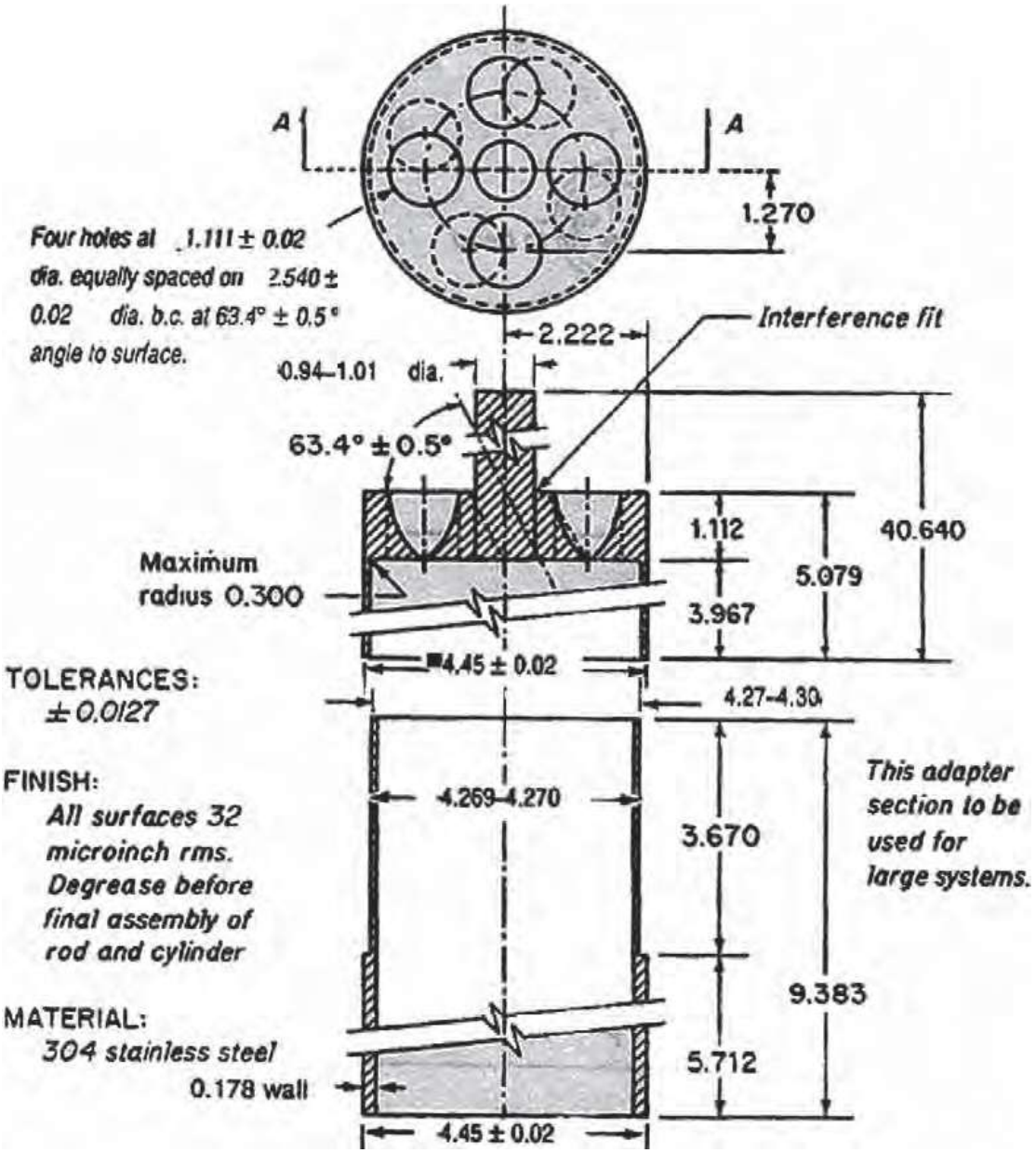


FIGURE. USP APPARATUS 6: ROTATING CYLINDER.

Centering	± 2 mm centerline
Speed	±4% of set speed
Vessel Temp.	32± 0.5 C
Time points	± 2% or 15 min of the specified time (lesser)

F. ROTATING CYLINDER

This apparatus utilizes the typical dissolution apparatus and vessel configuration with the exception of rotating cylinder stirring elements that are used in place of paddle or basket stirring elements. The shaft consists of a stainless steel cylinder stirring element with a removable extension, which is used for larger transdermal systems.

The dosage unit is placed on the cylinder at the beginning of each test. The distance between the inside bottom of the vessel and the cylinder is maintained at 25± 2 mm during the test.

To attach the transdermal system, remove the system from the package and remove the protective liner from the system. Place the adhesive side on a piece of Cuprophan that is not less than 1 cm larger on all sides of the system perimeter.

Place the system on a clean surface with the Cuprophan side down. Apply a suitable adhesive (Dow Corning, 355 medical adhesive 18.5% in Freon 113, or equivalent) to the exposed Cuprophan borders and back, if necessary. Carefully apply the adhesive-coated side of the system to the exterior of the cylinder with the long axis of the system fitting around the circumference of the cylinder.

The use of a soft pad or a computer mouse pad will help to attach the system to the cylinder without trapping air bubbles or damaging the patch. Place the cylinder in the dissolution apparatus, lower into the media, and immediately rotate at the specified speed.

CURRENT PHYSICAL PARAMETERS AND TOLERANCES:

Dimensions	per USP
Height	25 ± 2 mm
Centering	± 2 mm centerline
Speed	± 4% of set speed
Vessel Temp.	32 ± 0.5 C
Time points	±2% or 15 min of the specified time (lesser)

- **RECIPROCATING HOLDER**

Also known as the ‘‘Alza apparatus’’ and USP Apparatus 7, the reciprocating holder apparatus has evolved to handle not only transdermal products, but also other sustained-release products. The apparatus utilizes sets of volumetrically or gravimetrically calibrated

tubes and a mechanical device capable of reciprocating the specific holders vertically in the tubes containing dissolution medium. Typical vessel volumes of 50–75 mL are used. Vessels are usually 50 mL with an operational minimum around 25 mL. Adaptations have been made to accommodate 100- and 300-mL vessels. Products typically tested in the reciprocating holder apparatus are transdermal delivery systems, osmotic pumps, and other non-disintegrating extended- release dosage forms such as implants and drug-eluting stents.

The holder apparatus consists of five different reciprocating holder configurations.

The disk, cylinder, and angled disk are used for transdermal delivery systems.

The pointed acrylic rod operates with an osmotic pump dosage unit or implant glued to the tip. The spring holder contains a non-disintegrating dosage unit or osmotic pump as it reciprocates vertically in the vessel. Illustrations of the vessels and reciprocating holders are shown in Figure 28.

CURRENT PHYSICAL PARAMETERS AND TOLERANCES:

Temperature	32 ± 0.5 C
Dip rate	30 DPM
Stroke Distance	2 cm
Holder	per USP
Time points	±2% of specified time

- **INTRINSIC DISSOLUTION**

During the development process for new oral dosage pharmaceutical products, drug substances must be evaluated to show consistent physical characterization as represented by their dissolution rate constants. The drug substances, sometimes available from multiple techniques and sources, must demonstrate uniformity in the manufacturing process as well as in their physiochemical properties. The intrinsic dissolution rate is defined as the dissolution rate of pure substances under the condition of constant surface area. Intrinsic dissolution apparatus utilizes a constant surface method in the form of the modified Woods apparatus, which compresses the active pharmaceutical ingredient into a disk of known surface area (0.5 cm2). The Woods apparatus is found under *USP* General Chapter 1088 Intrinsic Dissolution. The dissolution rate obtained by this method is termed the *intrinsicdissolution rate,* and is characteristic of each solid compound in a given solvent under the fixed experimental

conditions. The modified Woods apparatus consists of a punch and die which contains the compressed pellet of bulk drug substance. The die is attached to the holder, which is inserted into the dissolution apparatus capable of holding the device. The test begins by lowering the intrinsic device containing the drug substance into the dissolution vessel and rotating.

Samples are obtained to a point where at least 10% of the drug contained in the device has dissolved. The intrinsic dissolution may be calculated by plotting the cumulative amount of the drug substance dissolved per the constant surface area against time until 10% has dissolved. The cumulative amount of drug substance dissolved for a specific area is obtained by dividing the amount dissolved at each time point by 0.5 cm2. Performing a linear regression on the data points up to the point that 10% has dissolved will yield the intrinsic dissolution rate from the slope of the regression line. The value obtained is based on the Noyes-Whitney equation and is generally expressed as milligrams dissolved per minute per centimeter squared (mg/min/cm2). Intrinsic rates greater than 1.0 mg/min/cm2 have negligible problems with dissolution rate limitations, but rates less than 0.1 mg/min/cm2 suggest problems with dissolution rate.

G. ADDITIONAL ‘‘UNOFFICIAL’’ APPARATUS: ROTATING BOTTLEAPPARATUS AND DIFFUSION CELLS

➢ ROTATING BOTTLE

Approximately ten years before the development of the rotating paddle and basket apparatus, the rotating bottle method was developed to study timed-release formulations. The original chapter in the *National Formulary* where references are made to the operation of the equipment is entitled *Timed-Release Tablets andCapsules In Vitro Test Procedure.* Originally, the apparatus used various ‘‘extracting fluids’’ composed of a mixture of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) over five pH ranges: 1.2, 2.5, 4.5, 7.0, and 7.5.

The apparatus is constructed to hold sets of bottles, 150 mm in length with a 30-mm diameter, containing a dosage unit and attached to a rotating bar while submersed in a water bath to maintain the extracting fluid within the bottle at 37.0 C.

After specified periods of time, the apparatus is removed from the water bath, the bottles are removed, and samples are generally acquired through total media exchange.

After samples are taken and 37.0 C media added back to the vessels, the bottles are recapped, reattached to the apparatus, and the apparatus is lowered back into the water bath.

The rotating bottle apparatus was first introduced as an official apparatus in the *United States National Formulary* but was not carried over to the *United StatesPharmacopeia,* which presently contains the seven ‘‘official’’ dissolution apparatus used in the United States. While it is no longer an official apparatus, it does possess several distinguishing characteristics. It is the only dissolution apparatus that has no evaporative loss and therefore can run for extended periods of time without loss of media. This is especially helpful for implants, which may need to run for days or even weeks to acquire in vitro dissolution data for regulatory approval or even quality control over the manufacturing process. The rotating bottle method is a labor-intensive method and this may be part of the reason that it has never become as popular as other, more efficient and easy to use apparatus.

- **DIFFUSION CELLS**

Over the past decade, the use of an in vitro release test to evaluate drug release from semisolid formulations has received increased attention. Two in vitro apparatus have evolved over this period, the Franz Cell and the Enhancer Cell. Both systems have been developed to monitor formulation performance or changes in batch-to-batch uniformity. Both apparatus produce similar results in response to formulation changes, but preference to using one device over the other depends on the application technique desired. The methodology consists of either diffusion cell, an appropriate synthetic membrane, appropriate receptor phase, at least 5 data points over a six-hour period to determine the release rate, and an analytical method to determine the concentration at a given time. These diffusion cells should be capable of providing manufacturing process control and ultimately a quality control test to ensure batch-to-batch uniformity. The diffusion cells are also quite useful in the development of transdermal systems. Both diffusion systems have various surface sizes depending on the analytical need.

- **FRANZ CELL**

The Franz cell is a vertical diffusion cell system developed by Dr. Thomas Franz. It has been used historically to determine the release of active from topical preparations and has been modified to analyze potential transdermal drug delivery formulations.

The glass cell system consists of a receptor chamber, donor chamber with clamp, sampling and media replacement ports, temperature-controlled heating jacket, and a stirrer. The Franz

cells may be sampled manually or automatically. A top cap seals the donor compartment from air to minimize reverse diffusion associated with automatic samplers.

- **ENHANCER CELL**

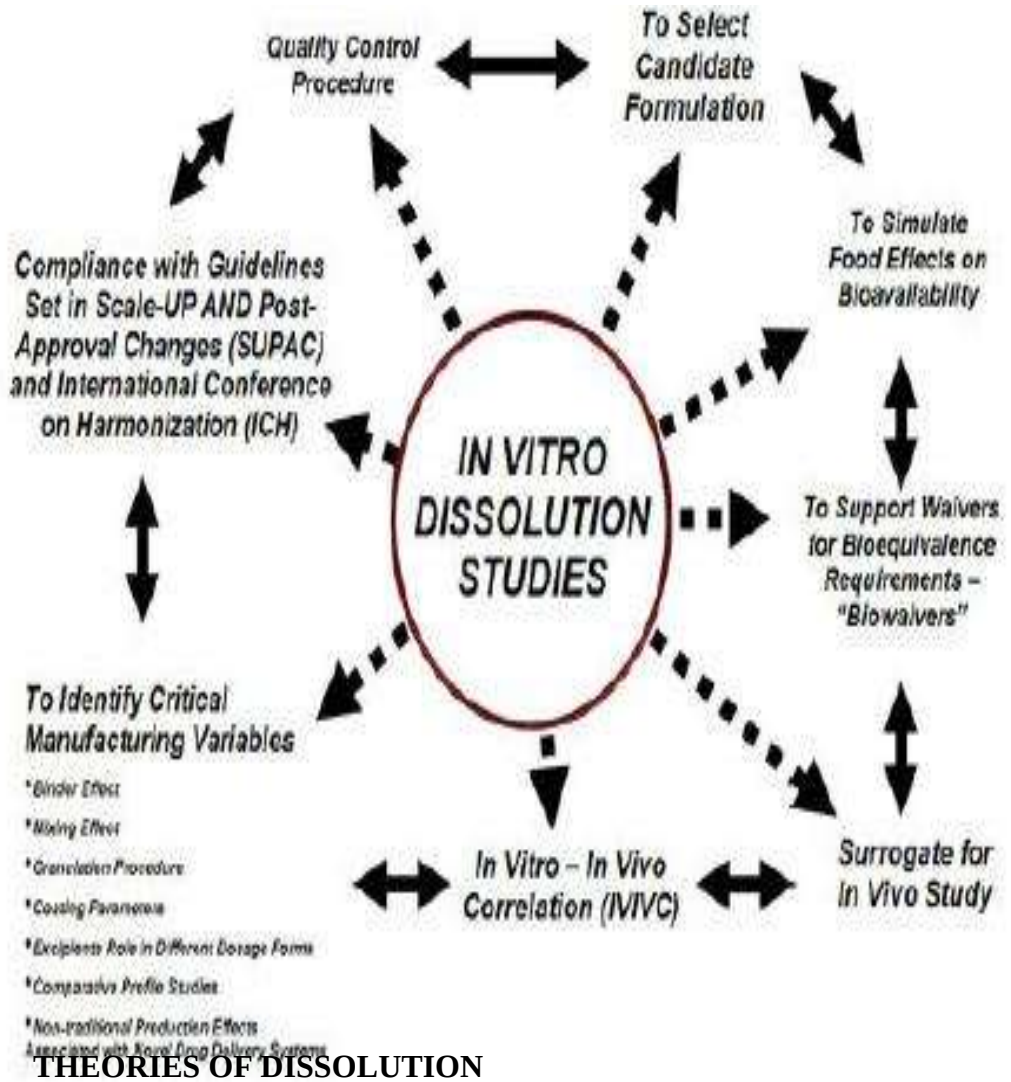
The Enhancer Cell is a device that can be used to study the drug release profiles of topical formulations. It is made of Teflon, an inert and non-reactive material. The main idea behind the development of the enhancer cell was to develop a simple, affordable, reliable, and reproducible quality control method that could be used to discriminate variations in the release characteristics of topical dosage forms.

The Enhancer Cell consists of a cap, a washer, membrane, an o-ring, and a drug reservoir. The outer diameter of the body and the solid ring are identical to theinner diameter of the cap, which aids in keeping the membrane in place whiletightening the cell. A typical six-spindle dissolution tester may be used. The apparatusmust be modified to hold 200-mL capacity vessels instead of the standard900-mL vessel. It is essential to use smaller receptor volumes to obtain sampleswith detectable concentration of drug for HPLC analysis. Additional requiredequipment includes an adapter plate to position the vessel in the center, an evaporationcover, a smaller-sized shaft, and collet to hold the shaft firmly in place.

The operational minimum volume is about 50 mL. Transdermal membrane candidates may also be evaluated with the Enhancer Cell. Regarding the qualification and use of ‘‘unofficial’’ dissolution apparatus, the FDA encourages the development and use of the most appropriate instrumentation. However, the use of rare or exotic systems not only places undue burden on the regulatory laboratory, but also may delay the approval process for new drug products. When noncommercial instrumentation is used, the instrumentation should be capable of being constructed from commercially available components at a reasonable cost, if possible. For unique methodologies or instrumentation requiring contract fabrication, the applicant’s cooperation with the FDA laboratories in helping facilitate duplication of the analytical equipment and procedure is important.

In addition to design and equipment specifications, complete performance assessment procedures should be provided. Such systems may be found suitable for regulatory use. However, unnecessary proliferation of alternative dissolution apparatus should not be encouraged, and alternatives or modifications to established dissolution apparatus should be considered on the basis of proven superiority for a particular product.

- **APPLICATION OF DISSOLUTION STUDIES**



Applications of in vitro dissolution studies.

H. THEORIES OF DISSOLUTION

1. Diffusion Layer Model (Film Theory)
2. Danckwert's Model (Penetration or Surface Renewal Theory)
3. Interfacial Barrier Model (Double Barrier Mechanism OR Limited Solvation Theory)

DIFFUSION LAYER MODEL (FILM THEORY):

It is a simplest model where dissolution of crystal, immersed in liquid takes place without involving reactive or electrical forces. Consist of two consecutive steps:

Solution of the solid to form a thin film or layer at the solid/ liquid interface called as stagnant film or diffusion layer which is saturated with the drug this step is usually rapid (instantaneous).

Diffusion of the soluble solute from the stagnant layer to the bulk of the solution this step is slower and is therefore the rate determining step in the drug dissolution. The model is depicted in following fig.

Fick's law covers only diffusions under steady state conditions. Modifying it Noyes & Whitney established another equation

$$\frac{dC}{dt} = k\,(Cs - Cb) \quad \text{——————} \quad (A)$$

$\frac{dC}{dt}$ = dissolution rate of the drug

k = dissolution rate constant (first order)

Cs = conc. of drug in stagnant layer(saturation or max. drug solubility)

Cb = conc. of the drug in bulk of the solutionat time t

Brunner &Tolloczko incorporated surface area 'A' in Noyes & Whitney Equation.Dc/dt = k1A (Cs – Cb). Afterwards Brunner, incorporated Fick's law of diffusion & expanded his given eq. to include diffusion coefficient 'D', thickness of stagnant diffusion layer 'h' & volume of dissolution medium 'v'.

$$\frac{dC}{dt} \quad \frac{D\,A\,k_{w/o}\,(Cs - Cb)}{V\,h} \quad \text{————} \quad (B)$$

D = difussion coefficient of the drug

A = surface are of dissolving solid

$k_{w/o}$ = water / oil partition coefficient of the drug considering the fact that dissolution body fluid are aqueous since the rapidity with which a drug dissolved depend on the $k_{w/o}$, it is also called as the instrinsic dissolution rate constant

V = `volume of disssolution medium

h = thickness of stangnant layer

(Cs - Cb) = conc. gradient for diffusion

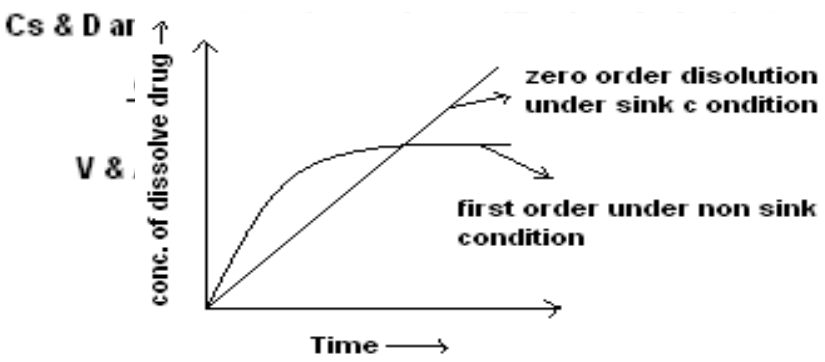
Thiseq.describesafirst–orderdissolutionkinetics.Itrepresentsdissolutionundernon-sinkconditions.

Ifvolumeisrelativelylarge suchthat

$$Cs >> Cb \text{ so,}$$

$$\frac{dC}{dt} = \frac{A\,k_{w/o}}{V\,h} Cs$$

Dissolution rate under sink condition follow zero order dissolution rate.



For obtaining IVIVC sink condition can be achieved by:

1. Bathing the dissolving solid in fresh solvent from time to time. Increasing the volume of dissolution fluid.
2. Removing the dissolved drug by partitioning it from the aqueous phase of dissolution fluid into the organic phase placed either above or below the dissolution fluid for e.g. hexane or chloroform.
3. Adding a water miscible solvent such as alcohol to the dissolution fluid.
4. By adding selected adsorbents to remove the dissolution drug.

In vitro sink condition is so maintain thatCb always less than 10% of Cs.

- **HIXON-CROWELL CUBE ROOT RELATIONSHIP**

Major assumptions in Noyes-Whitney relationship is that the S.A.(A) term remains constant throughout dissolution process. This is true for some formulations, such as transversal patches.However, size of drug particles from tablets, capsules and suspensions will decrease as drug dissolves.

This decrease in size of particles changes the effective S.A.

- Thus, Hixon& Crowell modified the eq. to represent rate of appearance of solute by weight in solution by multiplying both sides of volume term.

$W_0^{1/3} - W^{1/3} = kt$

W_0 = original mass of drug

W = mass of drug remaining to dissolve at time t

K = dissolution rate constant

- **DANCKWERT'S MODEL (PENETRATION OR SURFACE RENEWAL THEORY)**

o This theory assumes that solid-solution equilibrium is achieved at interface and mass transport is slow step in dissolution process.

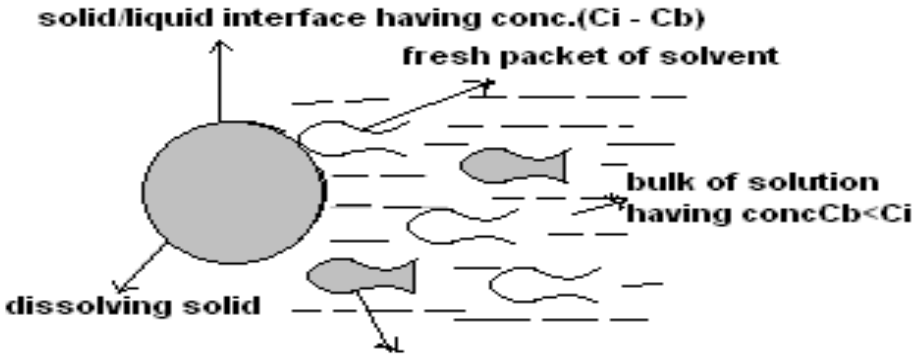
o The model could be visualized as a very thin film having a conc. Ci which is less than saturation, as it is constantly being exposed to fresh surfaces of liquid having a conc. much less than Ci. Acc. to model, the agitated fluid consist of mass of eddies or packets that are

continuously being exposed to new surfaces of solid and then carried back to bulk of liquid.

- Diffusion occurs into each of these packets during short time in which the packet is in contact with surface of solid.
- Since turbulence actually extends to surface, there is no laminar boundary layer and so no stagnant film exists. Instead, surface continually being replaced with fresh liquid

$$V\frac{dC}{dt} = \frac{dm}{dt} = A(Cs - Cb)\sqrt{rD}$$

where m = mass of solid dissolution
r = rate of surface renewal (or the interfacial tension)



Danckwert's model

- **INTERFACIAL BARRIER MODEL (DOUBLE BARRIER OR LIMITED SOLVATION THEORY)**

The Diffusion layer model and the Dankwert's model were based on two assumptions:

1. The rate determining step that controls dissolution is the mass transport.
2. Solid solution equilibrium is achieved at the solid/liquid interface.

According to interfacial barrier model, an intermediate conc. can exist at the interface as a result of salvation mechanism and is a function of solubility rather than diffusion.

 When considering the dissolution of the crystal will have a different interfacial barrier givenby following equation,

G = ki (Cs – Cb)

Where G = dissolution per unit area

Ki = effective interfacial transport constant

Table. Mathematical Models for Drug Dissolution Profile Analysis

Model	**Mathematical equation**	**Release mechanism**	**Theoretical RD[a]**	**Release class**
Zero-order	C_1t	Constant release rate	0.3	0
First-order	$C_2(1 – exp(–c_1))$	Fick's first law diffusion mechanism	1.0	1
Higuchi	$c_1t0.5$	Diffusion medium based mechanism	0.8	2
Hixson–Crowell	$C_2(1– (1– c_1t)^3)$	Erosion release mechanism	0.6	3
Korsmeyer–Peppas	$C2tc1$	Semi-empirical model, diffusion medium based mechanism		
Weibull	$c_3(1 – exp(–t/_{c1})C_2)$	Empirical model, lifetime distribution function		

In this theory, the diffusivity D may not be independent of saturation conc. Cs .The interfacial barrier model can be extended to both Diffusion layer model and the Dankwert's model.

1. EXPEIMENT

- **Study design***:* The study of in-vitro quality analysis of available alprazolam tablet brands in Bangladesh was studied by the evaluation of weight variation, hardness, friability, and disintegration time and dissolution profile. The study was conducted using various standard test methods related to estimate the quality of tablets.
- **Sample collection and identification*:*** Seven (7) brands of alprazolam tablets were purchased from various medicine shops. They were randomly marked from ALP01 to ALP07. The samples were properly checked for their manufacturing license numbers, batch

numbers and date of manufacture and expiry dates. The entire tablet brands were containing labeled shelf life of three years from the date of manufacture and before two years of labeled expiry date it was taken for the evaluation. The labeled active ingredient was 0.25mg of alprazolam and all were packaged in strip or in blister. Reference standard of alprazolam (99.87%) was collected from Incepta Pharmaceuticals Limited.

- **Analyticalmethods*:*** In this study, following quality control tests were performed for the evaluation of all the alprazolam tablet brands.
- **Weightvariationtest*:*** The acceptable range of weight variation for tablets should not exceed 10% or more having average weight of 80 mg or less (British

Pharmacopoeia, 2005). For each brand, ten tablets were randomly selected and weighed individually using an analytical balance. The average weights were determined using the following formula.

$$\text{Weight variation (\%)} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} \times 100$$

- **Hardness test**: Hardness of randomly selected ten tablets was determined for all the brands using 'Monsanto' type hardness tester. Finally the mean crushing strengths were determined.
- **Friability test:** In the study, it was determined by using Electrolab EF-2 Friabilator (USP) and the values of friability were expressed in percentage (%). From each selected brands ten tablets were individually weighed and transferred into friabilator which was operated at 25 rpm and continued up to 4 minutes (100 revolutions). Then the tablets weights were measured again and the percent (%)of friability was calculated using following formula.

$$\text{\% of Friability} = \frac{\text{Weight before test} - \text{Weight after test}}{\text{Weight before test}} \times 100$$

- **Disintegration time test**: The instrument used for this test was Disintegration tester – USP; (Electro lab EF 2L; with disc in distilled water medium. To test for disintegration time three tablets of each brand were placed in each tube and the basket rack is positioned in a 1 liter beaker of water at 37 ± 0.50c. The time required to break of each tablet into minute particles and pass out through the mesh was recorded. Then the mean disintegration time was

calculated for every brands.

- **Dissolution test:** For all brands of studied tablets, dissolution test was carried out using Dissolution Tester – USP Apparatus-1 (Basket type). Individually 3 tablets of each brand were placed in 3 different beakers in dissolution medium containing 900 ml of 0.1NHCl buffer (pH 7.4). The process was done at a speed of 100 rpm by maintaining temperature at 37±1ºC in each test. At regular time intervals of 10 minutes samples were withdrawn as 5 ml which was predetermined and same method was continued up to 30 minutes by replacing equal quantity of fresh dissolution medium. The filtered samples were diluted suitably and analyzed by using UV Spectrophotometer (UV Spectrophotometer: UV-1800-240V) at 260 nm for alprazolam and percentage (%) of drug release was calculated by measuring the absorbance.

- **Preparation of the stock solution**

10 mg of the alprazolam standard powder was weighted precisely and transferred to a 100 mL volumetric flask. A solvent mixture of methanol : water (9:1 V/V) was added to the flask and made the volume exactly to 100 mL. Therefore, a 0.1mg/mL or 100 µg/mL of the active ingredient was made. 1 mL of this solution was taken with microsyringe and transferred into a 100 mL volumetric flask and made the volume exactly to 100 mL with the above mentioned solvent mixture. Therefore, the final concentration of 1 µg/mL was obtained and used for the preparation of various concentration solutions necessary for plotting the calibration curve.

- **Preparation of the standard solutions**

For plotting the calibration curve, concentrations of 0.2, 0.4, 0.6, 0.8 and 1µg/mL were needed. From the above mentioned stock solution, 2, 4, 6, 8 and 10 mL were taken and each one was placed in an individual 10 mL volumetric flask, then made the volumes exactly to 10 mL by adding the solvent mixture of methanol:water (9:1 V/V) to each of the flasks. Therefore, solutions with concentrations of 0.2, 0.4, 0.6, 0.8 and 1µg/mL were obtained which would be used for plotting the calibration curve and injection into the HPLC instrument.

- **Determination of λ_{max} of alprazolam standard powder**

The UV spectrum of alprazolam standard powder in methanol:water (9:1 V/V) was taken. The $\lambda_{max}$was determined as 254 nm.

- **Plotting the standard calibration curve**

For plotting the standard curve, five times and each time 20 µLfrom each of the standard solutions prepared in **(6)** was injected into the HPLC instrument from the lowest to the highest concentrations. The chromatograms and the relevant data such as peak area, peak height, retention time, etc. were recorded and saved as Peak – Report tables in the soft ware program (Table 5). For the assurance of the accuracy and precision of the measurement method, the whole procedures for plotting the calibration curve were repeated three times within a day and twice between two consecutive days. Then, the calibration curve was plotted (Figure 2). On the basis of the calibration curve (Figure 2), the unknown samples were injected into the HPLC instrument and the chromatograms were recorded, then the amounts of the unknown samples were determined.[6]

TableHPLC data obtained from the injection of samples prepared from alprazolam standard powder with given concentrations

Concentration µg/mL	Retention time (t_R) min.	Height, mv	Area, mv*min
0.2	1.633	11.77± 0.18	0.98±0.050
0.4	1.633	23.57± 0.19	1.97±0.032
0.6	1.633	34 ± 0.45	2.85±0.030
0.8	1.633	46.01± 0.16	3.72±0.045
1.0	1.633	56.7 ± 0.29	4.67±0.030

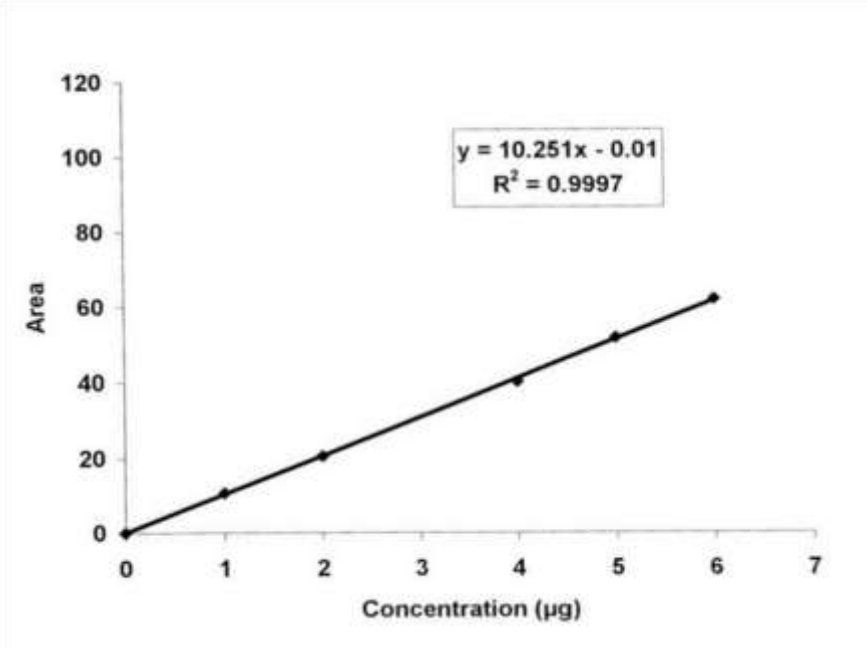


Figure. Calibration curve of alprazolam standard powder

Table Measurement of weight variation of different brands of Alprazolam tablet.

Alprazolam tab. brands	Minimum weight{g}	Maximum weight{g}	Average weight (g)	Standard deviation	Relative standard deviation
ALP 01	0.1344	0.1385	0.1364	0.0010	0.760
ALP 02	0.1529	0.1563	0.1543	0.0012	0.786
ALP 03	0.1328	0.1373	0.1353	0.0017	1.266
ALP 04	0.1326	0.1362	0.1347	0.0011	0.856
ALP 05	0.1154	0.1211	0.1188	0.0021	1.805
ALP 06	0.1317	0.1339	0.1323	0.0007	0.540
ALP 07	0.1335	0.1351	0.1343	0.0005	0.402

Table Results of hardness, friability, disintegration tests of different brands of Alprazolam tablets.

Alprazolam tab. brands	Hardness (kg-ft) (mean ± SD)	Friability (%)	Disintegration time (min)
ALP 01	3.26 ± 0.23	0.73	0.57 ± 0.45
ALP 02	2.50 ± 0.16	0.97	1.26 ± 0.07
ALP 03	4.21 ± 0.11	0.61	1.52 ± 0.31
ALP 04	1.50 ± 0.18	0.79	1.32 ± 0.11
ALP 05	1.64 ± 0.15	0.50	1.15 ± 0.13
ALP 06	2.43 ± 0.12	0.87	1.39 ± 0.12
ALP 07	3.53 ± 0.14	0.63	2.22 ± 0.23

ALP= Alprazolam, g=gram

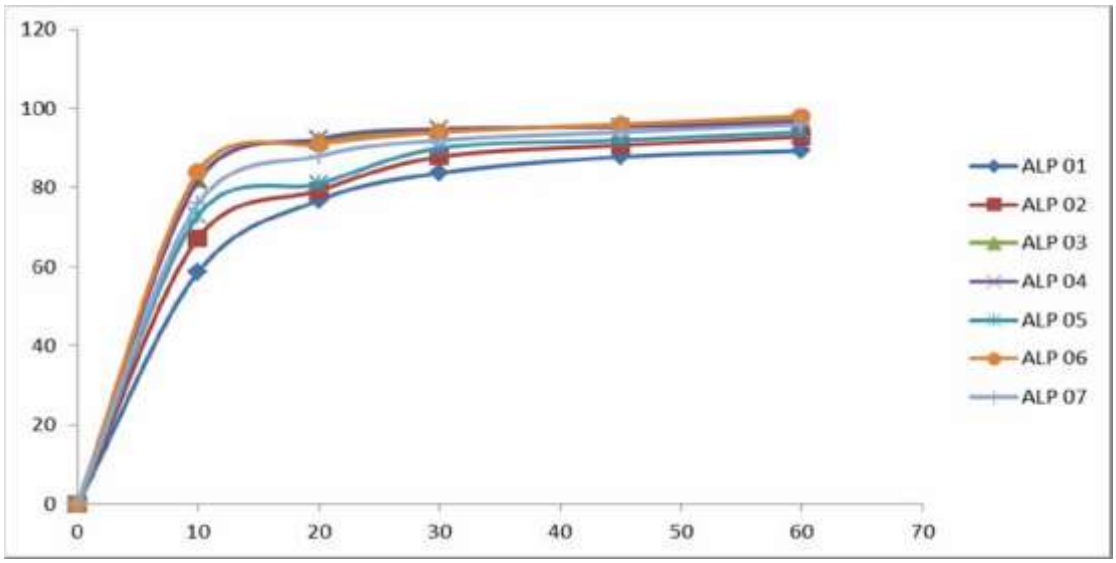


Figure. Drug release curve of different brands of Alprazolam tablet ALP= Alprazolam

2. RESULTS AND DISCUSSION

Alprazolam is most commonly used to relieve anxiety, nervousness, and tension associated with anxiety disorders. Alprazolam is also used to treat panic disorders. Regarding the efficacy of alprazolam and its rapid effect in patients, which is dependent largely upon the quality of the drug, it was decided to evaluate in vitro the following objectives between

different types of alprazolam tablets and make a comparison between the quality of these tablets:

1. Investigating and determining the extent of purity of the active ingredient of the imported standard powder.

2. Studying and determining the active ingredient of each type of the tablets.

3. Study the dissolution rate of each type of the tablets.

4. Study the degree of hardness, friability percentage, disintegration time and weight variation and uniformity content of each type of the tablets.

5. Finally, concluding about the efficacy and quality of these tablets.

For the determination of the active ingredient, content uniformity and dissolution rates of alprazolam tablets and standard powder, high performance liquid chromatography (HPLC) which is a rapid and precise technique was used.

Friability percentages of the tablets were calculated using the following formula:

$$\%\text{Friability} = [(W_1 - W_2)/ W_1] \times 100$$

Where W_1 is the initial weight of the 20 tablets and W_2 is the final weight of the 20 tablets. The maximum acceptable friability range should be within 0.5-1%, on condition that it does not affect the apparent shape of the tablet.[6]

For the determination of % release of the tablets, the following calculations were done:

i) $$\text{Concentration } (\mu g/mL) = \frac{\text{Active ingredient of the tablet used (mg)}}{\text{Total volume of the dissolution medium (mL)}}$$

ii) This concentration was considered as 100% drug release.

iii) $$\%\ \text{Release} = \frac{\text{The Amount (from the HPLC Peak Report data)}}{\text{Concentration } (\mu g/mL)} \times 100$$

Determination of the degree of hardness, friability percentage and disintegration time of the tablets were made by using the corresponding instruments. Weight variations were measured by analytical balance. The various results obtained in this research have shown that:

i)Alprazolam tablets manufactured by Upjohn (Xanax) of USA had the highest whereas those manufactured by Unichem laboratory had the lowest degree of hardness,

ii) Friability percentages of all seven types of the tablets were within the internationally well-knownpharmacopoeia acceptable range.

iii)Disintegration time of all seven types of the tablets were within the expected range.

CONCLUSION:

From the study it was identified that weight variation and friability test of alprazolam tablet brands met the specification of B.P. Variations were obtained in hardness, disintegration time and dissolution profile. On the other hand almost all alprazolam tablet brands showed better disintegration time but some were slight different in their dissolution profile which is related to its absorption property. Manufacturers should always maintain highest standard for all quality parameters of any medicine because better quality ensures better medicine to get desired therapeutic effect.

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Original Research Article **Volume 4 Issue 3**

FORMULATION, DEVELOPMENT AND EVALUATION OF TOPICAL LIPOSOMAL GEL OF FLUCONAZOLE FOR THE TREATMENT OF FUNGAL INFECTION

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Abstract:

The present work on the preparation of topical liposomal gel containing fluconazole is an attempt to utilize the immense potential of liposomes as a carrier to increase the permeability. Liposomal encapsulation showed more drug retention compared with plain drug gel .

Further, the physicochemical modification in the drug by means of phospholipid membrane also promises to prolong the drug action. A number of problem associated with drug molecule such as bioavailability, degradation, stability and side effects can be overcome by incorporating it into liposomes. The liposomes of fluconazole was prepared by film hydration method and evaluated. The *In vitro* release of the formulation was studied and found to be more efficient than plain gel. Creation of reservoir effect for drug in skin due to deposition of other components of liposomes with drug into the skin and thereby increasing the drug retention capacity into the skin. Liposomal formulations were more spherical with stable zeta potential and mono-disperse with no clumping.

Variables such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency. Liposomal dispersion and gels were found to increase the skin permeation and deposition compared to control. Drug release of all batches was studied. Stability studies performed for Liposomal gel indicates the prepared liposomes have more stability at freezing temperature than that of room temperature.

Fluconazole molecules could be successfully entrapped in liposomes with reasonable drug loading. Hence from results obtained it can be concluded that liposomal gel containing fluconazole has potential application in topical delivery for the treatment of fungal skin ailments.

1. INTRODUCTION

Fungal infection of skin is now-a-days one of the common dermatological problem. The physicians have a wide choice for treatment from solid dosage to semisolid dosage form and to liquid dosage formulation. Among all the topical formulation clear transparent gels have been widely accepted in both cosmetics and pharmaceuticals[1]. In the past decade, liposomal formulations have been extensively used to enhance the drug delivery efficiency through various routes of administration. In a number of instances, liposomal drug formulations in any dosage form, have shown to be markedly superior to the conventional dosage forms, especially for intravenous and topical route of administration of drugs. The major reasons of topical liposomal drug formulations being better to other dosage forms are, ability:

- To reduce serious side effects and incompatibilities that may arise from undesirably high systemic absorption of drug;
- To enhance the accumulation of drug specifically at the site of administration because of the lipid nature of both liposomes and the biological membrane.
- To readily incorporate a wide variety of both hydrophilic and lipophillic drugs.

In recent years, increasing attention is being paid to the development of controlled drug delivery system (CDDS). The advantages of CDDS over conventional therapy are numerous, including better plasma level profile, increased patient compliance, lower dosage and toxicity, possibility of targeting and more efficient utilization of active agent. Liposomes are one of the most suitable drug delivery systems to deliver the drug to the target organ and minimize the distribution of the drug to non-target tissues.[2]

Novel drug delivery systems (NDDS), bear great potential for topical delivery. Among them lipidic and non-lipidic vesicular systems like liposome, niosome, transferosome and ethosome have been used since last decade to overcome the shortcomings with conventional topical formulations. These vesicular systems are found to be much more effective as they provide controlled release of drug. It is so as they form a depot in skin that keeps the therapeutic drug concentration for long period of time.[3,4]

In further chapters of this paper topically applied liposomal formulations is discussed with emphasis on their application for fungal infections using fluconazole as the drug of choice. The topical antifungal formulation would be formulated using drug fluconazole for the deeper fungal infections like candidiasis. Liposomal topical gel is formulated due to its enormous advantages

over conventional dosage forms. The mechanism by which liposomes facilitate deposition of drugs into the skin, route of permeation and its potential applications are discussed.[5]

Topical drug delivery is a very useful and attractive route for both local and systemic treatment. The delivery of drugs onto the skin is identified as an effective means of therapy for local dermatological diseases. Fluconazole, a synthetic antifungal agent, is a triazole derivative. It is used in the treatment of oropharyngeal, esophageal, or vulvovaginal candidiasis as well as other serious systemic candidal infections. It is also effective against superficial fungal infections and dermatophytoses.[6]

Topical liposome formulations could be more effective than conventional formulations. Liposomal carriers, well known for their potential in topical drug delivery and their drug release efficiency have been chosen to help fluconazole molecules to permeate in the skin layers. These vesicles are expected to provide lipid enriched hydrating conditions to retain the drug molecules within the skin layers. With this objective fluconazole loaded liposomal systems will be prepared and their topical performance will be compared with non liposomal systems containing fluconazole.[9]

INTRINSIC WORTH OF TOPICAL LIPOSOME

The major problem which lies with the efficacy of topical drugs is that they have to reach the site of action i.e skin and to stay there in an effective therapeutic concentration for a certain period of time. Although the skin is the organ which can be reached directly; drug application on the surface of skin does not mean the drug is getting to the right site of action.[10] This is the problem with the conventional dosage forms like creams and ointments etc. so in these type of dosage forms penetration enhancers, e.g. dimethylsulphoxide (DMSO) or propylene glycol is used which, on the one hand, improves transport rate through the epidermal barrier but, on the other hand, produce unwanted effects due to an increased systemic drug level. Moreover, irritative and even toxic side effects are reported sometimes leading to the conclusion that addition of penetration enhancers does not really improve topical dosage form.[11,12]

Topical drug administration is a localized drug delivery system which is applied anywhere in the body through ophthalmic, rectal, vaginal, and skin as the topical routes. As discussed topical application have many advantages over the conventional oral or other dosage forms. In general, they are deemed more effective, and less toxic due to the bilayer composition, properties and structure.[12]

QUICK VIEW OF TYPES OF TOPICAL PREPARATIONS

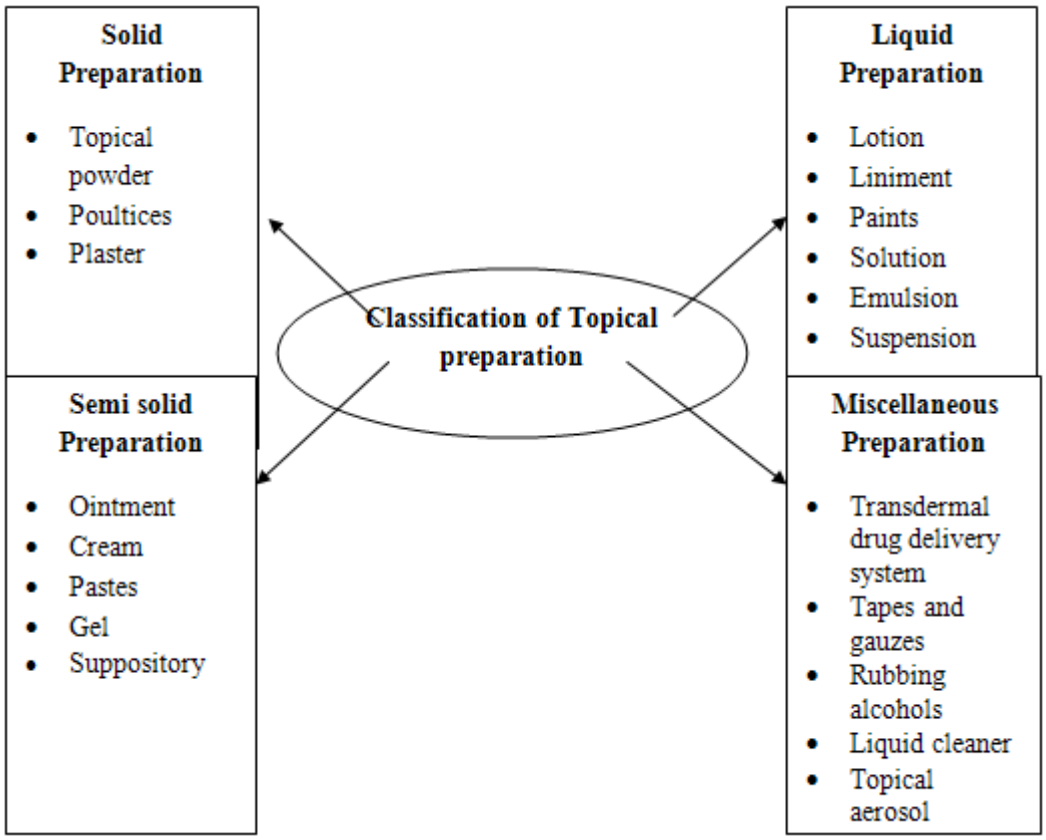

Figure 1: Types Of Topical Preparations

1.1 INTRODUCTION TO FUNGAL INFECTIONS

The fungal infections are incredibly familiar and can be current as well as systemic. The incidence of superficial fungal infections of skin, hair and nails has been increased in worldwide. It has been predictable that about 40 million people have suffered from fungal infections in developing and under de- veloped nations. The succession of fungal infections can be rapid and serious due to compromising with immune function. *Dermatophytes* are one of the the majority frequent causes of *tinea* and o*nchomycosis*. Candidal infec- tions are also among the most widespread superficial cu- taneous fungal infections. Even, *candida* can occupy deeper tissues as well as the blood which leads to life- threatening systemic candidiasis, when the immune system is weakened. The fungal infections can be treat by topically applied medicines as well as by oral administrations. However, oral use of medicine is not much significant in treating local fungal infections and also has systemic side effects.

Fungal infections of the skin are also known as 'mycoses'. They are universal and generally mild. However, in very sick or otherwise immune suppressed people, fungi can sometimes cause serious disease.[13]

1.1.1 CHARACTERISTICS OF FUNGI

Fungi are parasites or saprophytes i.e. they live off living or dead organic matter. Mycologists identify and classify fungi according to their exterior by microscopy and in culture, and by the process of reproduction, which may be sexual or asexual.

Growing fungi have divided filaments called hyphae, which make up the mycelium (like branches are part of a tree). Some fungi are compartmented by cross-walls (called septae).

Arthrospores are made up of fragments of the hyphae, breaking off at the septae. Asexual spores (conidia) form on conidiophores. The sexual reproductive phase of many fungi is unknown; these are 'fungi imperfecta' and include those which infect humans.

Yeasts form a subtype of fungus characterised by clusters of round or oval cells. These bud out comparable cells from their surface to divide and propagate. In some conditions they appearance a chain of cells called a pseudomycelium.

1.1.2 MAJOR FUNGAL INFECTIONS

The number of dissimilar kinds of fungi out there is vast, and, of course, some of them are pleasant to eat. Only a minute subset is capable of infecting humans.[14]

1.1.2.1 Superficial fungal infections

These influence the outer layers of the nails, the skin, and hair. The main groups of fungi causing superficial fungal infections are:

- Dermatophytes
- Yeasts i.e. candida, malassezia, piedra

1.1.2.2 Subcutaneous fungal infections

These occupy the deeper layers of the skin (the dermis, subcutaneous tissue and even bone). The causative organisms in general live in the soil or in rotting vegetation. They can get into the skin as a result of an injury but frequently stay localized at the site of injury. Deeper skin infections include:

- Mycetoma
- Chromoblastomycosis

1.1.2.3 Systemic fungal infections

Systemic mycoses may consequence from breathing in the spores of fungi, which usually live in the soil or rotting vegetation or as opportunistic disease in protected compromised individuals. Systemic fungal infections are more infectious as they are usually more difficult to diagnose, are

chronic in nature, and, in some cases, can become life-threatening. They occur more recurrently in individuals with compromised immune systems (cancer patients; AIDS patients; transplant patients). Prophylactic treatment is sometimes indicated in AIDS patients and bone marrow transplant patients, but risk of developing resistance is high. Life-threatening infections require the utilization of more potent but much more toxic antifungals.[15]

1.1.2.4 Inhaled fungal infection

Although uncommon, some may infect healthy individuals. The result is most often a mild infection and long lasting resistance to further attack, but occasionally these infections are more somber and chronic (especially in the immune suppressed).

1.1.3 A BRIEF DESCRIPTION OF SOME COMMON FUNGAL INFECTIONS

1.1.3.1 Candidiasis

Candidiasis is a fungal infection due to any types of *Candida* (a type of yeasts). When it affects the mouth, it is commonly called thrush. Symptoms embrace appearance of white patches on the tongue or other areas of the oral cavity i.e mouth and throat. Other symptoms may contain soreness in throat and problem in swallowing .If it affect the vagina, it is usually called as yeast infection. Symptoms include genital itching, burning sensation, and sometimes a white "cottage cheese-like" discharge from the vagina. Less commonly the penis may be affected, resulting in itchiness. Very rarely, the infection becomes invasive and spread throughout the body, consequential in fever sometimes.[16]

There are more than 20 types of *Candida* can cause infection, but *Candida albicans* is the most common. Infections of the mouth are the majority common among children less than one month old, the elderly, and those with weak immune systems. Conditions that result in a weaker immune systems include HIV/AIDS, after organ transplantation, diabetes, and the use corticosteroids. Other risks include dentures and following antibiotic therapy. Vaginal infections occur more commonly during pregnancy, in those with weak immune systems, and following antibiotic use. Risk for widespread infection includes being in an exhaustive care unit, , low birth weight infants, following surgery and those with weak immune systems.[10]

For infections of the mouth, treatment with topical clotrimazole or nystatin is usually effective. Oral or intravenous fluconazole, itraconazole, or amphotericin B may be used if these do not work. A number of topical antifungal medications are used for vaginal infections including clotrimazole. In certain groups at very high risk antifungal medications may be used preventatively.

Symptoms of candidiasis vary according to the area affected. Mainly candidial infections effect in minimal complications such as redness, itching, and discomfort, on the other offer the complications may be severe or yet fatal if left untreated. In immunocompetent persons, candidiasis is usually a very localized infection of the skin or mucosal membranes, including the oral cavity (thrush), the pharynx or esophagus, the gastrointestinal tract, the rectum, anus, perianal/perirectal or ano-rectal area (in men as well as women), the perineum, urinary bladder, the finger and toe nails (onychomycosis), and the genitalia (vagina, penis, ectcetera).

Candidiasis is a very common cause of vaginal irritation, or vaginitis, and can also occur on the male genitals. In immunocompromised patients, *Candida* infections can affect the esophagus with the potential of becoming systemic, causing a much more serious condition, called candidiasis.

Oral candidiasis is the most familiar fungal infection of the mouth, and it also represent the mainly common opportunistic oral infection in humans. They are commonly treated with antimycotics; antifungal drugs; these contain topical nystatin, fluconazole, topical clotrimazole, and topical ketoconazole.

1.1.3.2 Mycosis

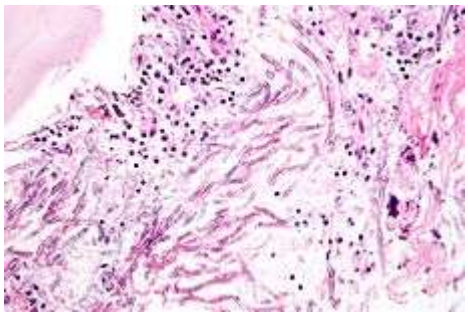

Figure 3: Micrograph showing a mycosis (aspergillosis).

Mycosis (plural: **mycoses**) is a fungal infection of animals, including humans. Mycoses are universal and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may instigate persistent infections; as a result, mycoses often start in the lungs or on the skin.

Individuals with weakened immune systems are also at risk of increasing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people, also, are groups at risk.[16]

1.1.3.2.1 Brief Classification

Mycoses are classified according to the tissue levels initially colonized.

- **Superficial mycoses**

External mycoses are limited to the outermost layers of the skin and hair.

An example of such a fungal infection is *Tinea versicolor*, a fungus infection that normally affects the skin of young people, especially the chest, back, and upper arms and legs. These fungus produce spots that are either lighter than the skin or a reddish-brown. This fungus exists in two forms, one of them causing visible spots. Factors that can cause the fungus to become more visible include high humidity, as well as immune or hormone abnormalities. However, almost all people with this very common condition are healthy.

1.1.3.3 ATHLETE'S FOOT

Athlete's foot is the term worn for a common disorder affecting the skin between the toes. The cleft between the fourth and fifth toes is the most frequently affected, with moist soft skin that peels off easily. Often the skin splits uncomfortably (a fissure). It may smell unpleasant. It is normally mild; very inflamed athlete's foot is generally due to resultant bacterial infection. The ordinary disease is the athlete's foot which most commonly affects children before puberty.

❖ **Causes**

- Athlete's foot can be due to one factor or a combination of factors.
- Bacterial infection (erythrasma), pseudomonas, Staphylococci and Streptococci).
- Mould infection.
- Soft corn (build-up of thick skin because the toes are pressing against each other).
- Injury e.g.vigorous removal of peeling skin.
- Skin conditions such as psoriasis, eczema or keratolysis exfoliativa.
- Fungal infection (tinea pedis).

❖ **Treatment**

Treatment for athlete’s foot begins with general measures:

- Dry carefully between the toes.
- Keep toes apart using a cotton or foam wedge.
- Should wear shoes that are loose around the toes or go bare foot.

- Apply a topical antifungal agent. These may also control many of the bacteria responsible for athlete's foot.
- Whitfield's ointment is particularly useful as it removes the surface layer of moist peeling skin (i.e. it is keratolytic) and eliminates bacteria and fungi.

1.1.3.4 CRADLE CAP (infantile seborrhoeic dermatitis)

Cradle cap is a patchy, greasy, scaly and crusty skin rash that occur on the scalp of newly born babies. Cradle cap is infantile seborrhoeic dermatitis that is restricted to the scalp. childish seborrhoeic dermatitis may also affect other areas of the body such as behind the ears, in the crease of the neck, armpits and diaper area.

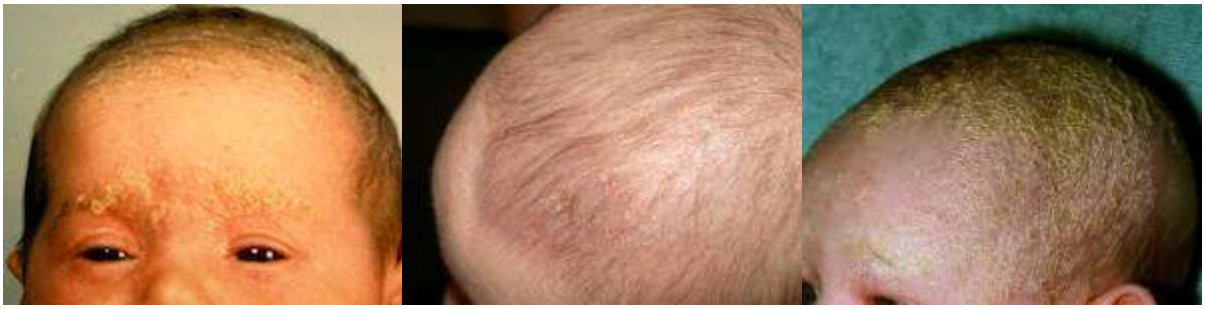

Figure 5: Cradle cap

1.3.3.6 ADULT SEBORRHOEIC ECZEMA

Seborrhoeic eczema affect scalp, face (creases around the nose, behind ears, within eyebrows) and upper trunk.

1.3.3.6.1 Diagnosis

Seborrhoeic eczema is diagnosed by its experimental appearance and behaviour. As *Malassezia* is a normal component of skin flora, its attendance on microscopy of skin scrapings is not diagnostic. Skin biopsy may be helpful but is rarely indicated.

1.3.3.6.2 Treatment

Treatment of seborrhoeic dermatitis often involve several of the following options.

- Keratolytics are used when essential, eg salicylic acid, lactic acid, urea, propylene glycol
- New antifungal agents are active against *Malassezia* eg ketoconazole, or ciclopirox shampoo or cream.
- Mild topical corticosteroids for 1-3 weeks to decrease inflammation in acute flare

- Topical calcineurin inhibitors (pimecrolimus cream, tacrolimus ointment) as required
- In resistant cases in adults, oral itraconazole, tetracycline antibiotics or phototherapy may be recommended.
- Ketoconazole or ciclopirox cream can be used once daily for 2 to 4 weeks, repeated as necessary.[18]

1.4 LIPOSOMES AS NOVEL DRUG DELIVERY SYSTEM

An ideal release system delivers drug at a specified rate in the body in a confident period of time for an effective treatment. Drugs used in the treatment of various fungal disease can be highly toxic to standard tissues. The toxicity of these drugs could be minimized by decreasing delivery to healthy cells and tissues.

Liposomes have been considered to be excellent models of cell membranes. They explain effective drug delivery which is frequently used in dermal applications. Liposomes were first described by British haematologist Alec D Bangham in 1961 (published 1964), at the Babraham Institute, in Cambridge. Liposomes are microscopic spherical vesicles together of one or more lipid bilayers with an aqueous core. Liposomes are lengthily used as carriers for numerous molecules in cosmetic and pharmaceutical industries. To grow delivery system that can entrap unstable compounds and to shield their functionality, food and undeveloped industries uses the liposome encapsulation.[22]

Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets. As a drug delivery system, liposomes, commercially, have increased rate of use because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues. Many studies have been conducted on liposomes with the aim of decreasing drug toxicity or targeting specific cells.[23]

One of the main aims of any behavior employing drug is to enlarge the therapeutic index of the drug and while minimizing its side effects. The clinical usefulness of most conservative chemotherapeutics is controlled either by the incapability to transport therapeutic drug concentrations to the target soft tissue and harmful toxic side effects on normal organs and tissues. Different approaches have been made to conquer these difficulty by providing the 'selective' delivery to the target area; the ideal answer would be to target the drug alone to those

cells, tissues, organs that are affected by the disease. The significance of liposomes lies in their composition, which makes them biodegradable and biocompatible.

The liposomes contain drugs can be administrated by many routes i.e. intravenous, oral, nasal, intramuscular, pulmonary, topical, and ocular. They can be delivered in many vesicles like creams, ointments, capsules, solutions, sprays, etc. Liposomes can be used for the treatment of many diseases:, endocrine, arthritis, cancer, bacterial, fungal, ocular, vaccines, fibrinolysis asthma, diabetes, diseases of immune system, drugs used to achieve relief from pain and topical anesthesia.

Liposomes are classified on the basis of structural parameters, and application in biology, biochemistry, cosmetics and medicine method of preparation, composition. Phospholipids which are capable of forming liposomes include both natural and synthetic phospholipids. The main sources of natural phospholipids are yolk egg and soya bean although they can be obtained from plant oils such as olive oil. Liposomes can be confidential as conventional, pH sensitive liposomes, cationic liposomes, immunoliposomes, temperature or heat sensitive liposomes, magnetic liposomes and sterically stabilized “stealth” liposomes.[23]

Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticuloendothelial system(RES). These liposomes are known as "stealth liposomes", and are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery instrument. Though, research currently seeks to investigate at what amount of PEG coating the PEG actually hinders binding of the liposome to the delivery site. In addition to a PEG coating, most stealth liposomes also have some sort of biological species attached as a ligand to the liposome in order to enable binding via a specific expression on the targeted drug delivery site.[24] These targeting ligands could be monoclonal antibodies (making an immunoliposome), vitamins, or specific antigens. Targeted liposomes can target nearly any cell type in the body and transport drugs that would naturally be systemically delivered.

The mainly common used phospholipids in preparation of liposomes are:

Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), dipalmitoylphosphatidylcholine (DPPC), dipalmitoyl-phosphatidylglycerol dioleoylphosphatidylethanolamine (DOPE). 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine, distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylserine.

The conservative liposomes are characteristically composed of phosphatidylcholine and cholesterol. On the other hand, "stealth" liposomes are lipid bilayers coated with polyethylene glycol(PEG). The "stealth" liposomes provide enhanced stabilization and additional protection of the entrapped substances. They allow longer circulation time for the drug delivery mechanism. The importance of a prolonged period of time can be achieved by continued release of the drug.

Liposomes are composed of accepted phospholipids that are biologically inert and feebly immunogenic, and they have low inherent toxicity. Furthermore, drugs with different lipophilicities can be encapsulated into liposomes: strongly lipophilic drugs are entrapped almost totally in the lipid bilayer, intensely hydrophilic drugs are situated entirely in the aqueous compartment.[25]

1.4.1 CLASSIFICATION OF LIPOSOMES BASED ON STRUCTURE PARAMETERS

1. Multilamellar Large vesicles > 0.5um
2. Oligolamellar vesicles (0.1-1um)
3. Unilamellar vesicles (All size range)
   a) Small unilamellar vesicles 20-100nm
   b) Medium sized unilamellar vesicles
   c) Large unilamellar vesicles >100nm
   d) Giant unilamellar vesicles >1um
4. Multivesicular vesicles >1um

1.4.2 STRUCTURE AND COMPOSITION OF LIPOSOME

Among the variety of novel drug delivery systems liposomes seem to have the best potential to accommodate both water and lipid soluble compounds to protect the liposome-encapsulated drug from metabolic degradation and to act as a delivery instrument, releasing active ingredients slowly and in a controlled manner.

Phospholipid, the component of the liposome lipid bilayer is usually extracted from egg yolk or soy bean oil consists of a hydrophilic head portion covalently attached to two hydrocarbon tails representing the lipophilic portion. Aggregation in a bilayer structure occurs by direction of the hydrophilic head groups towards the aqueous environment.[35] While keeping the lipophilic hydrocarbon chains sequestered inside. Formation of such a configuration provides the vesicle

with the lowest potential energy state during solvation of the polar head groups and hydrophobic interactions of the lipid chain.

Natural phosphatidylcholine extracted from egg yolk or soy bean oil or its semisynthetic derivatives represents the main constituent in various liposomal formulations. The chemical structure of naturally occurring phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or unsaturated. Each may have between 10 to 24 carbon atoms together forming the hydrophobic (lipophilic) portion of the particle. The phosphate and choline moieties form the hydrophilic "head".

The fatty acid chains, depending on their length and degree of saturation, can exist in the gel phase in which the lipids are rigid, impermeable and easily aggregated upon storage or in the more fluid liquid crystalline phase. The temperature at which the gel phase converts to the liquid crystalline phase is known as the transition temperature. Cholesterol is normally added in minute quantities to most liposomal formulations to increase the fluidity of the liposomal gel phase enhance the retention of hydrophilic particles and to steady the bilayer membrane in a manner similar to that of biological membranes.[26]

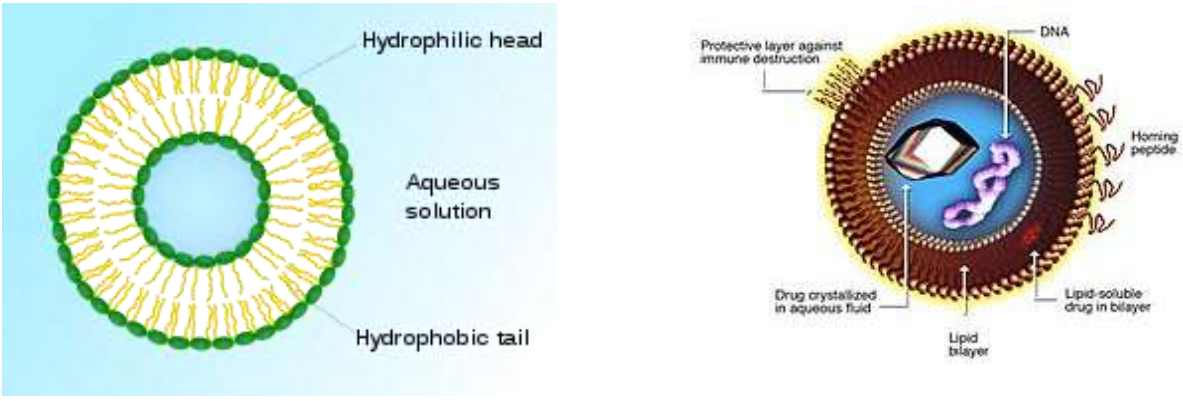

Figure 10: Structure of Liposomes.

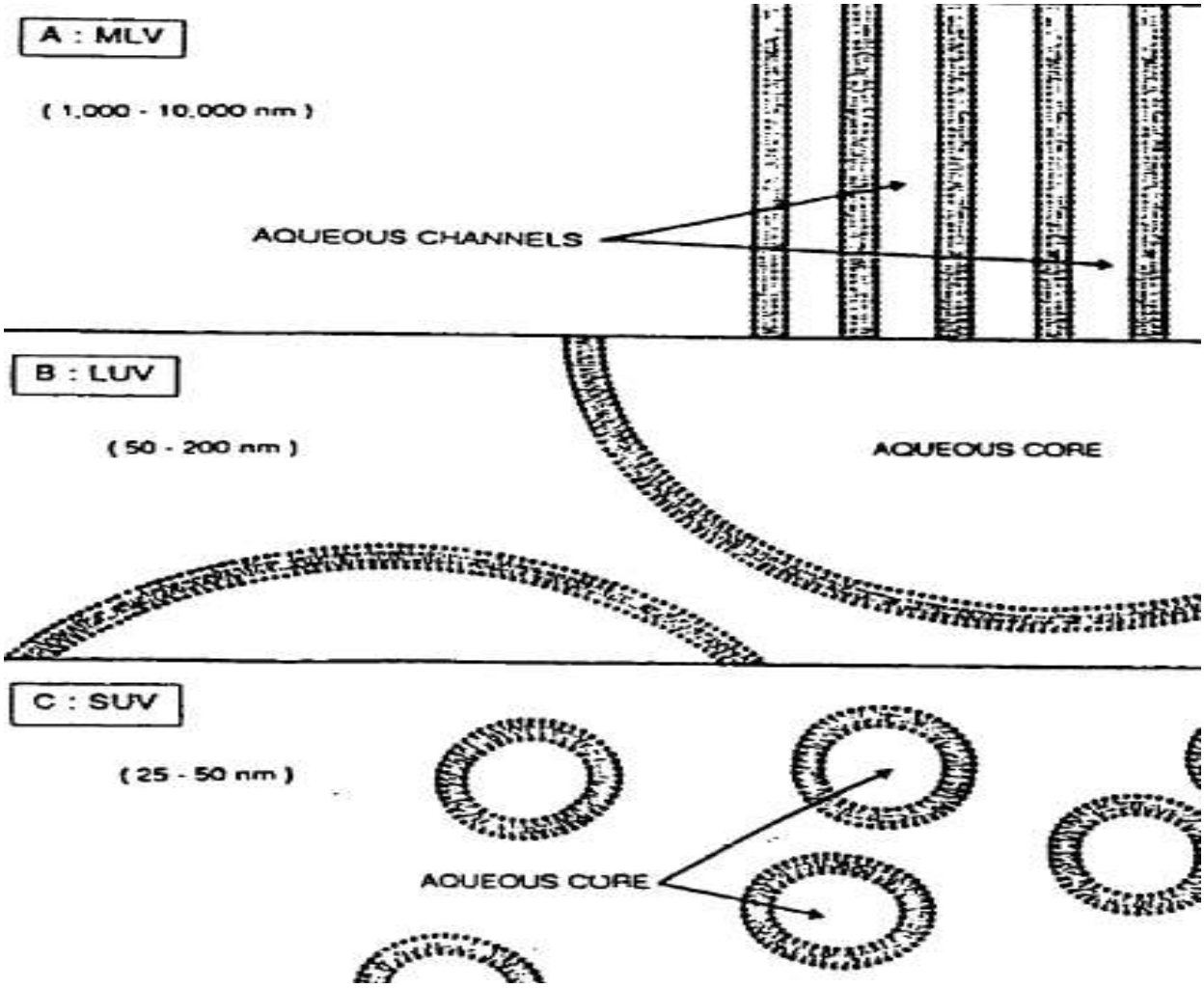

Figure 11: A diagrammatic representation of three main class of liposomes.(A) Multilamellar vesicles; (B) Large unilamellar vesicles; (C) Small unilamellar vesicles

1.4.3 METHODS OF PREPARATION OF LIPOSOMES

1.4.3.1 Multilamellar Liposomes (MLV)

1. Lipid hydration method:

This is the most widely used scheme for the research of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for a short time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the maximum melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities.MLV are simple to organize by this method and a variety of substances can been capsulated in these liposomes. The drawback of the process are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous.

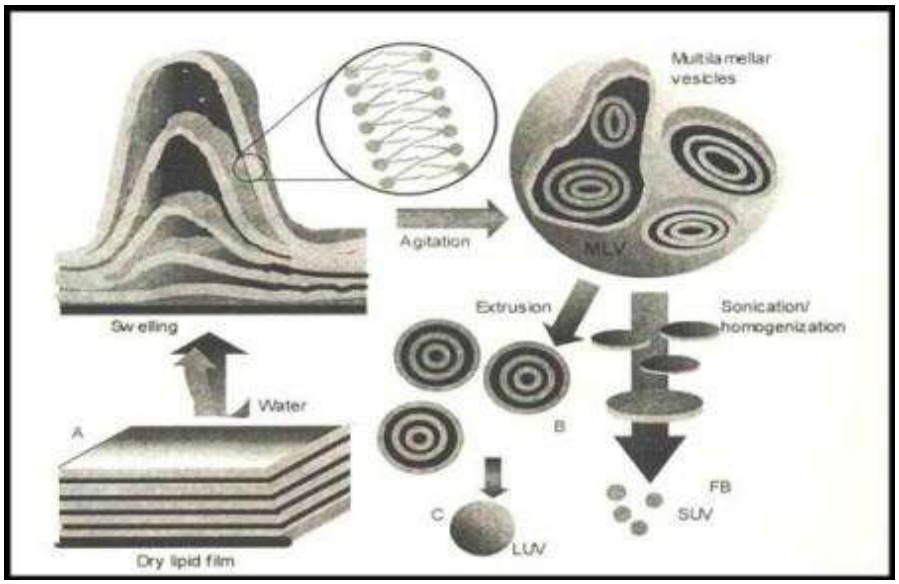

Figure 12: Multilamellar vesicles

2. Solvent spherule method:

A process for the preparation of MLVs of homogeneous size distribution .The procedure involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were created when controlled evaporation of organic solvent occurred in a water bath.

1.4.3.2 Small Unilamellar Liposomes (SUV):

1. Sanitation Method:

Here MLVs are solicited either with a bath type sonicator or a probe sonicator below an inert atmosphere. The main drawbacks of this method are very low interior volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulate, exclusion of large molecules, metal pollution from probe tip and existence of MLV along with SUV.

***2. French Pressure Cell Method**:*

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has a number of compensation over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs.

1.4.3.3 Large Unilamellar Liposomes (LUV)

They encompass high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

1. Solvent Injection Methods

a) Ether Infusion Method

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70- 190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature.

b) Ethanol Injection Method

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the populace is heterogeneous (30-110 nm), liposomes are very dilute, it is complex to remove all ethanol because it forms azeotrope with water and the possibility of various biologically lively macromolecules to inactivation in the presence of even low amounts of ethanol.

c) Reverse Phase Evaporation Method

First water in oil emulsion is formed by sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure.

With this method high encapsulation competence up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication.

d) Calcium-Induced Fusion Method:

This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structure in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs. The foremost advantage of this method is that macromolecules can he encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, even though of a assorted size range. The chief drawback of this method is that LUVs can only be obtained from acidic phospholipids.

e) Freeze-Thaw Method:

SUVs are rapidly frozen and followed by slow thawing. The concise sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is unpaid to the fusion of SUV during the processes of freezing and or thawing. This type of fusion is strongly repressed by increasing the ionic force of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained.

1.4.3.4 Giant Liposomes

The procedure for the arrangement of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methylglucoside detergent alongside an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm.[26,27]

1.4.3.5 The advantages of liposomes as drug carrier systems

1. Similar to biological membranes they can accumulate water-soluble and **lipophilic** substances in their different phases i.e. it readily incorporate a wide diversity of hydrophilic and hydrophobic drugs.
2. They are similar to the epidermis with respect to their lipid composition which enables them to penetrate the epidermal barrier to a greater extent compared to other dosage forms.
3. According to studies performed so far liposomes are biodegradable and non-toxic which is important to avoid side effects.
4. The new aspect with liposomes is that they are thought to act as “drug localizers” - not only as “drug transporters”. i.e. to enhance significantly the accumulation of drug at the site of administration as a result of the high retentivity of liposomes with biological membranes.[42]

5. The uptake of intact liposomes by the reconstructed epidermis, these vesicles do not penetrate through healthy skin. Yet, this is to be expected in diseased skin with no intact epidermal barrier. This is particularly important as far as drugs like glucocorticosteroids or retinoids are concerned which are known to create severe systemic effects when absorbed percutaneously to a greater extent. Vehicles which can transfer these drugs to the wanted site of action within the skin would thus prevent systemic absorption and consecutively unwanted effects. This is the reason why liposomes as a promising form for current drug delivery.[27]
6. Liposome may serve as a local store for the sustained release of dermally active compounds including antibiotics, corticosteroids or retinoic acid.
7. By virtue of penetration of individual phospholipid molecules or nonionic ether surfactants into the lipid layers of the stratum corneum and epidermis they may serve as penetration enhancer and facilitate dermal delivery leading to higher restricted drug concentrations.

1.4.4 MECHANISM OF ACTION OF TOPICAL LIPOSOME

The mode by which liposome facilitate transfer of drug into living skin strata and beyond has always been a topic of much interest. They propose a simple hypothesis of liposomal action that accounts for a majority of the effects observed. For a liposomal formulation to be effective, especially for hydrophilic drugs, it is critical that the deferment experience significant dehydration. Since in most studies reported the lipid concentration scarcely exceeds 100 mg/ml, the bulk aqueous medium constitutes roughly 90% of the formulation. Thus, without a high degree of dehydration, no compensation over simple aqueous solution can be governed by employing liposomal systems, especially if the drug action is anticipated to occur within few hours after application. The dehydration of liposomal suspension can either be complete or reach an equilibrium stage wherein a certain amount of water is always held within the bilayers.[28]

Drug Criteria for Topical Liposomal Drug Delivery System which groups of substances are considered to be especially interesting for liposomal encapsulation in the field of dermatology?

1. There are drugs which are known to have severe side-effects by the predictable way of topical administration, e.g. topical glucocorticosteroids.
2. There are substances which normally are helpful by systemic application but not by topical application, e.g. interferon.
3. There are drugs which only show inadequate effects when applied topically. e.g. hamamelis distillate.

1.4.5 Marketed Formulation Of Topical Liposome

❖ **Celadrin®:**

Celadrin® Topical Liposome Lotion- 4 oz. (a proprietary blend of esterified fatty acid carbons), MSM (Methylsulphonylmethane), *Arnica spp*. Flower Tincture and Natural Menthol (1.25%).

Celadrin® is a registered trademark of Imagenetix, Inc.

1.5 INTRODUCTION TO GEL DOSAGE FORMS

Gels are semisolid systems in which a liquid phase is inhibited surrounded by a three dimensional polymeric matrix, in which a high degree of corporal cross linking has been introduce. It consists of either natural or synthetic gums.[24] The structural material that is worn to form the gel network is composed of inorganic particles or organic macromolecules, primarily called as polymers. Cross link network can be shaped via chemical or physical interactions. This had lead to classification of gel into chemical and physical gel systems, respectively. Chemical gels are connected with permanent chemical covalent bonding between atoms while physical gels are coupled with relatively weaker and reversible inter molecular interactions such as hydrogen bonding, dipole dipole interactions, Vander Waals forces electrostatic interactions and hydrophobic interactions.[29]

DEFINITION - GEL (U.S.P.):

A semisolid system consisting of dispersion complete up of either small inorganic particles or large organic molecules enclosing and interpenetrated by continues phase liquid. Gels consist of two phase system in which inert particles are not dissolve but merely dispersed throughout the continuous phase and large organic molecules are dissolved in the continuous phase, arbitrarily coiled in the flexible chains. Gels are used dermatologically and have so many favorable properties such as thixotropy, easily removed, emollient, non-staining, greaseless, easily spreadable, and compatible with several excipients and water soluble or miscille, non grittiness. These all characteristics make them valuable in topical drug delivery system.[26,29]

Gels are defined as “the semisolid system in which a liquid phase is constrained within a polymeric matrix containing high degree of physical and chemical cross-linking”.

1.5.1 STRUCTURE OF GEL

The gel is inflexible due to the attendance of a network formed by the interlinking of particle gelling agent. The structure of network depends on nature of the particles and the type of force that determine the properties of gel.[28] The entity particles of hydrophilic colloid may consist of either spherical or an isometric aggregates of small molecules, or single macromolecules. In linear macromolecules the network is comprise of entangled molecules, the point of contact between which may either be relatively small or consist of several molecules aligned in a crystalline order.[31] The force of appeal responsible for the linkage between gelling agent particles may range from strong most important valencies, as in silicylic acid gels, to weaker hydrogen bonds and vander waals forces. The weaker nature of these latter forces is indicate by the fact that a slight increase in temperature frequently cause liquefaction of gel.

5.2 PROPERTIES OF GELS

1. Ideally, the gelling agent for pharmaceutical or cosmetic use should be inert, safe, and should not react with other formulation components.
2. The gelling agent incorporated in the preparation should create a reasonable solid-like nature during storage that can be easily broken when subjected to shear forces generated by shaking the bottle, squeezing the tube, or through topical application.
3. It ought to possess suitable anti-microbial to avert from microbial attack.
4. The topical gel be supposed to not be tacky.
5. The ophthalmic gel be supposed to be sterile.

1.5.3 CHARACTERISTICS OF GELS

a) Swelling

When a gelling agent is kept in make contact with with liquid that solvates it, then an appreciable amount of liquid is taken up by the agent and the volume increases. This method is referred to as swelling. This phenomenon occurs as the in the money penetrates the matrix. Gel-gel relations are replaced by gel solvent interactions.[29,30] The quantity of swelling depends on the number of linkages between entity molecules of gelling agent and on the strength of these linkages.

b) Syneresis

Many gels frequently contract spontaneously on standing and exude some fluid medium. This consequence is known as syneresis. The quantity to which syneresis occurs, increases as the concentration of gelling agent decreases. The incidence of syneresis indicates that the original gel

was thermodynamically unstable. The mechanism of contraction has been related to the reduction of elastic stress developed during the setting of the gels. As these stresses are relieved, the interstitial space available for the solvent is summary, forcing the liquid out.[31]

c) Ageing

Colloidal systems usually exhibit slow unprompted aggregation. This process is referred to as ageing. In gels, ageing results in measured formation of a denser network of the gelling agent.

d) Structure

The rigidity of a gel network is formed by the interlinking of particles of the gelling agents. The nature of the particle and the stress, straightening them out and lessening the resistance to flow.

e) Rheology

Solutions of the gelling agents and dispersion of flocculated solid are pseudo artificial i.e. exhibiting Non Newtonian flow behaviour, characterized by a decrease in viscosity with increase in shear rate. The tenuous arrangement of inorganic particles discrete in water is disrupted by functional shear stress due to breaking down of inter particulate association, exhibit a greater tendency to flow. Similarly, for macromolecules the applied shear stress aligns the molecules in the course of Organic phase (single phase system).

1.5.4 USES

- To deliver drug directly to the skin, mucous membrane or the eye.
- As long acting forms of drug injected intramuscularly.
- As binders in tablet granulation, protective colloids in suspensions, thickeners in oral liquid and suppository bases[32,33]

1.5.5 CLASSIFICATION OF GELS

Gels can be confidential based on colloidal phases, physical nature, nature of solvent used and rheological properties.

Table 3: Types of gel based on colloidal phases

Class	Description	Examples
Inorganic	Usually two phases system	Bentonite Magma
Organic	Usually single phases system	Carbopol, tragacanth

Hydrogel	Organic hydrogel natural and synthetic gums inorganic	Methyl cellulose, sodium carboxymethyl cellulose, pluronic,
Organogel	Hydrocarbon type animal, vegetable fats soap base greases hydrophilic organogel polar	Petrolatum, mineral oil/ polyethylene gel, cocoa butter aluminium stearate with heavy

1.5.5.1 Based on colloidal phases

They are classified into:-

- Inorganic (two phase system)
- Organic (single phase system)

1.5.5.1.1 Two phase system

If fractional size of the dispersed phase is relatively large and structure, in this organization is not always stable. They ought to be thixotropic-forming semisolids on standing and develop into liquid on agitation.

1.5.5.1.2 Single-phase system

These consist of large organic molecules existing on the twisted strands dissolved in a continuous phase. This larger organic molecule either accepted or synthetic polymers are referred as gel formers, they tend to tangle with each other their accidental motion or bound together by Vander walls forces.[31]

1.5.5.2 Based on nature of solvent

- Hydro gels (water based):

  Here they include water as their incessant liquid phase.

  E.g.: bentonite magma, Gelatin, , carpooler, cellulose derivatives and poloxamer gel.

- Organic Gels (with a non-aqueous solvent):

  These enclose a non-aqueous solvent on their continuous phase.

  E.g. plastibase (low molecular wt polyethylene dissolved in mineral oil & short Cooled) Olag (aerosol) gel and dispersion of metallic stearate in oils.

- Xerogels:

Solid gels with low in the chips concentration are known as xerogels. These are created by evaporation of solvent or freeze drying, leaving the gel structure behind on write to with fresh fluid, they swells and can be reconstituted.

E.g. Tragacanth ribbons, acacia tear β-cyclodextrin, dry cellulose and polystyrene.

1.5.5.3 Based on rheological properties

Usually gels display non-Newtonian flow properties.

They are classified into,

a) Plastic gels

b) Pseudo plastic gels

c) Thixotropic gels.

(a) Plastic gels

E.g. - Bingham bodies, flocculated suspensions of Aluminum hydroxide exhibit a artificial flow and the plot of rheogram gives the yield worth of the gels above which the elastic gel distorts and begins to flow.

(b) Pseudo-plastic gels

E.g.: - Liquid dispersion of tragacanth, sodium alginate, Na CMC etc. exhibits pseudo-plastic flow. The viscosity of these gels decreases with escalating rate of shear, with no yield value. The rheogram results from a shearing action on the long chain molecules of the linear polymers. As the shear stress is increased the disordered molecules begin to align their long axis in the direction of flow with release of solvent from gel matrix.[32]

(c) Thixotropic gels

The bonds between particle in these gels are very weak and can be broken down by shaking. The resultant solution will revert back to gel due to the particles colliding and linking together again (the reversible isothermal gel-sol-gel transformation). This occurs in colloidal system with non-spherical particles to build up a scaffold like structure.

E.g.: Kaolin, bentonite and agar.

1.5.5.4 Based on physical nature

(a) Elastic gels:

Gels of agar, pectin, Guar gum and alginates exhibit an elastic behaviour. The rubbery molecules being linked at the point of junction by quite weak bonds such as hydrogen bonds and dipole attraction. If the molecule possesses free –COOH group then supplementary bonding takes place by salt overpass of type –COO-X-COO between two adjacent strand networks.

E.g.: Alginate and Carbapol.

(b) Rigid gels:

This can be twisted from macromolecule in which the structure linked by primary valance

bond.

E.g.: In silica gel, silic acid molecules are held by Si-O-Si-O bond to give a polymer structure

possessing a network of pores.

1.5.6 PREPARATION OF GELS

Gels are in general in the industrial scale equipped under room temperature. Though a small number of polymer need special treatment before processing. Gels can be organized by following methods.

1. Thermal changes

2. Flocculation

3. Chemical reaction

1) Thermal changes

Solvated polymers (lipophilic colloids) when subjected to thermal changes causes gelatin. A lot of hydrogen formers are more soluble in hot than cold water. If the temperature is reducing, the degree of hydration is reduced and gelatin occurs. (Cooling of a concentrated hot explanation will produce a gel.

E.g.: - Gelatin, agar sodium oleate, guargummed and cellulose derivatives etc. In dissimilarity to this, some materials like cellulose ether have their water solubility to hydrogen bonding with the water. Raising the temperature of these solutions will interrupt the hydrogen bonding and reduced solubility, which will cause gelation. Hence this method cannot be adopted to prepare gels as a all-purpose method.[34][35]

2) Flocculation

Here gelation is produced by adding just adequate quantity of salt to precipitate to produce age state but insufficient to bring about complete precipitation. It is essential to ensure rapid mixing to circumvent local high concentration of precipitant.

E.g.: Solution of ethyl cellulose, polystyrene in benzene can be gelled by rapid mixing with suitable amounts of a non-solvent such as petroleum ether. The calculation of salts to hydrophobic solution brings about coagulation and relation is hardly ever observed. The gels formed by flocculation process are Thixotropic in behaviour. Hydrophilic colloids such as gelatin, proteins and acacia are only affected by high concentration of electrolytes, when the effect is to "salt out", the colloidal and gelation doesn't occur.

3) Chemical reaction

In this process gel is created by chemical interaction between the solute and solvent. E.g.: aluminium hydroxide gel can be equipped by interface in aqueous solution of an aluminium salt and sodium carbonate an augmented concentration of reactants will produce a gel structure.

PREPARATION OF CARBOPOL GEL AND INCORPORATION OF LIPOSOMES INTO CARBOPOL GEL

7.2.1 PREPARATION OF LIPOSOMES

Different weight ratio of phospholipids(soyalecithin): choleseterol and stearic acid was weighed in different ratios and dissolved in chloroform:methanol mixture (2: 1 v/v) in 250 ml round bottom flask. A thin film was formed on the inner side of round bottom flask by evaporating organic solvent under vacuum in rotary evaporator at 45-50 °C. Subsequently, the flask kept overnight under vacuum to ensure the complete removal of residual solvent. The dry lipid film was hydrated with 20 ml phosphate buffer solution (pH 7.4) containing fluconazole at a temperature of 60±2 °C. The dispersion will be left undisturbed at room temperature for 2-3 h to allow complete swelling of the lipid film and hence obtain vesicular dispersion.

7.2.2 PREPARATION OF CARBOPOL GEL

Aqueous liposomal formulations were prepared by conventional lipid film hydration method. As a vehicle for incorporation of liposomes for topical delivery, a carbopol gel was made.

Carbopol 934 (1 g) was dispersed in distilled water (88 g) by stirring at 800 rpm for 60 minutes. Then, propylene glycol (10 g) was added and the mixture was neutralised by

dropwise addition of tri-ethanolamine. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 5.5.[5,69]

Table 6: Table showing composition of Fluconazole loaded liposomal gel

Formulation	Fluconazole	Phospholipids	Cholesetrol	Stearic acid	Carbopol 934	Ratio P:Ch:S:C
F1	100mg	100mg	500mg	500mg	500mg	1:1:5:5:5
F2	100mg	200mg	500mg	500mg	500mg	1:2:5:5:5
F3	100mg	100mg	1000mg	1000mg	700mg	1:1:10:5:5
F4	100mg	200mg	1000mg	1000mg	700mg	1:2:10:10:7
F5	100mg	300mg	500mg	500mg	500mg	1:3:5:5:5
F6	100mg	300mg	1000mg	1000mg	700mg	1:1:10:10:7
F7	100mg	400mg	500mg	500mg	500mg	1:4:5:5:5
F8	100mg	400mg	1000mg	1000mg	1000mg	1:4:5:5:7

7.2.3 INCORPORATION OF LIPOSOMES OF OPTIMIZED BATCH INTO CARBOPOL GEL

Fluconazole loaded liposomes were prepared by thin film hydration technique using soya lecithin, cholesterol and drug in different weight ratios.[70] Liposomes containing fluconazole (separated from the unentrapped drug) were mixed into the 1% (w/w) Carbopol gel with an electrical mixer (25 rpm, 2 min), the amount of liposomes of optimized batch added into the gel, such that the prepared gel have 2% w/w fluconazole concentration (20 mg drug per 1gm of gel). Plain drug gels (2% w/w) were made under the same conditions. Instead of liposomes, those samples contained free fluconazole were incorporated.

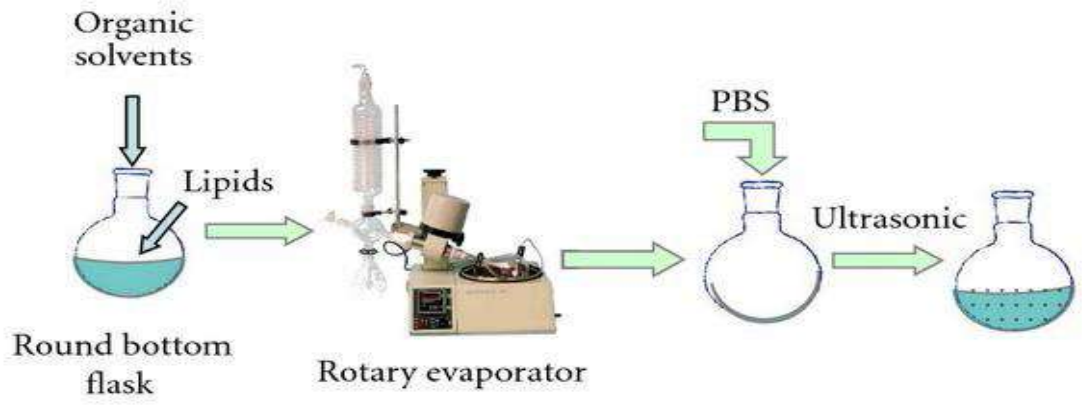

Figure 17: Assembly for thin film hydration method

7.3 EVALUATION OF LIPOSOMAL GEL

The characterization parameters for the purpose of evaluation was carried out in following categories, which include physical, chemical and biological parameters.[17]

Physical classification evaluates various parameters, including size, shape, surface features, lamellarity, phase behaviour and drug release profile.

Chemical characterization includes those studies which establish the purity and potency of various liposomal constituents.

Biological characterization parameters are helpful in establishing the safety and suitability of the formulations for the in vivo use or the therapeutic application.

Physical and chemical characterizations are very important for meaningful comparison of dissimilar liposomes arrangements for different batches. Biological consideration helps to ensure safety of use in humans.

A. Physicochemical Evaluation of liposomal gel

1) Physical examination: The prepared Liposomal gel was evaluated for colour, and transparency.

2) pH: The pH values of 1% aqueous solutions of the prepared gels were measured by digital pH meter.(Chameline model)

3) Drug Content uniformity:

Preparation of standard graph

Stock solution of Fluconazole: Stock solution of 100µg/ml was prepared by dissolving 10 mg of fluconazole in 100 ml of methanol. Dilution in the range of 10 to 40 µg/ml were scanned for determining λmax from 200-400 through UV spectrophotometer(Systronic model) and λmax was found to be at 260 nm for fluconazole.

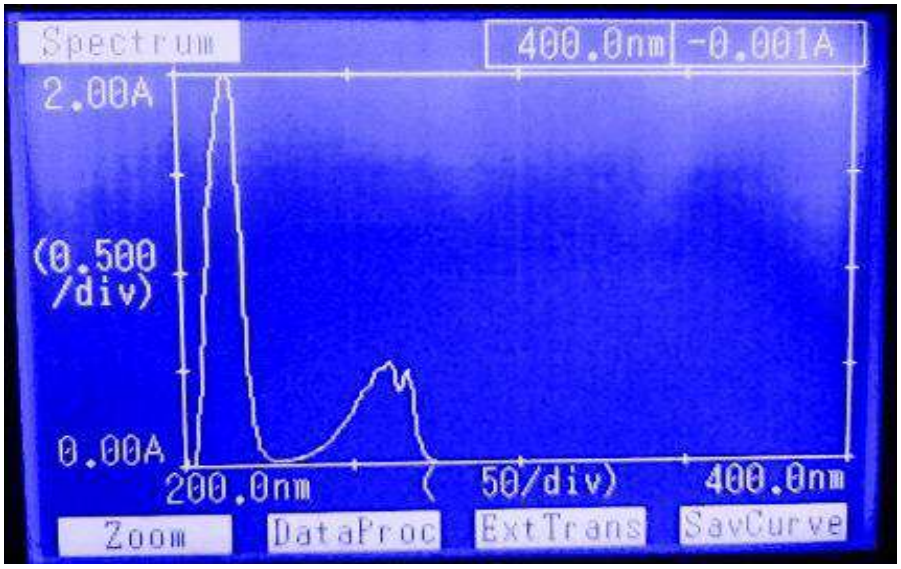

Figure 18: λmax of Fluconazole in methanol

Table 7: Absorbance of different dilutions of drug at 260 nm in methanol

S.No.	Concentration (µg/ml)	Absorbance (mean ± SD)(n=3)
1.	0	0
2.	10	0.131 ± 0.001
3.	20	0.262 ± 0.004
4.	30	0.355 ± 0.002
5.	40	0.448 ± 0.003

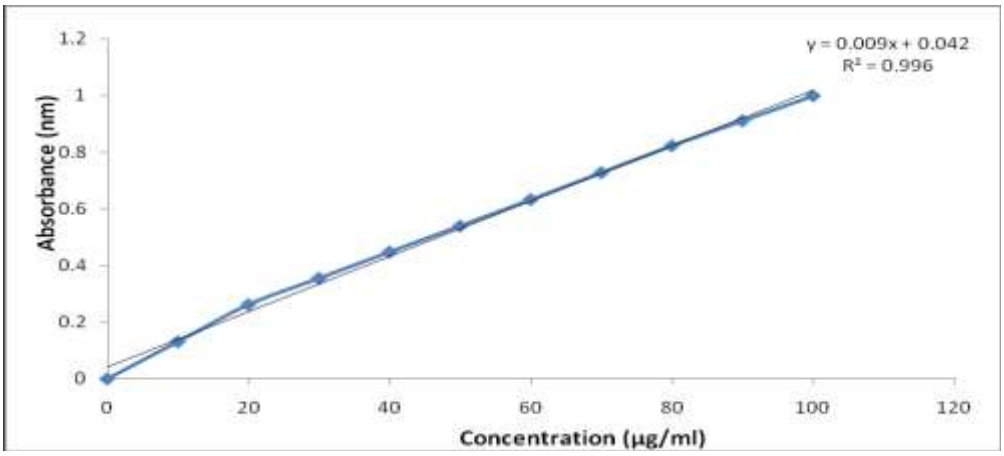

Figure. 19: Standard calibration curve of fluconazole at 260nm in methanol

The gel sample (100 mg) withdrawn and drug content was determined using a UV spectrophotometer at 260 nm. Similarly, the content uniformity determined by analyzing the drug concentration in gel taking from 3 to 4 different points from the container. In case of liposomal gel, it will be shaken with adequate quantity of methanol to extract the drug and then analyzed by using a UV spectrophotometer at 260 nm.

4) Spreadability: It was determined by wooden block and glass slide apparatus. Two glass slide of 20×20 cm were selected. The gel formulations were placed over one of the slides. The other slide was placed upon the top of the gel such that the gel was sandwiched between the two slides in an area occupied by a distance of 60. cm along 100g weight was placed upon the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gels adhering to the slide was scrapped off. The two slides in positioned were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 g weight was tied to upper slide carefully. The time taken for the upper slide to travel the distance of 6 cm and separate away from the lower slide under the direction of weight was noted. Weights were added to the pan and the time noted for upper slide (movable) to separate completely from the fixed slides. Spreadability intended by using the formula:

$S = M.L / T$

Where, S = Spreadability M = Weight tide to upper slide L = Length of glass slide

T = Time taken to separate the slide completely from each other

5) Homogenicity: Developed gel was experienced for homogenicity by visual inspection after the gel has been set in the container. This tested for their appearance and presence of any aggregates.

6) Viscosity Studies: Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. 50 g of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor.

7) Rheological study*:* The semisolid preparations should flow or deform after applying the force and regain its elasticity as the force is removed. Thus, to understand the rheological properties of liposomal gels rheological study has been performed.

8) *In-vitro* drug diffusion study

Rat abdominal skin was used for diffusion studies as an *in-vitro* experimentation. Subcutaneous fatty tissue will be unconcerned from the skin using a scalpel and surgical scissors. After the fatty tissue was removed, the surface of the skin will be cleaned with saline solution. [80]

Experiment

A Franz diffusion cell was used to perform the experiment. *In vitro* absorption studies are generally carried out in vertical franz diffusion cell. According to Food and Drug Administration (FDA) regulations, it is an ideal tool for quality control of topical preparations. It has a receptor and a donor chamber, which is filled with phosphate buffer medium solution from the receptor chamber. The jacketed cell embodied is stirred throughout the study at 500 rpm employing a magnetic stirrer.. The dialysis membrane is sandwiched between the two chambers and clamped in place tightly. Full shaved abdominal skin was mounted between the cells and the receptor compartment was 10 ml. The donor medium consisted of 1 gm liposomal gel. To maintain the sink condition Phosphate buffer pH (6.5): Ethanol (3:1) was used as receptor medium. Stirring rate and temperature were kept 400 rpm and 37°C respectively. At different intervals (1, 2, 4, 8, 12 and 24 h), the receptor samples were removed and replaced with fresh receptor medium. Receptor samples were then analyzed for drug content spectrophotometrically at 260 nm wavelengh. Cumulative amount of drug release was determined as a function of time and the release rate was calculated.

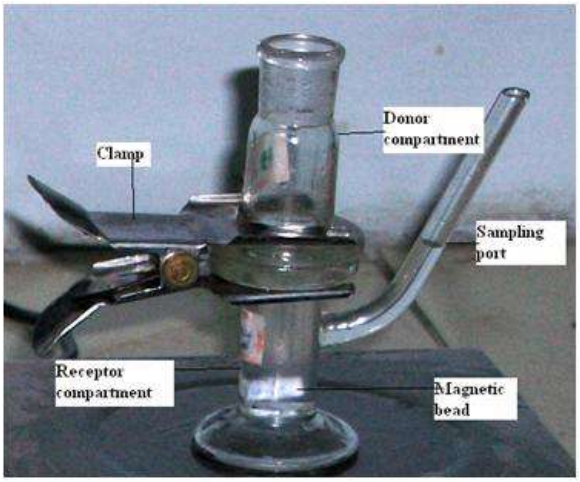

Figure 20: Franz diffusion cell

7.4 Physicochemical Evaluation Of Liposomes

1) Entrapment efficiency

Drug associated with liposome were divided from unentrapped drug using centrifugation method. Liposomes were centrifuged[70]. Supernatant contain unentrapped drug was withdrawn and measured UV spectrophotometrically against phosphate buffer saline (pH 7.4). The amount of drug entrapped in liposome determined as follow:

EE (%) = [(Cd–Cf)/Cd] 100

Where Cd is concentration detected of total drug and Cf is concentration of free drug.EE is entrapment efficiency.

2) Zeta potential (z) determination

Charge on empty and drug loaded vesicles surface will be determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined. Zeta potential is the measure of the magnitude of the electrostatic or charge repulsion or attraction between particles and, known to affect stability. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation, and can be applied to improve the formulation. Almost all particulate or macroscopic materials in contact with a liquid acquire an electronic charge on their surfaces [71]

3) Stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) will be assessed by keeping the liposomal suspensions and liposomal gel at two dissimilar temperature situation for particular period of time. Samples will be reserved periodically and analyzed for the drug content

and particle size for liposomal suspension and drug deposition for liposomal gel in the manner described under entrapment efficiency and particle size distribution studies.[72]

4) Vesicular morphology

The morphology of vesicles was studied by scanning electron microscope. For SEM one drop of each sample from liposome were mounted on a stub covered with clean glass respectively. The drop was spread out on the glass homogenously. A sputter coater was used to sputter coat the samples with platinium and samples were examined under Jeol 6480 LVJSM at an accelerating voltage 20kv.

RESULTS AND DISCUSSION

8.1 Preformulation Studies

8.1.1 FTIR studies

Fourier Transform Infrared (FTIR) studies of Fluconazole and polymer Carbopol were performed at Pinnacle Biomedical Research Institute Bhopal, India, in order to carry out drug polymer compatibility study (Figure 8.1 and 8.2) using UNIVERSAL Q 200 V 23.5 instrument.

The principle peaks of fluconazole were observed in the spectra of the drug / polymer physical mixtures indicating no interactions had been occurred.

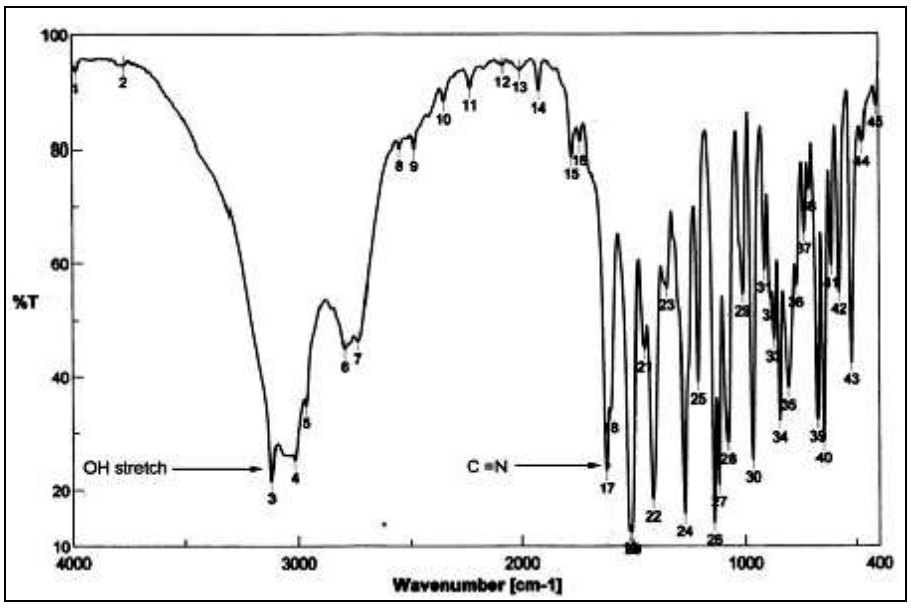

Figure 21: FT-IR of drug fluconazole

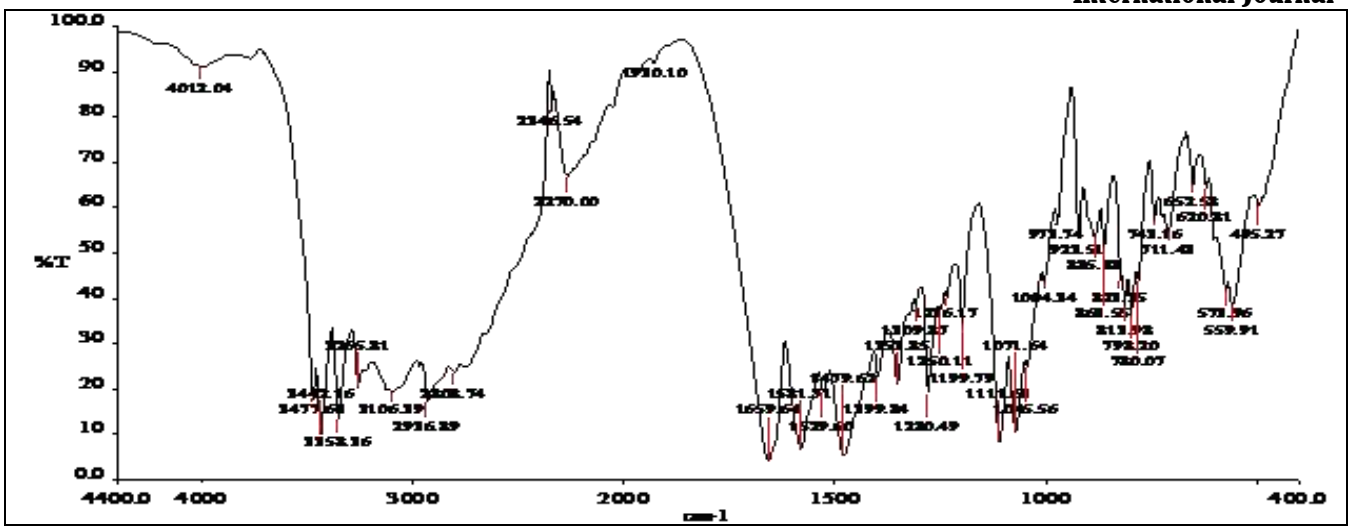

Figure22: FT-IR of polymer: Carbopol 934

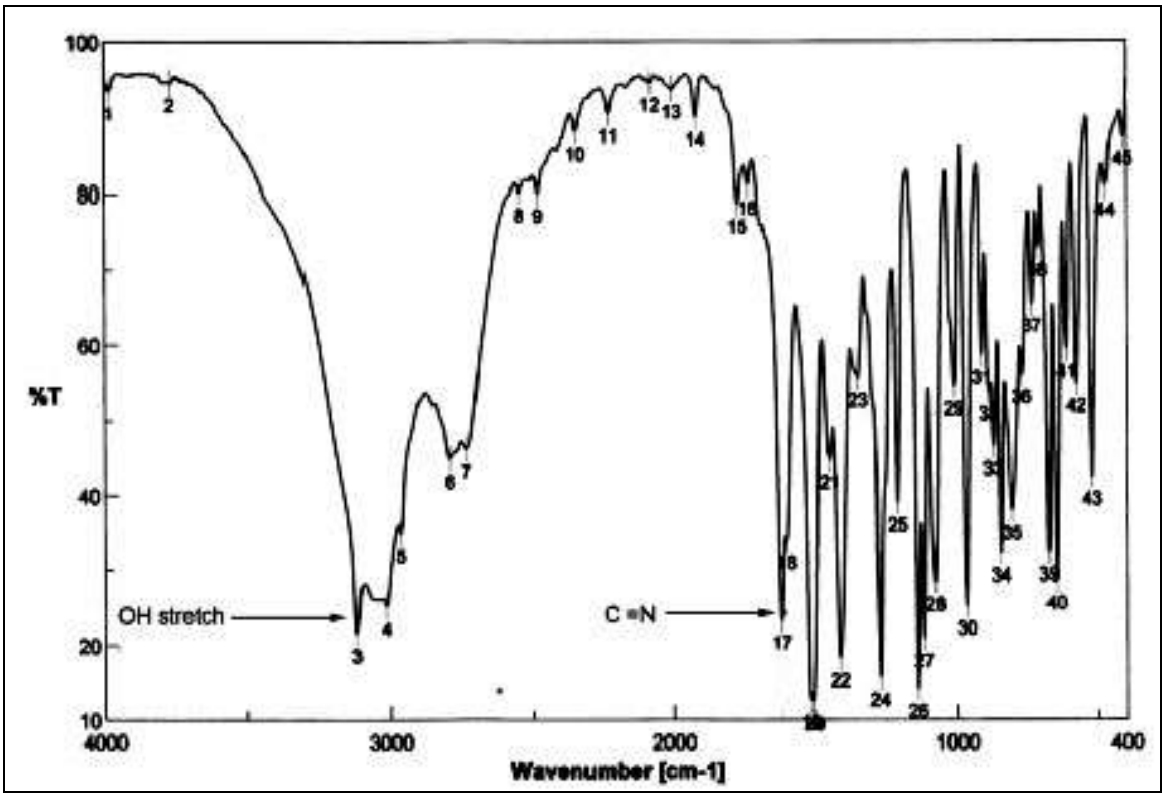

Figure 23: FT-IR of drug fluconazole and Carbopol 934

Table .8 FTIR interpretation in vibration mode for mixture of fluconazole and carbopol 934

Peak obtained in drug (frequency cm-1)	**Description**	**Peak obtained in mixture (frequency cm-1)**

3176.76	OH Stretching	3192.19
2817.36	CH2 Stretching	2880.41
2966.52	CH (Aromatic Stretching)	2956.87
1560.41	C = N Stretch	1562.34
1456.80	CH (Aromatic bending)	1456.20
1133.93	C - F Stretch	1139.39

8.1.2 Differential scanning calorimetry(DSC)

Methodology:

Differential Scanning Calorimetry (DSC) studies of drug Fluconazole and polymer Carbopol was carried out by heating the samples from 40°C to 240°C at the rate of 20°C/min, using UNIVERSAL Q 200 V 23.5 instrument.

Table. 9 Summary of DSC for decomposition of Fluconazole, carbopol 934 and mixture.

Parameters	**Pure Drug Fluconazole**	**Carbopol 934**	**Fluconazole in Mixture of Fluconazole & Carbopol**	**Carbopol 934 in Mixture of Fluconazole & Carbopol**
DSC Onset Temp. ^{0}C (Initial Temp.)	135.11 ^{0}C	90.8 °C	138.60 ^{0}C	100.55^{0}C
Final Temperature ^{0}C (Peak)	136.36 ^{0}C	93.5 °C	140.6 °C	102.3 ^{0}C
Enthalpy in KJ/Mole H	57	34	59	36
Characteristic of	Endotherm	Endotherm	Endotherm	Endotherm

Peak				

DSC was carried out for the drug fluconazole, for polymer carbopol and mixture. Pure fluconazole gave melting endotherms at 136.36 ^{0}C indicating that the drug is in crystalline form.

In the DSC thermogram of Carbopol 934, glass transition temperature (Tg) was observed near the range of 90-110 ^{0}C. The ***Carbopol 934*** sample gave an ***endothermic melting*** transition at 93.5 °C. Decomposition of Carbopol 934 was observed near 110 ^{0}C where Carbopol melted and decomposed sequentially.

DSC thermograms of physical mixtures showed the broad endotherms due to water removal at about 100 to 140.80C. The physical mixture of Fluconazole and polymer carried for DSC the melting of fluconazole observed between 100^{0}C and 102.3^{0}C and Carbopol showed onset temperature 100.5^{0}C and the peak transition temperature is 102.3^{0}C. It proves no interaction between drug and carbopol 934.

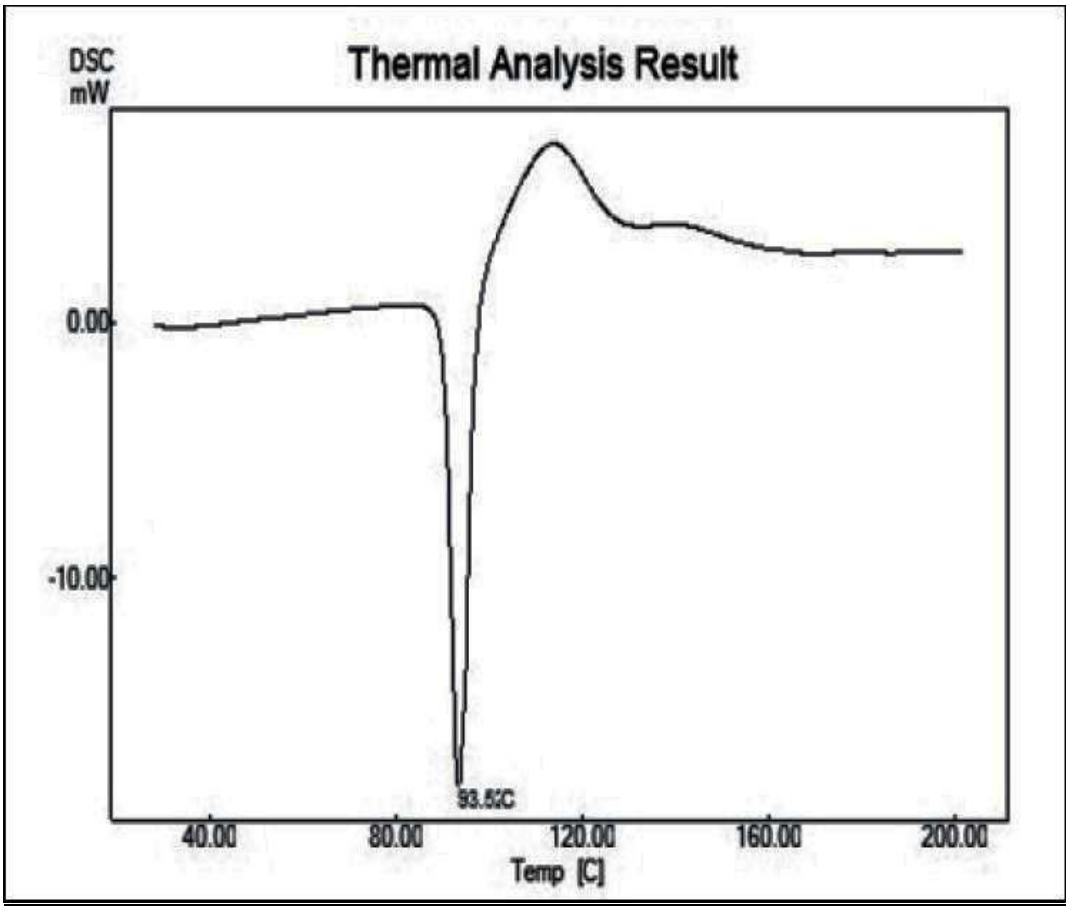

Figure 24: DSC of Polymer Carbopol 934

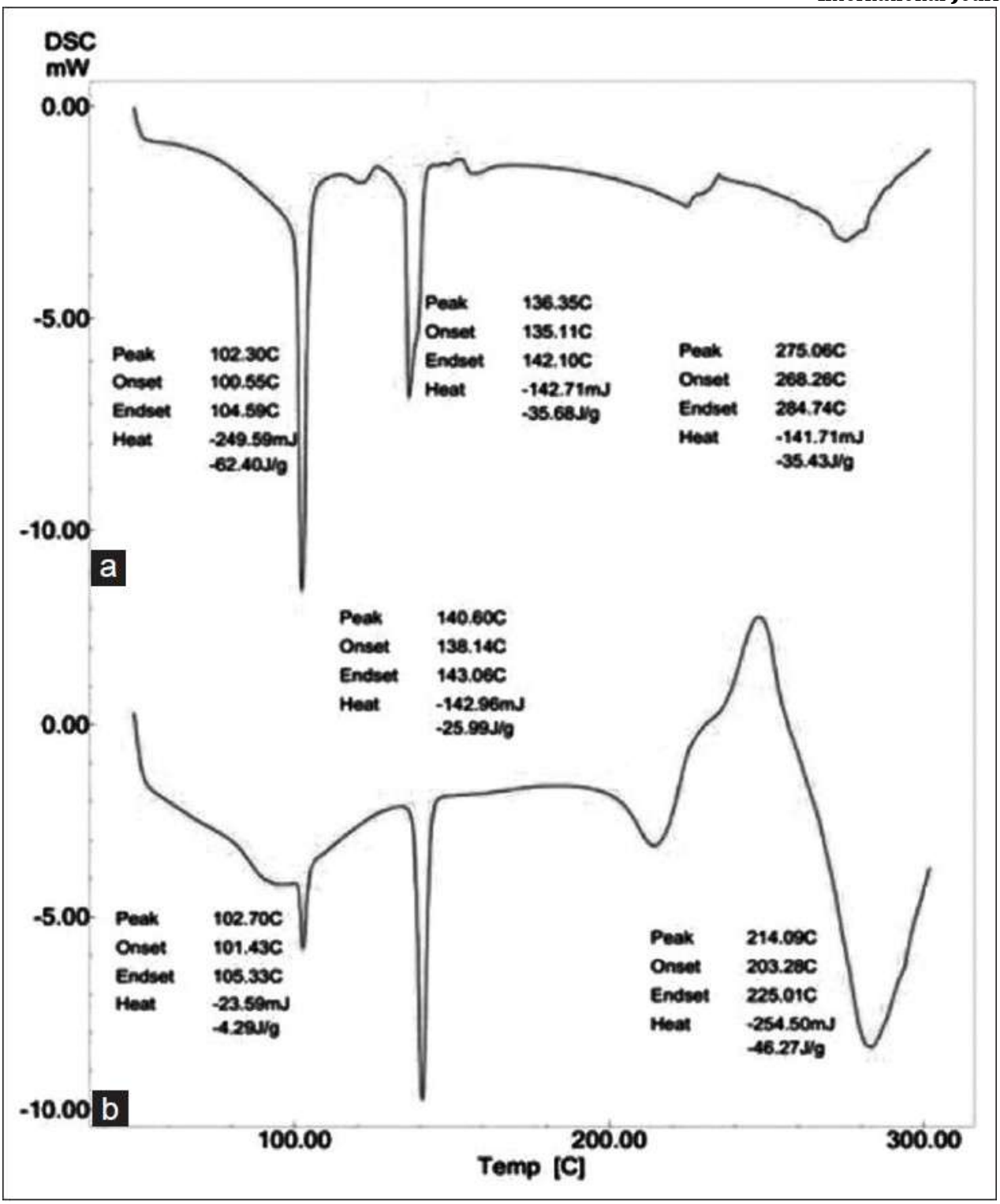

Figure 25: DSC of drug fluconazole and Polymer Carbopol 934 with drug fluconazole

8.2 Physicochemical Evaluation of liposomal gel

8.2.1.1 Physical examination: The prepared Liposomal gel was evaluated for colour, and transparency; the prepared gel was in acceptable limits.

8.2.1.2 pH: The pH values of 1% aqueous solutions of the prepared gels were measured by pH meter, the average pH of formulations were comes to 7.3 pH.

8.2.1.3 Drug Content uniformity: The gel sample (100 mg) withdrawn and drug content was determined using a UV spectrophotometer at 260 nm. Similarly, the content uniformity determined by analyzing the drug concentration in gel taking from 3 to 4 different points from the container. In case of liposomal gel, it will be shaken with adequate quantity of methanol to extract the drug and then analyzed by using a UV spectrophotometer at 260 nm.

There was no significant difference observed in the % drug at various locations, indicating that the method used to disperse the liposomal dispersion in the gel base is satisfactory.

8.2.1.4 Spreadability: It was determined by wooden block and glass slide apparatus. Weights were added to the pan and the time noted for upper slide (movable) to separate completely from the fixed slides. Spreadability intended by using the formula:

S = M.L / T

Where, S = Spreadability M = Weight tide to upper slide L = Length of glass slide

T = Time taken to separate the slide completely from each other

8.2.1.6 Homogenicity: Developed gel was experienced for homogenicity by visual inspection after the gel has been set in the container. This tested for their appearance and presence of any aggregates that comes in acceptable range.

8.2.1.7 Viscosity Studies: Viscosity of prepared gel was carried out by using Brookfield Viscometer. The rotation was varied from 30 to 50 rpm at 25±1 ◦C.

8.2.1.8 Rheological study*:* The semisolid preparations should flow or deform after applying the force and regain its elasticity as the force is removed. Thus, to understand the rheological properties of liposomal gels and for selection of optimum concentration of carbopol having desired rheological properties, different concentrations (1, 1.5, 2% w/w) of carbopol 934 were used to prepare liposomal gels at 25 °C with neutralization method. The rheologies of all samples were determined to identify the minimum concentration of carbopol required for the formation of gel with good visco-elastic properties.

8.2.2 *In-vitro* studies

Rat abdominal skin was used for diffusion studies as an ex-in-vivo experimentation. Subcutaneous fatty tissue will be unconcerned from the skin using a scalpel and surgical scissors.

After the fatty tissue was removed, the surface of the skin will be cleaned with saline solution.

Skin Permeation Test

Rat was sacrificed by exposing to excess chloroform. To the abdominal skin, depilatory was applied and kept for 10 m to remove the hair from the skin. After 10 m of application, skin was washed with water. Skin was excised from rat with scalpel and fatty layer was removed by keeping the skin in warm water at 0 60 C. After 2 m, fatty layer was peeled off gently and skin was washed with water and kept for saturation in phosphate buffer saline pH 7.4 for about 30 m before it was used for permeation studies. Fresh skin was used every time. Skin permeation studies with fluconazole containing liposome formulations were carried out using abdominal rat skin, employing modified Franz-diffusion 12 cells. The results obtained were compared with that of non-liposomal formulations of fluconazole. The skin was prepared by mounting on the receptor chamber with 2 cross-sectional area of 3.91 cm exposed to the receptor compartment. The receptor compartment was filled with phosphate buffer pH 7.4. It was jacketed to 0 maintain the temperature 37 + 0.5 C and was kept stirring at 50 rpm. Prior to application of formulations, the skin was allowed to equilibrate at this condition for 1 h. Liposomal or non-liposomal fluconazole formulation (amount equivalent to 5 mg of drug) was applied uniformly on the dorsal side of skin. Aliquots of 2 ml were withdrawn periodically and replaced with same amount of saline solution to maintain the receptor phase volume at a constant level. The samples were quantified spectrophotometrically at a wavelength of 260 nm. Max For determination of drug deposited in skin, cell was dismantled after a period of 8 h and skin was carefully removed from the cell. The formulation applied on skin surface was swabbed first with phosphate buffer pH 7.4 and then with methanol. The procedure was repeated twice to ensure no traces of formulation are left onto skin surface. The skin was then cut into small pieces and drug present in skin was extracted in phosphate buffer pH 7.4 using bath sonicator and determined spectrophotometrically after suitable dilution and filtration.[81]

Table 10(a): Drug release profile of fluconazole loaded liposome Formulation F1 to F4 and plain gel

Time(hr)	**F1**	**F2**	**F3**	**F4**	Plain Gel
1	17.3±1.98	23.4±0.98	14.6±1.42	14.0±1.24	14.8±2.00
2	17.5±1.31	23.5±1.08	14.9±1.55	14.2±1.22	15.0±2.12

3	19.9±1.33	24.2±1.21	16.0±1.32	16.3±1.32	16.7±1.98
4	22.4±1.42	26.7±1.24	19.8±1.57	19.0±1.36	20±1.22
5	29.3±2.01	29.9±0.99	22.6±1.67	23.0±1.52	26.7±1.35
6	32.5±1.98	30.6±1.35	23.8±1.28	25.0±1.35	33.0±1.87
8	40.0±2.22	57.3±1.75	41.3±1.98	32.7±1.87	39.7±1.98

Table 10(b): Drug release profile of fluconazole loaded liposome Formulation F5 to F8

Time(hr)	F5	F6	F7	F8
1	19.6±1.02	14.5±1.82	17.3±2.01	19.6±1.82
2	19.8±1.32	14.7±1.38	17.4±2.22	19.6±1.67
3	22.0±1.54	16.6±1.37	19.9±2.70	22.7±1.37
4	25.6±1.65	18.8±1.65	21.2±1.00	27.4±1.46
5	26.6±1.11	20.3±1.29	24.3±1.28	30.7±1.66
6	19.9±2.01	27.3±1.58	23.8±1.28	26.6±1.65
8	30.0±1.98	31.0±1.62	28.0±1.65	28.2±1.73

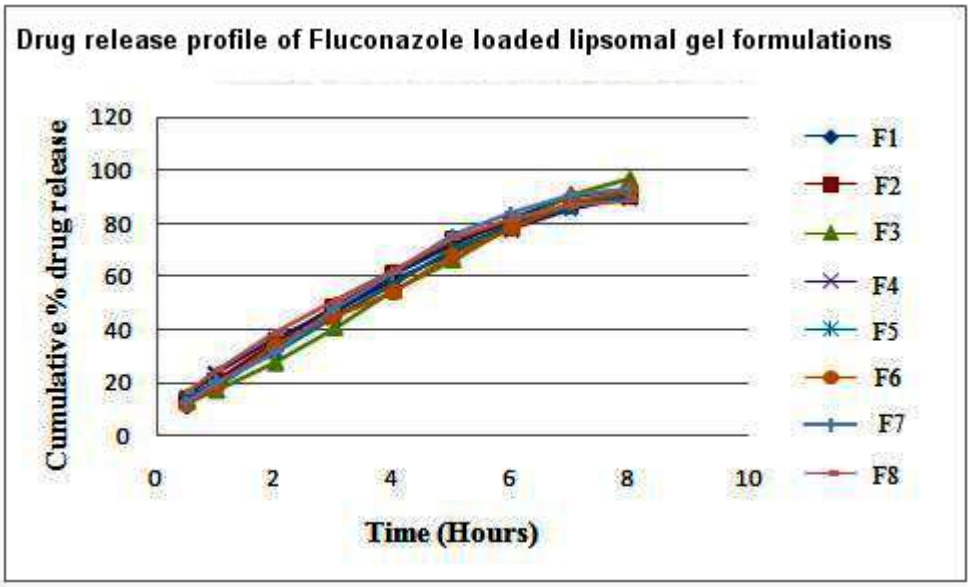

Figure 26: Drug release profile of fluconazole loaded liposomes

8.3 Physicochemical evaluation of liposomes

Vesicle Morphology

The various drug loaded formulations of liposomes (including the blank particles) appeared more or less spherical when observed by Scanning Electron Microscopy SEM.

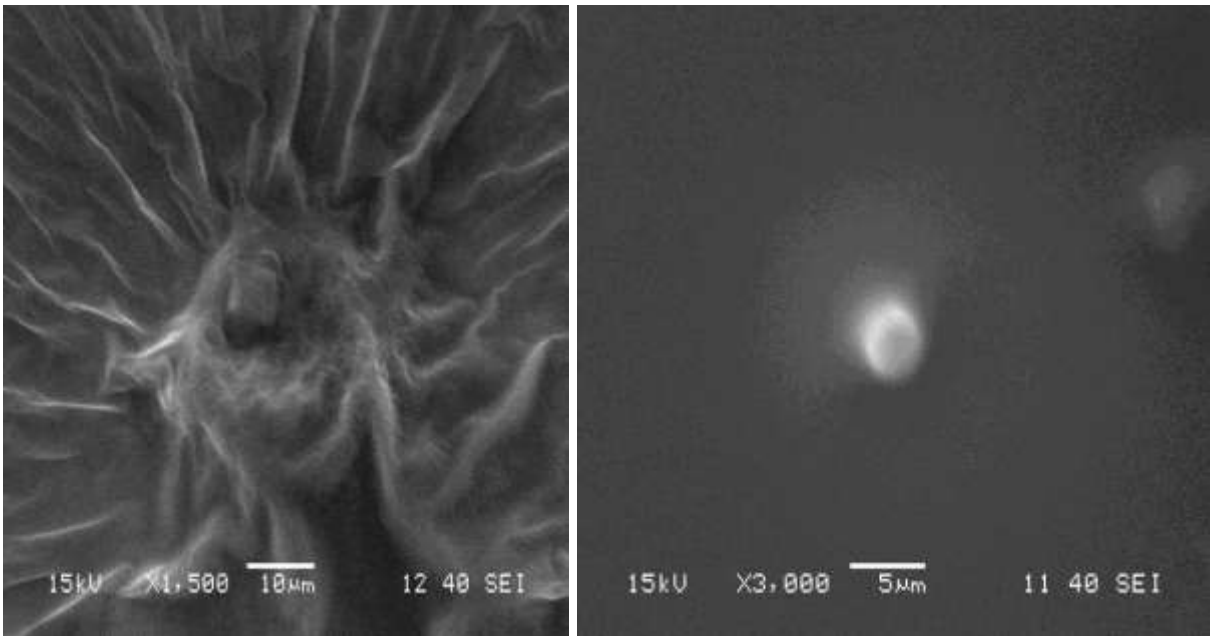

Figure 27: Scanning Electron Microscopy image of Liposome

8.3.1 Entrapment efficiency:

Drug associated with liposome were divided from unentrapped drug using centrifugation method. Liposomes were centrifuged. Supernatant contain unentrapped drug was withdrawn and measured UV spectrophotometrically against phosphate buffer saline (pH 7.4). The amount of drug entrapped in liposome determined as follow:

EE (%) = [(Cd–Cf)/Cd] 100

Where Cd is concentration detected of total drug and Cf is concentration of free drug.EE is entrapment efficiency.

8.3.2 Zeta potential ( ) determination:

Charge on empty and drug loaded vesicles surface will be determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined.[33]

In the present study the obtained for liposomes are shown in Table …. The values of potential (-54.1 63.6 mV for vesicles) showed prepared liposome have sufficient charge to avoid aggregation of vesicles.

The zeta potential of the liposomes ranged between -12.2 mV to -29.0 mV indicating incipient stability.

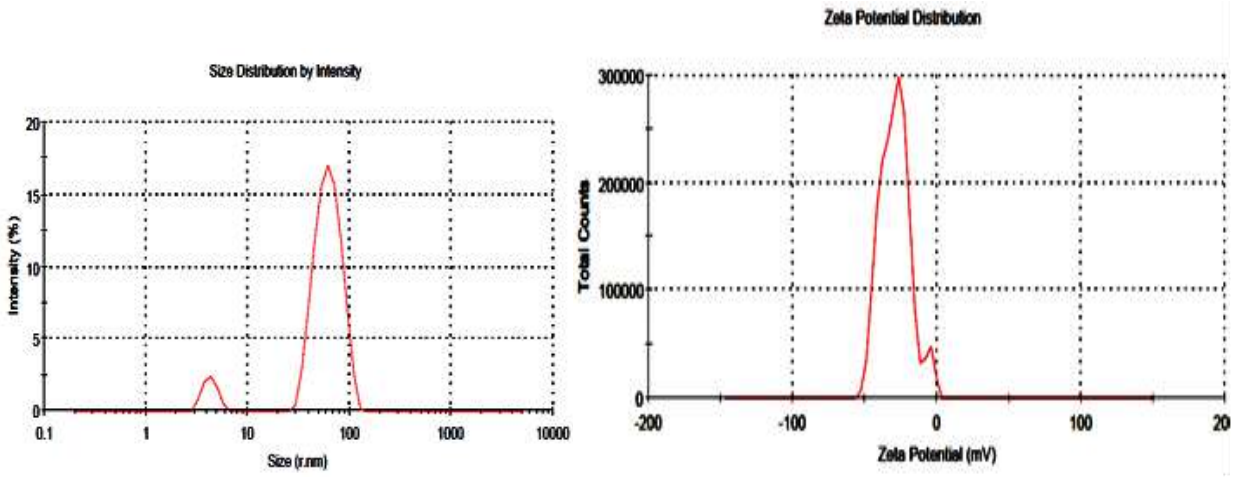

Figure 28: Size and zeta potential of liposome

8.3.3 Stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) will be assessed by keeping the liposomal suspensions and liposomal gel at two dissimilar temperature situation for particular period of time. Samples was withdrawn periodically and analyzed for the spreadibility,

viscosity, pH, and drug content particle size for liposomal suspension in the manner described under entrapment efficiency and particle size distribution studies.[33,76]

The liposomal gel were packed and kept for one month at 0^0C- 2^0C, 40^0C/ 75% RH in a stability chamber, 60^0C/80% in incubator . At the interval of 15 days gel were withdrawn and evaluated for physical properties like Spreadibility, pH, viscosity and content uniformity carried out.

Table 11: Stability studies of Liposomal Gel

				Storage Condition			
S. No	**Parameters**	**0°C**		**25°C±2°C, 60% RH**		**40°C±2°C, 75% RH**	
		15days	**30days**	**15days**	**30days**	**15days**	**30days**
1.	**Spreadability (gcm/sec) ±SD**	54.46±0.79	53.92± 0.78	55.65± 0.81	54.79± 0.47	53.55± 0.78	51.62 ± 0.74
2.	**Viscosity (Centipoise)**	15174	157678	16695	16687	13546	13959
3.	**pH**	5.47	5.49	5.48	5.48	5.53	5.58
4.	**% Drug Contents**	95.05± 0.44	95.10± 0.51	95.49± 0.45	95.55± 0.43	93.61± 0.65	93.08 ± 0.67

Table 12: Entrapment efficiency, Vesicle Size and Zeta Potential of various formulations

Batch No.	**Entrapment Efficiency**	**Vesicle Size**	**Zeta Potential**

	(EE ± SD,%)	**(Size ± SD, µm)**	**(z ±SD,mV)**
1	55.6±1.23	2.88±0..22	- 54.5 ± 0.77
2	57.3±0.54	3.46±0.83	- 55.9 ± 1.43
3	59.8±0.87	3.98±0.99	- 57.5 ± 1.73
4	61.02±1.23	4.06 ± 0.40	- 58.6 ± 2.41
5	62.88±0.71	3.50 ± 0.92	- 54.5 ± 1.57
6	63.92±1.67	4.28 ± 1.62	- 58.9 ± 1.43
7	64.07±0.93	5.83 ± 1.49	- 55.09 ± 0.87
8	65.22±0.66	7.15 ± 1.14	- 53.4 ± 2.97

Table 13: *In-vitro* skin permeation and skin retention of fluconazole from different formulations

Fluconazole formulations	**Mean cumulative % drug permeated**	**Permeation flux mg/ cm2 / h**	**% Drug retained in skin**
Liposomal gel	30.46	0.1754	8.21
Liposomal dispersion	32.47	0.1871	10.03
Marketed gel	23.41	0.1341	5.21
Carbopol plain gel	24.68	0.1531	3.64
Aqueous solution	27.2	0.1570	5.79

Table 14: Effect on entrapment efficiency, vesicle size for liposomal dispersion and drug deposition from liposomal gel during stability

	Entrapment efficiency %		Vesicle size (□ m)		Drug deposition %	
No.of days	4-8 ^{0}C	Room Temp.	4-8 0 C	Room Temp.	4-8 ^{0}C	Room Temp.
0	64.88	64.88	3.5	3.5	0.41074	0.41074
30	63.92	61.73	4.28	4.3	0.3981	0.3174
60	61.39	58.32	4.13	5.2	0.3127	0.2349

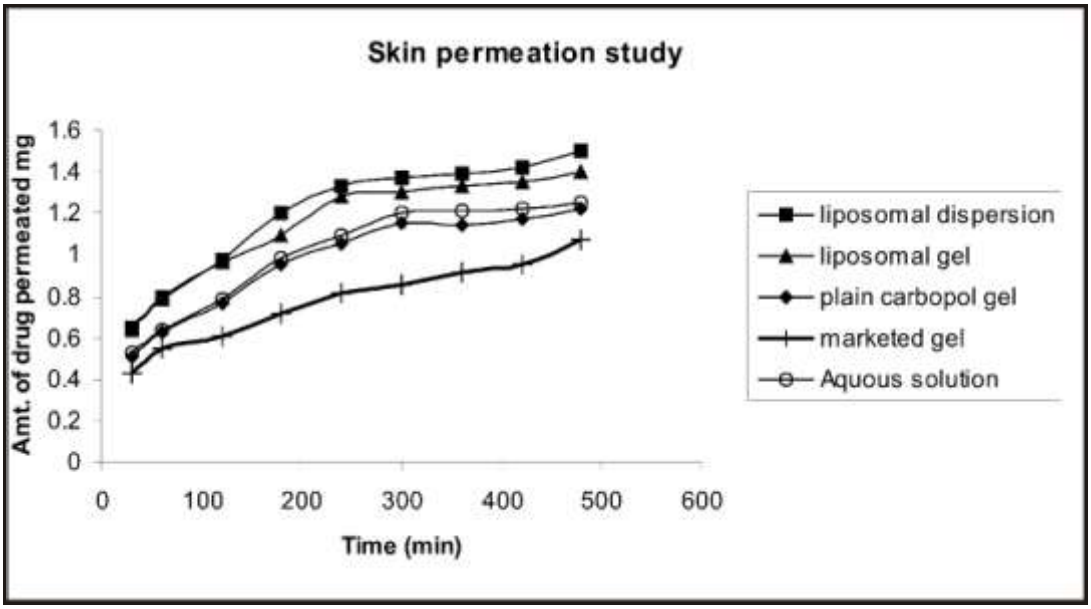

Figure 29: Permeation profile of different fluconazole containing systems

9. SUMMARY AND CONCLUSION

The present work on the preparation of topical liposomal gel containing fluconazole is an attempt to utilize the immense potential of liposomes as a carrier to increase the permeability. Liposomal encapsulation showed more drug retention compared with plain drug gel .

Further, the physicochemical modification in the drug by means of phospholipid membrane also promises to prolong the drug action. A number of problem associated with drug molecule such as bioavailability, degradation, stability and side effects can be overcome by incorporating it into liposomes. The liposomes of fluconazole was prepared by film hydration method and evaluated. The *In vitro* release of the formulation was studied and found to be more efficient than plain gel. Creation of reservoir effect for drug in skin due to deposition of other components of liposomes

with drug into the skin and thereby increasing the drug retention capacity into the skin. Liposomal formulations were more spherical with stable zeta potential and mono-disperse with no clumping.

Variables such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency. Liposomal dispersion and gels were found to increase the skin permeation and deposition compared to control. Drug release of all batches was studied. Stability studies performed for Liposomal gel indicates the prepared liposomes have more stability at freezing temperature than that of room temperature.

Fluconazole molecules could be successfully entrapped in liposomes with reasonable drug loading. Hence from results obtained it can be concluded that liposomal gel containing fluconazole has potential application in topical delivery for the treatment of fungal skin ailments.

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Original Research Article **Volume 6 Issue 1**

PREPARATION AND EVALUATION OF MULTIPLE-UNIT FLOATING DRUG DELIVERY SYSTEM OF CLARITHROMYCIN

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Abstract:

Multiple-unit floating beads of clarithromycin were prepared from sodium alginate solution containing hydroxypropylmethylcellulose (K100M) and sunflower oil using the technique of three variables at three levels (3 power 3) factorial design and twenty-seven possible batches were prepared. These beads were evaluated for entrapment efficiency, drug loading, buoyancy and in vitro drug release. All formulations showed floating lag time below 2 minutes and showed total floating duration more than 10 hours. The result of in-vitro dissolution studies revealed that the formulation F14 was showing sustained release pattern of clarithromycin. The release rate, entrapment efficiency, drug loading and buoyancy was greater with formulation containing 2 percent sodium alginate solution and 5 percent calcium chloride solution along with 5 ml sunflower oil.

Keywords: Floating alginate beads; emulsion gelation; clarithromycin; controlled release.

INTRODUCTION

Gastro-retentive dosage forms are particularly appropriate for drugs, (1) that are locally active to the gastric mucosa in the stomach; with an absorption window in the stomach or in the upper small intestine; that are unstable in the intestine; with low solubility at high pH values.[3] One of the approach for gastro- retentive system is floating dosage forms, which remain buoyant on gastric contents because they have a lower density than gastric fluids.[1]

Calcium alginate gel beads have been developed in recent years as a unique vehicle for drug delivery system. Various categories of drug have been encapsulated such as nonsteroidal anti-inflammatory drugs, enzymes, antibiotics, peptides/proteins, and acid labile drugs[5].

Materials and methods:

Sodium alginate, hydroxyl propyl methylcellulose (K100M) and calcium chloride were obtained from Colorcon Asia Pvt. Ltd. (Goa, India.). Clarithromycin was donated by Biochem Pharmaceutical (Daman, India.). All other chemicals used were of analytical grade.

Formulation of clarithromycin floating beads:

Clarithromycin floating beads were prepared using emulsion-gelation method. Sodium alginate and hydroxyl propyl methylcellulose (K100M) were dissolved in water with stirring. Sunflower oil was added to polymer solution followed by clarithromycin. The homogenized mixture was extruded into calcium chloride solution with gentle agitation at room temperature. The formed beads were allowed to stand for 30 min in the solution for curing then separated by filtration and dried at room temperature and used for further studies[9].

Process variables and process optimization:

To investigate the contribution of formulation variables on the release profile of clarithromycin from alginate beads, a 3^3 full factorial design was utilized and the different batches were produced. The process parameters investigated are concentration of sodium alginate, concentration of calcium chloride, amount of sunflower oil, % entrapment efficiency, % drug loading and buoyancy[10]. Three factors were evaluated at three levels and experimental trials were performed at all possible

levels and 27 formulations were prepared[10]. (Table 1). Actual physical values of coded values are given in Table 2.

Table 1. Formulations using 3^3 full factorial design.

Formulation Code	Amount of Clarithromycin (mg)	Amount of HPMC K100M (mg)	Amount of Sodium alginate	Amount of Sodium chloride	Amount of Sunflower oil (ml)
F1	250	500	1%	4%	2
F2	250	500	2%	4%	2
F3	250	500	3%	4%	2
F4	250	500	1%	5%	2
F5	250	500	2%	5%	2
F6	250	500	3%	5%	2
F7	250	500	1%	6%	2
F8	250	500	2%	6%	2
F9	250	500	3%	6%	2
F10	250	500	1%	4%	5
F11	250	500	2%	4%	5
F12	250	500	3%	4%	5
F13	250	500	1%	5%	5
F14	250	500	2%	5%	5
F15	250	500	3%	5%	5
F16	250	500	1%	6%	5
F17	250	500	2%	6%	5
F18	250	500	3%	6%	5
F19	250	500	1%	4%	10
F20	250	500	2%	4%	10
F21	250	500	3%	4%	10
F22	250	500	1%	5%	10
F23	250	500	2%	5%	10
F24	250	500	3%	5%	10
F25	250	500	1%	6%	10
F26	250	500	2%	6%	10
F27	250	500	3%	6%	10

Table 2. Actual physical values of the coded values.

Coded value	Concentration of sodium alginate (X1)	Concentration of calcium chloride (X2)	Amount of sunflower oil. (X3)
-1	1%	4%	2 ml
0	2%	5%	5 ml
1	3%	6%	10 ml

Evaluation of beads:

Determination of drug loading and encapsulation efficiency:

Drug loading was determined by dissolving 25 mg of floating alginate beads in 50 ml HCL buffer (pH 1.2.) The prepared solution was filtered through 45 µm filter paper and assayed spectrophotometrically at 760 nm. The drug lading was calculated according to formula;

% drug loading = (Amount of drug in beads/Amount of beads) × 100

Percentage encapsulation efficiency was calculated using following formula,

Percentage encapsulation efficiency = AQ / TQ × 1OO

Where- AQ is the actual drug content of beads and TQ is the theoretical quantity of drug present in beads[11].

Buoyancy study:

The time between the introduction of the floating alginate beads into the medium and the time taken to rise on the surface was measured as floating lag time and the duration for which the formulation constantly floated on the surface of the medium was measured as total duration of floating[12].

SEM of floating beads:

Morphological characterization of the floating alginate beads of clarithromycin was done by talking scanning electron micrograph (Model Jeol JSM-5200). Cross-sectional views were obtained by cutting the bead with a razor blade. The samples were coated to 200 Aº thickness with gold- palladium prior to microscopy. A working distance of 20 nm, a tilt of 0º and accelerating voltage of 15 kv were the operating parameters. Photographs were taken within the range of 50- 500 magnifications[2].

***In-Vitro* drug release studies:**

The in-vitro dissolution studies of floating alginate beads was carried out by using 900ml of 0.1N HCl(pH 1.2) maintained at 37±.0.5 ^{0}C at 100 rpm using USP XXIV dissolution test apparatus. The samples were removed periodically and assayed on UV spectrophotometer at 760 nm[13].

Kinetic modeling and mechanism of drug release:

To analyze the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order, Higuchi matrix, and Peppas model using PSP-DISSO – v2 software. Based on the r-value, the best-fit model was selected[4].

Results:

The floating beads of clarithromycin were prepared by emulsion-gelation method and influence of amount of sunflower oil on floating property and particle size of the beads, as well as concentration of hydroxypropylmethylcellulose (K100M) on the release profile of clarithromycin from floating alginate beads were studied.

Drug loading capacity of beads ranged from 28.11% to 39.99 % and encapsulation efficiency was in the range 75.97% to 91.44 %. The formulations F4, F11, F14 and F23 showed total floating duration more than 10 hr. Drug loading, encapsulation efficiency and total floating duration of the prepared floating beads of clarithromycin are shown in Table 3.

Table 3. Comparative study of pharmaceutical parameters of the floating beads.

Formulations	%DEE	%DL	B(hr)	Formulations	%DEE	%DL	B(hr)
F1	81.68	31.19	9	F15	78.56	34.68	9
F2	83.11	36.74	10	F16	80.55	29.77	7
F3	80.08	32.24	8	F17	79.94	34.56	8
F4	79.91	31.91	9	F18	77.23	32.55	7
F5	88.69	37.64	11	F19	78.44	31.66	6
F6	81.36	30.46	8	F20	81.58	34.16	7
F7	78.64	31.00	10	F21	75.97	31.24	8
F8	80.19	35.94	9	F22	87.20	38.11	6
F9	81.98	30.42	9	F23	82.53	33.69	11
F10	78.24	29.54	7	F24	80.14	30.70	10
F11	82.59	36.19	11	F25	80.04	28.91	6
F12	79.21	28.11	7	F26	82.63	34.12	6
F13	80.00	35.63	9	F27	79.55	29.33	7
F14	91.44	39.99	12				

% DEE % drug entrapment efficiency, % DL % drug loading, B buoyancy

SEM of floating beads:

The surface and cross-sectional SEM pictures for different formulations of floating beads are shown in Fig. 1. The SEM picture shows the presence of oil droplets throughout the alginate matrix.

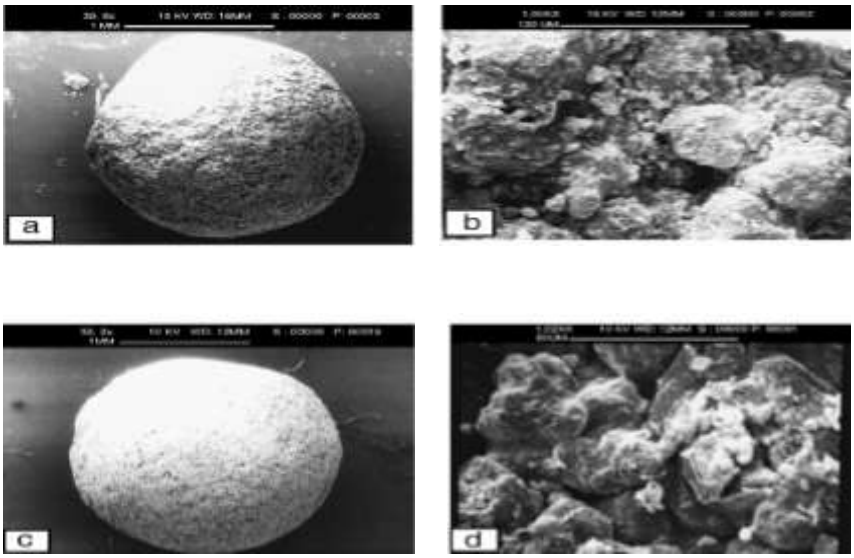

Fig. 1. SEM graphs of alginate beads (a) and (b) Surface morphology.

(c) and (d) cross-sectional view of floating alginate beads of clarithromycin.

***In-Vitro* drug release studies:**

From the results of in vitro dissolution studies (Table 4), it revealed that the floating alginate beads (F5, F8, F13 and F14) showed controlled release of clarithromycin for about 12 hr. Amongs the formulations, formulation F14 shows maximum % cumulative release within 1 hr, 6 hr and 12 hr. This suggested that formulation F14 was having the good sustained release of the clarithromycin up to the 12 hr. The beads showed excellent sustaining properties as compared to the conventional beads.

Table 4. Percent cumulative release of formulations.

Formulations	% cumulative release			Formulations	% cumulative release		
	Q1	Q6	Q12		Q1	Q6	Q12
F_1	19.54	38.11	89.36	F15	21.28	41.90	85.75
F_2	19.11	45.35	92.71	F16	17.72	46.49	19.74
F_3	21.56	43.33	87.91	F17	20.25	41.59	82.77
F4	18.32	43.79	88.20	F18	19.26	40.31	81.88
F5	20.10	47.08	93.11	F19	16.34	38.22	78.49
F6	19.77	42.57	85.40	F20	17.63	36.16	77.34
F7	18.44	41.14	80.79	F21	19.33	41.72	82.59
F8	21.46	47.63	91.64	F22	18.99	37.91	78.34
F9	19.71	39.55	79.17	F23	20.70	45.20	92.74
F10	19.36	38.10	78.50	F24	19.34	39.11	79.90
F11	20.74	45.25	92.00	F25	20.20	37.17	75.33
F12	18.59	38.67	79.20	F26	21.79	46.71	90.26
F13	20.23	47.76	93.22	F27	19.76	42.23	85.82
F14	21.78	46.28	94.11				

Kinetic modeling and mechanism of drug release:

The results of in-vitro dissolution data analysis (Table 5) revealed that formulation F1 to F9 showed best fit in Korsmeyer-peppas model. The values of release exponent (n) were in between 0.5 and 1.0, suggesting non – Fickian diffusion. The best fit model for formulation F10 to F18 was found to be zero order, with correlation coefficient (R^2) ranging from 0.9919 to 0.9897. The formulations from F20 to F27 also showed best fit in Korsmeyer-peppas model, with correlation coefficient ranging from 0.9923 to 0.9958.

Table 5. Kinetic modeling of formulations F1-F27

Formulation	Zero order	First order	Higuchi	Korsmeyer Peppas	Diffusional exponent(n)
F1	0.9872	0.8946	0.9730	0.9946	0.7672
F 2	0.8668	0.8797	0.9799	0.9923	0.5237
F 3	0.8733	0.8629	0.9640	0.9994	0.6977
F 4	0.9658	0.8860	0.9672	0.9986	0.6900
F 5	0.9215	0.9164	0.9634	0.9903	0.6312
F 6	0.9174	0.9215	0.9749	0.9854	0.7411
F 7	0.9208	0.9130	0.9661	0.9829	0.6550
F 8	0.9670	0.8626	0.9704	0.9873	0.6608
F 9	0.9708	0.9641	0.9704	0.9894	0.6608
F10	0.9919	0.9812	0.9763	0.9750	0.5347
F11	0.9923	0.9822	0.9758	0.9323	0.6458
F12	0.9994	0.9768	0.9662	0.9794	0.6683
F13	0.9904	0.9833	0.9574	0.9992	0.7592
F14	0.9988	0.9716	0.9808	0.9803	0.7221
F15	0.9954	0.9630	0.9770	0.9754	0.5277
F16	0.9929	0.9799	0.9808	0.9829	0.5092
F17	0.9973	0.9840	0.9419	0.9873	0.6632
F18	0.9897	0.9616	0.9323	0.9873	0.6485
F19	0.9029	0.9734	0.9794	0.9750	0.6290
F20	0.8660	0.9409	0.9902	0.9923	0.7330
F21	0.8915	0.9391	0.9827	0.9834	0.7476
F22	0.8991	0.9524	0.9803	0.9949	0.6620
F23	0.8830	0.9404	0.9754	0.9891	0.6894
F24	0.8776	0.9523	0.9829	0.9933	0.5788
F25	0.9241	0.9818	0.9873	0.9904	0.5540
F26	0.9452	0.9732	0.9797	0.9833	0.7210
F27	0.8830	0.9894	0.9029	0.9958	0.7485

Discussion:

The results obtained revealed that there was no considerable effect of amount of sunflower oil on drug loading and encapsulation efficiency of clarithromycin. The percentage efficiency was high because bead formation was carried out in distilled water in which clarithromycin is insoluble and with a lesser possibility of leaching of clarithromycin during encapsulation. The obtained results of microscopial examination indicated that as there is increase in the concentration of sunflower oil, the particle size of the beads increases. Low moisture content in all the floating alginate beads indicated the effectiveness of the adopted drying conditions. Low moisture level ensures better stability of the clarithromycin in the beads. The initial burst effect seen in SEM pictures was due to some amount of the drug, which might have been dragged to the surface during the processing. In- Vitro dissolution studied revealed that a new sustained release system of oil entrapped calcium alginate beads can be designed and prepared by an emulsion-gelation method. The sustaining properties of beads were due to incorporation of HPMC K100M. Thus, oil entrapment technique can become a useful tool for the development of multiparticulate system even for a highly water-soluble drug. In In-Vitro data analysis results formulations F10 to F18 showing zero order release kinetics suggesting that drug dissolution from the floating beads do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained.

Conclusion:

The emulsion gelation method was successfully utilized for formulation of floating alginate beads of clarithromycin. The formulated floating alginate beads have shown higher percentage of drug loading, encapsulation efficiency, particle size and very low moisture content. The scanning electron photomicrographs of floating alginate beads reveals that the beads are almost spherical and the matrix showed densely populated sunflower oil droplets, which provides floating property. In-vitro dissolution study showed that, amongst the formulations, formulation F14 released clarithromycin for prolonged duration (12 h).The optimized formulation F14 showed best fit in zero order model. The floating alginate beads showed good stability at 4°C and at room temperature.

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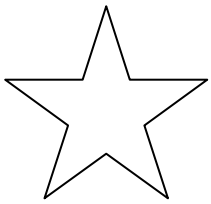

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Review Article **Volume 6 Issue 1**

A REVIEW ON BIOSENSOR

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Abstract

As the potential danger of bioterrorism increments, there is extraordinary requirement for an instrument that can rapidly, dependably and precisely distinguish sullying bio-specialists in the environment. Biosensors can basically fill in as minimal effort and very proficient gadgets for this reason notwithstanding being utilized as a part of other dayto-day applications. A biosensor is a detecting gadget contained a mix of a particular organic component and a transducer. A "particular natural component" perceives a particular analyte and the adjustments in the biomolecule are generally changed over into an electrical flag (which is thus aligned to a particular scale) by a transducer. In this article we exhibit the rudiments of biosensing gadgets which can fill in as an initial instructional exercise for perusers who are new to this field. In this way we give abnormal state portrayals of a couple of delegate biosensors as contextual analyses, trailed by a concise exchange of the real troubles the biosensor inquire about groups regularly experience.

Keywords: Introduction, Biosensor, techniques, application

INTRODUCTION

Biosensors are characterized as any measuring gadget that contains a natural component. It joins the perfect selectivity of science with the preparing force of present day microelectronics and optoelectronics to offer effective new scientific apparatuses with significant applications in the field of solution, ecological reviews, sustenance and handling businesses [1]. These scientific gadgets depend on the union amongst organic and physio-substance segments. Organic parts incorporate large scale atoms, for example, antibodies, compounds, tissue cuts which are utilized to perceive and cooperate with a particular analyte [1]. Physiochemical parts are normally alluded to as transducers which changes over the cooperations into signs; it is later increased concerning its centralization of analyte [1]. The transducer may utilize potentiometric, amperometric, optical, attractive, colorimetric gadgets [2]. An objective analyte in the outer film must have the capacity to enter the biosensor [2]. The outside layer of the biosensor must be penetrable to the analyte where the biosensor is delicate to it. The organic component inside the biosensor then collaborates with synthetic species through a biochemical response which thus delivers another concoction item and portrayed by change in mechanical, electrical properties. The yield flag might be an ordinary electrochemical flag contingent upon the sort of transducer it employments.

Standard:-

The essential standard of a biosensor incorporates a bioreceptor that is an immobilized touchy natural component, for example, chemical, DNAprobe, immunizer perceiving the analytes, for example, protein substrate, correlative DNA, antigen. They likewise incorporate antibodies, entire cells, including microbial, plant, and creature cells, subcellular organelles, tissue cuts, lectins, and various engineered particles with liking or reactant properties stretching out to those got through parallel blend and engraved polymers Although antibodies and oligonucleotides are generally utilized, catalysts are by a wide margin the most usually utilized biosensing components in biosensors. A transducer is utilized to change over biochemical flag coming about because of the collaboration of the analyte with the bioreceptor into an electronic one. The power of created flag is specifically or conversely corresponding to the analyte fixation. Electrochemical transducers are

frequently used to create biosensors. These frameworks offer a few favorable circumstances, for example, minimal effort, straightforward plan or little measurements. Biosensors can likewise be founded on gravimetric, calorimetric or optical recognition [1]. Biosensors is classified by the essential standards of flag transduction and biorecognition components. As indicated by the transducing components, biosensors can be named electrochemical, optical, piezoelectric, and warm sensors. Electrochemical biosensors are likewise delegated potentiometric, amperometric and conductometric sensors [3, 4].

Qualities of Biosensor:-

Selectivity is presumably the most essential component of a biosensor. Selectivity implies that sensor identifies a specific analyte and does not respond to admixtures and contaminants. Antigen-immune response connection has the most elevated selectivity, it is analyte-particular. Accuracy is a normal for any logical gadget that makes quantitative estimations. It is normally described as far as the standard deviation of estimations. Flag mistake in measured fixation. Flag security impacts the accuracy of sensor. It is an imperative normal for a sensor that performs consistent checking. Affectability demonstrates the insignificant sum or grouping of analyte that can be identified. Working extent is the scope of analyte focuses in which the sensor can work. Working scope of sensor ought to associate with the scope of conceivable fixations analyte in the measure. Reaction time will be time required to break down the examiner. Recovery time is the time required to give back the sensor to working state after association with the example. Number of cycles is the quantity of times the sensor can be worked. Debasement of natural material is unavoidable and it should be supplanted. In a few sensors (e.g. hand-held business glucose sensors) transducers are expendable, they should be changed after every estimation [5].

Development:-

So as to build a fruitful biosensor certain conditions must be met, for example, the biocatalyst must be exceptionally particular with the end goal of the examination, be steady under typical stockpiling conditions and demonstrate a low variety between tests. The response ought to be as autonomous as reasonable of such physical parameters as mixing, pH and temperature. This will permit examination of tests with negligible pre-treatment. In

the event that the response includes cofactors or coenzymes these ought to, ideally, additionally be co-immobilized with the chemical. The reaction ought to be exact, exact, reproducible and direct over the fixation scope of enthusiasm, without weakening or focus. It ought to likewise be free from electrical or other transducer prompted commotion. On the off chance that the biosensor is to be utilized for intrusive checking in clinical circumstances, the test must be small and biocompatible, having no dangerous or antigenic impacts. Besides, the biosensor ought not to be inclined to inactivation or proteolysis. For fast estimations of analytes from human specimens it is attractive that the biosensor can give continuous investigation. The total biosensor ought to be shabby, little, convenient and fit for being utilized by semi-gifted administrators [6].

Sorts of Biosensor:-

Electrochemical Biosensors

Regularly in (bio-) electrochemistry, the response under scrutiny would either create a quantifiable current (amperometric), a quantifiable potential or charge gathering (potentiometric) or quantifiably modify the conductive properties of a medium (conductmetric) between terminals. References are likewise made to different sorts of electrochemical recognition methods, for example, impedimetric, which measures impedance (both resistance and reactance), and field-impact, which utilizes transistor innovation to gauge present accordingly of a potentiometric impact at a door terminal. These estimation procedures will be presented here, and additionally a few gadgets that utilize varieties of these methods. Since responses are for the most part identified just in nearness to the terminal surface, the cathodes themselves assume a critical part in the execution of electrochemical biosensors. In view of the picked capacity of a particular terminal, the anode material, its surface change or its measurements enormously impact its discovery capacity. Electrochemical detecting ordinarily requires a reference cathode, a counter or assistant terminal and a working anode, otherwise called the detecting or redox anode. The reference cathode, ordinarily produced using Ag/AgCl, is avoided as much as possible from the response site keeping in mind the end goal to keep up a known and stable potential. The working anode fills in as the transduction component in the biochemical response, while the counter cathode sets up an association with the electrolytic arrangement so that a current can be connected to the working terminal. These terminals

ought to be both conductive and synthetically steady. In this manner, platinum, gold, carbon (e.g. graphite) and silicon mixes are regularly utilized, contingent upon the analyte [6]. Nanotechnology and Bioelectronicshave uncovered new conceivable outcomes to scale down and to upgrade existing microscale gadgets at the nanoscale. It is getting to be plainly conceivable to all the more precisely measure particular electrical properties in mix with different electrochemical transducers. The higher surface-to-volume proportion of nano-articles makes their electrical properties progressively vulnerable to outer impacts, particularly as these structures keep on shrinking toward as far as possible. Since the nanometer measurements of these items are practically identical to the extent of the objective biomolecules, higher estimation affectability may result and affectability may likewise increment because of higher catch proficiency. Nanostructures as of now speak to essential new segments in as of late created electrochemical biosensors, for example, the utilization of nanoparticles as electrochemical marks for DNA detecting. Nanowires, carbon nanotubes, nanoparticles and nanorods are simply a portion of the well known articles that are developing as contender to end up plainly essential components of future bioelectronic gadgets and biosensors [6].

Electrochemical biosensorsalso incorporates nanoparticles, nanotubes for detecting distinctive components which are utilized as a part of pharmaceutical fields for identifying different infections, pathogens. Detecting the natural reactions has expected extraordinary centrality in the present situation of ever element ecological improvements and comparing adjusted homeostatic happenings happening at both in vivo and in addition ex vivo levels. The examination of conduct of the perpetually changing materials has expected incredible essentialness in zones like pharmaceutical conclusion, screening sustenance quality, and ecological applications. A key segment of the biosensing is the transduction components which are in charge of changing over the reactions of bioanalyte associations in an identifiable and reproducible way utilizing the transformation of particular biochemical response vitality into an electrical frame using transduction systems. Nanomaterials can be magnificent officeholders in this measurement as they have high surface zone to volume proportions which permit the surface to be utilized as a part of a superior and much more differently useful way. Also, their electromechanical properties are the superb resources

for the biosensor innovation. The phrasing nanobiosensor is a misnomer as in it has the word nano prefixed to it. To get to the genuine innovation, one should soundly accumulate what a biosensor is. As nanoscience is interdisciplinary in nature so putting the word nano as prefix regularly infers the utilization or control at a scale identical to one-billionth of a meter. The primary segment incorporates analytes and bioreceptor. A bioreceptor is that part of a biosensor which fills in as a layout for the material to be identified. There can be a few materials which can be utilized as bioreceptors. For example, a counter acting agent is screened utilizing antigen and the other way around; a protein is screened utilizing its comparing particular substrate et cetera. The transformation of biochemical reaction into electrical flag is accomplished through transducer. The third segment is the locator framework. This gets the electrical flag from the transducer part and opens up it reasonably so that the comparing reaction can be perused and examined legitimately. Notwithstanding these parts, an extremely basic prerequisite of the nanobiosensors is the accessibility of immobilization plans which can be utilized to immobilize the bioreceptor to make its response with bioanalyte considerably more plausible and effective. Immobilization makes the general procedure of natural detecting less expensive, and the execution of the frameworks in light of this innovation is likewise influenced by changes in temperature, pH, obstruction by contaminants, and other physicochemical varieties.

Biomedical Detection through Nanobiosensors

Nanobiosensors are essentially the sensors which are comprised of nanomaterials. Nanomaterials are a one of a kind endowment of nanotechnology to the humanity. These are the materials which have one of their measurements in the vicinity of 1 and 100 nanometers. The size imperatives of these materials makes them exceptionally unique as they have a large portion of their constituent particles situated at or close to their surface and have all indispensable physicochemical properties exceedingly not quite the same as similar materials at the mass scale. They can assume extremely effective parts in the detecting instrument of the biosensor innovation. Incorporated gadgets of the nanomaterials with electrical frameworks offer ascent to nano-electro-mechanical frameworks (NEMS), which are exceptionally dynamic in their electrical transduction

systems. A few nanomaterials have been investigated on the premise of their electronic and mechanical properties for their utilization in enhanced natural flagging and transduction components. Some of such materials that are broadly utilized incorporate nanotubes, nanowires, nanorods, nanoparticles, and thin movies made up of nanocrystalline matter. Nanobiosensors have filled in as extremely powerful formative advances in the biosensor innovation, which has been conceivable just because of the marvels of nanotechnological ramifications of the matter. A wide assortment of biosensing gadgets that utilize nanoparticles or nanostructures have been explored in various reviews all through the world. These can be as various as utilizing amperometric gadgets for enzymatic location of glucose to utilizing quantum specks as fluo

Applications:-

Glucose observing is fundamental in diabetes patients. A typical case of a business biosensor is the blood glucose biosensor, which utilizes the catalyst glucose oxidase to separate blood glucose. In doing as such it first oxidizes glucose and utilizations two electrons to decrease the FAD (a part of the catalyst) to FADH2. This thus is oxidized by the anode in various strides. The subsequent current is a measure of the grouping of glucose. For this situation, the anode is the transducer and the chemical is the naturally dynamic part. As of late, varieties of a wide range of finder atoms have been connected in alleged electronic nose gadgets, where the example of reaction from the indicators is utilized to unique finger impression a substance. In the Wasp Hound smell indicator, the mechanical component is a camcorder and the organic component is five parasitic wasps that have been molded to swarm in light of the nearness of a particular substance. Current business electronic noses, in any case, don't utilize natural components. A significant number of today's biosensor applications are comparable, in that they utilize life forms which react to dangerous substances at much lower focuses than people can recognize to caution of their nearness. Such gadgets can be utilized as a part of natural observing, follow gas recognition and in water treatment offices. Light of a settled wavelength is reflected off the gold side of the chip at the edge of aggregate inward reflection, and distinguished inside the instrument. The point of episode light is changed keeping in mind the end goal to coordinate the fleeting wave spread rate with the proliferation rate of the surface plasmon

plaritons. This initiates the fleeting wave to infiltrate through the glass plate and some separation into the fluid streaming over the surface. The refractive list at the stream side of the chip surface impacts the conduct of the light reflected off the gold side. Official to the stream side of the chip affects the refractive file and along these lines natural communications can be measured to a high level of affectability with some kind of vitality. The refractive list of the medium close to the surface changes when biomolecules append to the surface, and the SPR point differs as a component of this change. Numerous optical biosensors depend on the wonder of surface plasmon reverberation (SPR) procedures. This uses a property of and different materials; particularly that a thin layer of gold on a high refractive list glass surface can ingest laser light, creating electron waves (surface plasmons) on the gold surface. This happens just at a particular point and wavelength of episode light and is exceptionally reliant on the surface of the gold, with the end goal that authoritative of an objective analyte to a receptor on the gold surface creates a quantifiable flag. Surface plasmon reverberation sensors work utilizing a sensor chip comprising of a plastic tape supporting a glass plate, one side of which is covered with a minute layer of gold. This side contacts the optical discovery contraption of the instrument. The inverse side is then reached with a microfluidic stream framework [11, 12, 13, 14]. The contact with the stream framework makes channels crosswise over which reagents can be passed in arrangement. This side of the glass sensor chip can be adjusted in various routes, to permit simple connection of atoms of intrigue. Ordinarily it is covered in carboxymethyl dextran or comparative compound. Other optical biosensors are primarily in light of changes in absorbance or fluorescence of a suitable marker compound and needn't bother with an aggregate inner reflection geometry. For instance, a completely operational model gadget identifying casein in drain has been manufactured. The gadget depends on identifying changes in retention of a gold layer. A broadly utilized research device, the smaller scale cluster, can likewise be viewed as a biosensor. Nanobiosensors utilize an immobilized bioreceptor test that is particular for target analyte particles. Nanomaterials are stunningly touchy synthetic and natural sensors. Nanoscale materials exhibit one of a kind properties. Their huge surface territory to volume proportion can accomplish quick and ease responses, utilizing an assortment of outlines. Natural biosensors regularly join a hereditarily altered type of a local protein or catalyst. The protein is arranged to identify a

particular analyte and the following sign is perused by a discovery instrument, for example, a fluorometer or luminometer [24, 25, 32].

Human services

Estimation of Metabolites:-

The underlying impulse for propelling sensor innovation originated from the social insurance range, where it is presently for the most part perceived that estimations of blood science are basic and permit a superior estimation of the metabolic condition of a patient. In serious care units, for instance, patients as often as possible show fast varieties in biochemical arrangement and levels that require earnest medicinal action.Also, in less extreme patient taking care of, more effective treatment can be accomplished by getting moment measures. At present, accessible moment investigations are not broad. By and by, these measures are performed by diagnostic research centers, where discrete specimens are gathered and dispatched for examination, regularly utilizing the more customary investigative methods.

Showcase Potential:-

There is an expanding interest for modest and solid sensors for use in specialist's workplaces, crisis rooms, and working rooms. At last, patients themselves ought to have the capacity to utilize biosensors in the checking of a clinical condition, for example, diabetes. It is most likely genuine that the major biosensor market might be found where a quick test is required. On the off chance that the expenses of research facility instrument upkeep are incorporated, then minimal effort biosensor gadgets can be attractive in the entire range of diagnostic applications from healing facility to home. Diabetes:-

The "work of art" and most broadly investigated case of shut circle sedate control is found in the improvement of a manufactured pancreas. Diabetic patients have a relative or outright absence of insulin, a polypeptide hormone created by the beta cells of the pancreas, which is basic for glucose take-up. Absence of insulin discharge causes different metabolic variations from the norm, including higher than typical blood glucose levels.In patients who have lost insulin-emitting islets of Langerhan, insulin is provided by

subcutaneous infusion. Notwithstanding, fine control is hard to accomplish and hyperglycaemia is frequently experienced. Further, even hypoglycaemia is some of the time instigated, bringing on hindered cognizance and the genuine long haul intricacies to tissue related with this discontinuous low glucose condition.

Insulin Therapy:-

Better strategies for the treatment of insulin-ward diabetes have been looked for and mixture frameworks for nonstop insulin conveyance have been produced. Be that as it may, paying little mind to the technique for insulin treatment, its acceptance must be made because of data on the present blood glucose levels in the patient. Three plans are conceivable (Figure 6.2.1), the initial two reliant on discrete manual glucose estimation and the third a "shut circle" framework, where insulin conveyance is controlled by the yield of a glucose sensor which is incorporated with the insulin infuser.In the previous case, glucose is evaluated in light of examination of finger-prick blood tests with a colorimetric test strip or all the more as of late with an amperometric pensize biosensor gadget by the patients themselves. Obviously, these indicative units must be effortlessly versatile, easy to utilize and require insignificant ability and simple translation. In any case, even with the capacity to screen current glucose levels, escalated traditional insulin treatment requires numerous day by day infusions. This open-circle approach does not foresee insulin measurements because of changes in eating routine and exercise. For instance, it was demonstrated that organization of glucose by subcutaneous infusion, a hour prior to a supper gives the Best glucose/insulin.

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Review Article **Volume 6 Issue 1**

INDIAN MEDICINAL PLANTS AS AN EFFECTIVE ANTIMICROBIAL AGENT

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Abstract:

India is a great country that is recognised for its rich culture and medicinal plants. Most of the people in India is reliant on herbal plant for their therapeutic needs. The present review will focus on Therapeutic plants from India along with its medicinal use.

Various medicinal plants have already proved their significance with curing diseases including bacteriological infections and some life threating serious diseases. Medicinal plants are rich in antioxidant and proved best as antimicrobial agents. Herbal drugs are achieving popularity as compared to allopathic drugs the reasons includes adverse effects of man-made antibiotics, prompt surge in contagious diseases, resistance of drug in microbes. Herbal plants shows slow recovery, still a great population is using it because it showed no side effects and low resistance in microbes. Antimicrobial status of various herbal plants has been reported. Therapeutic plants work as a potent antimicrobial. Herbal plants are used for its medicinal purpose throughout the world as herbal plants provide base material for various effective drugs. A great number of herbal plants has been used as drug in the form of crude extracts and extensively used for their therapeutic possessions. Huge number of plants has been examined for antimicrobial possessions, but still majority of plants have not been examined adequately.

So, the present review will focus on some of the selected medicinal plant along with its antimicrobial status.

Key words: Medicinal plants, antimicrobial agent, infections, herbal drugs, antifungal.

INTRODUCTION

The medical plants were in use since ages, Indian subcontinent uses plants for curing diseases and the stream of science which deals with plants and its therapeutic effects were governed by Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India. Today the pharmacologically active ingredients of many Ayurvedic medicines have been identified and their usefulness in drug therapy is being determined. It is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field.

As believed that Ayurveda exists in India since thousands of years. It employs various techniques to cure diseases. Ayurveda is totally dependent on herbal plants and its derivatives. According to World Health Organization, medicinal plants are the best source to obtain newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated for better understanding of their properties, safety and efficacy.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits.

In the present review we have tried to include some of the antibcterial and antifungal effects of medicinal plants, the methanol leaf extracts of *Tinosporacordifolia, Ziziphusmauritiana*, *Sidacordifolia, Acacia nilotica, Withaniasomnifer*have showed potent antibacterial activity against *Bacillus subtilis,E. coli, Pseudomonas fluorescens*, *Staphalococcus aureus* and *Xanthomonas axonopodis* and antifungal activity against *Aspergillus flavus*, *Dreschleraturcica* and *Fusarium verticillioides*. *Withaniasomnifer*is recognized as strong antibacterial, Methanol extract of *Withania somnifer* is effective against *Candidaalbicans*[1]. Organic extracts of *Cassia fistula* and *Acacia aroma* shows potent antibacterial and antifungal activities against various gram positive bacteria.

Azadirachtaindica popularly known as neem is effective against various infections and diseases, Neem shows antibacterial activity strongly against *Vibrio cholera* [2] Essential oil and organic extracts of *Ziziphoraclino podioides* shows antibacterial activity against a

huge class of bacteria including *Acidovoraxfacilis*, *Bacillusflexus*, *Bacillussphaericus*, *Brevibacillusbrevis*, *Corynebacterium*, *ammoniagenes*, *Enterobacter sakazakii*, *Moraxella catarrhalis* and *Xanthomonas*[3]. *Argemonemexicana*is reported to reduces bacterial infections of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* when used as crude extract with chloroform,[4]. As reported*Nepheliumlappaceum*methanolic extracts is effective against *streptococcus epidermidis*[5]. *Punicagranatum* is used as an effective agent against various antibacterial, anti-inflammatory and anti-allergic reactions against *Streptococcus aureus* and *Streptococcus epidermidis* [6].

In Asia people use plant extract of R*utagraveolens* and *Zingiberofficinale* and it inhibits the growth of *Bacillus cereus* species [7].Oil extracted from A*achilleamillefolium*'s leaves and stem represents higher antimicrobial activity compared to its organic extracts .Essential oil from the plant inhibits the growth of *Streptococcus pneumonia, Clostridium perfringes* and *Candida albicans* and it inhibit *Mycobacterium smegmatis*, *Acinetobacter lwoffii*and *Candida krussei*[8]

70% methanol extract from leaves of *Mikaniaglomerata* ("guaco"),guava, *Baccharistrimera* (carqueja), *Menthapiperita*(peppermint) and *Cymbopogoncitratus* (lemongrass), and *A. sativum* (garlic), *Syzygiumaromaticum* (clove) and *Zingiberofficinale* (ginger) worked as an antimicrobial ,all showed action against *Staphylococcus aureus* and satisfactory result in clove at the concentration of 0.36 mg/ mL and guava at 0.56 mg/mL.According to a study the hydroalcoholic extracts from *Vernoniapolyanthes* ("assa-peixe"), *Aristolochiatriangularis* ("cipó mil-homens"),*Tabebuiaavellanedae* (purple trumpet tree) and *Stryphnodendronadstringens* ("barbatimão") shows anti mycobacterial activity [9]

VernoniaPolyanthes extract shows potent inhibitory activity against Leishmania strains .In the same way under same condition *Baccharisdracunculifolia* oil ("alecrim-do-campo")at a concentration of 10-µL inhibits the microbial growth of *E coli, Staphylococcus aureus* and *P. aeruginosa*.[10] Alkaloid extract of *Phyllanthusdiscoideus* inhibits the growth of many pathogenic bacteria including *E. coli, E. faecium, P. aeruginosa, S. aureus and M. smegmatis*.[11]

Leaves of some medicinal plants including *Achyranthesaspera, Artemisia parviflora, Azadirachtaindica, Calotropis gigantean, Lawsoniainermis, Mimosa pudica,*

Ixoracoccinea, Partheniumhysterophorus and Chromolaenaodorata were examined for antimicrobial activity against various bacteria in different solvents and they show their maximum inhibition against *E.coli, S. aureus, X. vesicatoria.* Chloroform extract of *Curcuma amada* was effective against *bacillus cereus* and *bacillus subtilis* bacteria [12], a novel product named amadannulen from *curcuma amada* inhibits the bacterial growth. Crude methanolic extract of *Mallotuspeltatus* is reported to be effective against the bacterial growth of *Staphylococcus, Streptococcus, Bacillus* species. [13]

Emblica officinalis and *Nymphaeodorata* extract together is used to suppress the bacterial growth of *Staphylococcus aureus.Gallium Sativum*[14], commonly known as garlicis useful against various disease, it is rich in anti-oxidant. *Eucalyptus Globulus*is also known as eucalyptus is used to treat disorders of urinary and respiratory tract, it shows high level of antibacterial and anti-fungal properties.

*Bidenspilosa*L extract is used as antihelmintic and protozoaide agent, used for its antiseptic properties[15] ,It is rich in flavonoid[16],The ethanol leaf extract of *Bixaorellana*L shows antimicrobial activity against gram positive bacteria[17] *Candida albicans*is also used againstmalaria andleishmaniasis [18], Its seed contain carotenoids [19].The ethanol leaf extract of *Cecropiapeltata*L was effective as anti-bilious, cardio tonic and diuretic agent [20] and leafs are valuable as medicine against lennorrhea and warts[21][22]

Decoction of Leafs from *Cinchona officinalis* is found effective againstamebiasis. Dried bark is used to treat diseases caused by pathogenic strain of *P. falciparum*, and herpes [23].This extract is a rich source of quinoline alkaloid [24].Medicinal plant *Gliricidiasepium*is rich in antioxidant, Its branches and leafs are effective against fever ,employed against infections caused by *Microsporumcanis,Trichophyton mentagrophytes*, and *Neisseria gonorrohae [25.* Aqueous extract of *Jacaranda mimosifolia*is effective against *Pseudomonas aeruginosa,* the flowersof the plant contain flavones and flavonoids [27].The leafs of the plants are known to have triterpenes, flavones, and steroids [26]

*Justiciasecunda*is used as a disinfectant to treat scorpion wounds [27] while *Piper pulchrum*is found effective against snake bite [28]. Flowers from medicinal plant *Spilanthes Americana is* effective against infections of mouth and variety of herpes, they possess spilantol *[29]*

Carbazole alkaloid extracted from stem bark of *clausenaanisata* contain antibacterial and antifungal properties [30]. Alcoholic and acetonic leaf extract of*Cassia alata*is reported with antibacterial activity against*Staphylococcus aureus*, coagulase positive *Staplylococcus aureus, Bacillus subtilis, Bacillus stearothermophillus, Escherichia coli, Salmonella typhi* and *Salmonella dysentriae* while the alcoholic leaf extract of the same inhibits the growth of *Klebsiella pneumonia* and acetone extract inhibits the growth of *Vibrio cholerae*.[31]

Dry nuts of *semecarpusanacardium* is effective against various bacteria including 3 gram negative bacteria (*Escherichia coli, Salmonella typhi* and *proteusvulgeris* and gram positive strain(*Staphylococcus aureus* and *Corynebacterium diphtheriae*)[32]. Medicinal plant *amonaglabra* show potent antibacterial, antifungal modest insecticidal, sporicidal and cytotoxic activity. Hexane extract of the plant is used for the procedure. [33]

Antibacterial activity of plants like *Eugenia caryophyllus, Thymus vulgaris,Cinnamonumzeylanium*and*Cuminumcyminum*, hexane extract of these plants were examined on various gram negative and gram positive bacteria and *Thymus vulgaris* shows best antibacterial activity among all.[34]

CuminumCiminum popularly known as cumin reported to show high antibacterial and antifungal properties. Extracts from the bark of Walnut are effective against *pseudomonas* and *candida* microorganisms, it is active against all microbial infections. *Thymus Vulgaris* used against antibacterial activity, because it is rich in phenol, *AchilleaMillefolium* reported to show effectiveness in healing properties against antibacterial and antifungal infections, commonly it is used to cure wound, it is used as an extract in organic solvent. *PinusSilvestris*commonly known as pine used widely for its antiseptic activities, because of the presence of turpentine it is widely used against urinary tract infections and can be used against fungal infections.

Organic extract from *Peumusboldus, Agathosmabetulina,Echinacea angustifolia, Humuluslupulus, Glycyrrhizaglabra, Mahoniaaquifolium, Usneabarbata*and *Anemopsiscalifornica*s how activity against various microbial and fungal infections.

Discussion:

The above mentioned review clearly shows that medicinal plants are important link between diseases and drugs, they play active role in curing all disease and infections.

Almost all plants have medicinal belongings; the main aim of the article was to consider few therapeutic plants of Indian origin.

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