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PREPARATION AND EVALUATION OF SUSTAINED RELEASE MATRIX TABLETS

AIM: To prepare and evaluate sustained release matrix tablets.

FORMULA:

For matrix tablets:

S.no	ingredients	general formula
1	Ibuprofen	100mg
2	Carbapol	85mg
3	Dicalcium phosphate	40mg
4	Magnesium stearate	25mg

For conventional tablets:

S.no	ingredients	general formula
1	Ibuprofen	100mg
2	Corn starch	25mg
3	Dicalcium phosphate	90mg
4	Talc	10mg
5	Magnesium stearate	25mg

PRINCIPLE:

Sustained release, sustained action, prolonged action, controlled release, extended action, timed release dosage forms are designed to achieve prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose.

Advantages of sustained release dosage forms:

- Frequency of drug administration is reduced, patient compliance can be improved and drug administration can be more convenient as well.
- The blood level oscillation characteristic of multiple dosing of conventional dosage form is reduced, because a more even blood level is maintained.
- Total amount of drug administered can be reduced, thus maximizing availability with a minimum dose.
- The safety margin of high potency drugs can be increased and the incidence of both local and systemic adverse side effects can be reduced in sensitive patients.

  Overall, administration of sustained release forms enables increased reliability of therapy.

Disadvantages;

- Administration of sustained release medication does not permit the prompt termination therapy.
- The physician has less flexibility in adjusting dosage regimens.
- Sustained release forms are designed for the normal population, i.e on the basis of average drug biologic half lives. Consequently, disease states that alter drug disposition, significant patient variation, and so forth are not accommodated.
- Economic factors must also be assessed, since more costly process and equipment are involved in manufacturing many sustained release forms.

Two general sets of methods have been developed for implementation of practical sustained release dosage form design

1) Methods based on modification of physical and chemical properties of the drug.
2) Methods based on modification of the drug release rate characteristics of the dosage that affect bioavailability.

In second method the dosage forms can be classified in to three product types.

- Encapsulated slow release beads(granules).
- Tabletted mixed or slow release granulation.
- slow release (core) tablets.

Two general principles are involved in retarding drug release from sustained release formulation involving dosage form modification. These are embedded matrix and the barrier principle.

In the embedded matrix case the drug is dispersed in a matrix of retardant material, which may be encapsulated in particulate form or compressed into tablets. Release is controlled by combination of several physical process.

These include

- Permeation of matrix tablets.
- Leaching of drug from the matrix.
- Erosion of matrix material.

Alternatively, drug may dissolve in the matrix material and be released by diffusion through the matrix and material or partitioned between the matrix and extracting fluid matrices may be prepared from insoluble or erodible materials.

One of the least emplicated approaches to the manufacture of sustained release dosage forms involves the direct compression of blends of drugs retardant material and the additives to form a tablet in which drug is embedded in a matrix core of the retardant.

There are three retardant materials used to formulate matrix tablets.

1. Insoluble inert polymers: poly vinyl chloride, poly ethylene and acrylate co-polymers.
2. Insoluble erodible polymers: carbowax in formulation with stearyl alcohol and stearic acid.
3. Hydrophilic polymers: methyl cellulose, carboxy methyl cellulose, poly methyl cellulose.

PROCEDURE:

- Required amounts of ingredients was weighed both for conventional and matrix tablets.
- The weighed ingredients were taken in separate motors for conventional and matrix tablets and blended for uniform mixing.

- The punching machine(tablet press) dye was adjusted to get 250mg tablet and the hardness was adjusted to 4-6kg/cm^2 in the dye cavity.
- Then the tablets were punched using 9mm punches.
- The punched tablets were collected and evaluated.

EVALUATION:

Weight variation test:

10 tablets was weighed individually, each individual tablet weight was compared with average weight.

The weight variation limits uncoated tablets as per USP

Average weight(mg)	maximum percentage difference allowed
130 or less	10
130-324	7.5
>324	5

Hardness:

It is the force required to break a tablet on a diametric compression test. A tablet was placed between two anvils, force is applied to anvils and crushing strength that just causes the tablet to break was recorded.

Friability:

Tablets that tends to chip and fragment when handled can be detected by friability test. Roche friabilator subjects a number of tablets to the combined effect of abrasion and shock by utilizing a plastic chamber that resolves at 25rpm for 4min, dropping a tablet at a distance of 6 inches with each revolution.

Tablets were taken, dedusted and weighed, then subjected to 100 revolutions, after that the tablets were again dedusted and weighed.

Friability(f)=100 x (initial weight-final weight/initial weight)

Conventional compressed tablets that lose < 0.5 to1.0% of their weight are generally considered acceptable.

Dissolution test:

- Prepared tablets was taken and placed in dissolution apparatus containing dissolution medium (phosphate buffer 7.2 pH) and which was adjusted to 37 ± 0.5^{0}c and 50 rpm.
- Samples were collected predetermined intervals of 10 min,20 min, 30min, 1hr 2hrs, 3hrs, 4hrs for matrix tablets and 10mins, 20mins, 30mins, 40mins, 50mins, 60mins for conventional tablets in a clean and dry test tubes.
- The absorbance of samples was measured using UV-Visible spectro photometer at λ_{max} 272nm.
- The percentage release of the drug was calculated at each time interval using the standard graph and graph is plotted by taking amount of the drug release on y-axis and time on x-axis.
- The results of conventional and matrix tablets were compared.

REPORT;

PREPARATION AND EVALUATION OF TRANSDERMAL PATCH OF IBUPROFEN

AIM: To prepare and evaluate Transdermal patch of Ibuprofen.

GENERAL FORMULA:

Ibuprofen	–	100mg.
Ethyl cellulose : PVP	–	900mg (7:3)
Toulene : Ethanol	-	20ml (8:2)
Polyethylene glycol 400	–	0.3ml

THEORY:

Transdermal therapeutic systems are defined as self contained, discrete dosage forms which when applied to intact skin, deliver the drug to the skin at a controlled rate to systemic circulation.

BASIC COMPONENTS OF TRANSDERMAL PATCH:

1) POLYMER MATRIX:

Polymer matrix controls the release of the drug from device.

The polymer should be

- An inert drug carrier .
- Does not decompose on storage.
- Allow the diffusion of drug at desirable rate.

The most widely used polymers in the preparation of transdermal patch are polypropylene, poly vinyl carbonate, cellulose acetate nitrate, poly acrylo nitrate, ethylene vinyl acetate copolymer, hydroxyl propyl cellulose, polyethylene tetrapthalate and polyesters.

2) DRUG:

- Molecular weight of the drug should be less than 1000 daltons.
- Affinity to both hydrophilic and lipophilic phases.
- The drug should have low melting point i.e.,<200^{o}c.

- The half life of the drug should be short.
- The drug should not induce cutaneous irritation/allergic response.

3) PERMEATION ENHANCERS:

Permeation enhancers promote skin permeability by altering the behaviour of skin as a barrier to the flux of a desired product.

a) Solvents : They enhance the swelling of polar pathway or by fludising lipids.

Eg: Methanol, ethanol.

b) Surfactants : They enhance the poor pathway especially hydrophilic drugs.

Eg: sodium lauryl sulfate, sodium deoxy cholate.

c) Binary systems : Binary systems open up the heterogenous multilaminate pathway as well as continuous pathways.

Eg: propylene glycol, oleic acid.

d) Backing membrane :

This provides good bond to the drug reservoir prevent drug release from the top of the patch.

TYPES OF TRANSDERMAL SYSTEMS :

- Reservoir devices.
- Adhesive dispersion system.
- Matrix dispersion system.
- Micro reservoir system.

ADVANTAGES :

- Trandermal medication delivers a steady infusion of a drug over an extended period of time.
- Adverse or therapeutic failures frequently associated with intermittent dosing can also be avoided.
- Avoid hepatic or first pass metabolism.
- Patient compliance.
- Reduced dosage frequency.

LIMITATIONS :

If the drug dosage required for therapeutic value is more than 10mg/day the transdermal delivery will be very difficult if not impossible. The dose of less than 5mg/day are preferred.

PROCEDURE :

- Weighed quantities of ethylcellulose, PVP were dissolved in ethanol and kept for swelling upto 15min.
- The drug was dissolved in toulene and was added to polymeric solution.
- To the above solution PEG400 was added.
- The contents were mixed for about 10 min and sonicated to evolve the entrapped air.
- The above solution was poured into petriplate with aluminium foil and allowed to evaporate the solvent for about 8 to 12 hrs at room temperature.

EVALUATION:

1. **Thickness test:** Ten patches were taken and their thickness was measured by using screw guage and the average was calculated.

2. Weight variation: The patches were weighed individually and the average weight was calculated.

3. Hardness: The patch was folded several times at a same place until it breaks and the hardness was calculated in terms of number of folds.

4. Diffusion study:

- The patch was taken and sandwiched between two dialysis membranes and further it was placed in between two compartments of a diffusion cell.
- Phosphate buffer of pH 7.2 was taken in receptor compartment.
- The entire assembly was placed on magnetic stirrer and the experiment was carried out for 24hrs at 37ºC.
- 5ml of sample was collected at each interval and replaced it with same amount of buffer.
- The concentration in the sample was measured by using UV visible double beam spectrophotometer at 272nm.
- The amount of the drug diffused was calculated from the standard graph of Ibuprofen.
- Cumulative amount of drug was calculated and then plot a graph by taking time on X- axis and cumulative amount on Y- axis.

REPORT:

PREPARATION AND EVALUATION OF BUCCAL PATCH

AIM: To prepare and evaluate Ibuprofen buccal patch.

WORKING FORMULA:

Ibuprofen	- 50 mg
Dichloromethane	- 12.5 ml
Methanol	- 12.5 ml
HPMC 15	- 2.5 gm
Propylene glycol	- Q.S

THEORY:

A Bioadhesive is defined as a substance that is capable of interacting with biological materials and being retained on them or holding them for extended period of time.

In short, bioadhesive describes adhesion of polymer to a biological membranes and if adhesion is restricted to the mucus layer lining of the mucosal surface, it is termed as 'mucoadhesion'.

Mucoadhesive drug delivery systems utilize the property of bioadhesion of certain water soluble polymers which becomes adhesive on hydration.

Development of bond between a polymer and biological membrane occurs due to:

a) Initial contact between two surfaces,
b) Formation of secondary bonds due to non-covalent interactions.

Mechanistically bioadhesion involves the formation of hydrogen and electrostatic bonding at the mucus polymer interface. Immobilization of drug at the mucosal surface would result in prolonged residence time.

Localization of drug which ultimately results in better absorption and hence better bioavailability.

Buccal Delivery :

Buccal delivery is a potential alternative to the conventional therapy especially for the drugs that undergo degradation due to acidic environment and drugs that undergo extensive first pass metabolism and also drugs that have poor bioavailability when given by oral route.

Drug Selection Criteria:

1) Drugs should have high pharmacological activity at low dose requirements.
2) Site of dosage form should not exceed $12cm^2$ for buccal application or $3cm^2$ for sublingual or gingival application.
3) Drug with daily requirements of 25mg or less would make a good criteria.
4) Limited solubility or absorption through gastro intestinal membrane.
5) Drug susceptible to degradation and first pass metabolism.
6) The Half-life should be 2-8hrs.

PROCEDURE:

1. 10ml of Dichloromethane and Methanol were taken and 2.5gm of HPMC15 was added.
2. To this plasticizer was added and kept aside for 5-6hrs to swell.
3. Then to the remaining amount (i.e., 2.5 ml) of Dichloromethane and Methanol,the drug (Ibuprofen) was added.
4. Then this was added to above mixture.
5. This final mixture was poured into Petri plates and kept aside for about 12hrs at 37^oc for evaporation.
6. Then the patch was carefully removed and evaluation studies were performed.

EVALUATION:

1) Weight variation:

10 patches were weighed individually and compared with average weight to observe for variation in weight.

2) Thickness:

The thickness of patches were measured by using screw gauge.

3) Moisture adsorption studies :

Patches with uniform thickness were selected. Then 2% nutrient agar medium was prepared and solidified in Petri dish. Previously weighed patches were placed on the medium. For every 15minutes, patches were taken out and reweighed.

The % of moisture absorbed = $\frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$

4) Diffusion:

a. Diffusion was carried out using diffusion cell apparatus.
b. The receptor compartment of cell was filled with buffer and dialysis membrane was placed above this. The patch was placed on dialysis membrane and then donor compartment was placed above the patch.
c. Two compartments were fixed in a place using clamp.
d. A magnetic bead was placed in receptor compartment and the diffusion cell was placed on a magnetic stirrer.
e. The temperature was maintained at 37^{o}c.
f. At regular intervals, samples were withdrawn from receptor compartment up to 4hrs by replacing it with equal amount of buffer. Then the samples were analysed to determine the percentage drug release.

REPORT:

STUDY OF DRUG TRANSPORT ACROSS PORCINE BUCCAL MUCOSA

Objective: To study the permeation of hydrophilic drug pantoprazole sodium through porcine buccal membrane

Principle: Within the oral mucosal cavity, the buccal region offers an attractive route of administration for systemic drug delivery. The mucosa has a rich blood supply and it is relatively permeable.

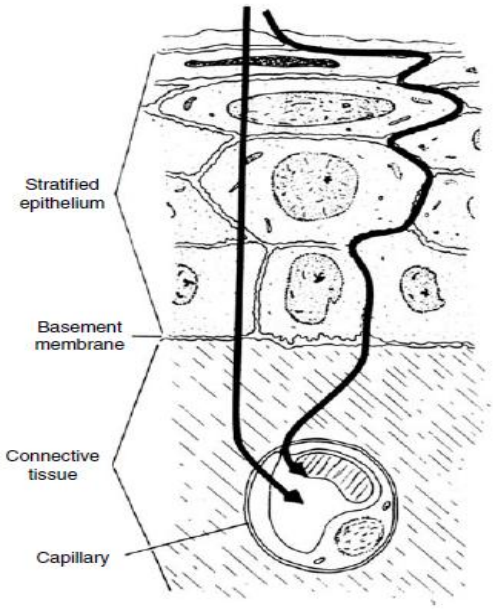
The delivery of drugs is classified into three categories:

(i) sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth
(ii) buccal drug delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa), and
(iii) local delivery, which is drug delivery into the oral cavity.

There are considerable differences in permeability between different regions of the oral cavity because of the diverse structures and functions of the different oral mucosae. In general, the permeabilities of the oral mucosae decrease in the order of sublingual greater than buccal, and buccal greater than palatal. This rank order is based on the relative thickness and degree of keratinization of these tissues, with the sublingual mucosa being relatively thin and non-keratinized, the buccal thicker and non-keratinized, and the palatal intermediate in thickness but keratinized

There are two permeation pathways for passive drug transport across the oral mucosa: paracellular and transcellular routes. Permeants can use these two routes simultaneously, but one route is usually preferred over the other depending on the physicochemical properties of the diffusant. Since the intercellular spaces and cytoplasm are hydrophilic in character, lipophilic compounds would have low solubilities in this environment.

The cell membrane, however, is rather lipophilic in nature and hydrophilic solutes will have difficulty permeating through the cell membrane due to a low partition coefficient. Therefore, the intercellular spaces pose as the major barrier to permeation of lipophilic compounds and the cell membrane acts as the major transport barrier for hydrophilic compounds. Since the oral epithelium is stratified, solute permeation may involve a combination of these two routes. The route that predominates, however, is generally the one that provides the least amount of hindrance to passage



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TRANSCELLULAR AND PARACELLULAR ROUTES OF DRUG ABSORPTION

Advantages of buccal mucosal drug delivery:

1.Avoids first pass effect thus enhancing bioavailabilty.

2.Ease of administration

3.Termination of therapy is easy.

4.Permits localization of the drug to the oral cavity for a prolonged period of time

5. A significant reduction in the dose can be achieved thereby reducing the dose dependent side effects.

6.Drugs which are unstable in the acidic environment of the stomach or which are destroyed by the enzymatic or alkaline environment of the intestine can be administerd through this route.

7. Drugs which show poor bioavailabilty via the oral route can be administers conveniently.

8. These can be administerd to patients with

a) Nausea and vomiting

b)gastrointestinal disorders or surgery.

c)swallowing difficulty.

Limitations of buccal drug administration:

1. Drugs which irritate the mucosa or have a bitter or unpleasant taste or an abnoxious odour cannot be administrated by this route.
2. Drugs which are unstable at buccal pH cannot be administered by this route.
3. Only drugs with small dose requirement can be administered.
4. Only those drugs which are absorbed by passive diffusion can be administered by this route.
5. There is an ever present possibility of the patient swallowing the tablet.
6. Sometimes they show unpredictable bioavailability.

Procedure:

A. In vitro Methods

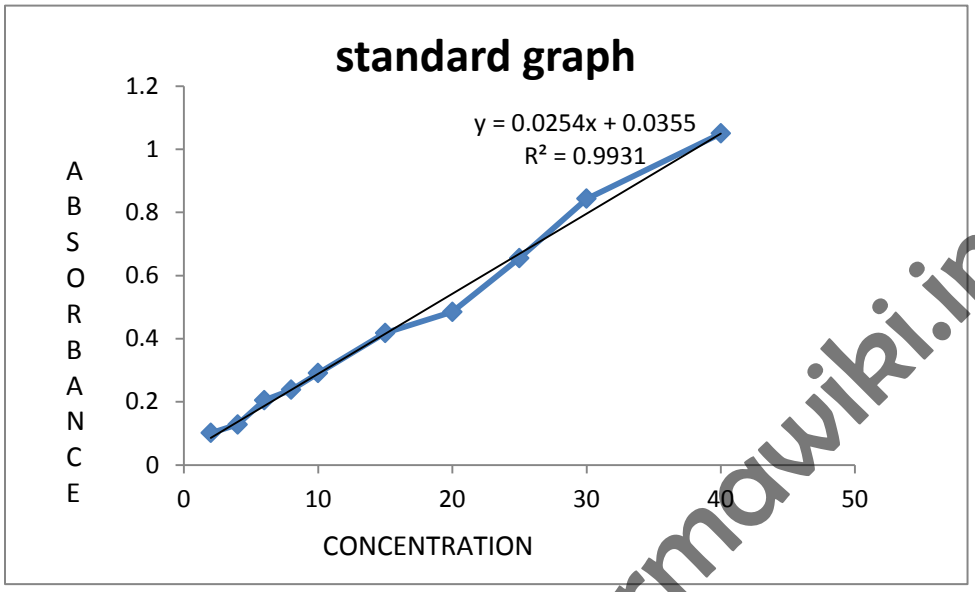
Isolation of porcine buccal memrbrane:

At the present time, most of the *in vitro* studies examining drug transport across buccal mucosa have used buccal tissues from animal models. Pigs are sacrificed immediately before the start of an experiment. Buccal mucosa with underlying connective tissue is surgically removed from the oral cavity, the connective tissue is then carefully removed and the buccal mucosal membrane is isolated. The membranes are then placed and stored in ice-cold (4°C) buffers (usually Krebs buffer) until mounted between side-by-side diffusion cells for the *in vitro* permeation experiments. The most significant questions concerning the use of animal tissues as *in vitro* models in this manner are the viability and the integrity of the dissected tissue. How well the dissected tissue is preserved is an important issue which will directly affect the results and conclusion of the studies. Despite certain gradual changes, the buccal tissue seems to remain viable for a rather long period of time. A decrease in ATP levels does not assure a drop in permeability characteristics of the tissue. The most meaningful method to assess tissue viability is the actual permeation experiment itself, if the drug permeability does not change during the time course of the study under the specific experimental conditions of pH and temperature, then the tissue is considered viable.

Standard graph of pantoprazole sodium:

Weigh accurately 100g of drug and dissolve it in distilled water and the volume was made upto 100ml in a volumetric flask(1000µg/ml)-Stock-I. From stock-I, 10 ml was taken and diluted to 100ml.(100µg/ml). This gives Stock-II solution. From stock-II, further dilutions were made by

taking 0.2ml,0.4ml,0.6ml,0.8ml,1ml,1.5ml,2ml,2.5ml,3ml and 4ml and diluting it to 10ml to give 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, 30µg/ml and 40µg/ml respectively.the absorbance of the obtained samples were determined spectrophotometrically at a λ_{max} of 287nm and a standard graph was plotted.



Preparation of drug solution:

8mg of drug was dissolved in 2ml of water.20 micro-liters of phenol red was added as a marker agent to determine the integrity of the membrane isolated.

Pantoprazole sodium buccal tablet and patch were prepared with the following composition to determine the drug permeation through the membrane

Pantoprazole sodium buccal tablet:

Formulation:

Pantoprazole sodium-15mg

HPMCK15-40mg

Pearlitol-63mg

Sodium stearyl fumarate-2mg

Total weight-120 mg

Preparation: They were compressed using direct compression method.

Pantoprazole sodium buccal patch:

Formulation:

Pantoprazole sodium-500mg

HPMC -4000mg

Poly ethylene glycol

Distilled water

Preparation: pantoprazole sodium buccal patches are prepare by solvent casting method.

Procedure for the buccal pearmeation studies:

25ml of distilled water was taken in the lower compartment of Franz diffusion cell. The porcine buccal membrane that was isolated was placed between the two compartments and are held together tightly with the help of a clamp. The three individual Franz diffusion cells containing drug solution, buccal tablet and buccal patch were placed on the upper surface of the membrane and the entire set up was placed on a multi-stationary magnetic stirrer and the rpm was set to 50. At regular intervals(0.5h,1h,1.5h,2h,4h) samples were withdrawn with the syringe through the side vent of the lower compartment and replaced with 1ml of water to maintain sink conditions. The absorbance of the obtained samples were recorded using UV-VIS spectrophotometer at λ_{max} of 287 nm.

Precaution: Remove the air bubbles, if any from the lower compartment by slightly tilting the cell.

OBSERVATIONS:

DRUG PERMEATED FROM THE DRUG SOLUTION

DRUG SOLUTION						
TIME POINTS	ABSORBANCE	CONC	AMT DISS	% REALEASE	FLUX	PERMEABILTY COEFFICIENT
0.5	0.307	10.88	2.72	34	8.722	1.09
1	0.537	20.08	5.02	62.75		
1.5	0.631	23.84	5.96	74.5		
2	0.66	25	6.25	78.125		
3	0.737	28.08	7.02	87.75		
4	0.754	28.76	7.19	89.875		
6	0.941	36.24	9.06	113.25		

Where,

FLUX=Amt of drug dissolved/(time*area)

PERMEABILTY COEFFICIENT=Flux/amt of drug

DRUG PERMEATED FROM THE PATCH:

TIME POINTS	ABSORBANCE	CONCN	AMT DISS	% RELEASE	FLUX	PERMETION COEFFICIENT
0.5	0.068	1.32	0.33	0.066	0.0173	
1	0.082	1.88	0.47	0.094		
1.5	0.08	1.8	0.45	0.09		
2	0.1	2.6	0.65	0.13		
3	0.143	4.32	1.08	0.216		
4	0.199	6.56	1.64	0.328		
6	0.302	10.68	2.67	0.534		

DRUG PEREMATED FROM THE TABLET:

TIME	ABSORBANCE	CONC	AMT DISS	%RELEASE	flux	Permeation coefficient
0.5	0.091	2.24	0.56	3.733333	0.21739	0.0144
1	0.089	2.16	0.54	3.6		
1.5	0.124	3.56	0.89	5.933333		

2	0.155	4.8	1.2	8		
3	0.305	10.8	2.7	18		
4	0.449	16.56	4.14	27.6		
6	0.748	28.52	7.13	47.53333		

RESULTS AND DISCUSSION:

EFFECT OF PERMEATION ENHANCERS ON DRUG PERMEATION THROUGH BIOLOGICALMEMBRANES

Aim: To study the effect of permeation enhancer on the permeation of drug through biological membrane.

Requirements:

Normal saline solution, processed rat skin, Diclofenac Sodium, petri dishes, aeration tubes, Franz diffusion cell, Tween80, Sodium Lauryl Sulphite, DMSO.

Theory:

Majority of drugs are absorbed by passive diffusion from the gastro intestinal track and their bio availability is closely related to their physicochemical properties . There are some indications that the efflux and influx pumps as well as metabolisms within the mucosal membrane of the GIT affects less than 10% of all orally administered drugs .

Biological membranes are composed of small amphiphilic molecules. Phospholipids with 2hydrophillic chains and cholesterols or other related structure which associate into lipoidal bi layers in aqueous media.

The membrane has a relatively hydrophilic exterior and a hydrophobic interior. Drug molecule must possess some lipophilicity to be able to permeate through biological membranes including biological barriers.

Drug permeation enhancers enhance drug delivery through biological membranes by causing some physicochemical changes within the lipophilic membrane barrier.

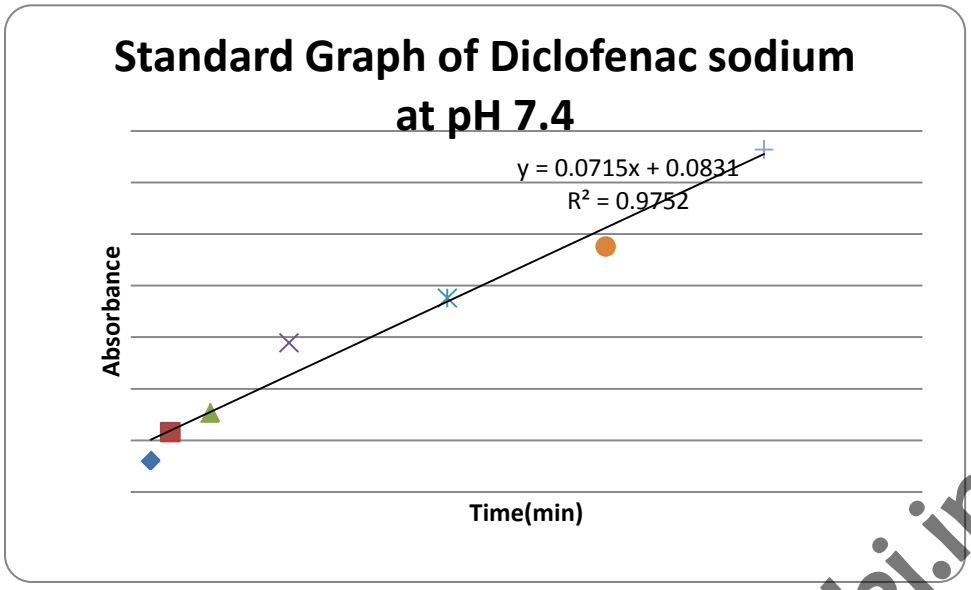
Diclofenac Sodium is a centrally acting analgesic used for treating moderate to severe pains.

Standard graph procedure:

Take 100mg of drug in 100mL volumetric flask and dissolve it in 10mL Methanol and diluted with buffer up to 100mL (stock I).

From stock I take 0.5, 1, 1.5, 2, 2.5, 3mL into different test tubes and make up to 10mL with buffer to give corresponding concentrations i.e., 5, 10, 15, 20, 25, 30µg/mL respectively. Then observe the absorbance of the above solutions at λ_{max} 276nm by using UV-Visible spectrophotometer.

A graph was plotted with concentration on x-axis and absorbance on y-axis.



DRUG PERMEATION STUDY:

PREPARATION OF KILLED BACTERIAL VACCINE

Aim: To prepare and submit killed bacterial vaccine.

Requirements: Bacillus subtilis, 24 hr culture, nutrient broth, nutrient agar, autoclave, incubator, petriplates.

Principle: vaccines are suspensions of killed, live or attenuated (having weakened virulence) cultures of microorganisms which are used as antigens to produce immunity against infections due to particular micro organisms.

Vaccination is the phenomenon of preservative immunization which protects the individual against future infections. The type of immunity produced by vaccinization is artificial activity immunity. It causes the stimulation of immune apparatus (system) to form antibodies and/or the production of immunologically active cells.

Active immunity develops after a latent period but once developed, it is long lasting.

Vaccines are several types namely.

Live vaccines:Non-virulent or weakened bacteria or viruses.

Eg: oral polio vaccine

Killed vaccines: dead bacteria or inactivated viruses.

Eg: typhoid, cholera, hepatitis vaccines.

Toxoids: modified toxins which have lost toxigenicity but retain antigenicity.

Eg: diphtheria, tetanus toxoids.

Viral fragments or bacterial molecules: sub unit vaccines.

In killed vaccine, the organisms are killed by heat, formation, phenol and alcohol;. They are preserved in phenol, N-methiolate and alcohol.

They possess antigens common to the original pathogen but do not replicate. Usually 3 doses of vaccines are required to have effective immune response.

Bacillus subtilis is a rod shaped gram +ve organism. It is one of the most potent and beneficial of all immune stimulating bacteria. The cell wall components of bacillus subtilis are able to activate nearly all systems of human defense, including the activation of three specific antibodies (IgA,IgM,IgG)which are highly effective against many of the harmful viruses, fungi and bacterial pathogens which regularly attempt to include and infect the human system.

Method of preparation: Bacterial vaccine of bacillus subtilis was prepared by “Heat method”.

Preparation of nutrient broth medium: 14 grams of nutrient broth was suspended in 1000ml of distilled water. The mixture is boiled and sterilized in autoclave at 15lb pressure and 121°c temperature for a period of15min.

Preparation of subculture of bacillus subtilis: Into 10ml of nutrient broth medium, a loopful culture of the bacterium was transferred under aseptic conditions and incubated for overnight.

Preparation of killed vaccine: The overnight culture as transferred to a centrifuge tube and centrifuged at 3000 rpm for 15-30 minutes. The supernatant was discarded and the obtained pellet was transferred to a test tube and suspended in normal saline solution.

The mixture was heated to 60°c for 1 hour and the resultant killed vaccine was transferred aseptically into vials and stored under refrigerator conditions for evaluation and for further use.

Evaluation:

Live test: a 2% solution of nutrient agar medium was prepared, sterilized and transferred aseptically into a petri dish. Then, the killed bacterial vaccine of Bacillus subtilis was streaked on to the plate and incubated for 24 hours and observed for microbial colonies.

If no colonies or growth is reported, then it indicates that the preparation contains no live organisms.

Report:

DEMONSTRATION OF P_{gp} ACTIVITY

AIM: Demonstration of role of P_{gp} as efflux transporter.

REQUIREMENTS: Tissue ringer solution, isolated rat intestinal segments, beakers, ice cold saline, petri plates, ligating thread.

PRINCIPLE: P_{-gp} is member of ABC (ATP Binding cassette) super family of membrane transport proteins. P_{-gp} is a trans membrane efflux transporter protein, encoded by the Multi-Drug resistance gene in human and mdr-1a and mdr-1 b genes in rodents.

P_{-gp} is found to be present in broad spectrum of tissues such as hepatocytes, intestinal mucosal cells, Gastro-intestinal epithelium, Blood capillaries of brain, Bone marrow stem cells etc.

The main function of P_{gp} is protection, by extruding toxins out of the organs or removes them once they have entered and protect the critical organs such as Brain and testis it is also known to confer multi Drug Resistance (MDR) to tumor cells. A drug with a high liquid partition co-efficient will be more easily removed from the lipid bi layer by p_{-gp} than one with lower lipid solubility, independent of their relative concentrations in the system.

PROCEDURE:

1) Anaesthetized rat was used for the experiment.
2) The abdomen of the rat was cut open to expose the stomach and intestine.
3) The duodenum, jejunum and ileum were isolated and immediately placed in tissue ringer solution with aeration, in order to maintain live conditions.
4) One end of the intestine was ligated with a thread and drug solution was poured in it and the other end was closed – normal sac.
5) This was introduced into a beaker containing buffer.
6) The intestine was everted with the help of glass-rod (Glass-rod technique) and one end of the intestine was ligated and drug solution was placed in it and the other end was closed. This was introduced into another beaker containing buffer – everted sac.
7) At regular time intervals, samples were withdrawn from both the breaker and analyzed for drug content.

CONCLUSION: If the introduced drug is a P_{-gp} substrate, it will not be diffused from the normal sac where as diffusion will be seen from the everted sac.

VALIDATION OF TABLET COATER

AIM: To validate tablet coater.

VALIDATION:

S.No	OPERATION	PERFORMANCE (YES/NO)	OBSERVATIONS
	INSTALLATION QUALIFICATION: Manufacturer Purchase order number Model number Location Utility requirements Calibration of Multi meter Air pressure guage Component material Coating pan Spray system		

	OPERATIONAL QUALIFICATION: Control function test Power start /stop push button Pan jog push button Heater on / off button Fan on / off button Exhaust plenum clamp switch Pan speed control knob Tablet coating rotation direction test Pan speed test Calibration and identification of Tachometer		

	Tablet coating pan operation Spray system test **PERFORMANCE QUALIFICATION:** Required material Test material Coating medium Batch size Pan performance test Supply temperature Exhaust temperature Supply air flow rate Pan differential air pressure Calibration of thermometer Pan speed Spray delivery rate Coating pan speed		

	Calibration of Tachometer		

REPORT:

VALIDATION OF LAMINAR AIR FLOW HOOD

AIM: To validate laminar air flow hood.

VALIDATION:

S.No	OPERATION	PERFORMANCE (YES/NO)	OBSERVATION
	INSTALLATION QUALIFICATION		
1.	Identification of instrument		
2.	Placed in an upright position		
3.	Identification number		
4.	Location		
5.	Utility requirements		
6.	Certification by QC/QA		
7.	Requalification		
	OPERATIONAL QUALIFICATION		

8.	Accessories a)variable speed blower motor control b) metal diffuser c) one or two extra prefilters		
9.	Electrical components		
10.	Fluorescent light components and switches		
11.	Motor blower and speed control		
	PERFORMANCE QUALIFICATION		
12.	HEPA filter integrity test		
13.	Checking material of construction		
	a) molybdenum test		
14.	Sterility test		

REPORT:

VALIDATION OF MEMBRANE FILTERS

AIM: To perform the validation of membrane filters.

REQUIREMENTS: Membrane filter, sterile syringe, sterile test tube, distilled water, incubator etc.

THEORY:

Membrane filters are also called 'microporous filters', and are used within fluid systems to trap or retain micro particles and microorganisms, which are larger than the membrane filters filtration gap or pore size. These filters have extremely small filtration gap sizes in the order of 0.08 to 6µm in size.

Membrane filters are available in standard and custom sizes, shapes and materials. Some common materials used are nitrocellulose, cellulose acetate, nylon, polycarbonate, glass, Teflon. Many membrane filters are placed within a autoclave for sterilization and post filtration processing. Filter material compatibility target and application of filter micro particles should be reviewed.

VALIATION:

Sterilizing grade membrane filters are defined by the FDA guideline on sterile drug products produced by aseptic processing by being able to retain 10^7 *Brevundimonas diminuta* (formerly *Pseudomonas diminuta*) organisms per square centimeter of filtration area at a differential pressure of 2 bars.

Such retention efficiency has to be validated using the actual drug product and the process parameters, due to the possibility of an effect of filters compatibility and stability and /or microorganisms' size and survival rate.

Performing these so-called product bacteria challenge tests become a regulatory demand and therefore, belong to a standard filter validation. Before these challenge tests can be performed, several parameters, for example, bacterial effects of the product, have to be evaluated.

Various tests that need to be performed are :

- Filter integrity test
- Bubble point test
- Pressure fold test
- Diffusive flow test
- Water intrusion test

The water intrusion test is used for hydrophobic vent and air membrane filters only. The upstream side of the hydrophobic filter cartridge housing is flooded with water. The water will not flow through the hydrophobic membrane. Air or hydrogen gas pressure is applied upstream which signifies a beginning of water intrusion into the largest (hydrophobic) pores, water being incompressible. The automated integrity tester is sensitive enough to detect the pressure drop. The measured pressure drop is converted into a measured intrusion value, which is compared to a set intrusion limit, which has been correlated to the bacteria challenge test. As with the diffusive flow test, filter manufacturers specify a maximum allowable water intrusion value. Above this value, a hydrophobic membrane filter is classified as non-integral.

PROCEDURE:

Organism: E.coli (E.coli has size less than the single use membrane filter of size 0.45µm.)

In nutrient broth medium, E.coli organisms were cultured. Nutrient agar medium was prepared and sterilized in autoclave and allowed to solidify in petriplate. 10ml of distilled water, around 1ml of E.coli culture was inoculated and incubated for 1hr. then it was filtered with the help of a syringe into a sterile test

tube. A loop full of culture from the test tube was streaked on the agar medium and incubated for 24hrs.

REPORT:

VALIDATION OF HOT-AIR OVEN

AIM: To validate the Hot-air oven.

REQUIREMENTS: Hot-air oven, thermometer, amber coloured bottles and loading material.

THEORY:

Hot – air oven is used for the dry heat sterilization. Substance that resist degradation at temperature above 140ºC (284ºF) may be rendered sterile by means of dry heat. Two hours exposure to a temperature of 180ºC (356ºF) or 40min at 260ºC normally can be expected to kill spores as well as vegetative forms of all micro-organisms.

Dry heat sterilization is recommended where it is undesirable or unlikely that steam under pressure will make direct contact with the material to be sterilized. It is useful in case of laboratory glassware as well as oils, powders etc.

PRINCIPLE INVOLVED IN THE VALIDATION OF HOT-AIR OVEN:

The key to validate hot-air oven is to prove its repeatability. This means that the unit can generate materials that are sterile and pyrogen free.

Repeatability in dry heat sterilization obviously involves consistency and reliability in attaining and maintaining a desired temperature in all the areas of the heating chamber. There will always be an area that represents a “cold spot” – an area that is most difficult to heat up to the desired temperature. This cold spot should be identified, so that validation studies involving thermocouples, monitoring of microbial challenges can be done at this location.

If certain key GMP features of the dry heat sterilizer are not followed, the cold spot will change and repeatability cannot be achieved.

As with any sterilization process, the first step in dry heat sterilizer validation involved qualification of all the equipment and instrumentation used. This step includes examination and documentation of all utilities, filters, control

values for the oven or tunnel unit and the calibration of the instrumentation used in validating and monitoring the process.

BATCH OVEN VALIDATION:

AIR BALANCE DETERMINATION:

In an empty oven, data are obtained for the flow rates of both intake and exhaust air. Air should be balanced so that a positive pressure is exerted to the non-sterile side when door is opened.

Air velocity across, up and down the opening of the door is $\pm$ 50 FPM of the average velocity. Efficiency of hot-air oven is determined primarily by 2 types of studies.

1. Heat distribution studies of an empty chamber to determine cold spot.
2. Heat penetration studies to determine the slowest heating point with load.

HEAT DISTRIBUTION STUDIES:

These are performed to ascertain whether or not the sterilizer unit is uniform throughout the sterilization process and during this test, the coolest heating position is located. For a new oven, this test is performed with an empty chamber to ensure that it is installed and operating properly. For existing ovens, the test is performed on standard loads to determine a standard load pattern. Thermocouples should be situated according to a specified predetermined pattern. Repeatability of temperature attainment and identification of cold spot can be achieved if the temperature range is $\pm$ 15ºC at all monitored locations.

Heat distribution studies can also be conducted as a function of variable air flow rates through the hood ducts and as a function of the gas flow rate to the sterilizing burner.

HEAT PENETRATION STUDIES:

The test should be performed on maximum load. Thermocouples are placed in the commodities located in the areas likely to present the greatest resistance to reach the desired temperature.

Minimum and maximum temperature as defined in the process specifications should be studied. Normally replicate cycles are run at each temperature. The cold spots represents the area to be used for the biological challenge studies.

Other variations in the cycle should also be performed which include

a. Test load variation
b. Temperature set point variations
c. Variations in the time of exposure

MECHANICAL REPEATABILITY:

During all these studies, mechanical repeatability in terms of air velocity, temperature, consistency and reliability, sensitivity of the oven and instrumental controls must be verified.

PROCEDURE:

Heat distribution studies were conducted in an empty chamber and in chamber with maximum load. Previously calibrated thermocouples or temperature recorders were selected and thermometers were labelled as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9. All the 9 were arranged in three trays at different locations, in such a way that they cover all the heat distribution points. An order of alignment of temperature recorder is predetermined. This arrangement was maintained for all temperature at which oven is validated which enable one to identify the cold spot easily, not only that but also observing the repeatability of temperature will be optimum. After arranging all the thermometers in a specified pattern, the door of the oven was closed properly and thermostat was adjusted to a desired temperature, the oven was stabilized for 1hr. After stabilization, the thermometers were checked to record the temperature in specified order.

PRECAUTIONS:

1. Thermometer should not touch the surface of the oven.
2. Location of the thermometers should be the same for all cycles.
3. Time for recording should be as minimum as possible.
4. Order of temperature recording should be maintained.

REPORT:

VALIDATION OF COATING PAN

OBJECTIVE: To establish a document evidence, that the coating pan is performing as per the manufacturers specifications and recommendations and quality attributes and complies with standard operating procedures (SPO'S) and current Good Manufacturing (CGMP) requirements.

Requirements:

The performance qualification shall be carried for establishing the performance and efficiency of the coating pan during tablet coating operation.

Process variables: whether the coating process is in a conventional pan system or in one of the perforated pan systems previously described, certain elements of the process need to be controlled to ensure consistent product quality. The process is an important as the coating solution formulation; consequently development of well-defined and well-controlled process should be a major concern of the formulator.

The variables to be controlled in pan-spray film coating process are:

1. Pan Variables---pan design/baffling, speed, pan load.
2. Process Air-----air quality, temperature, airflow rate/volume/balance.
3. Spray Variables--- spray rate, degree of atomization, spray pattern, nozzle-to-bed distance.

Since each listed variable is important to the overall success of the coating, further discussion is warranted.

Pan Variables: pan shape, baffling, rotational speed, and loading all affect the mixing of the tablet mass. Uniform mixing is essential to depositing the same quantity of film on each tablet. The tablet coating adds an approximate increase in weight of only 2 to 5 % to the tablet. Unacceptable color uniformity or enteric film integrity is encountered if the tablets are inadequately coated because of poor tablet movement in the coating pan.

Tablet shape can also affect mixing, but also the velocity at which the tablets pass under the spray. Speeds that are too slow may cause localized over wetting, resulting in the tablets sticking to the each other or to the pan. Speeds that are too high may not allow enough time for drying before the same tablets are reintroduced to the spray; this results in a rough coating appearance on the tablets. Pan speeds of 10 to 15 rpm are commonly used in the large pan coaters for nonaqueous film coating. Slower pan speeds (3 to 10 rpm) are used for aqueous film coating primarily to accommodate slower application rate and drying of the coating liquid. Selection of pan operating conditions depends on the equipment availability, type of tablets being coated, and the characteristics of the coating solution.

Spray variables: the spray variables to be controlled are the rate of liquid application, the pattern, and the degree of atomization. These three variables are inter dependent . In the airless, hugh pressure system, all the three variables are directly affected by fluid pressure and nozzle design. In the air atomized, low pressure system, the rate of liquid flow is most directly affected by the liquid pressure and liquid orifice size. The degree of atomization and spray pattern are most directly affected by atomizing air pressure, air volume, and the shape and design pressure, air volume, and the shape and design of the air jets in relation to the fluid stream. The proper rate at which the coating solution should be applied depends on the mixing and drying efficiency of the system, in addition to the coating formula and core characteristics. There is a range in which the coating rate must operate achieve the desired product quality or processing time. Over writing and over wetting must be avoided in all coating operations.

A band of spray should be spread evenly over the tablet mass. In larger pans, more nozzles must be added to cover the tablet bed width. A spray pattern that is too wide could result in the application of coating directly to the pan surface, producing lower coating efficiency and wasted material. If the spray pattern is too narrow, localized over wetting may result, and the tablet-to-tablet coating uniformity will be poor. Thus, tablets need to make many more passes through the spraying area to be adequately coated. During the coating operation, the spray width can be adjusted by moving the nozzles closer or farther away from the tablet bed. In the air pressure low-pressure systems, adjusting the air pressure and/or direction accomplishes the same effect. The distance to the nozzle is from the tablet bed affects not only the spray width, but also the quantity of coating applied to individual tablets per pass under the spray.

Atomization is the process whereby the liquid stream is finely subdivided into droplets. The degree of atomization-the size and size distribution of the droplets-obtained from the spray nozzle is not an easily controllable parameter. The relationships between the orifice size, nozzle configuration, fluid pressure, atomizing air pressure, air volume, and fluid viscosity vary with each coating formulation. Manufacturing literature may the droplet size range expected from a particular nozzle type based on water, however, this type of data is inadequate for optimizing the nozzle performance in relation to the variety of solution and suspensions used to coat tablets.

The degree of atomization, at present, can only be controlled empirically. Adjustments of either the fluid pressure on the airless high-pressure systems of the atomizing air pressure and air volume on the low-pressure systems ensures yield greater atomization. Atomization that is too fine causes some droplets to dry before reaching the tablet bed. This “spray-drying” effect can be readily detected as roughness on the tablet surface, especially in intagliations or as excess dust in the pan. Insufficient atomization may result in droplets that are too large reaching the tablet surface and causing localized over wetting, which could lead to sticking, picking or a rough “orange-peel” effect.

Process Air Variable: The temperature, volume, rate, quality, and balance are parameters of the process air that need to be controlled to obtain an optimum drying environment

for a particular coating process. The sensitivity of the film former and product core to heat largely determines the upper temperature at which the coating process is successful. In general, higher tablet bed and coating chamber temperatures are more conductive to rapid solvent evaporation, and consequently to faster coating rate. The limits to the air volume and rate depend on the overall design of the air-handling system and term's range is used most often. The more efficient the equipment design, the less air volume is needed for drying.

The balance between supply and exhaust air-flow should such that all dust and solvent are contained within the coating system.

LIST OF PARAMETERS TO BE STUDIED BEFORE AND AFTER COATING OPERATION:

- ✓ Average weight
- ✓ Weight variation of 20 tablets
- ✓ Hardness of 20 tablets
- ✓ Thickness of 20 tablets
- ✓ Diameter of 20 tablets
- ✓ Friability
- ✓ Appearance
- ✓ Gun to bed distance
- ✓ Gun to the side of the pan distance
- ✓ Spray rate
- ✓ Inlet air temperature
- ✓ Pan speed

ACCEPTANCE CRITERIA: There should be uniformity of coating and the increase in weight of tablet after coating should be only 2-5%.

PROCEDURE: After checking the machine for its cleanliness and necessary things for normal, set the pan with as per SOP and operate it according to instructions mentioned in the same SOP. Carry out the performance qualification (PQ) by using placebo tablets and pan is related at a speed o 100 RPM.

Performance qualification

Item	Specifications
Test material	Placebo tablets
Batch size	20 tablets

COATING PROCESS EXPERIMENTAL STUDIES:

Two categories of experimental studies in support of coating process validation. These include

1) Coating liquid studies

2) Coating process studies

1) Coating liquid studies: The parameters to be studies include

- ✓ Polymer-plasticizer ratio
- ✓ Viscosity
- ✓ Microbial count
- ✓ pH
- ✓ physical appearance

2) Coating process studies: it includes tablet attributes tested after coating.

- ✓ Friability
- ✓ Weight
- ✓ Disintegration
- ✓ Dissolution
- ✓ Hardness
- ✓ Weight variation
- ✓ Thickness and diameter.

OBSERVATIONS:

Sino	Before coating			After coating		

Individual weight variation:

sino	Tablet weight(mg) before coating	Tablet weight(mg) after coating

S.no	Appearance of tablets	Friability (%)	Average weight(mg)

Pan speed: speed at which the pan drum rotates usually in rpm. In this equipment, regardless of pan type and size, the pan speed is held constant and was found to be 100rpm.

Spray delivery rate: the quantity of coating solution sprayed per unit time, expressed as ml/min. spray gun was filled with 220 ml of coating solution. 50 sprays were given and the amount of the solution lost after spraying was 45 ml. a stop clock is required.

Hence, the volume of the coating solution per spray was found to be

GUN GEOMETRY:

Number of spray guns used—

Gun to bed distance—

Dry air nozzle to bed distance—

INLET AIR TEMPERATURE: the temperature of processing air used to dry the tablets in the coating pan was found to be

REVALIDATION: Revalidation once in a year and in case of change in critical parts or after major break down. Revalidation to be carried out for:

- RPM
- Any other suitable parameters related with the change.

CONCLUSIONS:

VALIDATION OF TABLET PUNCHING MACHINE

Objective: To establish document evidence that the Table ting machine is performing as per the design specifications, manufacturers, recommendations and process requirements, complies with standard operating procedures and Current Good Manufacturing Process (CGMP) requirements.

Construction and working of tablet punching machine:

Tablet compression machines: Tablets are made by compressing a formulation containing a drug or drugs with excipients on stamping machines called presses. Tablet compression machines or tablet presses are designed with the following basic components:

1. Hopper(s) for holding and feeding granulation to be compressed.
2. Dies that define the size and shape of the tablet.
3. Punches for compressing the granulation within the dies.
4. Cam tracks for guiding the movement of the punches.
5. A feeding mechanism for moving granulation from the hopper into the dies.

Tablet presses are classified as either single punch or multi-station rotary presses. All the compression is applied by the upper punch, making the single punch machine a “stamping press”.

Multi-station presses are termed rotary because the head of the tablet machine that holds the upper punches, dies, and lower punches in place rotates. As the head rotates, the punches are guided up and down by fixed cam tracks, which control the sequence of filling, compression, and ejection. The portions of the head that hold the upper and lower punches are called the upper and lower punches turrets respectively, and the portion holding the dies is called the die table.

At the start of a compression cycle, granulation stored in a hopper, showed in to the feed-frame, which has several interconnected compartments. These compartments spread the granulation over a wide area to provide time for the dies to fill. The pull-down cam guides the lower punches to the bottom of their vertical travel, allowing the dies to overfill. The punches then pass over a weight-control cam, which reduces the fill in the dies to the desired amount. A wipe-off blade at the end of their feed-frame removes the excess granulation and directs it around the turrent and back into the front of feed-frame. Next, the lower punches travel over the compression roll while simultaneously the upper punches ride beneath the upper compression roll. The upper punches a enter affixed distance into the dies, while the lower punches are raised to squeeze and compact the granulation within the dies. To regulate the upward movement of the lower punches, the height of the lower pressure roll is changed. After the moment of compression, the upper punches are with-drawn as they follow the upper punch raising cam; the lower punches ride up the cam, which brings the tablets flush with or slightly above the surface of the dies. The exact position is determined by a threaded bolt called the ejector knob. The tablets strike a sweep-off blade affixed to the front of the feed-frame and slide down a chute into

a receptacle. At the same time, the lower punches re-enter the pull down cam, and the cycle is repeated.

Design Qualification:

Equipment identification:

Manufacturer

Model

Capacity

Serial No

Equipment code

Location

Performance Qualification of Tablet Press:

The performance qualification shall be carried for establishing the performance (qualification shall be) and efficiency of the tablet machine during tablet compression operation.

Test for reproducibility of Hardness:

- Specific amount of granules were weighed and feed them to the die cavity according to SOP.
- Using the adjustable cam the hardness was set to a specified value and operated as per SOP.
- The hardness was decreased by adjusting the cam and compressed the granules region.
- The resulting tablets are checked for their hardness.
- The results were noted.

Acceptance criteria:

According to increase and decrease the corresponding (hardness) of the tablet should show the considerable proportionate change.

Test for reproducibility of weight:

- Specific amount of granules were weighed and feed them to the die cavity according to SOP.
- Using the adjustable cam the weight was set to a specified value and operated as per SOP.
- Tablets were produced, separately at following speeds- Low, medium and high.
- The weight was increased by adjusting the cam and compressed the granules again.

- The resulting tablets are analyzed for their weight.
- The results were noted

Acceptance criteria:

According to increase and decrease the corresponding (hardness) of the tablet should show the considerable proportionate change

List of parameters to be studied:

- Average weight
- Weight variation of 20 tablets
- Hardness of 20 tablets
- Thickness of 20 tablets
- Diameter of 20 tablets
- Friability
- Appearance (appearance checked for finish of tablet, edges, capping and chipping).

ACCEPTANCE CRITERIA: There should be uniformity of the weight and RSD of weight variation should not be more than 2%

OBSERVATION:

S.No	Speed of Machine	Appearance of tablets	Friability	Average weight
1	Low			
2	Medium			
3	High			

PHYSICAL PARAMETERS:

S.no	Speed of the machine (LOW)			Speed of the machine (MEDIUM)			Speed of the machine (HIGH)		

INDIVIDUAL WEIGHT VARIATION:

S.NO.	SPEED OF MACHINE LOW	SPEED OF MACHINE MEDIUM	SPEED OF MACHINE HIGH

S.NO	APPEARANCE OF TABLETS	FRIABILITY (%)	AVERAGE WEIGHT(mg)

CONCLUSION:

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VALIDATION OF ASEPTIC ROOM

AIM: To describe the procedure for the validation of aseptic area, to prevent cross contamination and demonstrate environmental control.

PRINCIPLE: The clean room with unidirectional airflow is more commonly known as laminar flow clean rooms. These are designed with an intention that air should move a single pass through the room. Air can enter through one valve and exit through the opposite.

In a conventional clean room HEPA filters are usually loaded in the mechanical room. They are located in the wall or ceiling of the room, so that only air passes through the filter, which is in the clean room. These systems (filters) must be sealed into the framing system, so that there can be no bypass of dirty air around them and into the clean room.

A laminar flow cabinet is a carefully enclosed bench, designed to prevent contamination of semiconductor wafers, biological samples or any particle sensitive device.The cabinet is usually made of stainless steel with no gaps or joints where spores might collect. The laminar flow cabinet may have an ultra violet lamp to sterilize the cell and its components when not in use (it is important to switch off the uv lamp while working, as it will quickly give any exposed sunburns and may cause contract)

PROCEDURE:

Validation parameters:

- Airborne microbial count
- Surface bio burden
- Lighting level

1. **Microbial (airborne) test:**

The test was performed to determine the airborne microbial contamination level.

Method:

- Petriplates used were sterilized prior to filling.
- The petriplates were filled adequately with solid bacteriological medium-nutrient agar, which was sterilized before filling.
- Sampling was done for a period of 20min at every location. The plates should be marked with identifications which include data, sampling location and plate number.

- Plates were kept in an incubator and maintained inverter to prevent condensation drop for a period of 18hrs to24hrs at 37c.
- After incubation, the number of colonies on each plate was counted using a standard bacterial colony counter.

2. Surface bioburden test:

The test was performed to determine the microbial contamination level on surfaces.

Method:

- The swab stick was taken and gently swabbed 25cm2 of area which includes walls, floors end the working bench.
- The number of colonies on each plate was counted, using a standard bacterial colony counter.

3. Lighting level test:

The test is performed to determine the effect of lights in maintaining sterility.

Method:

- Pre sterilized nutrient agar was poured into the petriplates which were sterilized prior to filling.
- Those plates were exposed to UV – germicidal lamp.
- Few plates were exposed to illuminous lights.
- All the plates were incubated and the number of colonies formed was determined using standard colony counter.

REPORT:

VALIDATION OF AUTOCLAVE

AIM: To validate an autoclave using chemical indicators.

REQUIREMENTS: Benzoic acid, Temperature indicator strips, capillary tubes, beakers and autoclave.

THEORY:

Sterility Assurance Level: The level of microbial inactivation can be described by an exponential function called sterility assurance level(SAL).

For example, a SAL of 10-6 means that the probability of a single viable micro organism being present on a sterilized item is 1in 1million after the sterilization process. The SAL required is determined by the intended use of the item.

Log Reduction: one log reduction means to decrease the microbial population by a factor of 10. The bio burden is the number and type of viable micro organism contaminating an item. A sterilization cycle that provides a SAL of 10-6 effectively means that the microorganisms that "could" be present (i.e. bio burden) are killed and additional 6log reduction safety factors has been provided.

The following provides an example of a cyclic achieving a SAL of 10 to 6.

- Bio burden(worst case) = 134CFU(colony forming units)
- To reduce the microbial population from 134 to 1 = log(134) = 2.13(i.e. a 2.13 log reduction is required to reduce the population from 134 to 1)
- Applying an additional6log reduction will theoretically reduce the microbial population from 1 to 0.000001. this provide a SAL of 10-6 or a one in 1million probability of a single surviving micro organism.
- Total log reduction = 2.13 + 6 = 8.13. Therefore to provide a SAL of 10-6 with the bio burden of 134 CFU requires a sterilization cycle that provides an 8.13 log reduction.

THERMAL RESISTANCE CHARACTERISTICS:

The thermal resistance of specific micro organisms is characterized by "D-values", "Z-values".

A D-value is the time in minutes, at a specific temperature, to reduce the surviving microbial population by 1-log.

A Z-value is the temperature change required to result in a 1-log reduction in D-value. Other time measurement variables pertaining to thermal resistance are "F-values" and "F_O Values ".

An F value is the number of minute to kill a specified number of micro organisms with a specified Z-value at a specific temperature. An F_O value is the number of minute to kill a specified number of micro organisms with a Z -value of $10^oC(50^OF)$ at the temperature of 121.1^oC.

BASIC VALIDATION APPROACH:

Installation qualification: The IQ process is intended to demonstrate that the autoclave has installed meats all specifications, is installed properly and that the supporting programs needed for ongoing operation (ex: SOP, maintenance program, etc..) are in place.

An IQ may include the following checks:

- Mechanical equipment specifications (chamber, valves, filters, regulators, condenser, vacuum pump, heat exchanger, etc..)
- Control and instrumentation specifications (programmable logic controller, operator interface, printer/recorder, control valves, pressure and temperature transmitters, resistance temperature devices , switches, etc..)
- Sight specifications/ utilities (power , water, air, clean steam, drain, isolation valves, electrical disconnect switches, etc..)
- Construction materials/Materials in contact.
- Approval documentation.
- Purchase orders
- SOPs.
- Operating maintaining manuals, etc..

Operational qualification:

The OQ process is intended to demonstrate that the components of the autoclave operate properly that the autoclave is deemed ready for performance or load testing.

Autoclave works on the principle of moist heat sterilization. The sterilizing medium is pressurized saturated steam. The typical operating temperature is 121c, but higher – lower temperatures often can be used. All autoclaves are intended for pharmaceutical industry, made up of stainless steel (316SS).

Moist heat sterilization causes coagulation of cell proteins at much lower temperature than dry heat.

Performance qualification:

The performance of an autoclave is tested by using biological indicators (Bacillus sterothermophillous), chemical indicators such as benzoin, benzoic acid, cinnamic acid, thio urea.

PROCEDURE:

- Chemical indicators such as benzoic acid,was filled in 4-5 different capillary tubes and tips sealed by using place
- Indicators indicator strips were placed at various parts of the inside of the autoclave
- The sealed capillaries along with the strip was placed in a beaker and was kept in an autoclave.
- The lid of the autoclave was closed and the mains were turned on.
- Temperature and pressure were monitored till the operating conditions were met
- After the stipulated amount of time the autoclave was turned off and was allowed to cool sufficiently before the samples were taken out for examination.

OBSERVATION:

REPORT:

VALIDATION OF TRAY DRYER

Aim: To carry out the validation of tray dryer.

Requirements: Tray dryer, thermometers, vials.

Theory:

Drying is defined as the removal of small amounts of water (or) other liquid from a material by the application of heat.

Drying is differs from the evaporation the product obtained is dry solid whereas concentrated solution (or) wet slurry (or) suspension incase of evaporation.

In a wet solid mass, water may present as bound water or unbound water.

Unbound water is the amount of water held by the material that exerts in equilibrium vapor pressure equal to that of pure water at the same temperature. Unbound water exists largely in the voids of the solid (non-hygroscopic material).

Bound water is the minimum water held by the material that exerts an equilibrium vapor pressure less than the pure water at the same temperature. Bound water is the present in the hygroscopic material.

$$\% \text{ loss on drying (LOD)} = \frac{\text{mass of water in sample (kg)}}{\text{total mass of wet sample (kg)}} \times 100$$

$$\% \text{ Moisture content (MC)} = \frac{\text{mass of water in sample (kg)}}{\text{Mass of the dry sample (kg)}} \times 100$$

$$\text{Drying rate} = \frac{\text{weight of water in sample (kg)}}{\text{Time (h)} \times \text{weight of the dry solid (kg)}}$$

Applications:

1. Preparation of bulk drugs & pharmaceuticals.
   E.g.: Dried aluminum hydroxide, Spray dried lactose Powdered extracts
2. Preservation of drug products.
3. Drying is necessary in order to avoid deterioration.Eg: Blood products, skin, tissue preventing from microbial growth. Effervescent tablets (Aspirin, Penicillin) preventing from chemical decomposition.
4. Improved handling: Drying → ↓ moisture content → ↓bulk and improve the handling of material by having light in eight → storage and transportation and easy.
5. Improved characteristics:
   Drying produces materials of light weight, spherical shape, uniform size, free flowing and enhanced solubility.

Validation is documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

Tray dryer is equipment used for removal of small amount of water or other liquids from a material by the application of heat. It is a static bed dryer in which there is no relative movement among the solid particles being dried, although there may be bulk motion of the entire drying mass.

Construction:

It consists of insulated walled rectangular chamber made up of stainless steel. Trays placed on a heating chamber, the number of trays varies with the size of the dryer. Laboratory size dryers contains minimum of 3 trays. Industry size dryer contains more than 20 trays. Each tray is rectangular in shape and about 1.2 to 2.4 m^2 (4-8 square feet) in area. Trays are usually loaded from 10 – 100mm deep. The distance between two trays must be 40 mm. In industry, trays are placed in trucks on wheels, which can be rolled into and out of chamber. These are called truck dryers. Usually a truck contains 18 or more trays. An air circulator or fan and a heater placed insid4e an A thermostat (temperature control device). An air inlet and outlet for introducing air removing exhausted air inside the chamber.

Working:

- Wet solid is loaded into the trays which are then placed in the chamber.
- Fresh air sis introduced through inlet, which passes through the heaters and gets heated up.
- The hot air is circulated by means of fans at velocity of 2 -5 meters per second.
- Turbulent flow lowers the partial vapor pressure in the atmosphere and also reduces the thickness of the air boundary layers. The water is picked up by air.
- As water evaporates from the surface, the water diffuses from the interior of the solid by capillary action.

- The event is very small and the discharged air 80 -90% is circulated back through fans. Only 10 – 20% of fresh air is introduced.
- Moist air is discharged through outlet.

Validation:

Design qualification: a documented review of the design, at an appropriate stage of stages in the project for conformance to operational and regulatory expectations.

(Designing the quality parameters required of the equipments and manufacturers).

The manufacturing equipments are custom made. Hence it is important that all the features of the equipment needs to be maintained to the fabricator.

The design qualification of tray dryer includes.

The dimensions: length_____cm, width_____cm.

Material of the construction contact part _____, noncontact part_____.

Model number_____.

Design qualification protocol must be attached.

Design qualification prepared jointly by the uses and the representatives of the engineering department and approved by engineering as well as qualification assurance department.

Identification and verification of documents:

Objective: To identify and verify all the documents associated with the tray dryer (3 dryers) are available.

Tools required: not applicable**.**

Test procedure: verify the availability of all documents, such as purchase order, tray dryer specification, installation, operation and maintenance manual, mechanical and electrical drawings, material of construction test certificates.

Record the document number, revision number, date and location (as applicable) in the table below.

Title	Document No.	Date	Location
Purchase order	136810	13.08.2005	Not applicable
Installation, operating, and maintenance man ual			
Control panel layout drawing & writing diagrams			
No. certificates			

Acceptance criteria:

All the documents listed above if available at the time of this execution of this protocol.

Comments:

Verified by: Date :

Verification of equipment details (major components & accessories)

Parameter/description	Specification	Observation	Discrepancy	Checked by/date
Equipment name plate	Should be clearly seen	Complies		
Model no.				
Capacity	½ kg/hour			
S.No				
Power records/ capacity	_____Watts			
Location				
Dimensions				
No. of Trays				
Maximum Temp.				

Comments:

Verified by: Date:

Installation qualification:

It is documented evidence that the premises, supporting utilities, the equipment have been built and installed an compliance with the design specifications.

The IQ is carried in accordance to previously approved protocols.

An ideal protocol shall document the following.

- Equipment identification
- Verification of equipment installation

- Materials in product contact
- Utility requirements
    - Electrical
    - Other utilities
- Grounding checkout
- Validation test instruments
- Documentation
- Drawings
- IQ check list
- Validation test instruments and calibrations
- Comments
- Acceptance criteria

Verification of equipment installation:

Objective: to verify that the tray dryer is installed (leveled, cleaned, earthed) properly as per the specifications.

Tools required: _____

Parameters/description	**Specification**	**Observation**	**Discrepancy**	**Checked by/ Date**
Leveling	Tray dryer is securely anchored & leveled properly			

Earthing & connections	All wires/ terminals identified & numbered properly. Required electric connections are connected properly & earthed			
Cleanliness	All access plate parts are examined and cleared off any debris			
Utility Requirements	Electricity supply of about _____			

Comments:

Verified by: Date:

Operational qualification and Performance Qualification:

An OQ evaluation should establish that the equipment can operate within specified tolerances and limits.

Follow the instructions specified in the Standard Operating Procedure and operate the equipment.

Performance qualification is the final phase of validation. This phase tests the ability of the process to perform over long periods of time within tolerance deemed acceptable.

An idle PQ protocol should include:

- Acceptance criteria
- Number of samples for analytical testing
- Location of sampling points
- Duration of testing
- Processing steps for testing
- Number of runs

Procedure:

Heat distribution studies:

- A thermometer was inserted into the hole provided at the top of the dryer to record the dryer temperature
- The door of the dryer was closed and the temperature of the pressure was set to desired level using temperature adjustment knob.
- After attaining the set temperature the thermostat was disconnected in 9 thermometers were taken and were placed on the shelves in the pattern shown to cover all the points in the dryer and the door was closed.
- The temperature was allowed to built up to a desired level and stabilized for 15 min.
- The temperature recorded by the 9 thermometers for every 15 min up to 1hour.

- The whole process was repeated by setting the temperature of the n dryer at 50°c, 60°c, 80°c and 100°c.
- The thermometer covering the area that shows the least temperature in the dryer represents the **cold spot**.

Heat penetration studies (with load):

- When the temperature in the dryer falls, the door of the dryer was opened.
- When the load (granules taken in vials), was introduced at 9 different points with thermometer inserted in them. The door was closed and the temperature was set to desired value.
- After attaining the desired the temperature and stabilized for 15 min temperature was recorded by thermometer were noted for every 15 min up to 1 hour.
- The area containing that the thermometer that shows the least temperature represents cold spot.

Results:

Conclusion: