East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo
Nagar PostBengaluru – 560049, Karnataka

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka
Bengaluru – 560041
India

LAB MANUAL

PHARMACEUTICS PRACTICAL-II

M. PHARM 2nd SEMESTER

EAST POINT COLLEGE OF PHARMACY
East Point Campus, Jnana Prabha, Virgo Nagar Post,
Bengaluru – 560049, Karnataka

M. Pharmacy (Pharmaceutics)	
Programme Outcome (PO)	
PO1	An ability to independently carry out research /investigation and development work.
PO2	An ability to write and present a substantial technical report/document
PO3	Students should be able to demonstrate a degree of mastery over the area as per the specialization of the program. The mastery should be at a level higher than the requirements in the appropriate bachelor's program
PO4	Graduates will demonstrate comprehensive knowledge and practical skills in advanced pharmaceutical development, encompassing drug analysis, drug formulation, and evaluation of novel drug delivery systems.
PO5	Students will acquire a deep understanding of regulatory processes and compliance, preparing dossiers for submission to regulatory agencies worldwide. They will navigate the intricacies of innovator and generic drug concepts, ensure adherence to global guidelines, and exhibit expertise in Biopharmaceutics& Pharmacokinetics.
PO6	Graduates will integrate technological advancements into pharmaceutical research and development, utilizing computational modelling, design of experiments, and prototype modelling.

Programme Specific Outcomes (PSO)	
PSO1	Apply appropriate tools and techniques for design and development of Pharmaceutical Dosage forms, cosmeceuticals and drug delivery systems
PSO2	Comprehend the pharmacokinetic parameters of drugs, dose calculations and biopharmaceutical approaches in problem solving
PSO3	Acquaint knowledge on investigational new drugs and regulatory submissions

Course Outcomes (CO's)	
Code: MPH205P Pharmaceutics Practical-II	
CO 1	Develop various techniques to enhance the dissolution characteristics of poorly soluble drugs, compare dissolution profile of two different marketed products, determine % protein binding
CO 2	Formulate and evaluate the various cosmetic products and address the problems associated with dry skin, acne, blemish, wrinkles bleeding gums and dandruff
CO 3	Able to explain basic principle of cosmetic formulation and principle to improve dissolution characteristic

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Table of Contents

EAST POINT COLLEGE OF PHARMACY
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Experiment No: 01

PREPARATION & EVALUATION OF PARACETAMOL SOLID DISPERSION BY MELTING METHOD

AIM: To prepare & evaluate Paracetamol Solid Dispersion by Melting Method

Requirements:

- **Apparatus-** Spatula, beaker, Sieve, Water bath, China dish, UV-Visible Spectrophotometer, Dissolution Apparatus
- **Chemicals-** Paracetamol, PEG 4000& 6000, Mannitol, Urea Sodium Hydroxide, Potassium dihydrogen phosphate, distilled water.

PRINCIPLE:

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles.

Methods of Preparation of Solid Dispersions:

1. Melting method
2. Solvent method
3. Melting solvent method (melt evaporation)
4. Melt extrusion method
5. Lyophilisation Technique
6. Melt Agglomeration Process
7. The use of surfactant
8. Electrospinning
9. Super Critical Fluid (SCF) Technology
10. Direct capsule filling
11. Dropping solution method
12. Co-precipitation method

Advantages of solid dispersions:

Generally, solid dispersion is mainly used:

- To reduced particle size.
- To improve wettability.
- To improve porosity of drug.
- To decrease the crystalline structure of drug in to amorphous form.
- To improve dissolvability in water of a poorly water-soluble drug in a pharmaceutical
- To mask the taste of the drug substance.
- To prepare rapid disintegration oral tablets.
- To obtain a homogenous distribution of small amount of drugs at solid state.
- To stabilize unstable drugs.
- To dispense liquid or gaseous compounds.
- To formulate a faster release priming dose in a sustained release dosage form.
- To formulate sustained release dosage or prolonged release regimens of soluble drugs using poorly soluble or insoluble carriers.

Disadvantages of solid dispersions:

Solid dispersions are not broadly used in commercial products due to mainly the problem of crystallization of the components from amorphous state during processing (mechanical stress) or storage (temperature and humidity stress). Moisture may increase drug mobility and promote drug crystallization and thus may hamper storage stability of amorphous pharmaceuticals. Phase separation, crystal growth or conversion of a product to more stable structure from metastable crystalline form during storage are also considered to be major hurdles to commercialize solid dispersions as they result in decreased solubility and thus dissolution rate

Paracetamol is a potent anti-inflammatory analgesic agent indicated for acute and chronic treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylytis. Paracetamol suffered from low and variable bioavailability which was attributed to its low water solubility. The increase in dissolution rate of poorly water soluble drugs from SDs can be attributed to one or combination of different factors Among the popular carriers used in the formulation of

SD are polyethylene glycols (PEGs). They are widely used because of their hydrophilicity, low melting point, and low toxicity.

Ingredients Table (Formula):

Formulation Code	Carrier	Drug: carrier ratio
SD1	PEG 4000	1:1
SD2		1:1.5
SD3		1:2
SD4	PEG 6000	1:1
SD5		1:1.5
SD6		1:2
SD7	Mannitol	1:1
SD8		1:1.5
SD9		1:2
SD10	Urea	1:0.5
SD11		1:1
SD12		1:1.5

Procedure:

A. Preparation of Solid Dispersions-

Solid dispersions were prepared by melting the accurately weighed amounts of carriers (PEG 4000, PEG 6000, Mannitol and Urea) in a water bath and the drug was dispersed in the molten solution. Solvent Evaporation method was used for the preparation of solid dispersions. Briefly appropriate amount of paracetamol was taken in china dish and required amount of carriers (PEG 4000, PEG 6000, Mannitol and Urea) were added to prepare required drug to carrier ratio for formulations. Then the mixture was heated under controlled temperature to melt drug and carrier with continuous stirring. The melted preparation was transferred to porcelain tile to solidify and cooled in an ice bath. The solid dispersions prepared were pulverized and sifted (80#) and stored in a desiccator.

B. Standard Graph of Paracetamol-

Stock solution 1: Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of paracetamol in 100 ml phosphate buffer pH 5.8 in 100 ml volumetric flask (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.

Stock solution 2: 10 ml of this (stock solution 1) is diluted to 100ml with phosphate buffer pH 5.8 to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.

Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml) in each test tube, add phosphate buffer 5.8 to make total volume of 10 ml to produce (2, 4, 6, 8, 10, 12 µg/ml) respectively.

The dilution was analysed in UV-Visible spectrophotometer and the absorbance were recorded. The standard plot was constructed and the standard equation was derived.

C. Evaluation of Prepared Solid Dispersion-

- **Drug content analysis (Assay):**

Preparations equivalent to 20 mg was weighed accurately and transferred to 100 ml volumetric flask and dissolved in phosphate buffer pH 5.8. The volume was made up with phosphate buffer pH 5.8 up to the mark. After suitable dilution, the absorbance of the above solution was measured at 243 nm using appropriate blank solution. The drug content of paracetamol was calculated using calibration curve.

- ***In vitro* release studies:**

Accurately weighed amount of sample was taken for dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analysed for drug release by measuring the absorbance at 243nm using phosphate buffer pH 5.8 as dissolution medium. The volume withdrawn at each time intervals were replaced with same quantity of fresh medium.

Observation & Calculation:

1. **Standard curve of Paracetamol:**

Sl. No	Concentration (µg/ml)	Absorbance (nm)
1	2	
2	4	
3	6	
4	8	
5	10	
6	12	

2. Drug Content Analysis:

Formulation Code	Concentration	Absorbance (nm)	Assay Value (µg/ml)
SD1	10 µg/ml		
SD2			
SD3			
SD4			
SD5			
SD6			
SD7			
SD8			
SD9			
SD10			
SD11			
SD12			

3. *In vitro* release studies:

Sl. No	Time (Mins)	Absorbance (nm)	Conc. In µg/ml	Conc. In µg/ 5 ml	Conc. In µg/ 900 ml	CDR in µg	CDR in mg	%CDR

Report: Paracetamol Solid Dispersions were prepared by Melting method. The Evaluation of the same was performed.

Experiment No: 02

PREPARATION & EVALUATION OF PARACETAMOL SOLID DISPERSION BY SOLVENT EVAPORATION METHOD

AIM: To prepare & evaluate Paracetamol Solid Dispersion by Solvent Evaporation Method

Requirements:

- **Apparatus- S**patula, beaker, Sieve, Water bath, China dish, UV-Visible Spectrophotometer, Dissolution Apparatus
- **Chemicals-** Paracetamol, Mannitol, Urea, Sodium Hydroxide, Potassium dihydrogen phosphate, distilled water.

PRINCIPLE:

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles.

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- To prepare rapid disintegration oral tablets.
- To obtain a homogenous distribution of small amount of drugs at solid state.
- To stabilize unstable drugs.
- To dispense liquid or gaseous compounds.
- To formulate a faster release priming dose in a sustained release dosage form.
- To formulate sustained release dosage or prolonged release regimens of soluble drugs using poorly soluble or insoluble carriers.

Disadvantages of solid dispersions:

Solid dispersions are not broadly used in commercial products due to mainly the problem of crystallization of the components from amorphous state during processing (mechanical stress) or storage (temperature and humidity stress). Moisture may increase drug mobility and promote drug crystallization and thus may hamper storage stability of amorphous pharmaceuticals. Phase separation, crystal growth or conversion of a product to more stable structure from metastable crystalline form during storage are also considered to be major hurdles to commercialize solid dispersions as they result in decreased solubility and thus dissolution rate

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SD are polyethylene glycols (PEGs). They are widely used because of their hydrophilicity, low melting point, and low toxicity.

Ingredients Table (Formula):

Formulation Code	Carrier	Drug: carrier ratio
SD1	Mannitol	1:0.5
SD2		1:1
SD3		1:1.5
SD4		1:2
SD5		1:3
SD6	Urea	1:0.5
SD7		1:1
SD8		1:1.5
SD9		1:2
SD10		1:3

Procedure:

A. Preparation of Solid Dispersions-

Weigh Paracetamol & different carriers, dissolve in suitable solvent (ethanol/ methanol/acetone). Add the drug & evaporate the solution to dryness. Finally, grind the dispersion, pass it through sieve no 60 & store in the desiccator for further studies.

B. Standard Graph of Paracetamol-

Stock solution 1: Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of paracetamol in 100 ml phosphate buffer pH 5.8 in 100 ml volumetric flask (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.

Stock solution 2: 10 ml of this (stock solution 1) is diluted to 100ml with phosphate buffer pH 5.8 to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.

Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml) in each test tube, add phosphate buffer 5.8 to make total volume of 10 ml to produce (2, 4, 6, 8, 10, 12 µg/ml) respectively.

The dilution was analysed in UV-Visible spectrophotometer and the absorbance were recorded. The standard plot was constructed and the standard equation was derived.

C. Evaluation of Prepared Solid Dispersion-

Drug content analysis (Assay):

Preparations equivalent to 20 mg was weighed accurately and transferred to 100 ml volumetric flask and dissolved in phosphate buffer pH 5.8. The volume was made up with phosphate buffer pH 5.8 up to the mark. After suitable dilution, the absorbance of the above solution was measured at 243 nm using appropriate blank solution. The drug content of paracetamol was calculated using calibration curve.

***In vitro* release studies:**

Accurately weighed amount of sample was taken for dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analysed for drug release by measuring the absorbance at 243nm using phosphate buffer pH 5.8 as dissolution medium. The volume withdrawn at each time intervals were replaced with same quantity of fresh medium.

Observation & Calculation:

1) **Standard curve of Paracetamol:**

Sl. No	Concentration (µg/ml)	Absorbance (nm)
1	2	
2	4	
3	6	
4	8	
5	10	
6	12	

2) **Drug Content Analysis:**

Formulation Code	Concentration	Absorbance (nm)	Assay Value (µg/ml)
SD1	**10 µg/ml**		
SD2			
SD3			
SD4			
SD5			
SD6			
SD7			
SD8			
SD9			
SD10			

***In vitro* release studies:**

Sl. No	Time (Mins)	Absorbance (nm)	Conc. In µg/ml	Conc. In µg/ 5 ml	Conc. In µg/ 900 ml	CDR in µg	CDR in mg	%CDR

Report: Paracetamol Solid Dispersions were prepared by Solvent Evaporation method. The Evaluation of the same was performed.

Experiment No: 03

COMPARISON OF DISSOLUTION PROFILE OF TWO DIFFERENT MARKETED PRODUCTS

AIM: To compare the dissolution profiles of two different marketed products

Requirements:

- **Apparatus-** UV-Visible Spectrophotometer, Dissolution Apparatus
- **Chemicals-** Marketed Product (Product 1, Product 2)

PRINCIPLE:

Dissolution-

A drug is expected to be released from solid dosage forms (granules, tablets, capsules, etc) and immediately go into molecular solution. This process is called dissolution.

- It is a critical step for performance of a drug as well as dosage form, because it is a prerequisite for the drug absorption.
- Absorption of drug is possible only when it is present in solution form, wherein the molecules are independent and assume molecular dispersion. Each molecules is absorbed independently through biological membranes.

Factors affecting dissolution-

- **Factors relating Dissolution apparatus and Dissolution test parameters (Instrumental factors): -**
  Temperature, Agitation speed, Dissolution medium & pH
- **Factors related to drug (physicochemical factors): -**
  Particle size, Shape, Surface area, Form (amorphous, crystalline) or state of drug (salt), Polymorphism
- **Factors related to dosage form: -**
  excipient related factors (diluent, disintegrant, binder, lubricant, surfactant, coating)
  processing related factor (method of granulation, compression force)

Dissolution test apparatus-

Dissolution test is specified for its compliance in the individual monographs of pharmacopoeias, particularly for tablets and capsules. A numbers of apparatus is available to conduct dissolution studies, because no single equipment is adequate for the study of all drugs and dosage forms. Therefore, the objectives of the test defined first.

Various official dissolution test apparatus

Type	I.P.	USP	B.P. / J.P.	E.P.
I	Paddle apparatus	Basket apparatus	Basket apparatus	Paddle apparatus
II	Basket apparatus	Paddle apparatus	Paddle apparatus	Basket apparatus
III	-	Reciprocating cylinder	Flow through cell apparatus	Flow through cell apparatus
IV	-	Flow through cell apparatus	-	-
V	-	Paddle over disk	-	-
VI	-	Cylinder	-	-
VII	-	Reciprocating holder	-	-

Procedure:

A. Standard Graph of Paracetamol-

Stock solution 1: Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of paracetamol in 100 ml phosphate buffer pH 5.8 in 100 ml volumetric flask (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.

Stock solution 2: 10 ml of this (stock solution 1) is diluted to 100ml with phosphate buffer pH 5.8 to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.

Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml) in each test tube, add phosphate buffer 5.8 to make total volume of 10 ml to produce (2, 4, 6, 8, 10, 12 µg/ml) respectively.

The dilution was analysed in UV-Visible spectrophotometer and the absorbance were recorded. The standard plot was constructed and the standard equation was derived.

B. *In vitro* release study of 2 different marketed paracetamol tablet (Product 1, Product 2)-

Two marketed tablets of paracetamol were taken and dissolution study was performed. Dissolution Studies were conducted in USP Apparatus 2 i.e. Paddle Apparatus (Elecrolab, Mumbai) with triplicate set for each brand. The dissolution medium was 900 ml of phosphate buffer pH 5.8. The paddle rotation speed was kept at 50 rpm for 60 minutes. Percentage of drug dissolved from the tablet was calculated. Aliquots of sample were withdrawn at predetermined intervals of time and analysed for drug release by measuring the absorbance at 243nm using phosphate buffer pH 5.8 as dissolution medium. The volume withdrawn at each time intervals were replaced with same quantity of fresh medium.

Observation & Calculation:

1) Standard curve of Paracetamol:

Sl. No	Concentration (µg/ml)	Absorbance (nm)
1	2	
2	4	
3	6	
4	8	
5	10	
6	12	

2) *In vitro* release studies:

Table 1: Dissolution Profile of Marketed Product 1:

Sl. No	Time (Mins)	Absorbance (nm)	Conc. In µg/ml	Conc. In µg/ 5 ml	Conc. In µg/ 900 ml	CDR in µg	CDR in mg	%CDR
1	5							
2	10							
3	15							
4	30							
5	45							
6	60							

Table 2: Dissolution Profile of Marketed Product 2:

Sl. No	Time (Mins)	Absorbance (nm)	Conc. In µg/ml	Conc. In µg/ 5 ml	Conc. In µg/ 900 ml	CDR in µg	CDR in mg	%CDR
1	5							
2	10							
3	15							
4	30							
5	45							
6	60							

Report:

The % CDR of two different marketed products were found to be:

- Product 1-…….
- Product 2-…….

Experiment No: 04

DETERMINATION OF PERCENTAGE PROTEIN BINDING OF DRUGS

AIM: To determine the percentage protein binding of Diclofenac sodium and paracetamol

Requirements:

- **Apparatus-** UV-Visible Spectrophotometer, Franz Diffusion cell, Dialysis Membrane
- **Chemicals-** Diclofenac sodium, Paracetamol, egg albumin,

PRINCIPLE:

A drug in blood exists in two forms: bound and unbound. Depending on a specific drug's affinity for plasma protein, a proportion of the drug may become bound to plasma proteins, with the remainder being unbound. If the protein binding is reversible, then a chemical equilibrium will exist between the bound and unbound states, such that:

Protein + drug ⇌ Protein-drug complex

Notably, it is the unbound fraction which exhibits pharmacologic effects when the drug undergoes metabolism in the liver whereas thebounded drug will accumulate and distribute into the tissues leading to a decrease in plasmaconcentration profile.

Determination of Protein Binding-

- **The Drug:**

  Physiological properties of the drug

  Total concentration of the drug in the body

- **The Protein:**

  Quantity of protein available for Drug-protein Binding.

  Quantity or Physiological nature of the Protein synthesized.

  The affinity between drug & Protein

  The magnitude of the dissociation constant

- **Drug Interaction:**

  Competition for the drug by others substances at a protein binding site.

- **The Pathophysiological Condition of Patient:**

  For example, drug protein binding may be reduced in uremic patients & in patients with hepatic disease.

Diclofenac is an NSAID used to treat the signs and symptoms of osteoarthritis and rheumatoid arthritis. It is almost completely absorbed after oral administration; it is subjected to first-pass metabolism so that about 50% of the drug reaches the systemic circulation in the unchanged form. More than 99% is bound to plasma proteins, primarily to albumin.

Human serum albumin is the primary protein present in human blood plasma. The main function of albumin is to maintain the oncotic pressure of blood. It binds to water, cations (such as Ca2+, Na+ and K+), fatty acids, hormones, bilirubin, thyroxine (T4) and pharmaceuticals (including barbiturates). Albumin represents approximately 50% of the total protein content in healthy humans. Human albumin is a small globular protein (molecular weight: 66.5 kDa), consisting of a single chain of 585 amino acids organized in three repeated homolog domains (sites I, II, and III). Each domain comprises two separate sub-domains (A and B).

Procedure:

A. Standard Graph of Diclofenac-

Stock solution 1: Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of paracetamol in 100 ml phosphate buffer pH 6.8 in 100 ml volumetric flask (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.

Stock solution 2: 10 ml of this (stock solution 1) is diluted to 100ml with phosphate buffer pH 6.8 to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.

Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml) in each test tube, add phosphate buffer 6.8 to make total volume of 10 ml to produce (2, 4, 6, 8, 10 µg/ml) respectively.

The dilution was analysed in UV-Visible spectrophotometer (absorption maxima-273 nm) and the absorbance were recorded. The standard plot was constructed and the standard equation was derived.

B. Standard Graph of Paracetamol-

Stock solution 1: Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of paracetamol in 100 ml phosphate buffer pH 5.8 in 100 ml volumetric flask (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.

Stock solution 2: 10 ml of this (stock solution 1) is diluted to 100ml with phosphate buffer pH 5.8 to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.

Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml) in each test tube, add phosphate buffer 5.8 to make total volume of 10 ml to produce (2, 4, 6, 8, 10, 12 µg/ml) respectively.

The dilution was analysed in UV-Visible spectrophotometer (absorption maxima-249 nm) and the absorbance were recorded. The standard plot was constructed and the standard equation was derived.

C. Preparation of Protein Solution-

Prepare 4% solution of albumin by dissolving 4 g of egg albumin in 100 ml of phosphate buffer pH 7.2. Allow albumin to swell with little quantity of phosphate buffer pH 7.2 & further dissolve slowly in phosphate buffer to avoid foaming of the solution. Keep the solution for incubation for 24 hrs.

D. Preparation of Protein-Drug Solution-

Divide the protein solution into two equal parts containing 50 ml each. Weigh and dissolve 50 mg 50 mg of drug to each of the above half. Study the % drug protein binding by following technique.

E. Centrifugation technique-

After incubation of 24 hrs. Place 8 ml of solution in centrifugation tubes. Albumin solution

will be takes as blank in another centrifuge tube, carry out centrifugation at 2000 rpm for 1 hr. Collect the supernatant liquid of the protein drug solution & dilute suitably to the Beer range & measure the absorbance at 273 nm for diclofenac & 249 nm for paracetamol.

F. Dialysis Method-

Drug release by diffusion will be studied using Franz Diffusion cell assembly, fill phosphate buffer pH 6.8/ 5.8 in the receiver compartment, close the mouth with a cellophane membrane, which is pretreated by soaking in acidic buffer. Fill 10 ml of the drug protein solution in the donor compartment at particular time intervals & replace with phosphate buffer pH 7.2. Dilute suitably & measure the absorbance at 273 nm for diclofenac & 249 nm for paracetamol.

Observation & Calculation:

1) Standard curve of Diclofenac:

Sl. No	Concentration (µg/ml)	Absorbance (nm)
1	2	
2	4	
3	6	
4	8	
5	10	

2) Standard curve of Paracetamol:

Sl. No	Concentration (µg/ml)	Absorbance (nm)
1	2	
2	4	
3	6	
4	8	
5	10	
6	12	

3) *In vitro* release studies:

Table 1: Release Profile of Diclofenac with 4% egg albumin:

Sl. No	Time (Mins)	Absorbance (nm)	Conc. In µg/ml	Conc. In µg/ 30 ml	CDR in µg	CDR in mg	%CDR
1	5						
2	10						
3	15						
4	30						
5	45						
6	60						

Table 2: Release Profile of Paracetamol with 4% albumin:

Sl. No	Time (Mins)	Absorbance (nm)	Conc. In µg/ml	Conc. In µg/ 30 ml	CDR in µg	CDR in mg	%CDR
1	5						
2	10						
3	15						
4	30						
5	45						
6	60						

Report:

The % protein bound Diclofenac was found to be ……

The % protein bound Paracetamol was found to be ……

Experiment No: 05

DETERMINATION OF BIOAVAILABILITY (BA) IN ANIMAL MODELS

AIM: To determine the bioavailability (BA) study of paracetamol (PCM) in animal model (mice).

Requirements:

- Chemical: PCM tablet
- Apparatus: Oral gavage needles syringes, blood collections & blood chemistry analyzer.
- Model: Mice.

PRINCIPLE:

The bioavailability of Paracetamol (PCM) is typically higher after intravenous administration than oral administration. This is because the drug is delivered directly into the bloodstream after the IV administration by passing the First pass metabolism that occurs in the liver after oral administration. The bioavailability (BA) of PCM can be determined by measuring the plasma conc. of PCM over time administration of the drug. The area under the plasma conc. time curve (AUC) is measures of bioavailability of drug:

$$\textbf{Bioavailability (BA)} = \frac{\text{AUCoral}}{\text{AUCIV}}$$

Procedure:

i. To follows the steps as following, take mice where weight was selected & housed in departmental animal house.
ii. Calculated their doses (PCM) based on their weight.
iii. Administer the PCM tablets to the mice orally using oral gavage needles.
iv. Collect blood samples from the mice at pre-determined time interval after administration of PCM tablet.
v. Analyze the blood sample for the con. of PCM.
vi. Calculate the bioavailability of PCM by composing the conc. of PCM in the blood after the oral administration to the con. od the PC in the blood after the intravenous administration.

$$\textbf{Bioavailability} = \frac{\text{Conc.of PCM blood after oral administartion}}{\text{Conc.of PCM blood after Intravenous administartion}} \textbf{ X 100}$$

$$\textbf{Bioavailability (BA)} = \frac{650\text{mg}}{153\text{ml}} \textbf{ X 100}$$

$$\textbf{Bioavailability (BA)} = \frac{650\text{mg}}{153\text{ml}} \textbf{ X 100 = 66. 4\%}$$

$$\textbf{For oral} = \frac{\text{AUCoral}}{\text{AUCIV}} = \frac{25}{3} \textbf{ x 100 = 673. 8\%}$$

Calculation:

Route of administration	Dose (mg/kg)	AUC (mg/mL x min.)	Tmax. (Min.)	Cmax. (mg/mL)	Bioavailability BA (%)
Oral	650	21.7	20-50	12.5	65-66%
Intravenous (IV)	153	32.5	0-2	36.8	95-100%

Pharmacokinetics Analysis: Non-compartmental including Vd, Cl, Cmax., Tmax, AUC etc.

Administration	Ka (hr^{-1})	Vd ($Lt\text{-}kg^{-1}$)	Cl_b ($Lt.kg^{-1}hr^{-1}$)	AUC
Oral	0.3-04.	1.22	0.67	1.5-1.8
Intravenous (IV)	0.7	0.7	1.32	3.72

$$\textbf{AUC} = \int \textbf{C(t). dt}$$

AUC is area under the curve, C is plasma conc. f drug at time t, dt is infinitely small incensement of time.

Peak conc. of PCM in blood (mg/mL) with the dose of PCM (mg):

Dose of PCM (mg)	Peak conc. of PCM in blood (mg/mL)
1000	100-150
2000	200-300
3000	300-450

Results:

The bioavailability of PCM tablet in animal mice is calculated 66.4% which accesses the effectiveness of PCM tablet in treating pain & fever in mice. The bioavailability of oral administration calculated 65-66.66% and after the administration will be calculated 95-100%

Reference:

- Simmaonds, E.A., et all, JR (2013), pharmacokinetics of PCM in mice for IV & oral administration, British journal of pharmacology, 6 (s3), page. No. 321-336.
- Agarwal, V. et all (2006), pharmacokinetics of PCM, "an review Journal of clinical pharmacy & therapeutics", 31 (2), page no. 99-113.

Experiment No: 06

CLINICAL DATA DEVELOPMENT MANUAL

AIM: To develop a Clinical Data Development Manual

INTRODUCTION:

The purpose of this document is to provide a Manual of Operating Procedures (MOP) template for principal investigators (PIs) of multisite clinical trials. The role of the MOP is to facilitate consistency in protocol implementation and data collection across participants and study sites. Use of the MOP increases the likelihood that the results of the study will be scientifically credible and provides reassurance that participant safety and scientific integrity are closely monitored. Investigators of single-site studies are encouraged to consider the template's contents. However, a MOP is not mandatory for these studies.

Definition:

A MOP is a handbook that details a study's conduct and operations. It transforms the study protocol into a guideline that describes a study's organization, operational data definitions, recruitment, screening, enrolment, randomization, follow-up procedures, data collection methods, data flow, case report forms (CRFs), and quality control procedures. The MOP is intended to serve as a study "cookbook" that facilitates adherence to study procedures. The MOP is developed before the study can commence.

During a study's planning phase, the PI and study staff drafts the protocol. The protocol must be approved by the IRBs of all Institutions participating in the study and by the Data and Safety Monitoring Board (DSMB). Prior to developing the MOP, the final protocol, CRFs, informed consent documents, and administrative forms (e.g., screening and enrolment log, protocol deviation log, etc.) should be finalized. Additionally, if the study is to be submitted to the Food and Drug Administration (FDA) under an Investigational New Drug Application (IND), an Investigator's Brochure (for investigational products) or Package Insert (for marketed drugs) must be included. The timeline for development of study materials must be planned for and typically takes approximately 6 months.

Procedure:

The MOP is a dynamic document that will be updated throughout the conduct of a study to reflect any protocol or consent amendments as well as the refinement of the CRFs and study procedures. The MOP should be maintained in a format that allows it to be easily updated, and is typically filed in a three-hole binder. For ease of organization, it is recommended that the MOP be subdivided into various sections separated by dividers or sheets of colour paper between each section. Further, each page of the MOP should contain the version number and date. As pages are revised, an updated version number and associated date will replace the original page(s) in the MOP. All previous versions should be archived.

Content:

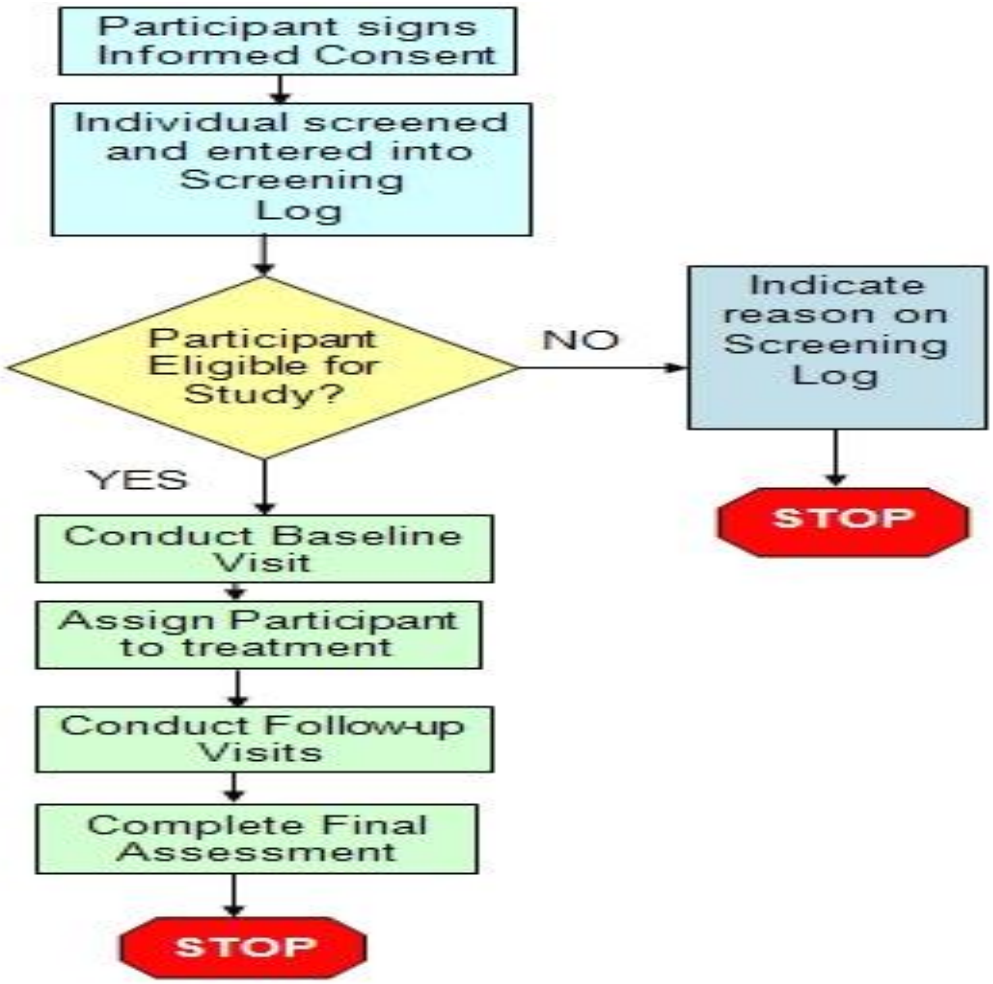
The MOP details the study procedures and describes the study-specific documents and must be adapted to each study's specific needs. It often includes the following sections:

- Study Protocol or Synopsis
- Staff Roster
- Study Organization and Responsibilities
- Training Plan
- Communications Plan
- Recruitment and Retention Plan
- Study Design Diagram
- Screening and Eligibility Criteria and Processes
- Informed Consent and HIPAA
- Study Intervention
- Blinding and Unblinding (Masking or Unmasking)
- Evaluations and Follow-up
- Concomitant Medications
- Safety Reporting
- Data and Safety Monitoring Responsibilities
- Study Compliance
- Data Collection and Study Forms
- Data Management

- Quality Control Procedures
- Study Completion and Closeout Procedures
- Policies
- MOP Maintenance

The MOP should include all of the relevant sections from this list that apply to the specific study. If a section does not apply (e.g., randomization for a study with no randomization), it is not included in the MOP. Additionally, if the study involves a drug intervention, either the Package Insert for an approved drug or the Investigator's Brochure for an investigational product must be included as an appendix.

Sample Study Flow Diagram:



Report: The Clinical Data Development Manual was Studied.

Experiment No: 07

FORMULATION & EVALUATION OF COLD CREAM

AIM: To prepare, evaluate and submit 20 gm of Cold cream.

Requirements:

- **Apparatus-**Spatula, Beaker, Glass rod, Water Bath
- **Chemicals-** Mineral oil, Bees wax, Borax, propylene glycol, paraffin wax, Distilled water, perfume

PRINCIPLE:

Cold cream is an example of water in oil type of emulsion. It is prepared by incorporating beeswax and alkali, which is usually borax as emulsifying agent. The term cold in conjugation with cold cream is due to cooling sensation caused by evaporation of water in the cream after itis applied to the skin. The consistency of cold cream can be adjusted by the wax content.

In making cream from beeswax, mineral oil, water and borax, the following factors should be considered; borax should not be less than 5% of beeswax used and not more than 8% depending on the acid number of beeswax. As the proportion of beeswax is increased the cream becomes harder. Mineral oil relationship to water has stiffness rather than softening effect upon the creamas the proportion is increased. About 60% of mineral oil, the cream shows signs of instability. Increase in water content softens the cream until the product becomes definitely liquid. When water is low, the cream may of w/o type. A high amount of water seems to lead to finer and grained creams. A good balance seems to be reached when water and oil are present in approximately equal amounts. However good products result when oil ratio varies from 1:2 to2:1.

Cold cream made with borax is more superior to those without it. Since the emulsion is whiter, smoother and more stable. Borax in excess is required for neutralization but this makes cream more alkaline without contributing to appearance and stability, while too little borax does not lead to desired smoothness.

Boil oil phase and aqueous phase should be mixed at equal temperature i.e. 70^0C to ensure using mixing of two phases. Perfume should be added when mixture has attained a temperature of 40-45^0C.

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity given (%)	Quantity taken for 20 gm
1	Mineral oil	47	
2	Bees wax	5	
3	Borax	0.2	
4	Water	32.8	
5	Propylene glycol	5	
6	Paraffin wax	10	
7	Perfume	q. s.	

Procedure:

A. Formulation of Cold Cream

Dissolve borax in water and heat the aqueous solution to 70^0C. Melt together beeswax, mineral oil, and paraffin wax, and heat the oil phase with constant stirring. Continue stirring until 45^0C and add the perfume at about 40^0C and stir till room temperature is attained.

B. Evaluation of Cold Cream

- **Physical properties:** The cream was observed for the colour, odour and appearance.
- **Washability**: The cream was applied on the hand and observed under the running.
- **pH**: The pH meter was calibrated with the help of standard buffer solution. Weigh 0.5 gm of cream dissolved itin 50.0ml of distilled water and its p H was measured with the help of digital pH meter.
- **Viscosity**: Viscosity of the cream was determined with the help of Brookfield viscometer at 20 rpm with the spindle no LV-4(64)
- **Spread ability test**: The cream sample was applied between the two glass slides and was compressed betweenthe two-glass slide to uniform thickness by placing 100 gm of weight for 5 minutes then weight was added to the weighing pan. The time in which the upper glass slide moved over the lower slide was taken as a measure of spread ability.

Spread ability=m *l/t

Where, M =weight tight to upper slide; L =length moved on the glass slide; T= Time taken

- **Irritancy test**: Mark an area (1sq.cm) on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs. and reported.

- **Saponification value:** Take 2 gm of the substance and reflux it with the 25 ml of 0.5 N alcoholic KOH for 30 minutes. Then add 0.1 ml of phenolphthalein as a indicator and titrate it with the 0.5 N HCL.

Saponifiction value=(b-a) *28.05/W

Where, A =volume of titrate; B =volume of titrate; W =weight of substances in gram

- **Acid value:** Take 10 gm of the cream dissolved in accurately weighed in 50 ml mixture of the equal volume of alcohol and solvent ether. Then attached the flask with the condenser and reflux it with the slow heating until the sample gets completely dissolve then add 1 ml of phenolphthalein and titrate it with 0.1 N NaOH until it gets faint pink color appears after shaking in 20 seconds.

Acid value=n*5.61/w

Where, W =weight of the substances; N =the number of ml in NaOH required.

- **Dye test**: The scarlet red dye is mixed with the cream. Place a drop of the cream on a microscopic slide then covers it with a cover slip, and examines it under a microscope. If the disperse globules appear red the ground colorless. The cream is o/w type. The reverse condition occurs in w/o type cream i.e. the disperse globules appearcolorless.
- **Homogeneity**: Homogeneity was tested via the visual appearance and test.

Observation:

- **Physical properties**: The physical properties of formulated cream were judged by color, odor and texture.

Sl. No	Parameter	Evaluation
1.	Color	
2.	Odor	
3.	Texture	

- **Washability**: The cream applied on skin was easily removed by washing with tap water

- **pH of the cream:** The pH of the cream was found to be in range of …….
- **Viscosity:** Viscosity of formulated cream was determined by brook field viscometer at 20 rpm using spindleno. LV-4(64). The viscosity of cream was in the range of ……
- **Spread ability test:** The spread ability test showed that the formulated cream has good spreadable property.

Formula	Average spreadbility
FI	

- **Irritancy test:** The formulated cream shows no redness, edema, irritation and inflammation during studies. The formulated cream is safe to use.

Irritancy test	Result
Irritation	
Edema	
Redness	
Swelling	

Label:

COLD CREAM **20 gm**	
Composition:	**BATCH NO.:** **MFG. DATE:**
Category: Emollient & Protective	**MFG. LIC NO.:** **EXPIRY DATE:**
FOR EXTERNAL USE ONLY.	
Use: It is used as emollient and protect to the skin. **Storage: Stored in a well closed container at a temperature not exceeding 25^0C**	
MFG BY: ABCD **Batch:** **Roll No.:**	

Report: 20 g of cold cream was prepared, evaluated, labelled and submitted.

Experiment No: 08

FORMULATION & EVALUATION OF VANISHING CREAM

AIM: To prepare, evaluate and submit 20 gm of Vanishing cream.

Requirements:

- **Apparatus-**Spatula, Beaker, Glass rod, Water Bath
- **Chemicals-** Stearic acid, KOH, Glycerine, Distilled water, Perfume

PRINCIPLE:

Vanishing cream is essentially an o/w emulsion which when applied on the skin gives a non-greasy appearance. The emulsifying agent being potassium or other stearate formed by action of caustic acid. A humectant such as glycerin is also present. The pH vanishing cream though variable is usually nearer to neutral.

The cream spreads easily on the skin surface giving a thin semi matt film which seemsto disappear/vanish after sometimes, hence the name vanishing cream. They are also used to counter act shine and to protect the skin from dust and wind. A conventional vanishing cream contains generally 4ingredients viz. Stearic acid, alkali, humectants and water. Additional ingredients such as perfume, preservative etc. are also added in the preparation. Stearic acid isthe principal film forming agent in the preparation, which imparts characteristics bloom and matteffect when the cream is applied on the skin. If The preparation contains too many Oils, fats, and waxes, the film will not vanish as effectively. Glycerin acts as humectant and also influences the product in container by reducing drying out of the cream and surface crest formation as well as providing pearly appearance

The choice and proportion of the alkali used in vanishing cream has much todo with the ultimate consistency texture and appearance of the cream. Various alkalis used include potassium hydroxide, ammonia, borax, potassium carbonate and triethanolamine. Of these potassium Hydroxide is used generally because it makesthe cream of fine texture and excellent consistency without excessive harshness. The carbonates are not favored as they liberate carbon. Ammonia is effective but objectionable to turn yellow with age. Borax is used in combination with JOH/TEA because it produces a very white emulsion. The crystal luster which gives pearly is due to formation of potassium Stearate.

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity given (%)	Quantity taken for 20 gm
1	Stearic acid	15	
2	Potassium hydroxide	0.7	
3	Glycerin	8	
4	Water	76.3	
5	Perfume	q.s	

Procedure:

A. Formulation of vanishing cream

Dissolve potassium hydroxide in water and then add glycerine. Heat this aqueous mixture to about 70^0C. Take stearic acid in china dish and heat to this oily mixture to about 70^0C in a water bath. Add the aqueous phase to the oily phase with constant stirring. At 40°C and perfume and cool till room temperature is attained.

B. Evaluation of vanishing cream

- **Physical properties:** The cream was observed for the colour, odour and appearance.
- **Washability**: The cream was applied on the hand and observed under the running.
- **pH**: The pH meter was calibrated with the help of standard buffer solution. Weigh 0.5 gm of cream dissolved itin 50.0ml of distilled water and its p H was measured with the help of digital pH meter.
- **Viscosity**: Viscosity of the cream was determined with the help of Brookfield viscometer at 20 rpm with the spindle no LV-4(64)
- **Spread ability test**: The cream sample was applied between the two glass slides and was compressed betweenthe two-glass slide to uniform thickness by placing 100 gm of weight for 5 minutes then weight was added to the weighing pan. The time in which the upper glass slide moved over the lower slide was taken as a measure of spread ability.

  Spread ability=m *l/t

  Where, M =weight tight to upper slide; L =length moved on the glass slide; T= Time
- **Irritancy test**: Mark an area (1sq.cm) on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs. and reported.

- **Saponification value:** Take 2 gm of the substance and reflux it with the 25 ml of 0.5 N alcoholic KOH for 30 minutes. Then add 0.1 ml of phenolphthalein as a indicator and titrate it with the 0.5 N HCL.

Saponifiction value=(b-a) *28.05/W

Where, A =volume of titrate; B =volume of titrate; W =weight of substances in gram

- **Acid value:** Take 10 gm of the cream dissolved in accurately weighed in 50 ml mixture of the equal volume of alcohol and solvent ether. Then attached the flask with the condenser and reflux it with the slow heating until the sample gets completely dissolve then add 1 ml of phenolphthalein and titrate it with 0.1 N NaOH until it gets faint pink color appears after shaking in 20 seconds.

Acid value=n*5.61/w

Where, W =weight of the substances; N =the number of ml in NaOH required.

- **Dye test**: The scarlet red dye is mixed with the cream. Place a drop of the cream on a microscopic slide then covers it with a cover slip, and examines it under a microscope. If the disperse globules appear red the ground colorless. The cream is o/w type. The reverse condition occurs in w/o type cream i.e. the disperse globules appearcolorless.
- **Homogeneity**: Homogeneity was tested via the visual appearance and test.

Observation:

- **Physical properties**: The physical properties of formulated cream were judged by color, odor and texture.

Sl. No	Parameter	Evaluation
1.	Color	
2.	Odor	
3.	Texture	

- **Washability**: The cream applied on skin was easily removed by washing with tap water
- **pH of the cream:** The pH of the cream was found to be in range of …….
- **Viscosity:** Viscosity of formulated cream was determined by brook field viscometer at 20 rpm using spindleno. LV-4(64). The viscosity of cream was in the range of ……

- **Spread ability test:** The spread ability test showed that the formulated cream has good spreadable property.

Formula	Average spreadbility
FI	

- **Irritancy test:** The formulated cream shows no redness, edema, irritation and inflammation during studies. The formulated cream is safe to use.

Irritancy test	Result
Irritation	
Edema	
Redness	
Swelling	

Label:

<table>
<tr><td colspan="2">VANISHING CREAM
20gm</td></tr>
<tr><td>Composition:
Stearic acid
Potassium hydroxide,
Glycerin, Perfume,
Purified water</td><td rowspan="2">BATCH NO.:
MFG. DATE:
MFG. LIC NO.:
EXPIRY DATE:</td></tr>
<tr><td>Category: Cosmetics</td></tr>
<tr><td colspan="2">FOR EXTERNAL USE ONLY.</td></tr>
<tr><td colspan="2">Use: As foundation to other cosmetics and for beautification
Storage: Stored in a well closed container at a temperature not exceeding 25^0C</td></tr>
<tr><td colspan="2">MFG BY: ABCD Batch: Roll No.:</td></tr>
</table>

Report: 20 g of vanishing cream was prepared, evaluated, labelled and submitted.

Experiment No: 09

FORMULATION & EVALUATION OF TOOTHPASTE

AIM: To prepare, evaluate and submit 20 gm of Toothpaste.

Requirements:

- **Apparatus**-Spatula, sieve, Mortar & Pestle
- **Chemicals-C**alcium carbonate, Sodium lauryl sulphate, Peppermint oil, Sodium saccharine, Calcium phosphate, CMC, Glycerine, water

PRINCIPLE:

Tooth paste made up of:

1. Abrasive: abrasives are used to clean the surface of teeth. At the same time, it shouldhave no harmful effect on the enamel. It should be non-toxic and non-irritant.
Eg: calcium carbonate, Di and tri calcium phosphate.

2. Surfactant: they are also referred as foaming agents. They bring about wetting anddispersion of powdered material with lowering of interfacial tension.
Eg: 1) Sodium lauryl sulphate (S.L.S)

2) Sodium n-lauryl sarcosinate – It has an anti-enzyme activity.

3. Humectant: To prevent of tooth paste.

Eg: Glycerin is used as humectants. It is sweet, non- toxic and solvent action. Other examples are sorbitol and propylene glycols are used as humectants.

4. Binding / Gelling agent: Gelling agents are used to make the tooth paste high solidsuspension stable. It modifies dispensability, and consistency.
Eg: Natural – Irish moss and tragacanth.

Synthetic – Cellulose derivatives.

Synthetic – cellulose, sodium derivatives, sodium CMC, Hydroxy ethyl cellulose and methyl cellulose.

5. Flavours: To get fresh, clean sensation.
E.g. spearmint oil, peppermint oil.

Other ingredients:

1) Corrosion inhibitors. e.g. sodium silicate.

2) Colour and bleachers (to remove stain) E.g. sodium per borate, magnesium peroxide, a small amount of sodium fluoride can be incorporated (1000 parts / million parts).

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity given (%)	Quantity taken for 20 gm
1	Carboxy methyl cellulose	1	
2	Calcium carbonate	25	
3	Sweetener (saccharin)	0.1	
4	Sodium lauryl sulphate	1	
5	Flavor (Peppermint Oil)	1	
6	Calcium phosphate	25	
7	Glycerin	25	
8	Water	q. s.	

Procedure:

A. Formulation of Toothpaste

- Hydration of gelling agent by adding carboxyl methyl cellulose, glycerine and part of water with continuous stirring.
- Add the fine powders to the gelling agent with constant stirring.
- Add the surfactant, sodium lauryl phosphate followed by the flavour.
- Mix to a paste.

B. Evaluation of Toothpaste

- **Drying Tendency:** All the formulated batches were evaluated for their drying tendency at room temperature for a week.
- **Organoleptic Characters:** Formulated batches were also evaluated for their organoleptic characters.
- **Determination of Grittiness:** The presence of hard, sharp-edged abrasive particles were evaluated by extruding near about 15 to 20 mm length paste from a collapsible tube of each sample on butter paper then pressed it along its entire length by finger.

- **Determination of pH:** In 100 ml cleaned beaker, accurately weighed 5 gm of sample was transferred. To this freshly boiled and cooled water was added and stirred well to get a uniform suspension. The pH was determined within 5 min by using a pH meter (M/s. Systronics Ltd. Ahmedabad). Results were tabulated in **Table 5**.
- **Determination of Foaming Power:** In 100 ml glass beaker near about 5 gm of sample was taken. To this 40 ml, water was added, and the beaker was allowed to stand for 30 min by covering with a watch glass for dispersion of toothpaste in water. Then the content was stirred with glass rod and slurry was transferred to a 250 ml graduated measuring cylinder. Precaution was taken at the time of transfer that no loss was produced. The reaming residue in the beaker was transferred with 5 to 6 ml of another portion of water. The volume make up to 50 ml by adding sufficient quantity of water and the temperature of the content is maintained near about 30 ºC, meanwhile stirring was continued to ensure uniform suspension. When the temperature of the content was reached to 30 ºC the stirring was stopped and 12 complete shakes were given and allowed to stand for 5 min. The foaming power was determined by measuring volume of foam with water (V1) and water only (V2) was noted for all samples.

Foaming power = V1 – V2

V1= Volume in ml of foam with water V2= Volume in ml of water only.

Observation:

- **Drying Tendency, Organoleptic Characters & Determination of Grittiness:**

Batch	Evaluation parameters					
	Dryness test	Colour	Appearance	Extrudability	Texture	After taste

- **Determination of pH & Foaming Power:**

Batch	Evaluation Parameters	
	Foaming power	pH

Label:

<table>
<tr><td colspan="2">TOOTHPASTE
20gm</td></tr>
<tr><td>Composition:
Calcium carbonate, Sodium lauryl sulphate, Peppermint oil, Sodium saccharine, Calcium phosphate, CMC, Glycerine, water</td><td>BATCH NO.: MFG. DATE:

MFG. LIC NO.: EXPIRY DATE:</td></tr>
<tr><td>Category: Dentifrices</td><td></td></tr>
<tr><td colspan="2">FOR INTERNAL USE ONLY.</td></tr>
<tr><td colspan="2">Use: Cleaning the surface of the teeth
Storage: Stored in a well closed container at a temperature not exceeding 25^0C</td></tr>
<tr><td colspan="2">MFG BY: ABCD Batch: Roll No.:</td></tr>
</table>

Report: 20 g of toothpaste was prepared, labelled and submitted.

Experiment No: 10

FORMULATION & EVALUATION OF SHAMPOO

AIM: To prepare, evaluate and submit 20 ml of Shampoo.

Requirements:

- **Apparatus-** Beaker, Water bath, Glass rod
- **Chemicals-** Coconut oil, Castor oil, Potassium hydroxide, perfume, Borax, Glycerine, water

PRINCIPLE:

Shampoos are one which is designed to provide sufficient cleaning powder to get adequate foam, to remove the oil from the hair and the scalp without reducing the natural oiliness, so that the hair is left with a natural gloss, soft and in manageable condition.

Properties / characteristics of shampoo

1. It should remove all the build-up from the hair as well as the scalp.
2. It should give adequate foam so that satisfies requirement of the user.
3. It should be easy to remove when washed with water.
4. It should give lustre to the hair.
5. Shampoo should retain pleasant fragrance /smell / perfume.
6. It should be non- staining.
7. It should be non – irritating especially to the eyes.
8. It should be highly stable physically as well as chemically.
9. It is important to adjust the pH of the shampoo (pH should be neutral or slightly acidic)

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity given (%)	Quantity taken for 20 ml
1	Coconut oil	18% v/v	
2	Castor oil	4%v/v	
3	Potassium hydroxide	5.3%w/v	
4	Glycerin	4%v/v	
5	Perfume	0.2%v/v	
6	Borax	0.5%w/v	
7	Purified water (q.s.)	68%v/v	

Procedure:

A. Formulation of Shampoo

1. Dissolve potassium hydroxide in small quantity of water.
2. Transfer measured amount of coconut oil and Castor oil into a dry beaker and keep it in hot waterbath.
3. Pour alkaline solution in a stream to the oil phase with continuous stirring.
4. Continuous stirring to complete the saponification process until the soap is formed.
5. After saponification, add glycerine, Borax and stir for 10 min. NOTE: add alcohol into the beaker if required.
6. Transfer into a container, label and submit.

B. Evaluation of Shampoo

- Physical appearance/visual inspection

  The formulations were evaluated in terms of their clarity, color, odor and texture.

- **Determination of pH**

  pH of your 10% shampoo solution. Dip one strip of pH paper in the solution and compare the color of the strip to key. pH meter can also be used after calibration.

  Most shampoos are neutral or slightly acidic. Acidic solutions cause the cuticle (outer layer) of the hair to shrink and lay flatter on the shaft of the hair. Basic solutions cause the cuticle to swell and open up. Acidic solutions make the hair seem smoother. Basic solutions make hair seem frizzier.

Neutral pH = 7 Acidic pH < 7 Basic pH >7

- **Dirt dispersion**

  Two drops of shampoo were added in a large test tube contain 10 ml of distilled water. 1 drop of India ink was added; the test tube was stoppered and shakes it ten times. The amount of ink in the foam was estimated as None, Light, Moderate, or Heavy.

  Shampoos that cause the ink to concentrate in the foam are considered poor quality. The dirt should vstay in the water portion. Dirt that stays in the foam will be difficult to rinse away. It will redeposit on the hair.

- **Determination of percentage solid content**

  A clean dry evaporating dish was weighed and added 4 grams of shampoo to the evaporating dish. The dish and shampoo was weighed. The exact weight of the shampoo was calculated only and put the evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the shampoo only (solids) after drying was calculated. If a shampoo has too many solids it will be hard to work into the hair or too hard to wash out. If it doesn't have enough it will be too watery and wash away quickly. A good shampoo will be between 20% – 30% solids.

- **Surface tension measurement**

  Measurements were carried out with a 10% shampoo dilution in distilled water at room temperature. Thoroughly clean the stalagmometer using chronic acid and purified water.

  Because surface tension is highly affected with grease or other lubricants. The data calculated by following equation given bellow:

  R2 = (w3-w2) n1 R2 (W2-w2)n2

  Where, W1 is weight of empty beaker, W2 is weight of beaker with distilled water, W3 is Weight of beaker with shampoo solution, n1 is no. of drops of distilled water, n2 is no. of drops of shampoo solution, R1 is surface tension of distilled water at room temperature, R2 is surface tension of shampoo solution

- **Cleaning action**

  5 grams of wool yarn were placed in grease; after that it was placed in 200 ml. of water containing 1 gram of shampoo in a flask. Temperature of water was maintained at 350C. The flask was shaked for 4 minutes at the rate of 50 times a minute.

  The solution was removed and sample was taken out, dried and weighed. The amount of grease removed was calculated by using the following equation:

  DP = 100 (1-T/C)

  In which, DP is the percentage of detergency power, C is the weight of sebum in the control sample and T is the weight of sebum in the test sample.

- **Wetting time**

  The canvas was cut into 1-inch diameter discs having an average weight of 0.44 g. The disc was floated on the surface of shampoo solution of 1% w/v and the stopwatch started. The time required for the disc to begin to sink was measured acutely and noted as the wetting time.

- **Foaming ability and foam stability**

  Cylinder shake method was most widely used for determining foaming ability. 50 ml of the 1% shampoo solution was put into a 250 ml graduated cylinder and covered the cylinder with hand and shaken for 10 times. The total volumes of the foam contents after 1-minute shaking were recorded. The foam volume was calculated only. Immediately after shaking the volume of foam at 1 minute intervals for 4 minutes were recorded.

Observation:

- **Evaluation of Formulation for physical appearance, pH**

Sl. No	Formulation	Physical Appearance	PH
1			
2			
3			

- **Evaluation of Formulation for Surface tension and % solid contents**

Sl. No	Formulation	Surface Tension	% solid contents
1			
2			
3			

- **Evaluation of Formulation for wetting time, cleaning, and Detergency**

Sr. No	Formulation	Wetting time (sec)	% cleaning	% detergency
1				
2				
3				

- **Foam stability of shampoos**

Time (mins)	Foam volume		
	F1	F2	F3
1			
2			
3			
4			
5			

Label:

<table>
<tr><td colspan="2">CLEAR LIQUID SHAMPOO
20 ml</td></tr>
<tr><td>Composition:
Coconut oil, Castor oil, Potassium hydroxide, Glycerin, Perfume, Borax, water</td><td>BATCH NO.: MFG. DATE:

MFG. LIC NO.: EXPIRY DATE:</td></tr>
<tr><td>Category: Hair Cleanser</td><td></td></tr>
<tr><td colspan="2">FOR EXTERNAL USE ONLY.</td></tr>
<tr><td colspan="2">Use: It is used as a clean the hairs and to remove dirt, dust and sebum from the surface
Storage: Stored in a well closed container at a temperature not exceeding 25^0C</td></tr>
<tr><td colspan="2">MFG BY: ABCD Batch: Roll No:</td></tr>
</table>

Report: 20 ml of clear liquid shampoo was prepared, evaluated, labelled and submitted.

Experiment No: 11

ADDRESSING DRY SKIN, ACNE, BLEMISH, WRINKLES, BLEEDING GUMS AND DANDRUFF

AIM: To address Dry Skin, Acne, Blemish, Wrinkles, Bleeding Gums and Dandruff

Requirements:

- **Apparatus-** Beaker, Water bath, Glass rod
- **Chemicals-** Stearic acid, Lanolin, beeswax, Mineral oil, Triethanolamine, Ethyl alcohol, Bezyl peroxide, HPMC etc

PRINCIPLE:

A. Dry Skin

Xeroderma, also known as dry skin, xerosis cutis, or asteatosis, is a prevalent condition resulting from inadequate hydrolipids in the skin. This deficiency can manifest as roughness, tightness, flaking, and scaling of the skin, resulting from various factors such as age, underlying medical conditions, medications, or environmental changes. This activity provides learners with insights into the evaluation and holistic management of xeroderma, including strategies to alleviate pruritus, minimize the risk of skin infections, and ultimately improve patient outcomes.

B. Acne

Acne is an inflammatory disorder of the skin, which has sebaceous (oil) glands that connects to the hair follicle, which contains a fine hair. In healthy skin, the sebaceous glands make sebum that empties onto the skin surface through the pore, which is an opening in the follicle. Keratinocytes, a type of skin cell, line the follicle. Normally as the body sheds skin cells, the keratinocytes rise to the surface of the skin.

Acne causes several types of lesions, or pimples. Doctors refer to enlarged or plugged hair follicles as comedones. Types of acne include: Whiteheads, Blackheads, Papules, Pustules or pimples, Nodules, Severe nodular acne

C. Wrinkles

Wrinkles are creases in the skin. The medical term for wrinkles is rhytids. Most wrinkles come from aging. Aging of the skin, hair and nails is a natural process.

Frequent or prolonged exposure to sunlight results in early skin wrinkles and dark areas. It also increases the chances of getting skin cancer. Exposure to cigarette smoke can also make the skin wrinkle sooner. Common causes of wrinkles include: Genetic factors (family history), Normal aging changes in the skin, Smoking, Sun exposure

D. Blemish

A blemish is the term for any mark on the skin. There are many different types of blemish, including acne, papules, and changes in pigmentation. They can appear anywhere on the body, including the face. Most blemishes are harmless, but some people may wish to treat them for cosmetic reasons.

E. Dandruff

Dandruff is a scalp condition that causes flakes of skin to appear. There may also be itching. Most people experience dandruff at some point, but it is more common from the teenage years up to midlife. There are various possible causes, including seborrheic dermatitis, allergic reactions, psoriasis, and eczema.

Procedure:

A. Procedure for preparation of Moisturizing Cream

- Weight the quantity of steric acid, lanolin & mineral oil. Place it in 150 ml beaker
- Heat the beaker in a water bath until all ingredients melt.
- Measure 48 ml of water in 250 ml beaker. Add the required amount of triethanolamine
- Heat the mixture to a temp 80º to 90º C. Add the triethanolamine slowly to the melted mixture with constant stirring

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity taken
1	Stearic Acid	10 g
2	Lanolin	7 g
3	Mineral Oil	10 g
4	Triethanolamine	2 ml
5	Water	Q.S.

B. Procedure for preparation of Acne Cream

- The oil phase consists of stearic acid & other oil soluble compounds such as cetyl alcohol & liquid paraffin were dissolved.
- The oil phase is placed in the beaker in the water bath at temp 75º C.
- The water soluble components & preservatives were dissolved in aqueous phase & heated in same water bath at temp 75º C.
- After heating, aqueous phase is added in position to oil phase with continuous stirring until the cooling of emulsifies.

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity taken
1	Benzyl peroxide	5.5%
2	Water	40.7%
3	Ethyl alcohol	44.1%
4	Laurath 20	6%
5	Magnesium sillicate	2.5%
6	HPMC	1%
7	Citric Acid	0.05%
8	Fragrance	Q. S.

C. Procedure for preparation of Anti-Wrinkle Cream

- Melt beeswax, emulsifying wax & oils together
- Add orange flavor water drop by drop with constant stirring
- Add tincture of benzoin & orange oil with constant stirring

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity taken
1	Bees wax	10 g
2	Emulsifying Wax	10 g
3	Almond oil	40 ml
4	Lanolin	20 g
5	Coconut oil	20 g
6	Orange flower oil	30 ml
7	Tincture benzoin	0.5 ml
8	Orange oil	0.5 ml

D. Procedure for preparation of cream for Blemish

- O/W emulsion of 20% drugs were formulated
- The emulsifying wax & other oil soluble components were dissolved in oil phase (Part A) & heated upto 80ºC
- Extract & water soluble components were dissolved in Part B & heated upto 80ºC
- After heating, the aqueous phase was added in portion to the oil phase with constant stirring until cream is formed.

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity taken
1	Extract	2 g
2	Petroleum Jelly	4.3 g
3	Hard paraffin	2 g
4	Cetyl alcohol	0.5 ml
5	Glyceryl monostearate	0.5 g
6	Methyl paraben	0.4 g
7	Propyl paraben	0.3 g
8	Fragrance	Q. S.
9	Activated charcoal	0.01 g

E. Procedure for preparation of Anti-Dandruff Shampoo

- The anti-dandruff shampoo was formulated using simple mixing process.
- Formulation were made by using two antidandruff agents such as Sulphur & benzoic acid.
- The other ingredients are being mixed later.

Ingredients Table (Formula):

Sl. No	Ingredients	Quantity taken
1	Sulpher	0.25 g
2	Benzoic acid	2 ml
3	Sodium Lauryl sulphate	20 g
4	Urea	1 g
5	Ctrice acid	0.5 ml
6	Sodium EDTA	0.05 g
7	Guar gum	0.2 g
8	Tween 80	0.5 g
9	Distilled water	Q.S.

Report: Cosmeceuticals for Dry Skin, Acne, Blemish, Wrinkles, Bleeding Gums and Dandruff were prepared and addressed.

Vision and Mission of the Institution

Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

Mission

M1	Create pharmacy graduates through **quality education**
M2	Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**
M3	**Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning
M4	**Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.