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Optimization in Pharmaceutical Experimentation:

Concepts, Applications and Demonstration

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Contents

- Need for optimization
- Concept of optimization
- Advantages of optimization
- Designs of optimization
- Terminology used in optimization
  - Factorial designs
  - Analysis of Variance

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Optimization Techniques

- Models of experimental design
- They are for producing "**a good and effective product**" in a reproducible manner.

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Pharmaceutical production

- Product must be **safe and effective**
- Production process must be **reproducible** when its quality is determined by **specific criteria**.

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Optimization

- Optimization has to do with this "**production process**".
- Today the regulatory environment is very strict and when the Drug Control Department inspectors come for inspection when a new drug is being introduced, they ask for a "**development report**" for both formulation and process.

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Optimization techniques

- Optimization techniques offer a **rational approach** for the selection of the several excipients and also for selecting different manufacturing steps for a given product.
- Optimization is not just a screening technique.

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Optimization Techniques

- Utilizing an experimental design helps us develop the best product under given conditions and also to submit proof that it is the best.

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Advantages

Designs for optimization give advantage over traditional experimentation because:

- In traditional experimentation we study **one factor at a time (OFAT)** and thus miss some interactions.
- We take the components into a system, by trial and error basis and sometimes miss the exact best composition, by not being able to go deep enough.

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Definition

- The word "**Optimize**" is defined by Webster's New Collegiate Dictionary 1974, as follows: "to make as perfect, effective or functional **as possible**".

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Questions

- Perfect by whose definition?
- Perfect by what criteria?
- Perfect under what conditions?

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Answers

- Researcher's definition
- Researcher's criteria
- Researcher's experimental conditions

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Traditional

- We use the word optimization in the context of process development and formulation.
- **Traditionally**, we carry out formulation by a **trial and error manner** and declare a product as optimized- the one which comes out as perfect in our evaluation, as per our criteria.
- In testing for process variables, we test **one factor at a time (OFAT).**

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Concept

- But under these conditions the best one is simply the last one prepared.
- After we finish experimentation, someone else may come up with changed conditions of experimentation and a more "perfect" product.

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Concept

- No matter how rationally designed, the trial and error method can be improved upon.
- But in optimization procedures, **we use a model to prove that under the given conditions, and judging by the given criteria, "one particular product" is the perfect one**.

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Optimization

- We are preparing a map of the responses (as judged by the criteria) obtained when the **variables are changed in a defined way** and from the map, we can choose the response we want and thus proceed to fixing the conditions needed for that response.

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- In traditional experimentation, dependent variable, y is taken as a simple function of X_1 and X_2.
- In optimization the designs take into consideration,
  - **the interactions between the components and**
  - **give us a multiple regression equation and**
  - **a map with a surface**.

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- The optimization designs, when fed with the ranges of the independent variables and the required ranges of the dependent variables, give us the compositions of the independent variables with which we have to work and report the dependent variables.
- They give us the compositions for the trials.
- The number of trials are usually less than the number which we do, if we are working on trial and error basis.

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Co-acervation phase separation

- We can prepare microcapsules by co-acervation phase separation method.

Example:

- We determine the point at which co-acervation is taking place and we take the **gelatin, sodium sulphate and water** in the quantities indicated by the point of coacervation.
- To determine that point, we do the following experiment.

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Experimental

- We take 10 test tubes.
- We take 2 ml of 5% gelatin solution into each tube.
- We take 10% sodium sulphate solution into a graduated pipette; add 0.5 ml of it to the first test tube of gelatin, add 1 ml of it to the second gelatin tube, add 1.5 ml to the third, ... and 5 ml to the last tube.
- We shake them thoroughly and put them back in the rack of test tubes in clear ascending order.

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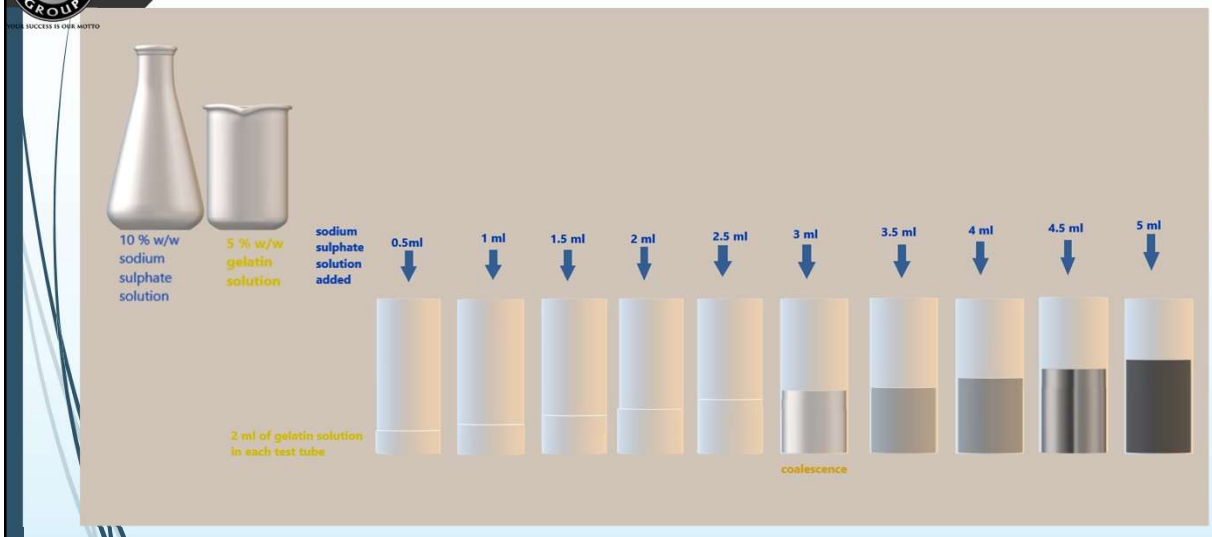

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- In the first tubes, there would be no co-acervation, and in the last tubes, there would be precipitation
- If we look at the tubes in the ascending order, at some point a cloud would be starting and gradually it would be more and more.
- Let us assume, in our case, **cloud is clearly observed in 3 ml sodium sulphate added tube, but there is nothing in 2.5 ml sodium sulphate added tube**.
- We record the observation, and remove the tubes.

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Pin-pointing

- Take 8 clean test tubes, put in each tube, 2 ml of 5 % w/w gelatin solution.
- Add to the first tube, 2.4 ml of 10% w/w sodium solution, 2.5 ml to the next, 2.6 ml to the next and so on – 3.1 ml to the 8th tube.
- Shake them thoroughly and keep them in the rack. Observe closely where co-acervation started.
- Let us say, there is nothing with 2.7 ml and there is a slight suspicion of a cloud at 2.8 ml.
- We record that **2.8 ml of 10% w/w solution of sodium sulphate is giving co-acervation with 2 ml of 5 % w/w solution of gelatin**.

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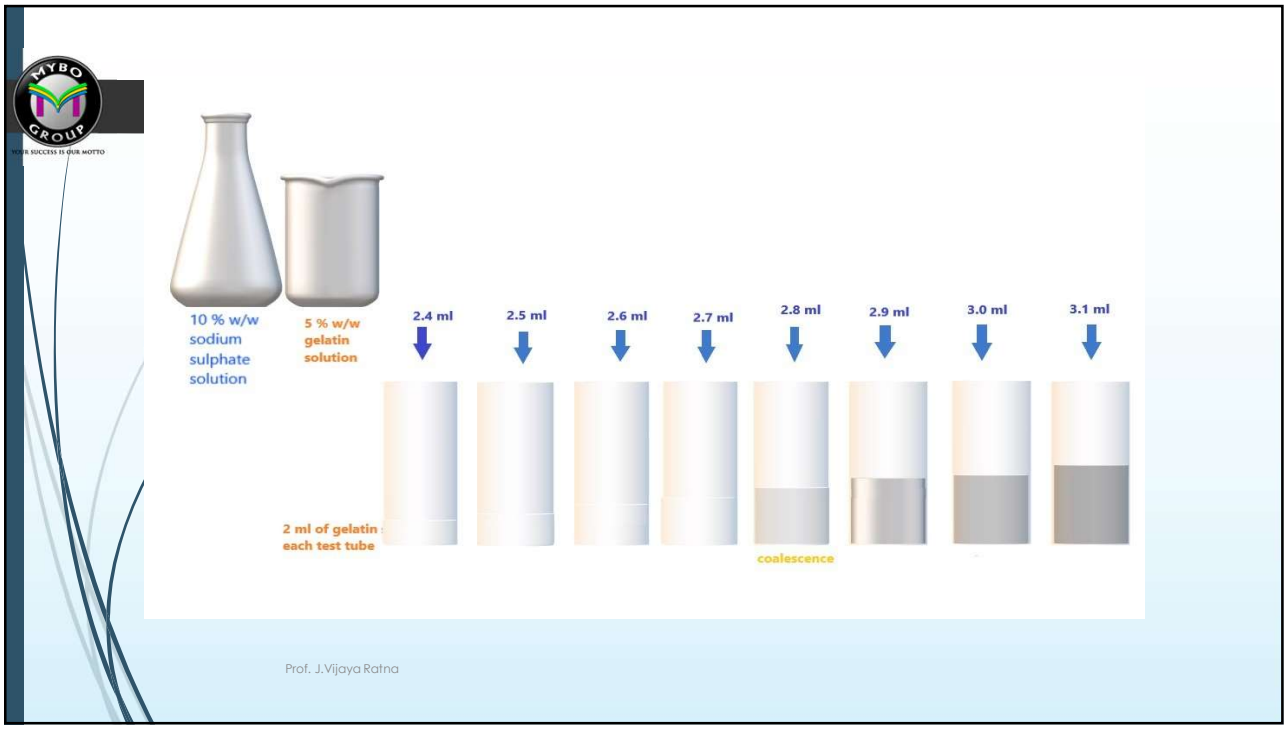

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Preparation of microcapsules

- Now, we take the quantities indicated by the solution in the test tube containing 2 ml gelatin and 2.8 ml sodium sulphate solution and with that composition go for the preparation of microcapsules.
- The assumption is that such a composition would give fine microcapsules of the drug taken with a thin coat.

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- Now, I was operating with a graduated pipette having up to 0.1 ml gradation.
- Suppose someone is working with a graduated pipette that has graduations up to 0.05 ml, then they can pin point the point of co-acervation much better and their microcapsules would be more accurate or nearer to the expected ideal.
- So, in this method, the last product prepared is the best product. Someone can come up with an idea and make a more perfect product anytime.

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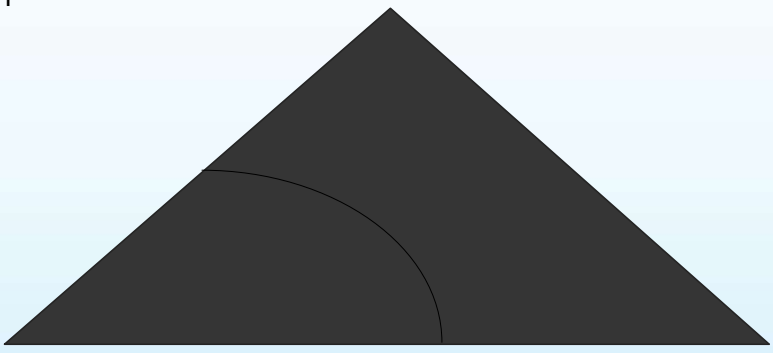
Gelatin- sodium sulphate-water system

- Objective is to draw the binodal curve (it is the boundary of the two phase system) in a triangular graph



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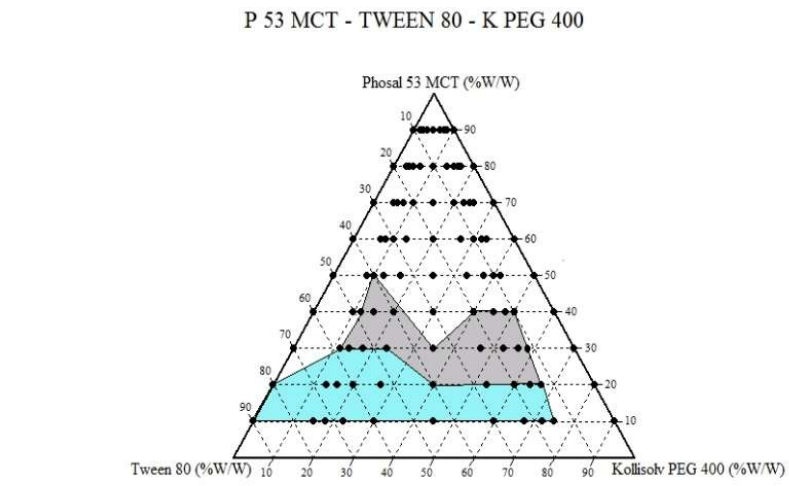

Figure 3.10: Phase diagram of Phosal 53 MCT – Tween 80 – Kollisolv PEG 400 system

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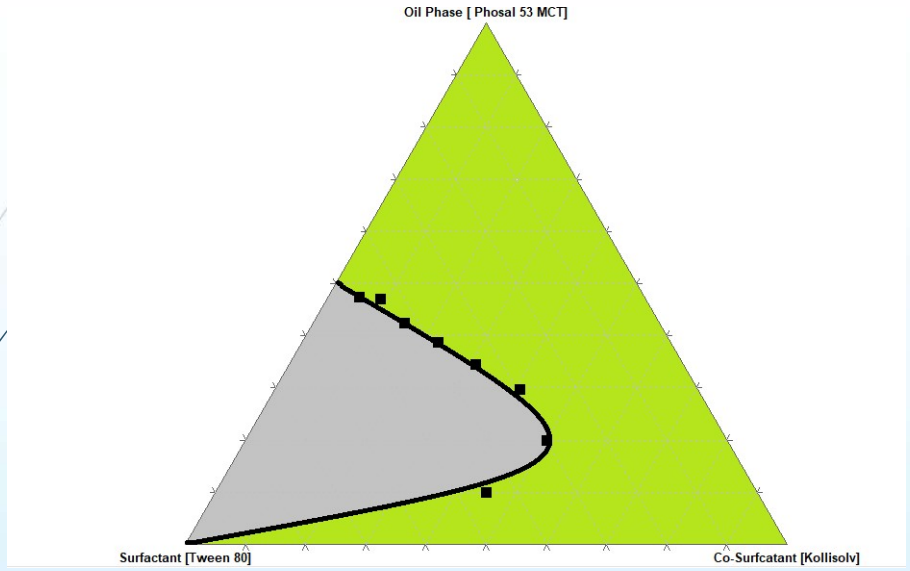

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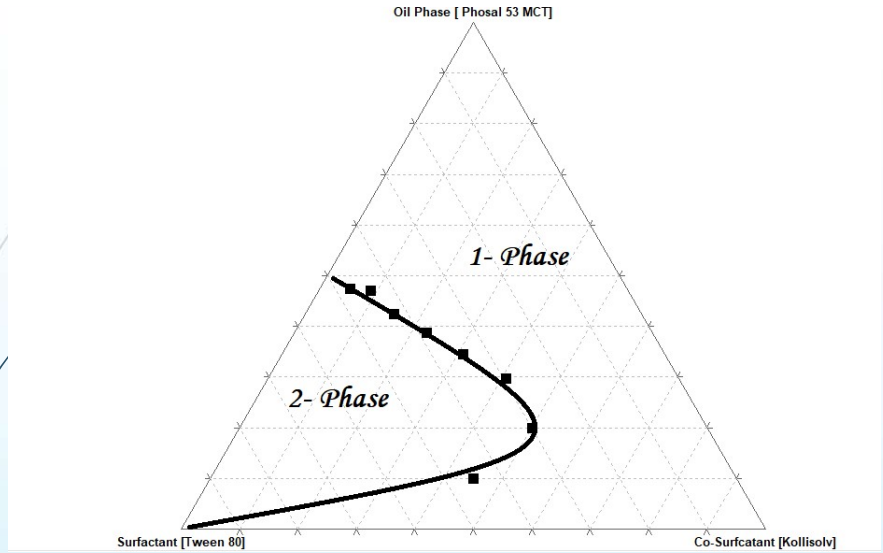

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Optimization

- If we could put our values into a system or a design (where we take:

independent variables as: gelatin, water and sodium sulphate
dependent variables as: size of microcapsule and encapsulation efficiency

- which could get a map, a three dimensional map which gives a response surface, and take the values of gelatin, sodium sulphate and water for microcapsules of a given size and encapsulation efficiency from it – and prepare microcapsules - that would be optimization as it is being done now.

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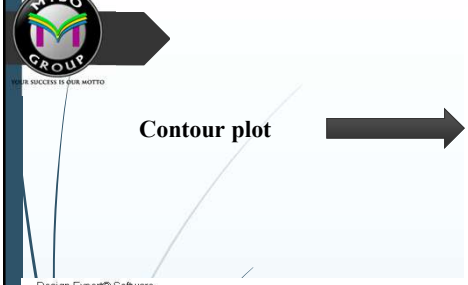
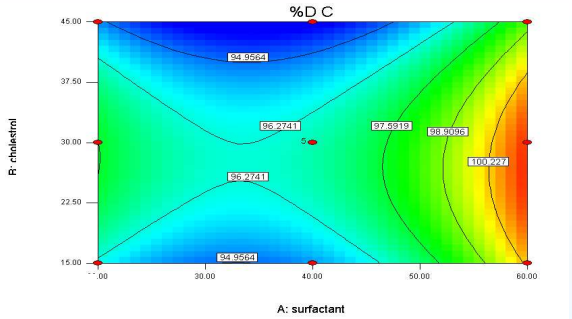
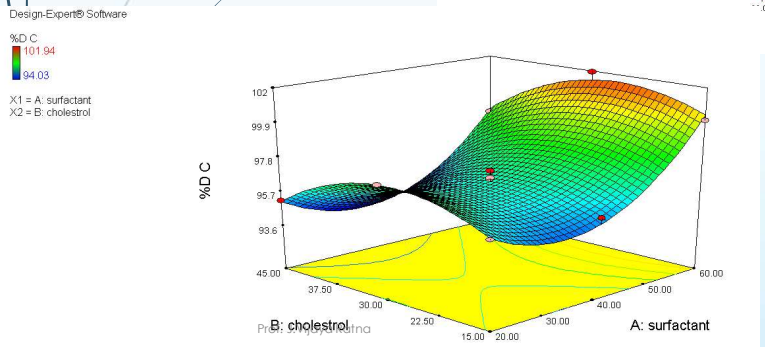
Plots showing the effect of surfactant and cholesterol on drug content of tamsulosin hydrochloride loaded niosomes

Contour plot







Response surface plot

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Optimization

- Now, this optimization by following a design- is giving us the most perfect product for the given weights of materials and for the given criteria defined. No one can improve it further and say- this is more perfect.
- In a three dimensional area map,
  - whatever depth you want, with respect to dependent variables, you can go, and
  - you would get the values of the independent variables for it
  - interpolation is at its best
  - every point on the map defines the product coming out with the quantities of independent variables suggested by that point in a most perfect manner.

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- **So, what are the designs doing?**
- When we give the ranges of our independent variables and dependent variables (by our experience), the design by certain complex calculations, coming out with the experiments that we have to do.
- When we do those experiments, each with three replications, and fill the data in the design area, they are coming out with response surface plots that give the elements of the products with different levels of the criteria.

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- We prepare our optimised product from this response surface plot
- Evaluate it
- Find out whether it is really as perfect as predicted by the plot or not.
- **We find the error between the suggested one and the practical one and this should be zero**.

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Differences

No.	TRADITIONAL	OPTIMIZATION
1	One factor at a time	All factors in every trial, factors studied simultaneously
2	Cannot study interactions	Studies interactions, separates main effects and interaction effects
3	Trial and error method	Trials are given by the design based on computations, based on a rationale
4	Graphs and plots (two dimensional)	Contour plots, response surfaces, overlay plots (two dimensional and three dimensional plots)

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Differences

No.	TRADITIONAL	OPTIMIZATION
5	Result and analysis constrained by the accuracy to which our equipment can reach (interpolation limited)	Analyses our results and gives maps which help us go to any point we want (interpolation at its best)
6	Proof of "optimized" limited to our trials	Can prove that "optimized" is the best under the given conditions
7	Dependent variable y is taken as a simple function of independent variables X_1 and X_2	Multiple regression equations are used
8	Number of trials are usually more	Number of trials are usually limited and lesser

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Differences

No.	TRADITIONAL	OPTIMIZATION
9	Time, expense and effort are more	Time, expense and effort are lesser
10	Generated data is not completely used in analysis	Maximum use of generated data is done
11	Modern Regulators do not prefer this mode of development from manufacturers	Modern Regulators prefer this mode of development because it is rational and can prove its validity.

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Constrained/ Unconstrained

- There are two varieties of problems that we encounter in optimization,
  - Constrained and
  - Unconstrained.

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Constrained

- In this case restrictions are placed on our experimentation and we cannot alter the variables or conditions as we like.
- For example, if we are homogenizing an emulsion using a colloid will, if we increase the time of homogenization we get a very small globule size which is very good for the product.

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- But increasing the homogenization time also increases the temperature of the product.
- So that is a restriction on the procedure.
- To give another example, we may be selecting excipients and process steps with a view to make a very hard tablet.

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- If we say "make the hardest possible tablet, but it should disintegrate within 15 minutes"; we have a constrained optimization procedure before us.

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Unconstrained

- If there are no restrictions on our product or process, we are doing unconstrained optimization.
- In pharmaceutical research we are usually dealing with constrained optimization as we have restrictions imposed by time, economy, product safety, efficacy, regulatory considerations and market considerations.

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- The development of a pharmaceutical formulation and the fine tuning of the process for that formulation involve
  - (1) changing the variables (factors) at different levels and
  - (2) measuring the dependent variables or responses (effects).

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Variables

- The independent variables are the formulation and process variables which the experimenter can change as per his judgement.
- Examples are
  - (1) the quantities of excipients in a formulation
  - (2) the time taken for homogenization
  - (3) the force exerted in punching a tablet; and so on.

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- The dependent variables are the responses or results or effects.
- Examples are
  - (1) the hardness or disintegration time of a tablet
  - (2) the viscosity or globule size of an emulsion
  - (3) the sedimentation rate of a suspension

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Classical Optimization

- Classical Optimization techniques result from application of calculus to the basic problem of finding the maximum or minimum of a function.

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Statistical Designs

There are two varieties of statistical optimization designs.

1. Experimentation continues as optimization study is done.
   - Evolutionary Operations Procedure or EVOP and
   - Simplex methods.

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2. Experimentation is completed before the optimization takes place.
   - Classic mathematical and
   - Search methods.

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Designs of Experiments

- Statistical Designs
- EVOP
- Simplex Lattice
- Factorial Design
- Response Surface Methodology

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Optimization techniques help us to

- (1) select experimental points so that the entire area of interest is covered or considered and
- (2) separate the effects of the variables, i.e. we can tell which variable caused a particular result.

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Steps in the procedure

- Suppose we are preparing some matrix tablets, we have to select the
  - weight of the polymer (X_1) and
  - weight of the diluent (X_2)

  in order to get tablets of
  - particular hardness (Y_1) and
  - time for 100 % dissolution (Y_2).

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1. We do some preliminary experimentation and determine the range of the two excipients to get the dependent variables as we want them.
2. We select our DoE, may be Central Composite Design, and enter our values into the template of the design (in software).
3. The design will ask us to do certain experimental trials.

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4. We will carry out those trials, prepare the tablets as per those compositions and give the results from the evaluation into the design (entered into software).
5. The design will give us plots which are analysis of our data. From these plots, we take those compositions which have desirability values of 1.
6. These are candidates for optimization.

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- We prepare these products, evaluate them and check whether their "Y" values are exactly agreeing with the "Y" values predicted by the design.
- If error is near zero, we have an optimized product.

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Factorial Design

- Factorial Design is the most widely used method of optimization.
  - We set up the design,
  - we carry out the experiments,
  - generate the data and
  - we write multiple regression equations that express the relationships between the variables.
- These equations are the basis of optimization and they define the response surface for the system under investigation.

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- When the effects of different factors, each at different levels, on the results of the experiments are to be elucidated, FACTORIAL EXPERIMENTS are carried out.

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Pharmaceutical examples

Experiments to determine the effect of

- pressure and lubricant on hardness of tablets,
- disintegrant and lubricant concentration on tablet dissolution

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Factorial Designs

- Designs of choice for simultaneous determination of the main effects and interaction effects of several factors.

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Definitions

Factor:

- A factor is an assigned variable such as concentration, temperature, lubricating agent , drug treatment or diet. A factor can be quantitative or qualitative.

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Factors

- A **quantitative factor** has a numerical value assigned to it.

  Ex: Concentration: 1%,2%,3%

- A **qualitative factor** has a name assigned to it rather than a value.

  Ex: Treatments, diets, batches of materials, laboratories, analysts or tablet diluents

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- Single factor designs fit the category of one way ANOVA design.

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Levels

- The levels of a factor are the values or designations assigned to the factor.
  - For the factor "temperature", levels may be 30° or 40°.
  - For the factor "concentration", levels may be 0.1 molar or 0.2 molar.
  - For the factor "drug treatment" levels may be drug and placebo.

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Runs or Trials

- The runs or trials that comprise factorial experiments consist of all combinations of all levels of all factors.
  - Ex: Investigation of the effects of polymer concentration and lubricant concentration on dissolution time of a tablet
- If both factors were at two levels (two concentrations for each factor), four runs (dissolution determinations for four formulations) would be required

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Effects

- The effect of a factor is the change in response caused by varying the levels of the factor.
- The main effect is the effect of a factor averaged over all levels of the other factors.
- In the example the main effect due to drug would be the difference between the average response when drug is at the high level (runs b and ab) and the average response when drug is at the low level (runs (1) and a).

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Interaction

- Interaction may be defined as a "**lack of additivity of factor effects**".
- Ex: In a two factor experiment if factor A has an effect equal to 5 and if factor B has an effect of 10, additivity would be evident if an effect of 15 were observed when both A and B are at their high levels.

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Interaction

- If the effect is greater than 15 when both factors are at their high levels, the result is synergistic with respect to the two factors.
- If the effect is less than 15 when both factors are at their high levels, an antagonistic effect is said to exist.
- The drug effect measured when the lubricant is at the low level (a-(1)) is DIFFERENT from the drug effect measured when the lubricant is at the high level (ab-b).

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- Factorial designs are the designs of choice for simultaneous determination of the effects of several factors and their interactions.

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- When a particular experiment involves more than one independent variable, the traditional method is to keep one variable (A) constant and change the other (B).
- After establishing the effect of B, in the next cycle B is kept constant and A is changed.
- The effects of changing of A and B on the dependent variable Y are then separately reported.
- The problem in this type of experimentation is, we cannot detect interaction effect

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Example:

- Imagine an old balance with a zero error of 1kg.
- A fat man comes and stands on it and the balance says 122 kg. We announce his weight as 121 kg.
- A child comes and stands on the balance and it shows 23 kg. We announce that the child's weight is 22 kg.

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- In this experiment we could have missed some interaction effect.
- The balance's zero error may be changing with weight.

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So what should be done is this.

- Take the weight readings
  - Empty balance: (1)
  - Balance + child: (a)
  - Balance + fat man: (b)
  - Balance + child + fat man: (ab)

The weight of the child $= \frac{((ab)-(b) + ((a)-(1)}{2}$

The weight of the fat man $= \frac{(b-1) + (ab-a)}{2}$

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Interaction effect

If (ab-b) = (a-1)

- then there is no interaction.
- The difference between these two is the interaction effect.

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Advantages

- In the absence of interaction, factorial designs have maximum efficiency in estimating main effects.
- If interactions exist, factorial designs are necessary to reveal and identify the interactions.
- Since factors' effects are measured over varying levels of other factors, conclusions apply to a wide range of conditions.

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- Maximum use is made of the data since all main effects and interactions are calculated from all of the data.
- Factorial designs are orthogonal, all estimated effects and interactions are independent of effects of other factors.

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Applications

- The results of a factorial experiment may be used to help interpret the mechanism of an experimental system.
- To determine whether the main effects and the interaction effects are significant or not.
- To recommend implementing in a practical procedure or in a pharmaceutical industry.

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- The name of a factorial design depends on two points:
  - The number of independent variables
  - The number of levels of each independent variable

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- The effect of a single variable is known as a main effect
- The effect of two variables considered together is known as an interaction
- For the two-way between groups design, an F-ratio is calculated for each of the following:
  - The main effect of the first variable
  - The main effect of the second variable
  - The interaction between the first and second variables

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Optimization Procedure

- In this procedure we construct an equation that describes the experimental results as a function of the factor levels.
- A polynomial equation can be constructed, in the case of a factorial design, where the coefficients in the equation are related to the effects and interactions of the factors.
- If we describe a factorial design as 2^n,
  - "n" stands for the number of factors
  - "2" stands for the number of levels.

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The polynomial equation for a 2^n design will be of the form.

$$Y=B_0+B_1X_1+B_2X_2+B_3X_3+B_{12}X_1X_2+B_{13}X_1X_3+B_{23}X_2X_3+B_{123}X_1X_2X_3+$$

- Where Y is the measured response X_i is the level of the i^{th} factor, B_i, B_{ij} etc. represent coefficients computed from the responses of the formulations in the design.

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- For a **2^3** experiment, i.e., an experiment in which
  - **3 factors** or independent variables are being tested,
  - each at **2 levels**, the following are the steps for the development of a polynomial equation and using it in optimization.

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Factorial Design

- Example of 2^3 i.e., three factors with two levels; preparation of ion exchange resinates with maintenance of three factors pH, drug concentration and time period

		Level-1	Level-2
Factor 1: pH	:	4	8
Factor 2: Drug Conc	:	100 mg	200 mg
Factor 3: Time period	:	20 min	40 min

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Step-1:

- We define the three factors (X_1, X_2 and X_3) and their two levels, say,

		Low level	High level
X_1 (starch)	:	0	2
X_2 (colloidal silica)	:	0	1
X_3 (drug)	:	0	5

- So, total number of experiments = $2^3 = 8$

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Step-2:

- We carry out the experiments, i.e. we prepare 8 formulations.

Formulation No.	X_1	X_2	X_3
1	0	0	0
2	2	0	0
3	0	1	0
4	2	1	0
5	0	0	5
6	2	0	5
7	0	1	5
8	2	1	5

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Step-3:

- We determine the result Y, it may be the hardness or disintegration time of the tablet.
- So we have 8 different Y values for 8 different formulations.

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Step-4:

- We write 8 different simultaneous equations.

For example

- (1) $0X_1+0X_2+0X_3=Y_1$ (some Y value determined)
- (4) $2X_1+1X_2+0X_3=Y_4$ (some determined Y value)

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Step-5:

We solve these 8 equations (with the help of a computer) to get the coefficients

- B_0, B_1, B_2, B_3, B_{12}, B_{23}, B_{123} etc.

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Step-6:

Then we write the polynomial equation of the form

$Y = B_0+B_1X_1+B_2X_2+B_3X_3+\ldots\ldots +B_{12}X_1X_2+B_{13}X_1X_3+\ldots\ldots +B_{123}X_1X_2X_3$

Step-7:

Using this equation we can calculate the response Y for unknown X_1, X_2, X_3 values.

Step-8:

Using this equation we can also develop a response surface.

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Step-9:

Utilizing the equation and the graph

- we can predict the response for a different set of X_is and
- we can select that set of X_is which can give us the "optimum"
- or "as perfect as possible under the given conditions" response Y.
- **This is optimization using factorial design.**

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Example of a 2^2 factorial

- We want to prepare a tablet which has a high dissolution rate.
- So dissolution rate is response Y.
- We know that two factors are having effect on this response Y, they are
  - (X_1) drug concentration and
  - (X_2) lubricant concentration.

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We decide to test each factor at two levels.
The design of the experiment is like this

Drug	lubricant	Symbol	Formulation
X_1	X_2		
Low	Low	(1)	Low drug and low lubricant concentration
Low	High	(a)	Low drug and high lubricant concentration
High	Low	(b)	High drug and low lubricant concentration
High	High	(ab)	High drug and high lubricant concentration

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- We prepare the four formulations.
- We determine the dissolution rates for them.
- Then we find the effects like this:

Main effect of drug = $\frac{[b+ab-(1)-a]}{2}$

Main effect of lubricant = $\frac{[ab+a-(b)-(1)]}{2}$

- Interaction effect will be the difference between (ab-b) and (a-1)
- and the difference between (ab-a) and (b-1)

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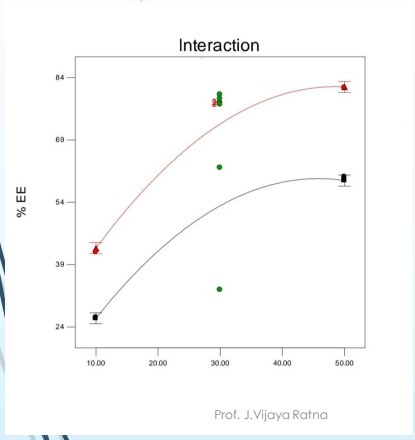
- If there is **no interaction** the graphs will look like this
- Graphs **Parallel**



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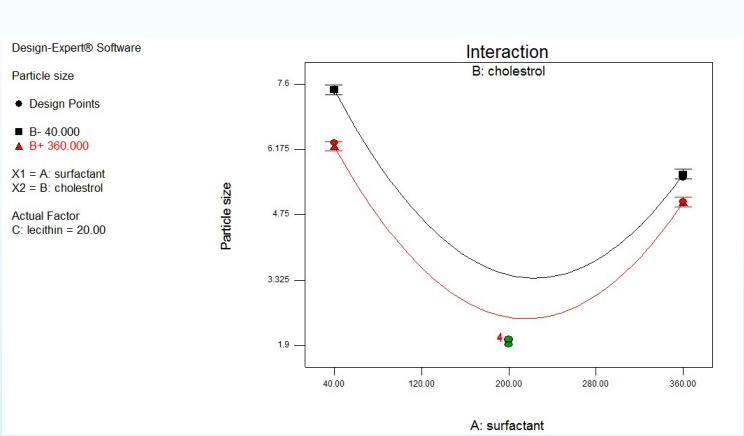

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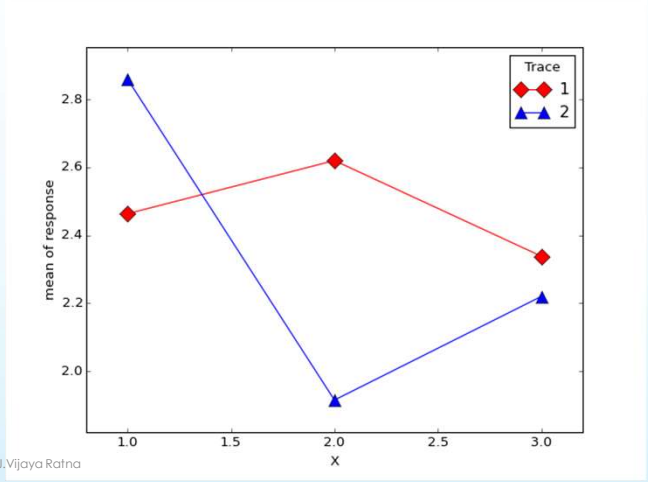
- If there is **interaction** the graphs will look like this
- Graphs **Not Parallel**



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Interaction

- So by this step we can tell what is the main effect of each of these two factors and whether interaction exists between these two factors and if exists to what extent.

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The next step is to write a regression equation like this :

- $Y = B_0+B_1X_1+B_2X_2+B_{12}X_1X_2$
- We have four equations involving X_1 and X_2 and the corresponding Y S.
- By solving these four equations
- we find out coefficients B_0, B_1, B_2 and B_{12}.

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- Then for different values of X_1 and X_2 we can calculate Y.
- In a reverse manner for a Y value that we want we can select X_1 and X_2 values.
- **This is optimization**.

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ANALYSIS OF VARIANCE (ANOVA)

- Analysis of variance is a **statistical procedure** designed **to analyze the difference between the means of more than two samples**.
- **Rationale:** Total variance of all the data in an experiment can be separated and attributed to two sources, variance between groups and variance within groups.
- Variance within groups is also called **error variance**.
- If the experimental treatment has been effective, the between groups differences will be expected to be greater than can be accounted for by chance.

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ANOVA TABLE

Source	SS	df	MS	F.value	P.value	Remark
Model	400.38	5	80.08	53.07	<0.0001	Significant
A	17.17	1	17.17	11.38	<0.0119	Significant
B	78.69	1	78.69	52.15	0.0002	Significant
AB	21.30	1	21.30	14.12	0.0071	Significant
A^2	39.14	1	39.14	25.94	0.0014	Significant
B^2	265.87	1	265.87	176.21	<0.0001	Significant
Residual	10.56	7	1.51			
Lack of fit					0.1121	Not significant
Total	410.94	12				

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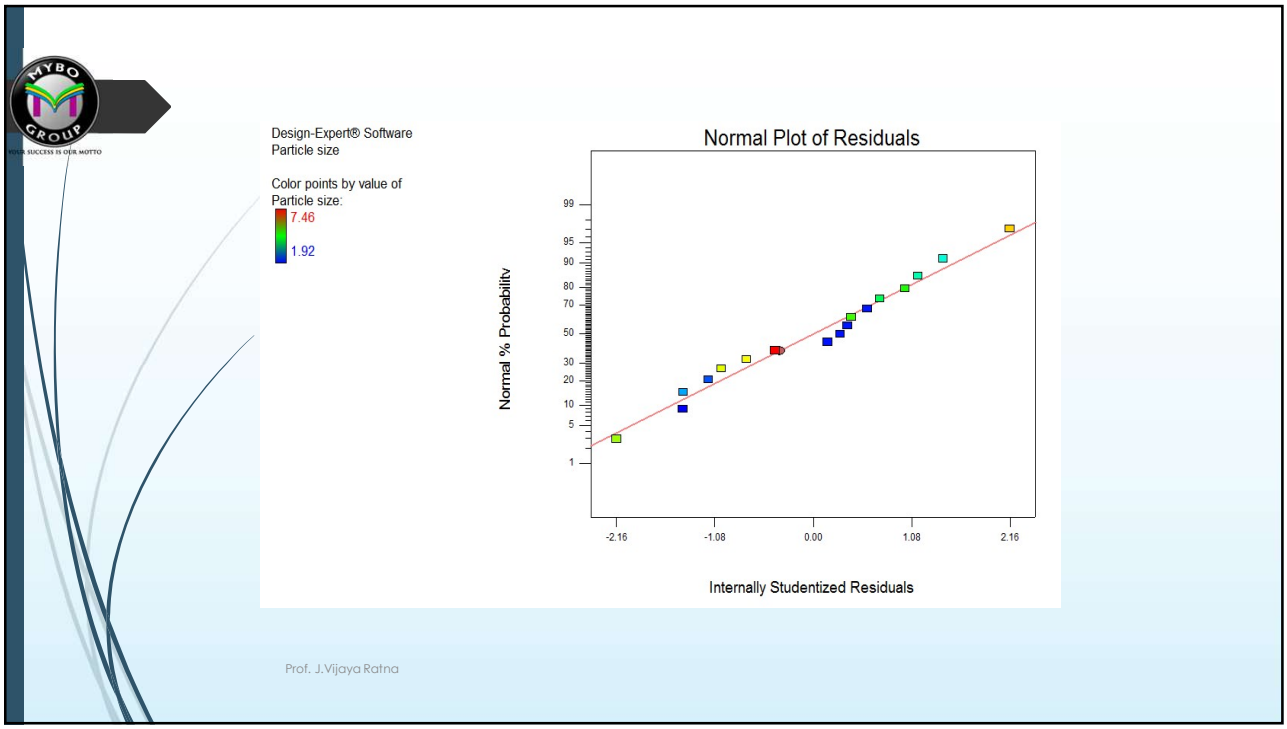

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Terms

- **Source of variation:** effects coming from factors-main as well as interaction
- **Sum of Squares:** term indicating the extent of variance. Concept- Sum of squares of deviation from mean
- **Degrees of freedom:** has to do with the number of repetitions of each factor
- **Mean sum of squares:** Sum of squares divided by corresponding degrees of freedom

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F value

- F is defined as greater variance/ lesser variance
- In ANOVA it is:

  Ex: MS of A /Residual MS = 17.17 / 1.51 = 11.38
- If F value is greater than the Table value, we declare that the treatment is significant.

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Probability

- Probability: If it is less than 0.05, we declare the treatment to be significant.
- P of Factor A is 0.0119. Implies the probability of this much effect coming due to chance is extremely low, i.e., 0.0119.
- **Significant:** This term stands to tell that the effect of the factor concerned is considerable/ important. Important word in statistics. Results that have significance have high value.

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Table Experimental range and levels of independent variables in Box-Behnken design

Run No.	Variable level in coded form		
	X_1	X_2	X_3
1	+1	-1	0
2	+1	0	-1
3	+1	0	+1
4	0	0	0
5	0	-1	-1
6	-1	0	-1
7	+1	+1	0
8	0	0	0
9	0	+1	+1
10	0	0	0
11	0	0	0
12	0	+1	-1
13	-1	0	+1
14	-1	+1	-1
15	-1	-1	-1
16	0	0	-1
17	0	-1	+1

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Factors	Levels used, Actual (coded)		
	Low (-1)	Medium (0)	High (+1)
X1 = Span 60 (mg)	360	200	40
X2 = Cholesterol (mg)	360	200	40
X_3 = Lecithin (mg)	40	20	0

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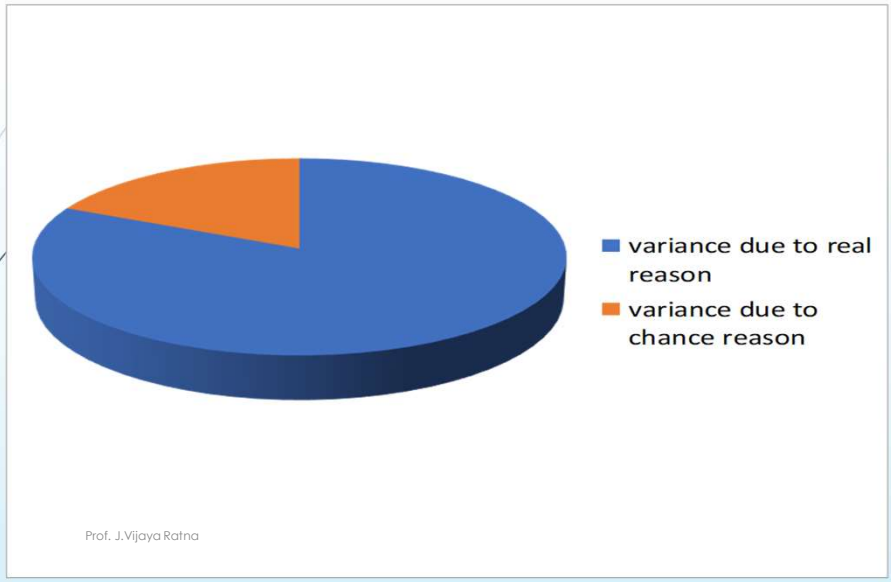
RATIONALE



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PARTITIONING

Variance is partitioned between two sources,

- variance between groups and
- variance within groups.

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Example

- Tablets are being punched out by three different machines.
- All granulation formulae are same.
- We expect that tablets coming from the same machine are having less variability and tablets coming from different machines are having more variability in between them.

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Tablet machines

- We calculate Sum of squares as a measure of variability.
- A ratio of the mean sum of squares of between groups and the mean sum of squares within groups must be lesser than that given by the tables (that comes due to chance reasons only at the given degrees of freedom.
- If the ratio is less than the table F value then we declare that the difference is not significant

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Chance Variance

- Chance Variance
- A variance which occurs due to chance reasons, which is very small in scale and which occurs on both positive and negative sides.
- Also called as error variance.

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Real Variance

- Real variance is due to a real reason and often is the effect of a treatment.
- When a treatment is effective there is a clear, significant difference in the result.
- So ANOVA consists of separating the variance into two categories, that which is due to a real reason and that which is due to a chance reason.

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PURPOSE OF ANOVA

- To establish whether variation between groups is likely to be a function of chance or not.
- Example: Comparison of three batches of tablets with their dissolution times

Tablets	Dissolution times				
Batch I	77	81	71	76	80
Batch II	72	58	74	66	70
Batch III	76	85	82	80	77

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Computational formulas for sums of squares

$$SS_T = \sum (X - \overline{X}_T)^2 = \sum X^2 - \frac{(\sum X)^2}{N}$$

SS_T → Total sum of Squares
X → Each Individual score
$\overline{X}_T$ → Grand mean of all the N cases
N → Total Number of observations
$= n_1 + n_2 + n_3$
Σx^2 → Sum of the squares of each raw score
$(\Sigma x)^2$ → Square of the sum of the raw scores

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Computational formulas for sums of squares

Sum of squares between groups

$$SS_b = \frac{(\Sigma X_1)^2}{n_1} + \frac{(\Sigma X_2)^2}{n_2} + \text{-----} \frac{(\Sigma X_k)^2}{n_k} - \frac{(\Sigma X)^2}{N}$$

SS_b Sum of squares between the groups

ΣX_1 Sum of the first group

N_1 Number in the first group

ΣX Sum of all the scores

N Total number of scores.

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Computational formulas for sums of squares

- Within groups sum of squares

$$SS_w = \Sigma X_1^{\,2} - \frac{(\Sigma X_1)^2}{n_1} + \Sigma X_2^{\,2} - \frac{(\Sigma X_2)^2}{n_2} + \text{-----} + \Sigma X_K^{\,2} - \frac{(\Sigma X_k)^2}{n_k}$$

SS_W = Sum of squares within the groups

ΣX_1^2 = Sum of the squares of the scores in the first group.

n_1= the number in the first group

$SS_W = SS_T - SS_b$

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SCHEMATIC OF ONE – WAY ANOVA

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F
Treatments (Between)	K-1	SS_{Tr} or SS_b	$MS_b = SS_b/K-1$	$MS_b/MS_{W=}$ F
Error (Within)	K (n-1) or N-K	SS_E or SS_W	$MS_W = SS_W$ / N-K	
Total	$K_{n-1 \text{ or }}$ N-1	SS_T		

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ANOVA Table for ex. problem

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square F	
Treatments (Between Groups)	2	390	195	8.48
Error (Within groups)	12	276	23	
Total	14	666		

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Problem

Tablet Batches	Dissolution times (Coded by Subtracting 70)				
Batch I	7	11	1	6	10
Batch II	2	-12	4	-4	0
Batch III	6	15	12	10	7

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Test Procedure

- 1. **Null hypothesis:** $\mu_1 = \mu_2 = \mu_3$
  Alternative hypothesis : μ's are not all equal.
- 2. **Level of significances:** $\alpha = 0.05$
- 3. **Criterion:** Reject the null hypothesis if F>3.89, the value of F0.05 for K-1=3-1=2 and N-K=15-3=12 degrees of freedom where F is to be determined by an analysis of variance, otherwise accept it.

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Calculations

- 4.**Calculations:**

$$SS_T = \sum X_i^2 - \frac{(\sum X_i)^2}{N}$$

$$SSb = \frac{(\sum X_i)^2}{n1} + \frac{(\sum X_2)^2}{n2} + \text{--------} \frac{(\sum X_i)^2}{n}$$

$$SS_W = SS_T - SS_b$$

- 5.**ANOVA table:**
- 6.**Decision:** If F value obtained exceeds 3.89 reject H_0, otherwise accept H_0

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TWO WAY ANOVA

- When we are trying to find out whether the effects of treatments in two directions, are significant or not.
- Sums of squares in two directions, column wise and row wise are calculated.
- The total variance in the data is subdivided into three categories, one- due to treatments (columns), two- due to blocks (rows), and three – due to chance.

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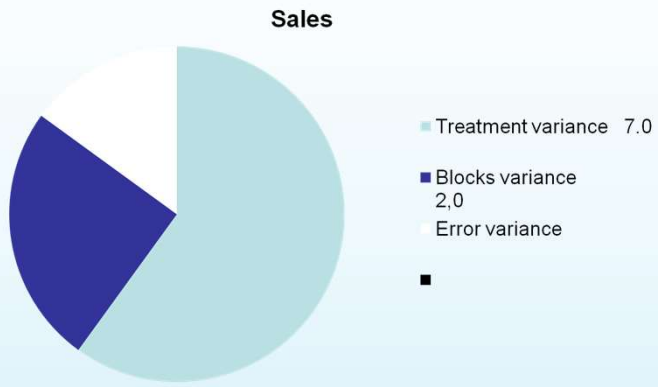
Two way ANOVA



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Two way ANOVA

- In two way ANOVA, two F ratios are calculated.
- First one is the ratio of treatments (columns) sum of squares to error or chance sum of squares.
- Second one is a ratio of blocks (rows) sum of squares to chance sum of squares.

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Two way ANOVA

- Two decisions are taken,
- one w.r.t. significance of difference between treatments (columns)
- the second w.r.t. significance of difference between blocks (rows).

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TWO WAY ANALYSIS OF VARIANCE

- $SS_T = SS\ (T_r) + SSB + SSE$
- $SSE = SST - [SS\ (T_r) + SSB]$
- SSB → Sum of squares between Blocks
- $SS\ (T_r)$ → Sum of squares between Treatments
- SSE → Error sum of Squares

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SCHEMATIC OF TWO WAY ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F
Treatments	K-1	SS (T_r)	MS (T_r) = SS(T_r)/ K-1	$\frac{MS(T_r)}{MSE}$
Blocks	n-1	SSB	MSB = SSB/n-1	$\frac{MSB}{MSE}$
Error	(K-1) (n-1)	SSE	MSE = $\frac{SSE}{(K-1)(n-1)}$	
Total	(N-1)	SST		

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Example for TWO WAY ANOVA

Patients	Low dose	Medium dose	High dose
Old	71	92	89
Young	44	51	85
Children	50	64	72
Infants	67	81	86

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ANOVA table for Two way ANOVA

Source of variation	Degrees of freedom	Sum of Squares	Mean Square	F
Treatments	3	1.260	420	6.21
Blocks	2	1.256	628	9.28
Error	6	406	67.67	
Total	11	2.922		

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Two way ANOVA

Source	SS	df	MSS	F
SS_{bc}	561.8	1	561.8	561.8 / 33.275 = 16.88
SS_{br}	352.8	1	352.8	352.8 / 33.275 = 10.60
SS_{rc}	180	1	180	180 / 33.275 = 5.409
SS_{w}	532.4	16	33.275	
SS_{T}	1627	19		

- $F_{1,16}$ table value is 4.49 (0.05)
  8.53 (0.01)

  SS_{bc}, SS_{br} are significant at 0.01 also
  SS_{rc} is significant at 0.05 but not at 0.01

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Two way ANOVA

- Females sleep longer than males
- Drug A has a longer duration of hypnotic action than drug B.
- Drug A causes especially long sleep in females or especially brief sleep in males.
- Drug B causes especially long sleep in males or especially brief sleep in femals.

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Three way ANOVA

- In Three way ANOVA, the total variability in the data is subdivided into four categories- rows, columns, treatments, and chance.
- Three F ratios are calculated.
- Three decisions are taken.
- Significance of column effect, row effect and treatment effect are determined.

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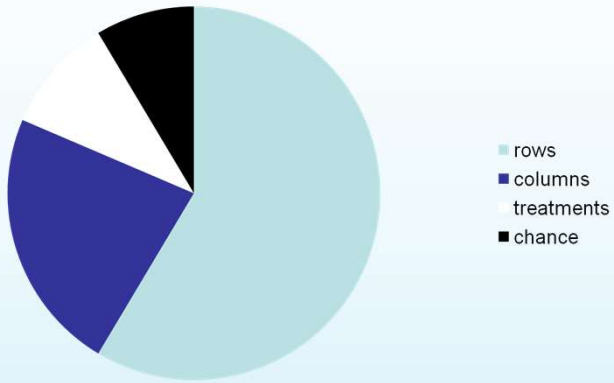
Three way ANOVA



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THREE WAY ANALYSIS OF VARIANCE

$$SS_T = \sum(X_1)^2 - \frac{(\sum Xi)^2}{N}$$

$$SS_{rows} = \frac{(\sum X_{1r})^2}{n_{1r}} + \frac{(\sum X_{2r})^2}{n_{2r}} + \ldots\ldots\ldots\frac{(\sum X_i)^2}{N}$$

$$SS_{columns} = \frac{(\sum X_{1c})^2}{n_{1c}} + \frac{(\sum X_{2c})^2}{n_{2c}} + \ldots\ldots\ldots\frac{(\sum X_i)^2}{N}$$

$$SS_{Treatments} = \frac{(\sum X_{1tr})^2}{n_{1tr}} + \frac{(\sum X_{2tr})^2}{n_{2tr}} + \ldots\ldots\ldots\frac{(\sum X_i)^2}{N}$$

$$SS_E = SS_T - [SS_R + SS_C + SS_{tr}]$$

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SCHEMATIC OF THREE WAY ANOVA

Source of Variation	Degrees of freedom	Sum of squares	Mean square	F
Rows	r-1	SSR	$MSR = \frac{SSR}{r-1}$	$\frac{MSR}{MSE}$
Columns	r-1	SSC	$MSC = \frac{SSC}{r-1}$	$\frac{MSC}{MSE}$
Treatments	r-1	$SS(T_r)$	$MS(T_r) = \frac{SS(T_r)}{r-1}$	$\frac{MS(T_r)}{MSE}$
Error	(r-1) (r-2)	SSE	$MSE = \frac{SSE}{(r-1)(r-2)}$	
Total	r^2-1	SST		

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Example for Three way ANOVA

	Subject 1	Subject 2	Subject 3	Subject 4
1st Week	A 3	B 2	C 4	D 2
2nd Week	B 2	C 2	D 3	A 4
3rd Week	C 4	D 3	A 3	B 2
4th Week	D 3	A 2	B 2	C 4

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Example for Three way ANOVA

- T_{max} values
- H_{01} : There is no difference between the four weeks
- H_{02} : There is no difference between the four boys
- H_{03} : There is no difference between A, B, C and D.

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Example for Three way ANOVA

- Total Sum of Sqaures :

$$SS_T = \sum X_i^2 - \frac{(\sum X_i)^2}{N}$$

$$\sum X_i^2 = 38 + 21 + 38 + 40 = 137$$

$$SS_T = 137 - \frac{(45)2}{16}$$

$$= 137 - \frac{2025}{16} = 137 - 126.6 = 10.4$$

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Example for Three way ANOVA

$$SS_{rows} = \frac{(\sum X_{1r})^2}{n_{1r}} + \frac{(\sum X_{2r})^2}{n_{2r}} \ldots\ldots - \frac{(\sum X_i)^2}{N}$$

$$= \frac{(11)^2}{4} + \frac{(11)^2}{4} + \frac{(12)^2}{4} + \frac{(11)^2}{4} - 126.6 = 0.15$$

$$SS_{columns} = \frac{(12)^2}{4} + \frac{(9)^2}{4} + \frac{(12)^2}{4} + \frac{(12)^2}{4} - 126.6 = 1.65$$

$$SS_{Tr} = \frac{(12)^2}{4} + \frac{(8)^2}{4} + \frac{(14)^2}{4} + \frac{(11)^2}{4} - 126.6 = 4.65$$

$$SS_E - SS_T - (SS_r + SS_c + SS_{tr})$$

$$= 10.4 - (0.15 + 1.65 + 4.65)$$

$$10.4 - 6.45 = 3.95$$

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SCHEMATIC OF THREE WAY ANOVA

Source of Variation	Degrees of freedom	SSR	MSSR	F
Rows	r-1=3	0.15	0.05	0.05 / 0.658 = 0.075
Columns	r-1=3	1.65	0.55	0.55 / 0.658 = 0.835
Treatments	r-1=3	4.65	1.55	1.55 / 0.658 = 2.355
Error	(r-1) (r-2) =6	3.95	0.658	
Total	r^2-1=15			

H_{o1} is accepted
H_{o2} is accepted
H_{o3} is accepted

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Conclusions

- Optimization helps in getting the perfect product with the help of designs of experiment.
- It has many advantages over traditional methods.
- Its tools include multiple regression equations, analysis of variance and several types of plots.

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