International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Formulation and Evaluation of Microspheres of Diclofenac Sodium

[1]Reetu Rani*, [2]Devender Chauhan, [3]Birender Singh, [4]Vineet Vashisth, [5]Sunil K. Batra

[1,2,3,4] *Faculty, Hindu College of Pharmacy, Sonipat, Haryana*

Submitted: 20-02-2023 Accepted: 28-02-2023

I. INTRODUCTION

[1] Oral drug delivery is the most preferred and convenient option as the oral route provides maximum active surface area among all drug delivery system for administration of various drugs. Usually conventional dosage form produces wide range of fluctuation in drug concentration in the bloodstream and tissues with consequent undesirable toxicity and poor efficiency. The maintenance of concentration of drug in plasma within therapeutic index is very critical for effective treatment. These factors as well as factors such as repetitive dosing and unpredictable absorption lead to the concept of oral controlled release drug delivery systems.

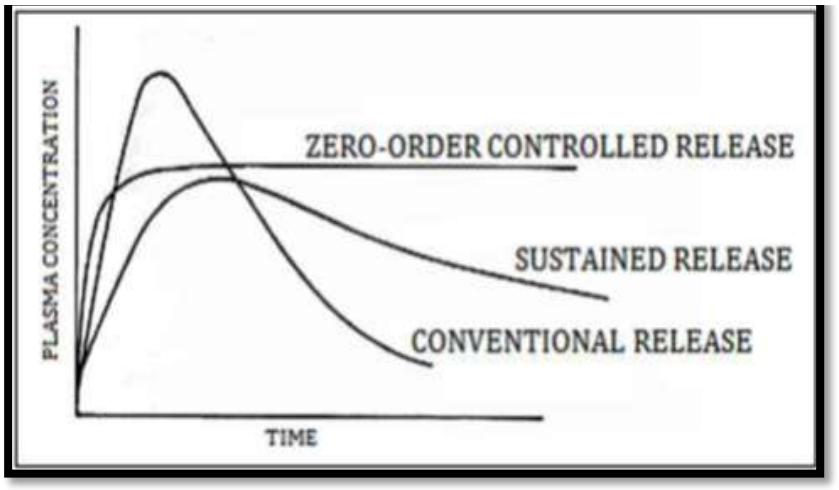


Figure 1: Plasma concentration time profile curve

[2] Controlled drug delivery system: Controlled release drug delivery (CRDD) has become the norm in dosage form design and intensive research has been undertaken in achieving better drug product effectiveness, reliability and safety. The purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under and overdosing. While these advantages can be significant, the potential disadvantages cannot be ignored viz. the possible toxicity or non bio-compatibility of the materials used, undesirable by-products of degradation, any surgery required to implant or remove the system, the chance of patient discomfort due to the delivery device, and the higher cost of controlled-release systems compared with traditional pharmaceutical formulations. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. Different types of controlled drug delivery systems are Liposomes, Niosomes, Nanoparticles, and Microspheres.

Sustained Controlled Drug Delivery System: Sustained release dosage forms are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose. The main aim of preparing sustained release formulations was intended to modify and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

improve the drug performance by increasing the duration of drug action, decreasing the frequency of dosing, decreasing the required dose employed and providing uniform drug delivery. Sustained release dosage form is a dosage form that releases one or more drugs continuously in predetermined pattern for a fixed period of time, either systemically or locally to specified target organ. Sustained release drug delivery system can be a major advance toward solving the problem concerning drugs that have a short half-life are eliminated quickly from blood circulation require frequent dosing. To avoid this problem, oral sustained release formulations have been developed in an attempt to release the drug slowly into the g.i.t and maintain a constant drug concentration for long period of time. There are various approaches for delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs.

[3, 4] Microspheres: Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system. Microspheres are solid spherical particles ranging in size from 1-1000µm. They are spherical free flowing particles consisting of proteins or synthetic polymers. The microspheres are free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature. There are two types of microspheres. Microcapsules and Micrometrics.

Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microspheres matrix.

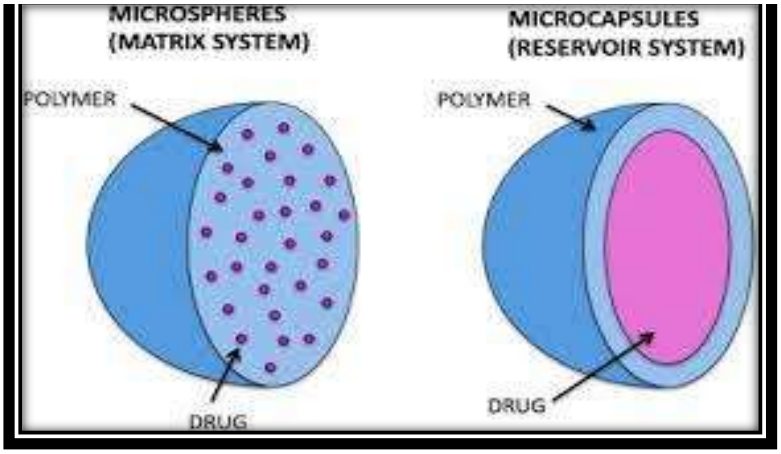


Figure 2: Types of microspheres

Advantages of microspheres

1. Reduce the dosing frequency and thereby improve the patient compliance.
2. Microsphere morphology allows a controllable variability in degradation and drug release.
3. Convert liquid to solid form & to mask the bitter taste.
4. Protects the GIT from irritant effects of the drug.
5. Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.
6. Controlled release delivery biodegradable microspheres are used to control drug release rates thereby decreasing toxic side effects, and eliminating the inconvenience of repeated injections.

[5, 6] Types of microspheres

1. **Bioadhesive microsphere:** Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc can be termed as bio adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and cause intimate contact with the absorption site and produce better therapeutic action. Bioadhesion is a term which broadly includes adhesive interactions with any

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

biological or biologically derived substance, and mucoadhesion is used when the bond is formed with a mucosal surface. Mucoadhesive drug delivery systems contain a mucoadhesive polymer that adheres to the gastric mucosal surface and prolong its gastric retention in the git. Mucoadhesive polymers are very useful excipients in the mucoadhesive DDS because of the capability to adhere to the mucous gel layer. The adhesion of polymers with mucous membrane may be mediated by hydration, bonding, or receptor mediated.

Mechanism of Bioadhesion:

Stage-1: An intimate contact between a bioadhesive and a membrane either from a good wetting of the bioadhesive and a membrane or from the swelling of bioadhesive.

Stage-2: Penetration of the bio-adhesive into the service of the tissue takes place.

Stage-3: Inter penetration of the chains of the bioadhesive with mucous takes place. Low chemical bounds can then settle.

The bonding between the mucus and the biological substance occurs chiefly through both physical and chemical interactions resulting from enlargement of the adhesive material and chemical bonds due to electro static interaction, hydrophobic interactions, hydrogen bonding and dispersion forces.

2. **Floating microspheres:** In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate for a prolonged period of time. This is mostly used approach of gastroretntive drug delivery system .Floating drug delivery systems (FDDS) are also known as hydrodynamically balanced systems. While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the stomach. After the release of the drug, the residual system is emptied from the stomach. This results in an increase in the gastric retention time and a better control of fluctuations in the plasma drug concentration in some cases. It also reduces chances of striking and dose dumping. These dosage forms are also known as gas powered system, which can float in the contents of the stomach and release the drug in a controlled manner for prolonged periods of time. The FDDS become an additional advantage for drugs that are absorbed primarily in the upper segments of GI tract, i.e., the stomach, duodenum and jejunum.

Table 1: Polymers used for the development of FDDS (26)

Delivery system	Polymer Type	
	Cellulosic hydrocolloids	**Gel-forming hydrocolloids and matrix former**
Microspheres/ Microparticles	Ethyl cellulose	Eudragit, polyacrylate, polymethacrylate, chitosan, gelatin, alginate
Tablets Tablets	HPMC, HPC, HEC, MC, NaCMC	Carbopol, carrageenan, gum guar, gum arabic, sodium alginate, polyarcylates
Capsules	HPMC, HPC, HEC, NaCMC	Sodium alginate, carbopol, agar

3. **Polymeric microspheres:** The different types of polymeric microspheres can be classified as follows and they are Biodegradable polymeric microspheres and Synthetic polymeric microspheres.

a. **Biodegradable polymeric microspheres:** The natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bio adhesive in nature. Biodegradable polymers prolong the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium, results in gel formation. The main drawback is in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release. However they provide wide range of application in microsphere based treatment.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

b. **Synthetic polymeric microspheres:** The synthetic polymeric microspheres are widely used in clinical application. Moreover they are used as bulking agent, fillers, embolic particles, drug delivery vehicles etc and proved to be safe and biocompatible. But the main disadvantages of these kinds of microspheres are they tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

4. **Radioactive microspheres:** Radio embolization therapy microspheres sized 10-30 nm are larger than the diameter of the capillaries and get tapped in first capillary bed when they come across. They are injected in the arteries that leads them to tumour of interest so all these conditions lead radioactive microspheres which deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery system, as radio activity is not released from microspheres but acts from within a radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, β emitters and γ emitters.

5. **Magnetic microspheres:** This kind of delivery system is very much important which localises the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials. Materials that are used for magnetic microspheres are chitosan, dextran etc.

Methods of preparation for microspheres: Different methods of preparation of microspheres are given below:

1. Single emulsion technique
2. Double emulsion technique
3. Polymerization techniques
4. Ionic gelation method
5. Phase separation coacervation technique
6. Spray drying and spray congealing method

[7] Experimental design: Design Expert is a piece of software designed to help with the design and interpretation of multi-factor experiments. In polymer processing, we might use the software to help us design an experiment to see how a property such as tensile strength varies with changes in the processing conditions - e.g. changes in rotor speed or ram pressure. The software offers a wide range of designs, including factorials, fractional factorials and composite designs The basic idea is to change all relevant factors over a set of planned experiments and then connect and interpret the results using mathematical models.

The various steps of the experimental design procedure are:

First, it is necessary to define the objective of the experimental design. It may be:

i. Screening design, in which the significant factors that influence the responses are identified.

ii. Optimization design, in which two or more significant factors are simultaneously optimized in order to find optimal experimental conditions.

The second step is concerning the selection of factors which are usually made based on the literature search, preliminary experiments and instrumental limitations.

The third step is to choose a response. Generally, responses measured may be both safety and effectiveness.

- **Selecting a Design:** Design Expert offers a large number of different classes of design. Design Expert offers a wide range of analytical and graphical techniques for model fitting and interpretation like Box-Behnken, central composite, factorial design etc.
- **Box-Behnken design:** A Box-Behnken experimental design was employed to statistically optimize the formulation parameters of microsphere preparation for maximum entrapment, optimum diameter and controlled release. The Box-Behnken design was specifically selected since it requires fewer treatment combinations than other design in cases involving 3 or 4 factors. The box-Behnken design is also rotable and contains statistical "missing corners" which may be useful when the experimenter is trying to avoid combined factor extremes. This property prevents a potential loss of data in those cases. Generation and evaluation of the statistical experimental design can be performed with the STATEASE, Design-Expert® version 11. A design matrix comprising of 17 experimental runs was conducted. An interactive second order polynomial was utilized to evaluate both the response variables:

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

$$Y= B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_1X_1^2 + B_2X_2^2 + B_3X_3^2.$$

Where B_0-B_3 are regression coefficients, X_1-X_3 are the factors studied and Y is the measured response associated with each factor level combination.

II. MATERIALS

Materials used in the formulations are Diclofenac sodium, Chitosan, Sodium tripolyphosphate, Ethanol, Glacial acetic acid, Potassium-dihydrogen orthophosphate, Di-sodium hydrogen phosphate. Diclofenac sodium belongs to group of medicines called non-steroidal anti-inflammatory drugs (NSAIDs). It is widely prescribed for mild to moderate pain, rheumatoid arthritis, osteoarthritis and other joint diseases. The selection of drug should be done on the basis of low aqueous solubility of drugs, High dosage frequency of drugs, Short half-life, Controlled drug delivery suitable drugs, higher adverse drug reaction drugs. It is nonselective cyclo-oxygenase inhibitor. It is poorly soluble in simulated gastric fluid and highly soluble in simulated intestinal fluid suggesting that the pH affects the solubility and absorption of diclofenac.

[8] Mechanism of action: Diclofenac sodium is the sodium salt form of diclofenac, a benzene acetic acid derivate and non steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory activity. Diclofenac sodium is a non-selective reversible and competitive inhibitor of cyclooxygenase (COX), subsequently blocking the conversion of arachidonic acid into prostaglandin precursors. This leads to an inhibition of the formation of prostaglandins that are involved in pain, inflammation and fever. Inhibition of COX-2 is thought to mediate the anti-pyretic, analgesic and anti-inflammatory actions of NSAIDs, but the simultaneous inhibition of COX-1 results in unwanted side effects, particularly those leading to gastric ulcers, most common side effect associated with nonselective COX inhibitors.

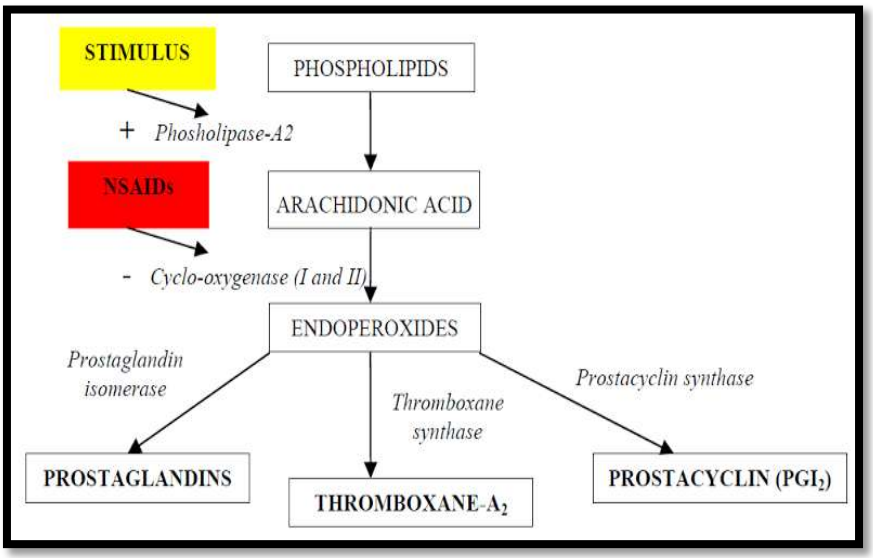


Figure 3: Formulation of prostaglandin via both cyclooxygenase enzymes

[9, 10] Identification of drug

- **Organoleptic properties of drug:** The organoleptic properties like general appearance, color, odor of drug were performed by visual observations and compared with standard of drug given in pharmacopoeia for identification of drug.
- **Solubility studies:** The solubility determination was made by adding solvent to glass tube containing accurately weighed quantity of solute. The solute was dissolved in each of investigating solvent at room temperature in tightly closed glass tubes. The system is vigorously shaken and examined visually for any undissolved solute particles. The solubility is expressed in terms of ratio of solute and solvent. The solubility study of diclofenac sodium was performed in methanol, ethanol, 0.1 N HCl, distilled water, phosphate buffer of pH 6.8 and 7.4 separately by keeping

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

the drug containing test tube on vortex mixture.

- **Determination of partition coefficient:** Partition coefficient was determined by shaking equal volumes of organic phase (n-octanol) and aqueous phase in a separating funnel. Since diclofenac sodium is a water insoluble drug, the drug solution was prepared in aqueous phase. A drug solution of 1 mg/ml was prepared in phosphate buffer pH 6.8 and 50 ml of this solution was taken in a separating funnel and shaken with an equal volume of n-octanol for 10 mins and allowed to stand for 24 hrs with intermittent shaking. The concentration of drug in aqueous phase was determined by UV spectrophotometer at 276 nm to get the partition coefficient value.

$$\text{Partition coefficient} = \frac{\text{Concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}}$$

- **Melting point determination:** Melting point of drug sample was determined by capillary method. The small amount of drug was taken in a capillary tube whose one end was sealed by flame. The capillary tube was placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed thrice and average value was taken.
- **Differential scanning colorimetry:** Differential scanning colorimetry was performed on a DSC Q200 V24 Build 116 with a thermal analyzer. Under nitrogen flow of 20 ml/min, sample weights 2 mg for diclofenac sodium were sealed in aluminium pan, and heated at a scanning rate of 10 ºC/min from 40 ºC to 300 ºC. An empty aluminium pan was used as reference.
- **Drug – excipients compatibility study:** 100 mg of each polymer / excipient was weighed accurately. To each of them 100 mg of drug was added. Four sets of prepared physical mixture were placed in glass vials which were then tightly sealed. Vials were kept at 25°C and 40°C for 4 weeks, after which the vials were opened and observed for caking, liquefaction, discoloration and odor or gas formation.
- **FT-IR spectroscopy:** In the preparation of microspheres, drug and excipients may interact as they are in close contact with each other, which could lead to the instability of drug. Preformulation studies regarding the drug excipients interaction are therefore very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility between diclofenac sodium and selected polymers. The pure drug, excipiens and drug with excipients were scanned separately by FT-IR spectrophotometer (Bruker). The spectrum was scanned over a frequency range 4000-400 cm^{-1}. All the powder samples were dried prior to obtaining any spectra in order to remove the influence of residual moisture.

Analytical method development

- **Standard calibration curve in methanol:** Accurately weighed quantity of diclofenac sodium (100 mg) was dissolved in 100 ml of methanol to get the stock solution Ⅰ (1000µg/ml). Further 1 ml of stock solution Ⅰ was diluted upto 10 ml to get the stock solution Ⅱ (100µg/ml). Now 1 ml of resulted solution was taken and diluted upto 10 ml to get the stock solution Ⅲ (10µg/ml). This stock solution was used to prepare further dilutions of standard solution. Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml of stock solution were transferred into a series of 10 ml of volumetric flasks and each were diluted upto 10 ml with methanol to produce the concentration ranging from 2-12 µg/ml. The absorbance of these solutions were measured at λ_{max} 276 nm by using methanol as blank.

- **Standard calibration curve in 0.1 N HCl:** Accurately weighed quantity of diclofenac sodium (100 mg) was dissolved in 5 ml of methanol and diluted upto 100 ml with 0.1 N HCl to get the stock solution Ⅰ (1000µg/ml). Further 1 ml of stock solution Ⅰ was diluted upto 10 ml to get the stock solution Ⅱ (100µg/ml). This stock solution was used to prepare further dilutions of standard solution. Aliquots of 0.5, 1, 1.5, 2, 2.5, 3 ml of stock solution were transferred into a series of 10 ml of volumetric flasks and each were diluted upto 10 ml with 0.1 N HCl to produce the concentration ranging from 5-30 µg/ml. The absorbance of these solutions was measured at λ_{max} 276 nm by using 0.1 N HCl as blank.

- **Standard calibration curve in phosphate buffer (pH 6.8):** Accurately weighed 100 mg of diclofenac sodium was dissolved in 100 ml of methanol to get the stock solution Ⅰ (1000 µg/ml). Further 1ml of stock solution Ⅰ was diluted up to 50 ml with pH 6.8, phosphate

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

buffer to get stock solution II (20µg/ml). This stock solution was used to prepare further dilutions of standard solution. Aliquots of 1, 2, 3, 4, 5, 6 ml were taken in 10 ml volumetric flasks and each was diluted upto 10 ml with pH 6.8, phosphate buffer to get the solutions of concentration ranging from 2-12 µg/ml. The absorbance of these solutions were measured at λ_{max} 276 nm keeping phosphate buffer (pH 6.8) as blank using double beam UV spectrophotometer.

- **Standard calibration curve in phosphate buffer pH 7.4:** Accurately weighed 100 mg of diclofenac sodium was dissolved in 100 ml of methanol to get the stock solution I (1000 µg/ml). Further 1ml of stock solution I was diluted up to 50 ml with pH 7.4, phosphate buffer to get stock solution II (20µg/ml). This stock solution was used to prepare further dilutions of standard solution. Aliquots of 1, 2, 3, 4, 5, 6 ml were taken in 10 ml volumetric flasks and each was diluted upto 10 ml with pH 7.4, phosphate buffer to get the solutions of concentration ranging from 2-12 µg/ml. The absorbance of these solutions were measured at λ_{max} 276 nm keeping phosphate buffer (pH 7.4) as blank using double beam UV spectrophotometer.

III. PREPARATION OF MICROSPHERES

The microspheres of diclofenac sodium were prepared by using chitosan as polymer and tripolyphosphate (TPP) as cross linking agent via ionotropic gelation technique. The ethanol and tween 80 were used as solvent system and surfactant respectively.

The ionic gelation process is commonly used to prepare chitosan nanoparticles because it is a very simple and mild method. These positively charged groups in chitosan can be chemically cross-linked with dialdehydes such as glutaraldehyde and ethylene glycol diglycidyl ether or physically cross-linked with multivalent anions derived from sodium tripolyphosphate (TPP), citrate, and sulphate. Both glutaraldehyde and ethylene glycol diglycidyl ether are toxic and can cause irritation to mucosal membranes. Non-toxicity and quick gelling ability of TPP are the important properties that make it a favorable cross-linker for ionic gelation of chitosan. Drug release from chitosan microparticles could be controlled by crosslinking the matrix using chemical crosslinking agents such as glutaraldehyde, NaOH and ethylene glycol diglycidyl ether. However, these chemical crosslinking agents have possibility of inducing undesirable effects**.** Various amounts of chitosan solutions were prepared by dissolving it in 1% acetic acid and tween 80 (2% v/v) was added into the solution as a surfactant. Core material, diclofenac sodium was dissolved in ethanol (2:10) due to its water-insoluble behavior and then this oil phase was mixed with aqueous phase (chitosan solution) by homogenizer at 5000 rpm for 20 min. The ratio of oil and aqueous phase was 1:10. O/W emulsion was dropped into TPP solutions by spray gun. After the crosslinking time, microparticles were washed with distilled water repeatedly and then vacuum dried for 12 h

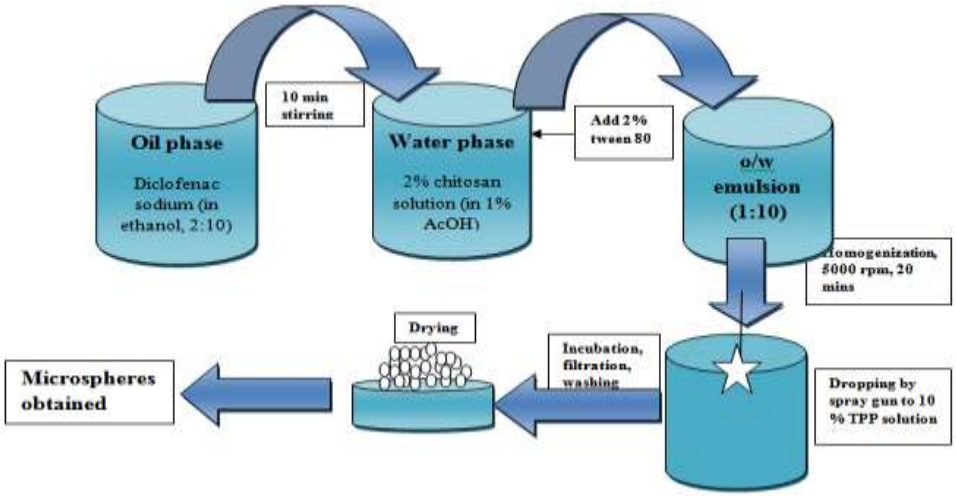


Figure 4: Process of preparation of microspheres

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Method of Preparation of microspheres

- **Preliminary trial for independent variables:** Chitosan concentration (A), tripolyphosphate concentration (B) and cross-linking time (C) were tried as independent variables for the preparation of microspheres.
- **Design of experiment (DOE) for preparation of preparation of microspheres:** A Box-Behnken design was used for exploring quadratic response surfaces and constructing polynomial models with Design-Expert® version 11. The three independent variables such as chitosan concentration (A), tripolyphosphate concentration (B) and cross-linking time (C) were selected on the basis of the preliminary studies carried out before the experimental design being implemented. The experimental design was applied to investigate the effect of different independent variables such as A, B and C. The interaction term shows how the response changes when three factors are changed simultaneously. The polynomial term is included to investigate non-linearity.

Table 2: Independent variables in Box-Behnken design

Correlation of actual and coded values				
Factor	**Coded value**	**Actual value**		
		Chitosan concentration (%)	**Tripolyphosphate Concentration (%)**	**Cross-linking time (min)**
Low	-1	0.5	5	20
Medium	0	1.25	10	40
High	+1	2	15	60

Table 3: Formulation selected using Box-Behnken design

S. No	Formulation code	Independent variables		
		A	**B**	**C**
1	F1	-1	-1	0
2	F2	1	-1	0
3	F3	-1	1	0
4	F4	1	1	0
5	F5	-1	0	-1
6	F6	1	0	-1
7	F7	-1	0	1
8	F8	1	0	1
9	F9	0	-1	-1
10	F10	0	1	-1
11	F11	0	-1	1
12	F12	0	1	1
13	F13	0	0	0
14	F14	0	0	0
15	F15	0	0	0
16	F16	0	0	0
17	F17	0	0	0

Table 4: Composition of different formulations

Formulation code	Drug (mg)	Chitosan (%w/v)	Tripolyphosphate (%w/v)	Ethanol (ml)
F1	100	0.5	5	0.5
F2	100	2	5	0.5
F3	100	0.5	15	0.5
F4	100	2	15	0.5
F5	100	0.5	10	0.5
F6	100	2	10	0.5
F7	100	0.5	10	0.5

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

F8	100	2	10	0.5
F9	100	1.25	5	0.5
F10	100	1.25	15	0.5
F11	100	1.25	5	0.5
F12	100	1.25	15	0.5
F13	100	1.25	10	0.5
F14	100	1.25	10	0.5
F15	100	1.25	10	0.5
F16	100	1.25	10	0.5
F17	100	1.25	10	0.5

[11, 12] Evaluation of formulation

- **Percentage yield:** The percentage yield was measured as actual weight of obtained microspheres divided by the total amount of all non-volatile material and drug that was used for the preparation of the microspheres.
- **Particle size analysis:** The particle size of the albumin microspheres were first evaluated using an optical microscope fitted with a calibrated eyepiece micrometer under a magnification of 403. The particle diameters of about 50 microspheres were measured randomly and the average particle size was determined.
- **Determination of encapsulation efficiency:** A quantity (100 mg) of the microparticles was placed in a beaker containing 100 ml of phosphate buffer (pH 6.8). The dispersion was vortexed repeatedly to break up the microparticles and cause them to discharge their contents completely. The solution was then filtered and analyzed spectrophotometrically at a wavelength of 276 nm using a UV-Vis spectrophotometer. The drug concentration in each batch of the microparticles was calculated from a Beers' plot previously determined for diclofenac sodium. An average of four determinations was taken as the mean drug content for each batch of microparticles

Flow properties

a) Angle of repose: It is defined as the angle of heap to the horizontal plane. Angle of repose was determined by using fixed funnel method. Specified amount of powder drug was transferred to the funnel keeping the orifice of the funnel blocked by thumb. When powder was cleared from funnel then measured its angle of repose.

Angle of repose (θ) = $\tan^{-1}$ h/r

Where, h is height of the heap, r is radius of the microspheres heap that is formed after making the microspheres flow from the glass funnel.

b) Bulk density & tapped density: A fixed weight of the powder prepared microspheres was poured in a 25 ml graduated cylinder, the powder was allowed to settle with no outer force and the volume occupied was measured as V_B (initial bulk volume).The cylindrical graduate was then tapped on a plan surface at a one inch distance till a constant volume was obtained. The tapped volume of the powder was then recorded as (V_T). The initial and tapped bulk densities were then calculated according to the following equation:

Bulk Density = M / V_B

Tapped Bulk Density = M / V_T

Where, M is mass of microspheres

c) Compressibility index or Carr's Index: Compressibility index or Carr's Index value of microspheres was computed according to the following equation:

$$\textbf{Carr's index} = \frac{\textbf{Tapped density} - \textbf{Bulk density}}{\textbf{Tapped density}} \times 100$$

Lower value of compressibility indicated better flow.

d) Hausner's ratio: Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation. It is an indirect index of ease of measuring of powder flow. Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25).

$$\textbf{Hausner's ratio} = \frac{\textbf{Tapped density}}{\textbf{Bulk density}}$$

In vitro Drug Release: The diclofenac sodium release study of the microspheres from each formulation was performed in the simulated gastrointestinal condition by the pH-change method at 37°C. The media of pH 1.2 (0.1N HCl) was chosen to represent the gastric condition and the condition in the small intestine was represented by

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

pH 6.8. Microspheres (100 mg) were enclosed in a teabag and placed into a beaker that contained 900 ml of the dissolution medium. The beaker was placed on a horizontal shaking water bath maintained at 37^0C at a speed of 50 rpm. In the dissolution model with pH-change, the pH of the dissolution medium was kept at 0.1N HCl (pH 1.2) for the first 2 hours. Then, the dissolution medium was changed to phosphate buffer 6.8 and maintained up to 12 hrs. 5ml aliquot was withdrawn from the dissolution medium at specific time intervals and analyzed by UV-visible spectrophotometer for diclofenac contents at a wavelength of 276nm. After every withdrawal, 5ml of freshly prepared pre-warmed dissolution medium was added to the vessel in dissolution apparatus to keep its volume constant. All experiments were performed in triplicate. The amount of diclofenac sodium released was calculated by interpolation from a calibration curve containing increasing concentrations of diclofenac sodium. A cumulative correction was made for the previously removed sample to determine the total amount of drug release.

[13,14] Drug release kinetics: In the present study, raw data obtained from the in vitro release studies were analyzed, wherein data were fitted to different equations and kinetic models to calculate the percent drug release and release kinetics of diclofenac sodium from microspheres. The results of in vitro release profile of optimized batch were fitted into four models of data treatment as follows:

- Cumulative percent drug released versus time (zero - order kinetic model)
- Log cumulative percent drug remaining versus time (first order kinetic model)
- Cumulative percent drug released versus square root of time (higuchi's model)
- Log cumulative percent drug released versus log time (korsmeyer's-peppas model)

Zero order release kinetics: It refers to the process of constant drug release from a drug delivery device independent of the concentration. A zero order release would be predicted by the following equation:

$$\mathbf{Q = Q_0 + K_0 t}$$

Where = amount of drug released
Q_0= initial amount of drug in solution
K_0 = zero order release constant
The data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero order release kinetics, with a slope equal to K_0.

First order release kinetics: The first order equation describes the release from system where release rate is concentration dependent. A first order release would be predicted by the following equation:

$$\mathbf{Log\ C_t = Log\ C_0 - k_t / 2.303}$$

Where, C_0 = initial concentration of drug
C_t = concentration of drug in solution at time t
K_t= first order rate constant
The equation predicts a first order dependence on the concentration gradient between the static liquid layer next to the solid surface and the bulk liquid. When the data plotted as log cumulative percent drug remaining versus time yield a straight line, it indicates that the release follows first order kinetics.
Application: This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices.

Higuchi's model: The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1963. This model is applicable to study the release of water soluble and low soluble drugs incorporated in semisolid and solid matrices.
This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always attained in the release environment.
Accordingly, model expression is given by the equation:

$$\mathbf{ft = Q = A\sqrt{D(2C - Cs)\,CsT}}$$

where, Q is the amount of drug released in time t per unit area A, C is the drug initial concentration, Cs is the drug solubility in the matrix media and D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance.

Korsmeyer - Peppas Model: Korsmeyer et al (1983) derived a simple relationship which described drug release from a polymeric system. The release rate can be calculated by the equation:

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

$M_t / M_\alpha = Kt^n$
Where,
M_t / M_α = fraction of drug released at time t
K= rate constant incorporating structural and geometric characteristics of the delivery system
n = release exponent indicative of the mechanism of transport of drug through the polymer.

Surface Morphology : The scanning electron microscope (SEM) is a type of electron microscope that gives images of the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The microparticles were coated uniformly with gold palladium by using a sputter coater after fixing the sample in individual stubs. The operating parameters were an acceleration voltage of 10.0 kV and chamber pressure of 15.9 mm.

Comparison of optimized formulation with marketed formulation: For comparison the in vitro dissolution of optimized formulation was compared with the marketed formulation (voltaren).

IV. RESULTS AND DISSCUSSION

Preformulation study

- **Organoleptic properties of diclofenac sodium:** The organoleptic studies of diclofenac sodium were performed for physical description.

Table 5: Physical description of drug

Nature	Soft powder
Color	White
Odour	Odorless

- **Solubility analysis:** The solubility of pure drug in 10 ml of solvent was carried out and it was found that the drug is freely soluble in methanol, soluble in ethanol (95%) and practically insoluble in HCl, ether, chloroform and toluene.

- **Differential scanning calorimetric (DSC):** The DSC thermo gram of pure diclofenac sodium is shown in figure 5.2. The thermo gram shows an exothermic peak at 281.6°C.

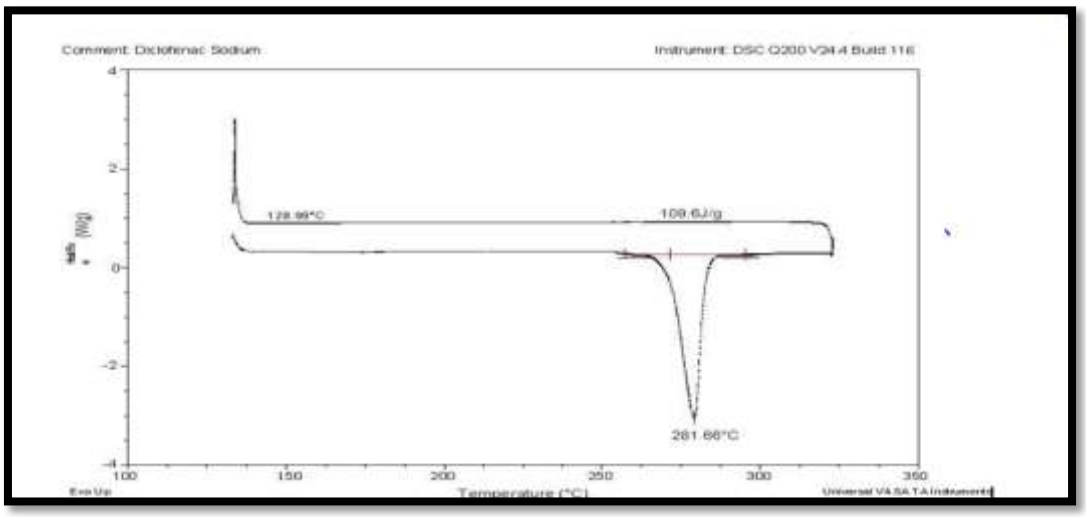


Figure 5: DSC thermo gram of diclofenac sodium

- **Drug – excipients compatibility study:** Drug was subjected to the compatibility study with different excipients at 25°C/60% RH and 40°C/75% RH and was observed for physical changes (color change, liquefication, lump formation and odor). After 2-4 weeks, no physical changes was observed in the vials containing drug and excipient which shows that drug is compatible with selected excipients.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Table 6: Drug – Excipients compatibility study

At temperature 25°C/60% RH					At temperature 40°C/75% RH			
Drug + excipients (1:1)	**1st week**	**2nd week**	**3rd week**	**4th week**	**1st week**	**2nd week**	**3rd week**	**4th week**
Drug + Chitosan	NC	NC	NC	NC	NC	NC	NC	NC
Drug + TPP	NC	NC	NC	NC	NC	NC	NC	NC
Drug + Chitosan +TPP	NC	NC	NC	NC	NC	NC	NC	NC

Drug: Diclofenac sodium
NC refers to no change
No physical incompatibilities were found with any of the excipients.

- **Compatibility study by FT-IR spectroscopy:** Chemical interaction between drug and polymeric material was studied by using FT-IR spectroscopy. The IR spectra of pure drug, chitosan, tripolyphpsphate (TPP) and physical mixture are shown in figure 5.2 to 5.5, which indicates no chemical interaction between diclofenac sodium and excipients when compared with the standard IR spectra of pure drug and excipients. From IR spectra, it is clearly evident that there were no interactions of drug with the polymer as the functional groups responsible for therapeutic action was unaltered. All the peaks were observed in the finger print region of FT-IR spectra. This proves that there is no drug – excipients interaction.

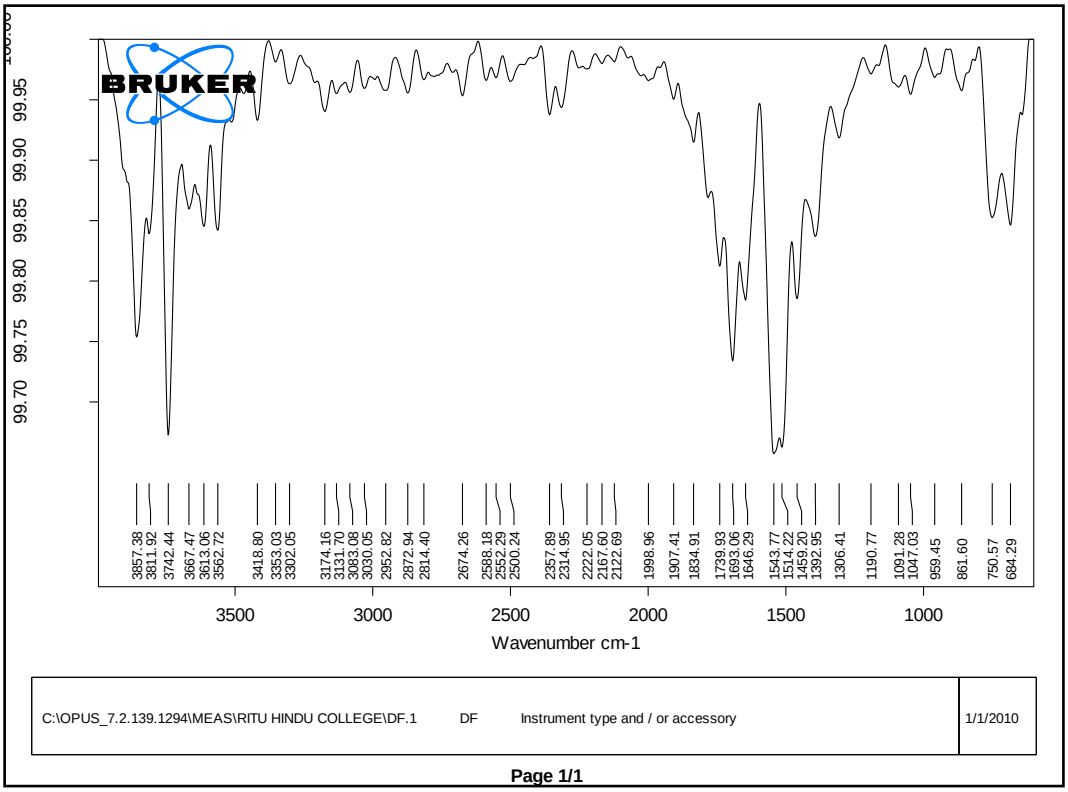


Figure 6: FT-IR spectra of diclofenac sodium

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Table 7: Interpretation for the IR absorption bands of diclofenac sodium, chitosan, TPP

Sample	Standard IR peaks (cm^{-1})	Obtained IR peaks (cm^{-1})	Assignment
Diclofenac sodium	1500-1700	1543.77	C=O group
	1180-1360	1306.41	C-N stretching
	1400-1600	1514.22	C=C stretching
	550-850	750.57	C-Cl group
	1050-1250	1190.77	C-O-C stretching vibration

Analytical method development

- **Determination of absorption maxima (λ_{max}) of diclofenac sodium in methanol:** The solution containing 10 µg/ml was scanned between 200-400nm.The λ_{max} was found to be 276 nm which indicates purity of drug diclofenac sodium. Absorption maxima of diclofenac sodium.

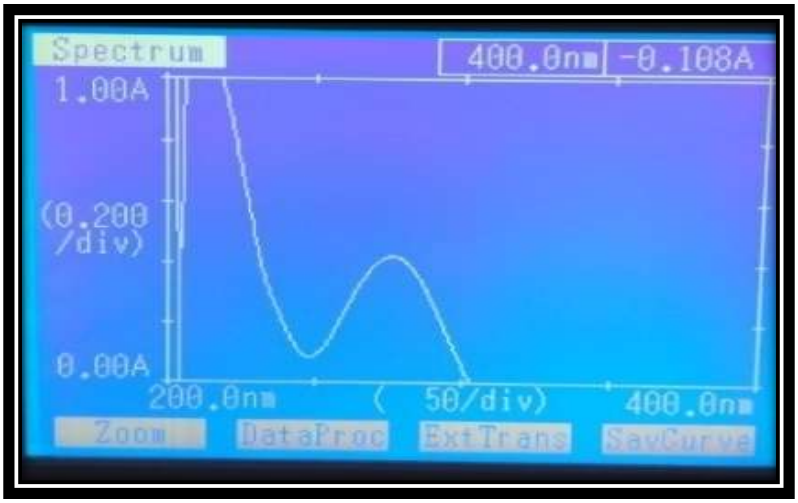


Figure 7: λ_{max} of diclofenac sodium in methanol

The absorbance of the each dilution was measured at 276 nm in Shimazdu UV- 1700 spectrophotometer against methnol as blank.

Table 8: Concentration and corresponding absorbance in methanol

S. No.	Concentration (µg/ml)	Absorbance at 276 nm
1	2	0.065 ± 0.001
2	4	0.128 ±0.002
3	6	0.2 ± 0.01
4	8	0.261 ± 0.001
5	10	0.336 ± 0.003
6	12	0.389 ± 0.001

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

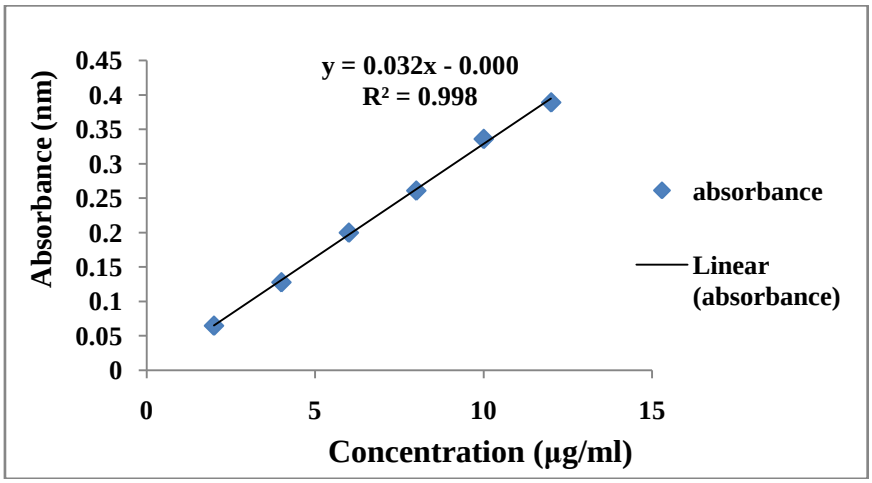


Figure 8: Standard plot of diclofenac sodium in methanol

The graph obeyed beer lamberts law in this selected concentration range. The calibration equation for straight line was observed to be y = 0.032x with correlation coefficient as 0.998, which was further used for determination of unknown samples.

- **Determination of absorption maxima (λ_{max}) of diclofenac sodium in 0.1 N HCl:** UV scan of diclofenac sodium was done in 0.1 N HCl and λ_{max} was observed at 276 nm.

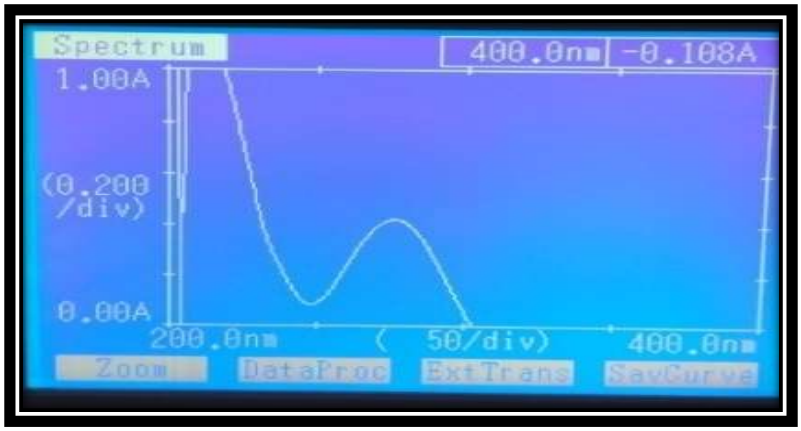


Figure 9: λ_{max} of diclofenac sodium in 0.1 N HCl

he absorbance of the each dilution was measured at λ_{max} 276 nm in Shimazdu UV- 1700 spectrophotometer against 0.1 N HCl as blank.

Table 9: Concentration and corresponding absorbance in 0.1 N HCl

S. No.	Concentration (µg/ml)	Absorbance at 276 nm
1	5	0.146 ± 0.001
2	10	0.304 ± 0.003
3	15	0.452 ± 0.002
4	20	0.621± 0.003
5	25	0.727± 0.0005
6	30	0.887± 0.004

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

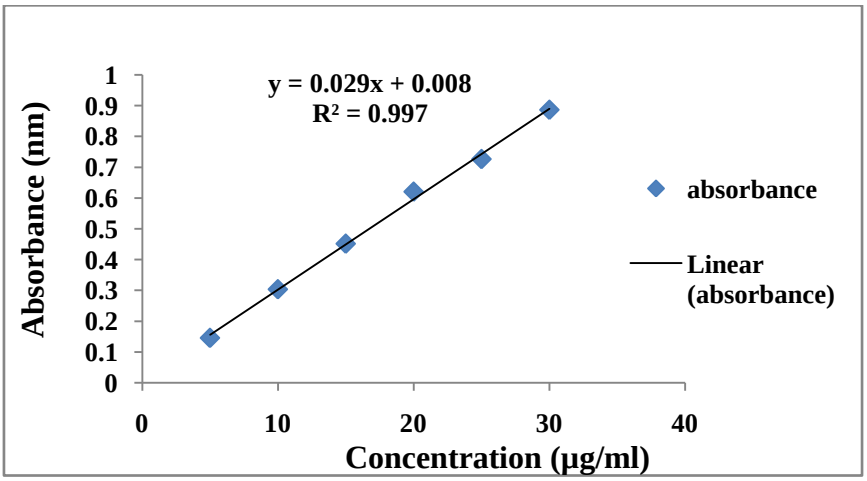


Figure 10: Standard plot of diclofenac sodium in 0.1 N HCl

The graph obeyed beer lamberts law in this selected concentration range. The calibration equation for straight line was observed to be y = 0.029x+0.008 with correlation coefficient as 0.997, which was further used for determination of unknown samples.

- **Determination of absorption maxima (λ_{max}) of diclofenac sodium in Phosphate buffer (pH 6.8)**

The solution containing 20 µg/ml was scanned between 200-400nm.The λ_{max} was found to be 276 nm which indicates purity of drug diclofenac sodium. Absorption maxima of diclofenac sodium was represented in figure:

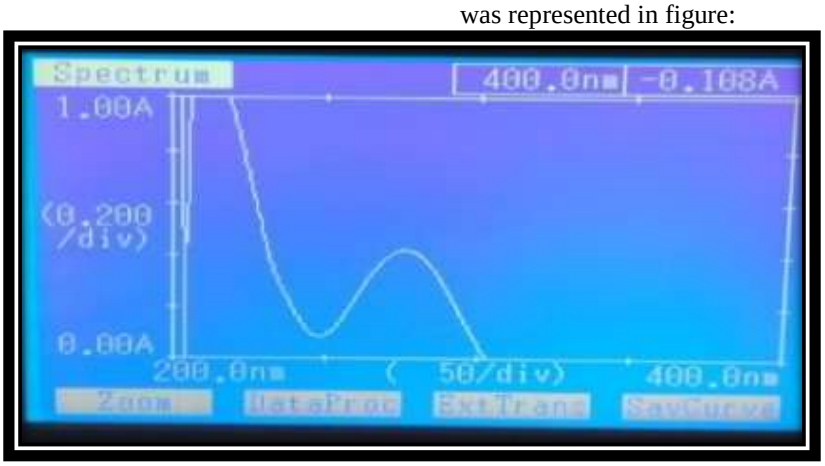


Figure 11: λ_{max} of diclofenac sodium in Phosphate buffer (pH 6.8)

The absorbance of the each dilution was measured at 276 nm in Shimazdu UV- 1700 spectrophotometer against phosphate buffer (pH 6.8) as blank.

Table 10: Concentration and corresponding absorbance in Phosphate buffer (pH 6.8)

S.No.	Concentration (µg/ml)	Absorbance at 276 nm
1	2	0.077 ± 0.001
2	4	0.17 ± 0.01
3	6	0.255 ± 0.003
4	8	0.357± 0.001
5	10	0.432 ± 0.004
6	12	0.513 ± 0.002

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

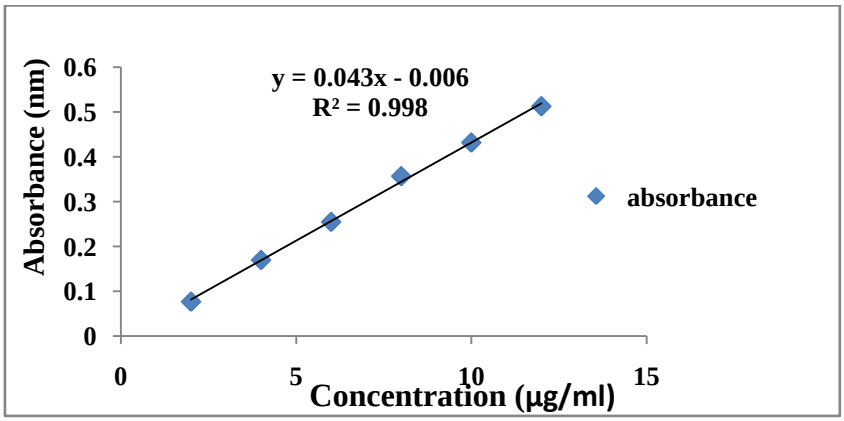


Figure 12: Standard plot of diclofenac sodium in Phosphate buffer (pH 6.8)

The graph obeyed beer lamberts law in this selected concentration range. The calibration equation for straight line was observed to be y = 0.043x-0.006 with correlation coefficient as 0.998, which was further used for determination of unknown samples.

- **Determination of λ_{max} of diclofenac sodium in phosphate buffer (pH7.4):** The solution containing 20 µg/ml was scanned between 200-400nm.The λ_{max} was found to be 276 nm which indicates purity of drug diclofenac sodium. Absorption maxima of diclofenac sodium was represented in figure:

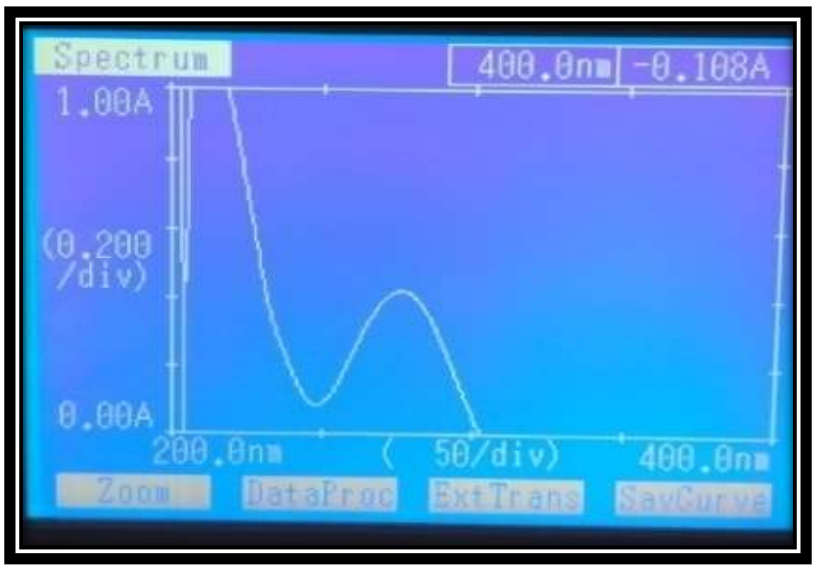


Figure 13: λ_{max} of diclofenac sodium in Phosphate buffer (pH 7.4)

The absorbance of the each dilution was measured at 276 nm in Shimazdu UV- 1700 spectrophotometer against phosphate buffer (pH 7.4) as blank.

Table 11: Concentration and corresponding absorbance in Phosphate buffer (pH 7.4)

S.No.	Concentration (µg/ml)	Absorbance (nm)
1	2	0.06 ± 0.004
2	4	0.125 ± 0.002
3	6	0.18 ± 0.01
4	8	0.242 ± 0.003
5	10	0.306 ± 0.001

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

6	12	0.378 ± 0.003

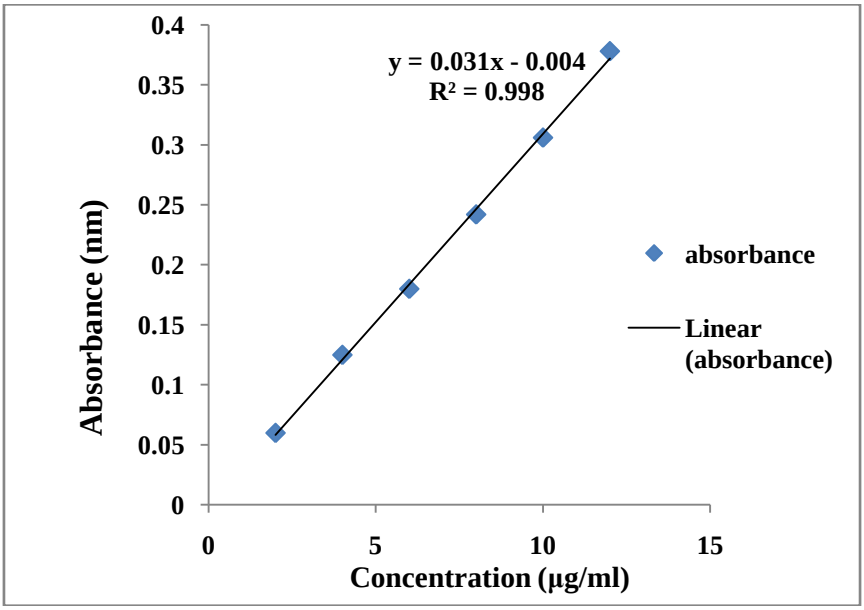


Figure 14: Standard plot of diclofenac sodium in Phosphate buffer (pH 7.4)

The graph obeyed beer lamberts law in this selected concentration range. The calibration equation for straight line was observed to be y = 0.031x-0.004 with correlation coefficient as 0.998, which was further used for determination of unknown samples.

Preliminary trials for independent variables: The important factors affecting the efficiency of microspheres were selected from literature and their working ranges were selected by performing trials. Based on literature, the main three independent variables of microspheres were selected viz. chitosan concentration, tripolyphosphate concentration and crosslinking time.

Formulation using RSM: Experimental design is a powerful and efficient tool for the development of a formulation. The experimental design allows for studying various processing parameters influencing the selected responses with lowest number of experiment, thereby reducing the time required in the development of work. Response surface methodology (RSM) is a widely employed approach in the development and optimization of drug delivery system. The design of experiment (DOE), methodology involving various types of experimental design, generation of polynomial equations and mapping of the responses over the experimental domain is used to determine the optimum formulations. RSM is widely used when only a few significant factors are involved in optimization. The technique requires minimum experimentation and time, thus providing to be far more effective and cost effective than the conventional methods of formulating dosage form.

Evaluation tests of microspheres

- **Percentage yield:** The percentage yield of all the 17 formulations was found to be in range 70.2 % to 84.6%.
- **Particle size** The particle size increases with increase in polymer concentration but size decreases with increase in cross-linking time. Particle size of all 17 formulations was in range of 192 to 307µm.
- **Entrapment efficiency:** As the concentration of chitosan increases, the drug entrapment efficiency also increases but as the tripolyphosphate concentration and crosslinking time increases, the entrapment efficiency decreases. The entrapment efficiency of different batches was in range 30.6 to 65.5%.
- **Angle of repose:** Angle of repose for all the formulations was found to be in range 24.8° to 27.7° which indicates good flow property
- **Bulk density:** The bulk density of all the 17 formulations was in range 0.540 to 0.670 g/cm^3.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

- **Tapped density:** The tapped density of all the 17 formulations was in range 0.578 to 0.769 g/cm^3.
- **Carr's index:** The Carr's index of all the 17 formulations was found to be in range 6.57 to 18.4 which is well acceptable within the limits and indicates good flow ability.
- **Hausner's ratio**: Hausner's ratio of all the 17 formulations was in range 1.06 to 1.22 which is well acceptable within the limits i.e. <1.25.

In-vitro drug release: Release studies are required for predicting the reproducibility of rate and duration of drug release. The importance of polymer dissolution on drug release from the matrices has been known for ensuring the sustained release performance. For in vitro release studies we employed paddle type dissolution apparatus. The results indicates that increase in the concentration of chitosan slows the drug release. The optimized formulation showed best results in 12 hrs.

Figure 15: Dissolution apparatus

Table 12: In-vitro drug release of F1 to F17 formulations

% Cumulative drug release												
Formulation code	**Time in hrs**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
F1	12.2	14.8	18.9	22.8	25.6	30.08	34.7	38.2	42.5	47.8	52.6	60.4
F2	12.6	14.2	18.3	22.6	25.2	31	34.2	38.1	42.1	47.2	52.7	60.2
F3	13	14.3	18.6	22.4	25.3	31.5	34.5	38.6	42.6	47.9	52.4	59.8
F4	7.02	9.08	11.8	15.2	19.5	22	26.4	30.9	35.2	38.2	44	48.0

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

F5	7.4	9.8	12.2	15.5	19.8	22.2	26.7	31.2	35.8	38.9	44.5	48.9
F6	7.8	9.2	11.9	15.9	20	22.4	26.9	32.4	35.6	39	44.2	48.6
F7	7.6	9.6	11.4	15.7	20.2	22	26.3	32.5	36.2	39.9	44.6	49
F8	8.1	10	12.5	16.02	20.6	22.8	26,6	32.8	36.5	39.5	44.8	49.2
F9	8.3	10.02	12.3	16.1	20.9	23.2	26.5	32.3	36.8	39.2	44.5	49.7
F10	8.6	10.05	12.5	16.3	21	23.6	26.8	32.5	37	39.9	45.0	50.2
F11	8.9	10.08	12.8	16.5	21.2	23.8	27.1	32.8	37.2	40	45.2	50.6
F12	9.2	10.1	13	17.2	21.5	24	27.5	33.2	37.9	40.5	45.9	51.5
F13	10.0	12.1	17	20.6	23.4	28.2	32.7	35.9	39.5	42.8	47.2	52
F14	10.1	12.3	17.2	20.4	24.2	28.7	32.9	36	39.9	43.0	47.8	52.0
F15	10.3	12.5	17.5	20.8	23.9	28.5	33	36.2	39.6	43.4	47.6	52.3
F16	10.5	12.8	17.4	21	23.6	28.8	33.4	36.5	39.9	43.8	48	52.9
F17	10.8	13.2	17.8	21.2	24.4	29.0	33.6	36.3	40	43.9	48.5	52.6

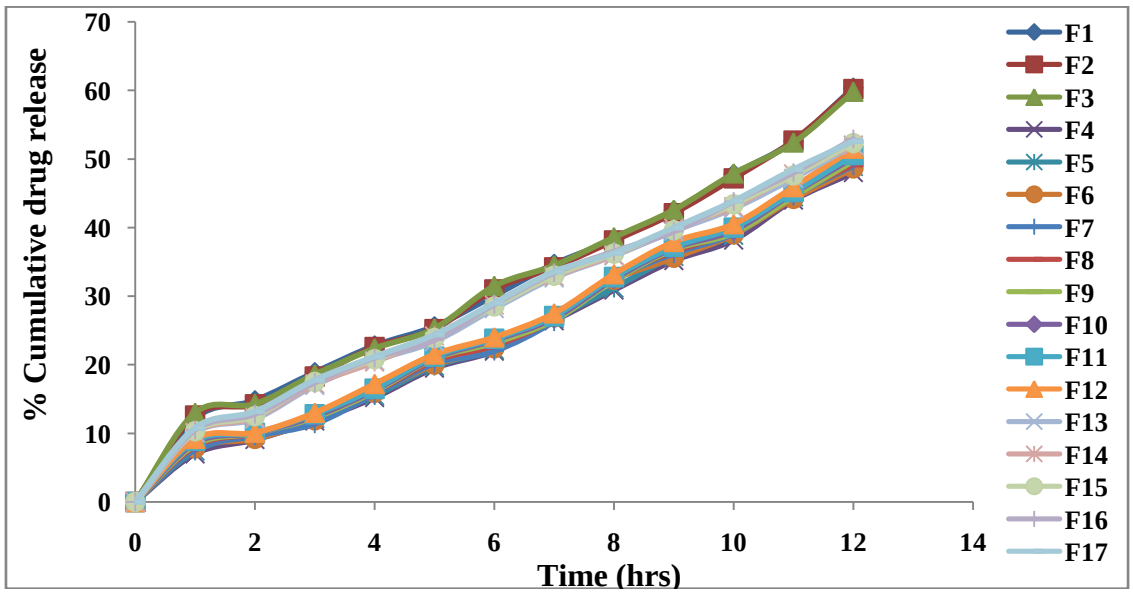


Figure 16: In- vitro dissolution profile of microspheres of diclofenac sodium during 12 hrs for all 17 formulations

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Effect of polymer concentration on drug release: The release of drug from microspheres prepared with the 1.25% and 2% chitosan solution (F13 and F4) were lower than those produced with the 0.5% chitosan solution (F1) as shown in figure 5.25. These results indicate that the release behavior of drug is relative to viscosity of chitosan solution. The increased viscosity of chitosan solution forms relatively strong walls of microparticles upon interaction with TPP. High crosslinking density of TPP-chitosan matrix resulted in less swelling ability, therefore the release of drug decreased.

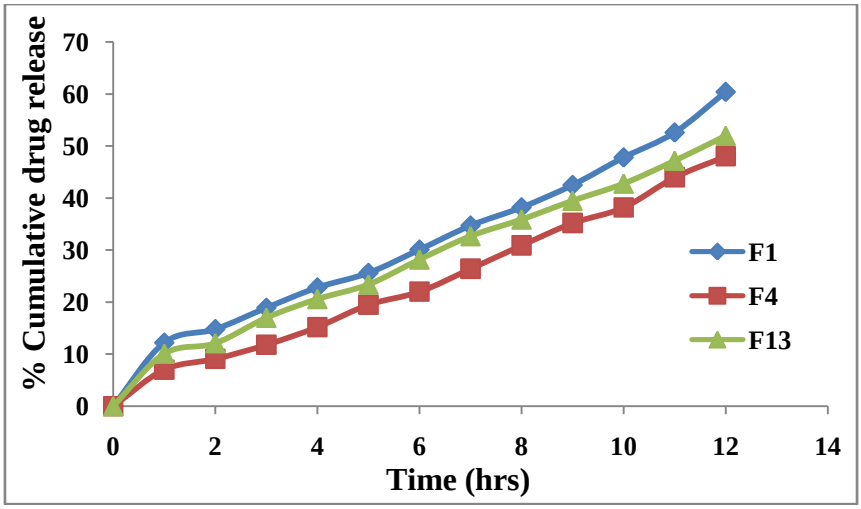


Figure 17: Effect of chitosan concentration on release profile of microspheres of diclofenac sodium during 12 hrs for formulation F1, F4 and F13

Response analysis of optimization: Statistical validation of polynomial equation generated by Design- Expert version 11 was established on the basis of ANOVA provision in the software. A total of 17 runs (F1-F17) were generated. The 3D response surface plots were drawn using this software. The resultant experimental data of response properties were compared with that of predicted values.

Table 13: The composition and observed responses from randomized runs in Box-Behnken Design

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
		A: chitosan conc. %	B: TPP conc. %	C: crosslinking time min	Particle size µm	Entrapment efficiency %
1	7	-1.000	-1.000	0.000	219.4	44
2	9	1.000	-1.000	0.000	275.4	55
3	10	-1.000	1.000	0.000	192.4	30.6
4	16	1.000	1.000	0.000	276	62
5	14	-1.000	0.000	-1.000	231	47.9
6	17	1.000	0.000	-1.000	299.3	63.3
7	4	-1.000	0.000	1.000	218.6	32.2
8	3	1.000	0.000	1.000	307	62
9	15	0.000	-1.000	-1.000	262.3	62
10	6	0.000	1.000	-1.000	248.6	58.8
11	5	0.000	-1.000	1.000	251.5	53.1
12	13	0.000	1.000	1.000	240.3	38.1
13	2	0.000	0.000	0.000	278.3	65.5
14	12	0.000	0.000	0.000	282	64.2
15	11	0.000	0.000	0.000	282.4	62.2
16	1	0.000	0.000	0.000	284.7	59.4

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

17	8	0.000	0.000	0.000	281.2	58.8

- **Fit Summary Response 1: Particle size**

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0008	0.0003	0.6447	0.5314	
2FI	0.8762	0.0001	0.5675	0.1751	
Quadratic	**< 0.0001**	**0.1103**	**0.9880**	**0.9351**	**Suggested**
Cubic	0.1103		0.9946		Aliased

- **Sequential Model Sum of Squares [Type I]**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs. Total	1.155E+06	1	1.155E+06			
Linear vs. Mean	11373.98	3	3791.33	10.68	0.0008	
2FI vs. Linear	293.00	3	97.67	0.2260	0.8762	
Quadratic vs. 2FI	**4238.01**	**3**	**1412.67**	**117.53**	**< 0.0001**	**Suggested**
Cubic vs. Quadratic	62.75	3	20.92	3.91	0.1103	Aliased
Residual	21.39	4	5.35			
Total	1.171E+06	17	68859.03			

- **Model Summary Statistics**

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	18.84	0.7114	0.6447	0.5314	7491.72	
2FI	20.79	0.7297	0.5675	0.1751	13189.62	
Quadratic	**3.47**	**0.9947**	**0.9880**	**0.9351**	**1037.46**	**Suggested**
Cubic	2.31	0.9987	0.9946		*	Aliased

Case(s) with leverage of 1.0000: PRESS statistic not defined.
Focus on the model maximizing the **Adjusted R^2** and the **Predicted R^2**.
Select the highest order polynomial where the additional terms are significant and the model is not aliased.

- **Lack of Fit Tests**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	4593.77	9	510.42	95.46	0.0003	
2FI	4300.77	6	716.79	134.06	0.0001	
Quadratic	**62.75**	**3**	**20.92**	**3.91**	**0.1103**	**Suggested**
Cubic	0.0000	0				Aliased
Pure Error	21.39	4	5.35			

The selected model should have insignificant lack-of-fit.

- **ANOVA for Quadratic model**

Response 1: Particle size

Table 14: ANOVA results for particle size

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	15905.00	9	1767.22	147.02	< 0.0001	significant
A-chitosan conc.	10974.21	1	10974.21	912.99	< 0.0001	
B-TPP conc.	328.96	1	328.96	27.37	0.0012	
C-crosslinking time	70.81	1	70.81	5.89	0.0456	
AB	190.44	1	190.44	15.84	0.0053	
AC	101.00	1	101.00	8.40	0.0230	
BC	1.56	1	1.56	0.1300	0.7291	
A^2	803.02	1	803.02	66.81	< 0.0001	

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

B^2	3094.54	1	3094.54	257.45	< 0.0001	
C^2	65.20	1	65.20	5.42	0.0527	
Residualv	84.14	7	12.02			
Lack of Fit	62.75	3	20.92	3.91	0.1103	not significant
Pure Error	21.39	4	5.35			
Cor Total	15989.14	16				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**
The **Model F-value** of 147.02 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.
P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
The **Lack of Fit F-value** of 3.91 implies the Lack of Fit is not significant relative to the pure error. There is a 11.03% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

- **Fit Statistics**

Std. Dev.	**3.47**	R^2	**0.9947**
Mean	260.61	**Adjusted R^2**	0.9880
C.V. %	1.33	**Predicted R^2**	0.9351
		Adeq Precision	42.3504

The **Predicted R^2** of 0.9351 is in reasonable agreement with the **Adjusted R^2** of 0.9880; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 42.350 indicates an adequate signal. This model can be used to navigate the design space.

- **Coefficients in Terms of Coded Factors**

Table 15: Coefficient influencing particle size

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	281.72	1	1.55	278.05	285.39	
A-chitosan conc.	37.04	1	1.23	34.14	39.94	1.0000
B-TPP conc.	-6.41	1	1.23	-9.31	-3.51	1.0000
C-crosslinking time	-2.98	1	1.23	-5.87	-0.0765	1.0000
AB	6.90	1	1.73	2.80	11.00	1.0000
AC	5.03	1	1.73	0.9259	9.12	1.0000
BC	0.6250	1	1.73	-3.47	4.72	1.0000
A^2	-13.81	1	1.69	-17.81	-9.81	1.01
B^2	-27.11	1	1.69	-31.11	-23.11	1.01
C^2	-3.93	1	1.69	-7.93	0.0603	1.01

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

- **Final Equation in Terms of Coded Factors**

Particle size =

+281.72

+37.04 A

-6.41 B

-2.98 C

+6.90 AB

+5.03 AC

+0.6250 BC

-13.81 A^2

-27.11 B^2

-3.93 C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

- **Final Equation in Terms of Actual Factors**

Particle size =

+118.47472

+78.96111 chitosan conc.

+17.85550 TPP conc.

+0.157000 crosslinking time

+1.84000 chitosan conc. * TPP conc.

+0.335000 chitosan conc. * crosslinking time

+0.006250 TPP conc. * crosslinking time

-24.55111 chitosan conc.^{2}

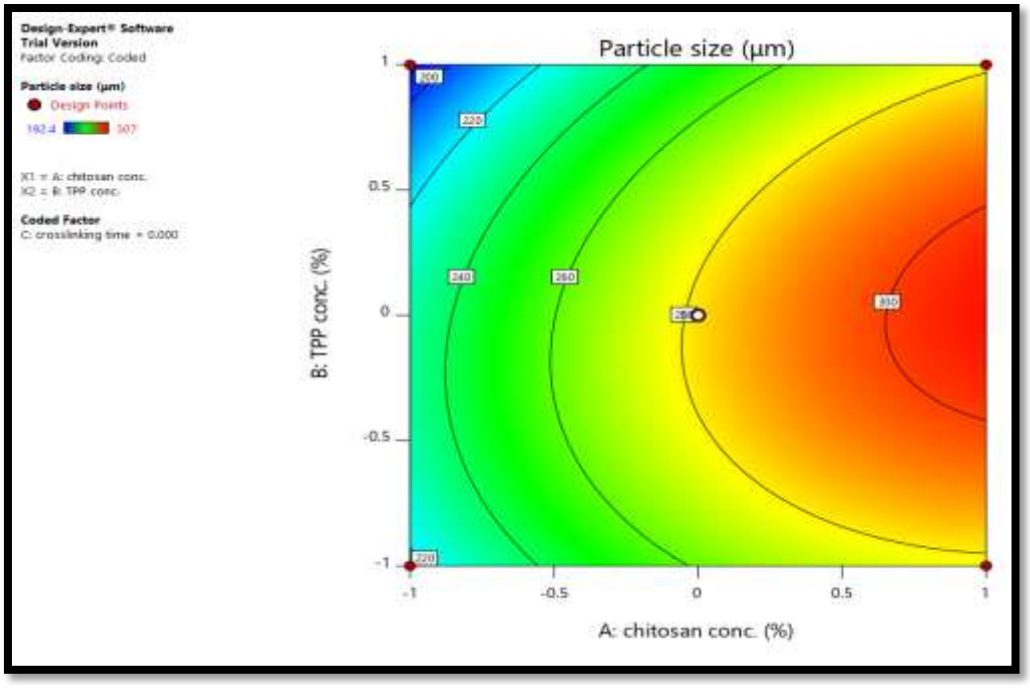
-1.08440 TPP conc.^{2}

-0.009837 crosslinking time^2

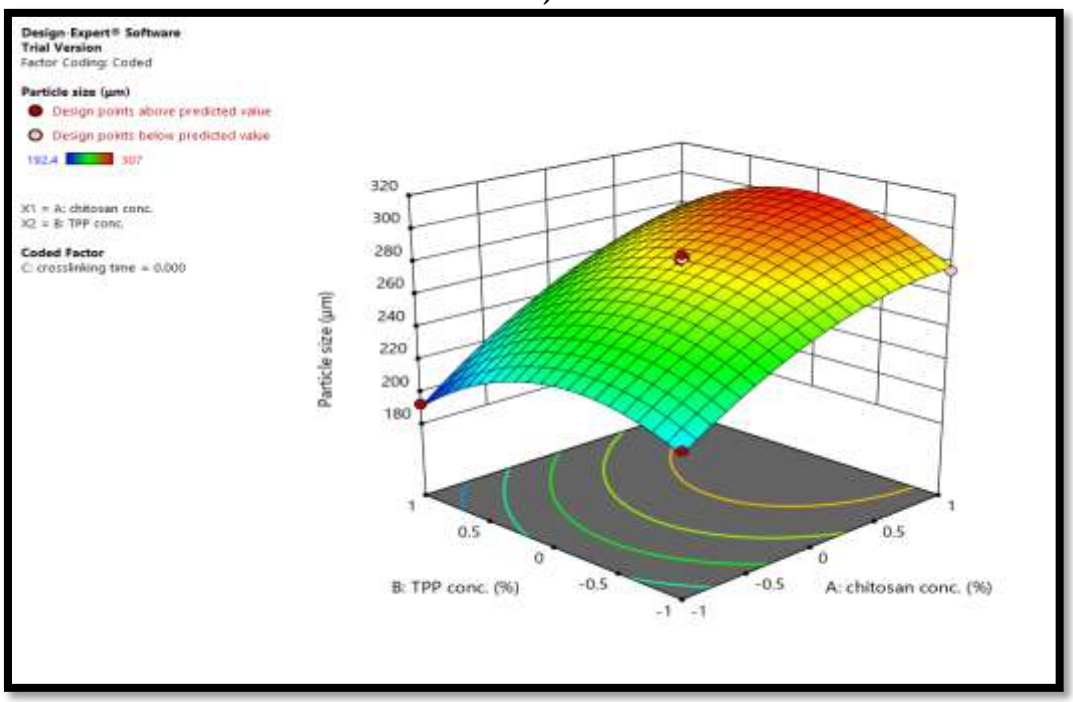
The equation in term s of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

a)



b)



International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

c)

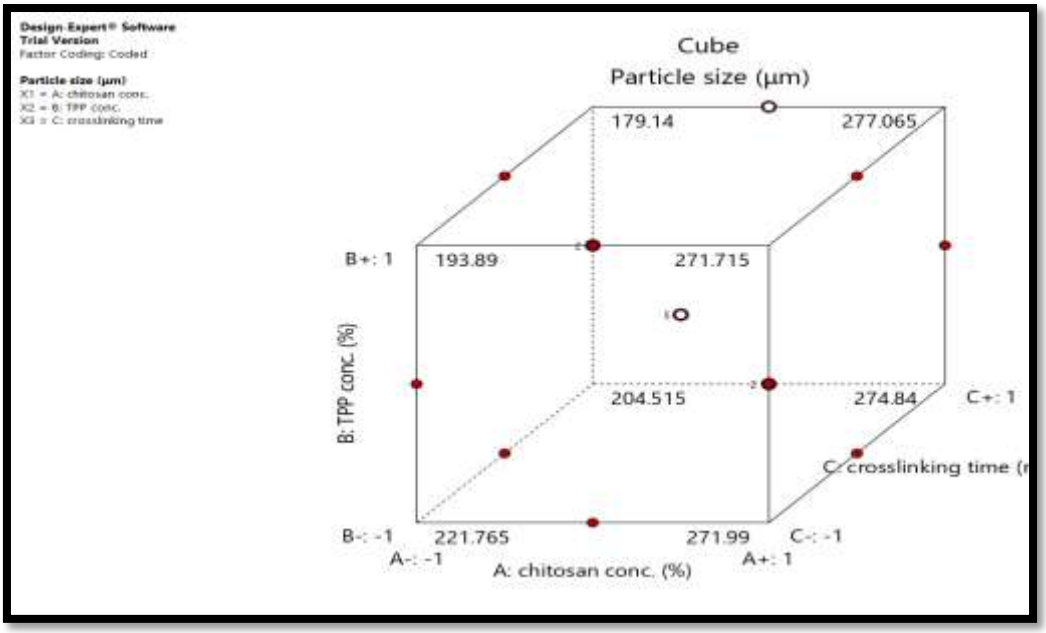


Figure 18: Various plots showing influence of chitosan concentration, tripolyphosphate concentration and crosslinking time on particle size a) Contour plot b) Response surface plot c) Cube plot

Warning: The Cubic model is aliased.

- **Fit Summary**

Response 2: Entrapment efficiency

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0039	0.0221	0.5452	0.3897	
2FI	0.3918	0.0195	0.5561	0.2529	
Quadratic	**0.0016**	**0.3443**	**0.9202**	**0.6790**	**Suggested**
Cubic	0.3443		0.9341		Aliased

- **Sequential Model Sum of Squares [Type I]**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs. Total	49690.87	1	49690.87			
Linear vs. Mean	1306.31	3	435.44	7.39	0.0039	
2FI vs. Linear	190.69	3	63.56	1.11	0.3918	
Quadratic vs. 2FI	**502.46**	**3**	**167.49**	**16.20**	**0.0016**	**Suggested**
Cubic vs. Quadratic	38.23	3	12.74	1.49	0.3443	Aliased
Residual	34.13	4	8.53			
Total	51762.69	17	3044.86			

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

- **Model Summary Statistics**

	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	7.67	0.6305	0.5452	0.3897	1264.34	
2FI	7.58	0.7226	0.5561	0.2529	1547.94	
Quadratic	**3.22**	**0.9651**	**0.9202**	**0.6790**	**665.01**	**Suggested**
Cubic	2.92	0.9835	0.9341		*	Aliased

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

- Case(s) with leverage of 1.0000: PRESS statistic not defined.

Focus on the model maximizing the **Adjusted R²** and the **Predicted R²**.

- **Lack of Fit Tests**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	731.38	9	81.26	9.52	0.0221	
2FI	540.69	6	90.12	10.56	0.0195	
Quadratic	**38.23**	**3**	**12.74**	**1.49**	**0.3443**	**Suggested**
Cubic	0.0000	0				Aliased
Pure Error	34.13	4	8.53			

The selected model should have insignificant lack-of-fit.

- **ANOVA for Quadratic model**

Response 2: Entrapment efficiency

Table 16: ANOVA results for entrapment efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1999.46	9	222.16	21.49	0.0003	significant
A-chitosan conc.	959.22	1	959.22	92.80	< 0.0001	
B-TPP conc.	75.65	1	75.65	7.32	0.0304	
C-crosslinking time	271.44	1	271.44	26.26	0.0014	
AB	104.04	1	104.04	10.06	0.0157	
AC	51.84	1	51.84	5.02	0.0601	
BC	34.81	1	34.81	3.37	0.1091	
A^2	261.78	1	261.78	25.33	0.0015	
B^2	163.69	1	163.69	15.84	0.0053	
C^2	32.66	1	32.66	3.16	0.1187	
Residual	72.36	7	10.34			
Lack of Fit	38.23	3	12.74	1.49	0.3443	not significant
Pure Error	34.13	4	8.53			
Cor Total	2071.82	16				

Factor coding is **Coded**.
Sum of squares is **Type III – Partial**

The **Model F-value** of 21.49 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise.
P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
The **Lack of Fit F-value** of 1.49 implies the Lack of Fit is not significant relative to the pure error. There is a 34.43% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

- **Fit Statistics**

Std. Dev.	**3.22**	R^2	**0.9651**
Mean	54.06	**Adjusted R^2**	0.9202
C.V. %	5.95	**Predicted R^2**	0.6790
		Adeq Precision	14.4979

The **Predicted R²** of 0.6790 is not as close to the **Adjusted R²** of 0.9202 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 14.498 indicates an adequate signal. This model can be used to navigate the design space.

- **Coefficients in Terms of Coded Factors**

Table 17: Coefficients influencing entrapment efficiency

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	62.02	1	1.44	58.62	65.42	
A-chitosan conc.	10.95	1	1.14	8.26	13.64	1.0000
B-TPP conc.	-3.07	1	1.14	-5.76	-0.3871	1.0000
C-crosslinking time	-5.82	1	1.14	-8.51	-3.14	1.0000
AB	5.10	1	1.61	1.30	8.90	1.0000
AC	3.60	1	1.61	-0.2012	7.40	1.0000
BC	-2.95	1	1.61	-6.75	0.8512	1.0000
A^2	-7.89	1	1.57	-11.59	-4.18	1.01
B^2	-6.24	1	1.57	-9.94	-2.53	1.01
C^2	-2.78	1	1.57	-6.49	0.9200	1.01

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

- **Final Equation in Terms of Coded Factors**

Entrapment efficiency =
+62.02
+10.95 A
-3.07 B
-5.82 C
+5.10 AB
+3.60 AC
-2.95 BC
-7.89 A^2
-6.24 B^2
-2.78 C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

- **Final Equation in Terms of Actual Factors**

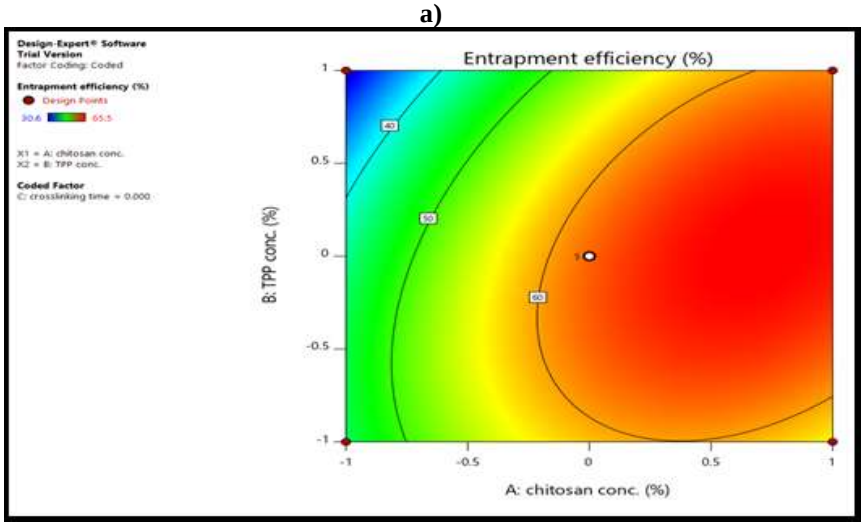
Entrapment efficiency =
+20.78722
+26.44444 chitosan conc.
+3.85300 TPP conc.
+0.260750 crosslinking time

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

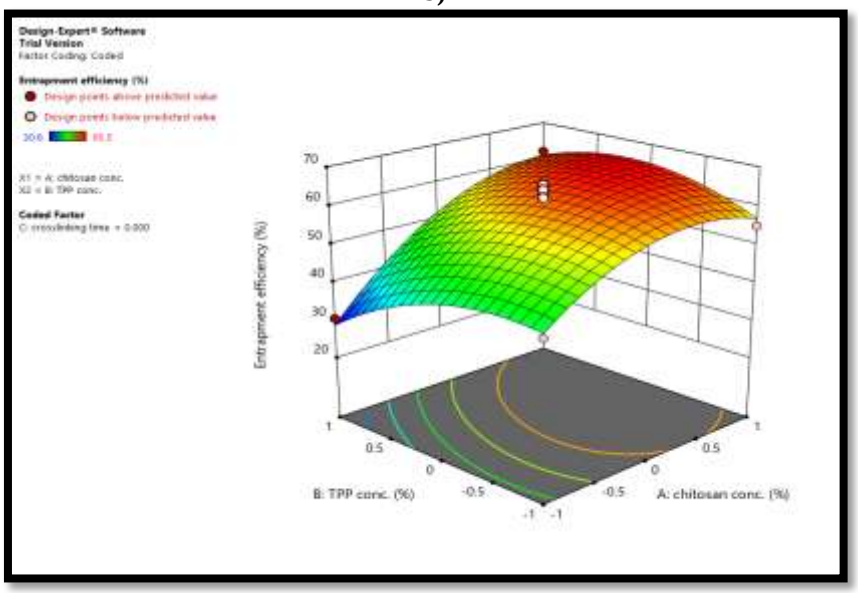
+1.36000	chitosan conc. * TPP conc.
+0.240000	chitosan conc. * crosslinking time
-0.029500	TPP conc. * crosslinking time
-14.01778	chitosan conc.^{2}
-0.249400	TPP conc.^{2}
-0.006963	crosslinking time^2

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

a)



b)



International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

c)

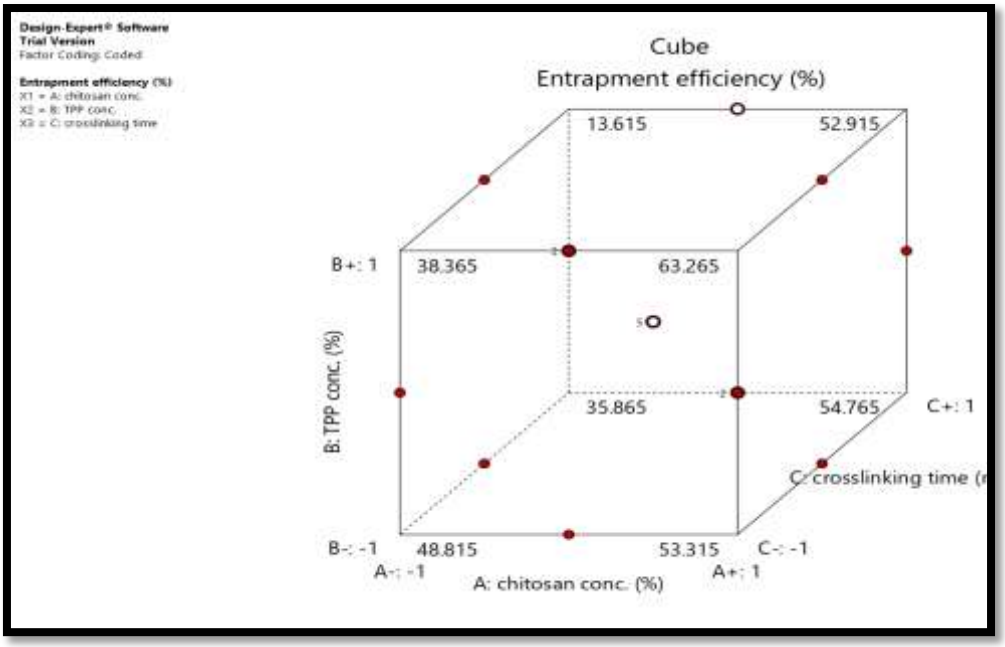


Figure 19: Various plots showing influence of chitosan concentration, tripolyphosphate concentration and crosslinking time on entrapment efficiency a) contour plot b) response surface plot c) cube plot

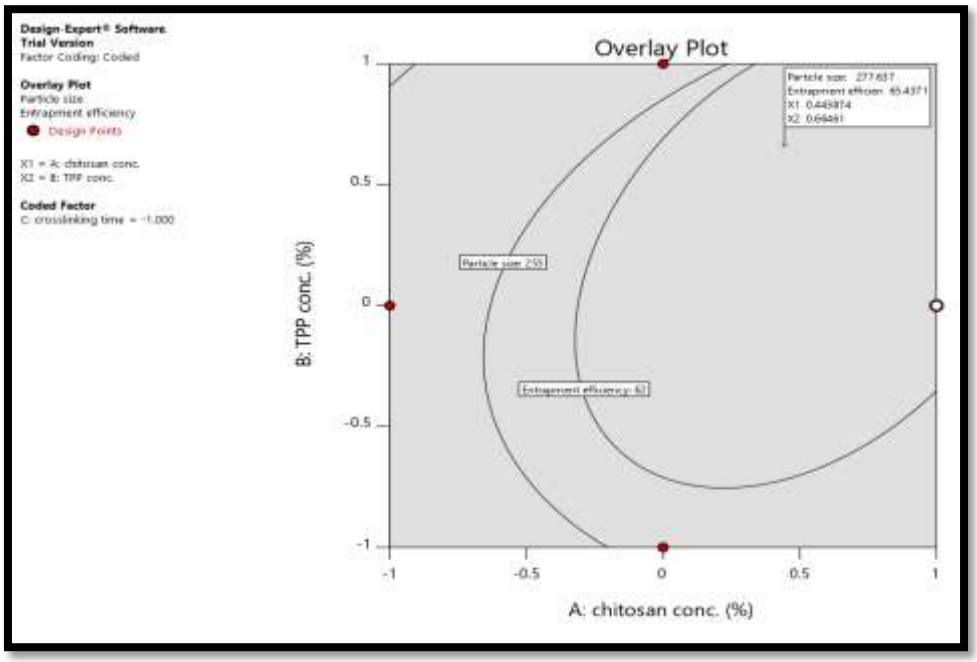


Figure 20: Contour overlay graph for optimum desired response variables for different concentration of polymer-chitosan, cross linker-tripolyphosphate and crosslinking time

Optimization data analysis: Analysis of variance (ANOVA) and estimated regression coefficients were applied to evaluate response 1 (particle size), response 2 (entrapment efficiency). A series of experiments was carried out by considering a Box-Behnken design. Various statistical data (standard error of estimate, sum of squares of the errors, F statistics and P value) were examined.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

Particle size = 281.72+37.04A-6.41B-2.98C+6.90AB+5.03AC+0.6250BC-13.81A^2-27.11B^2 3.93C^2

Entrapment efficiency = 62.02+10.95A-3.07B-5.82C+5.10AB+3.60AC-2.95BC-7.89A^2-6.24B^2-2.78C^2

A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and response. It is evident that all the independent variables, namely the concentration of chitosan (A), concentration of TPP (B), cross-linking time (C) have interactive effects on the two responses namely particle size and entrapment efficiency respectively.

Table 18: Predicted and observed values of responses of optimized formulation

	Predicted value	**Observed value**
Particle size	277.632µm	275.7
% Entrapment efficiency	65.4363%	65.1

Evaluation tests of the optimized formulation

The various tests like percentage yield, particle size, entrapment efficiency, angle of repose, bulk density, tapped density, Carr's index and hausner's ratio was carried out for the optimized formulation and the results are shown in table 5.5:

Table 19: Evaluation tests of the optimized formulation

S. No.	**Tests**	**Observed values**
1	% Percentage yield	84.37 ± 0.71
2	Particle size	275.7 ± 0.50
3	% Entrapment efficiency	65.1 ± 0.20
4	Angle of repose	24.2° ± 0.27
5	Bulk density	0.618 ± 0.003
6	Tapped density	0.717 ± 0.004
7	Carr's index	13.8
8	Hausner's ratio	1.16

In vitro release study of optimized formulation

The optimized formulation showed negligible release in gastric fluid of pH 1.2 during 2 hrs but sustained release up to 50.2 % in intestinal fluid of pH 6.8 during next 10 hrs.

Table 20: % Cumulative drug release from optimized formulation

Time (hrs)	**% Cumulative drug release**
0	0
1	10.08
2	11.8
3	17.04
4	20.9
5	23
6	27.7
7	32.4
8	35.7
9	38.9
10	42.8
11	46.2
12	50.2

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

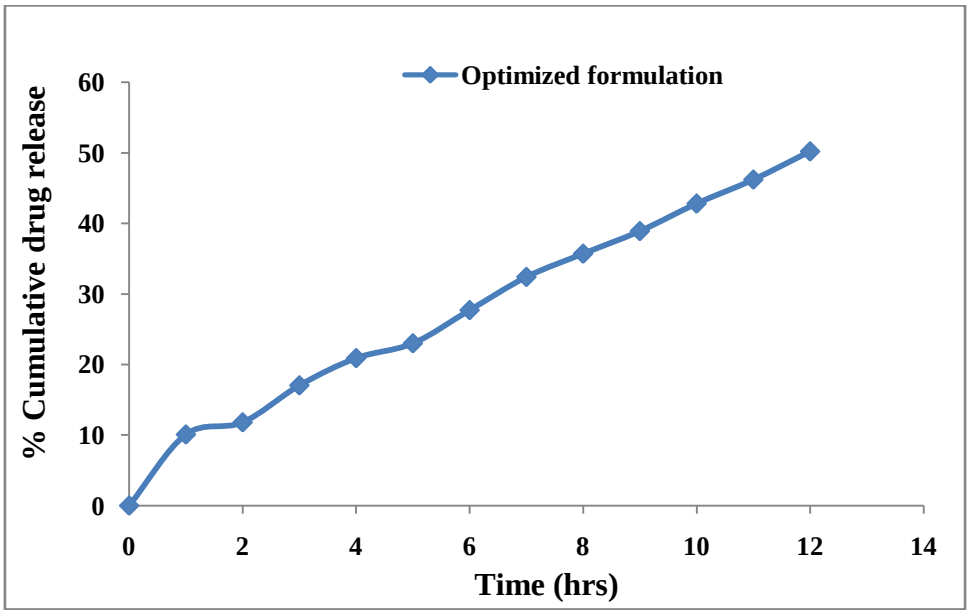


Figure 21: In vitro dissolution profile of optimized formulation during 12 hrs

5.9 Drug release kinetics

The drug release kinetics was done for the optimized formulation of diclofenac sodium microspheres. It was observed that zero order release kinetic was best suited based on R^2 value.

Table 21: Observation of drug release kinetics of optimized formulation

S. No.	Time (hrs)	Log time	Square root of time	% Cumulative drug release	% Log cumulative drug release	% Cumulative drug remaining	Log % Cumulative drug remaining
1	1	0	1	10.08	1.003	89.92	1.953
2	2	0.301	1.414	11.8	1.071	88.2	1.945
3	3	0.477	1.732	17.04	1.231	82.9	1.918
4	4	0.602	2	20.9	1.320	79.1	1.898
5	5	0.698	2.236	23	1.361	77	1.886
6	6	0.778	2.449	27.7	1.442	72.3	1.859
7	7	0.845	2.645	32.4	1.510	67.6	1.829
8	8	0.903	2.828	35.7	1.552	64.3	1.808
9	9	0.954	3	38.9	1.589	61.1	1.786
10	10	1	3.162	42.8	1.631	57.2	1.757
11	11	1.041	3.316	46.2	1.664	53.8	1.730
12	12	1.079	3.464	50.2	1.7007	49.8	1.697

Table 22: Regression coefficient (R^2) values of drug release data obtained from various kinetic models

S. No.	Model	Regression coefficient (R^2)	Release constant (K_0)
1	Zero order release	0.997	3.708
2	First order release	0.991	-0.0227
3	Higuchi's model	0.972	16.485
4	Korsmeyer's – peppas model	0.974	0.6730

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

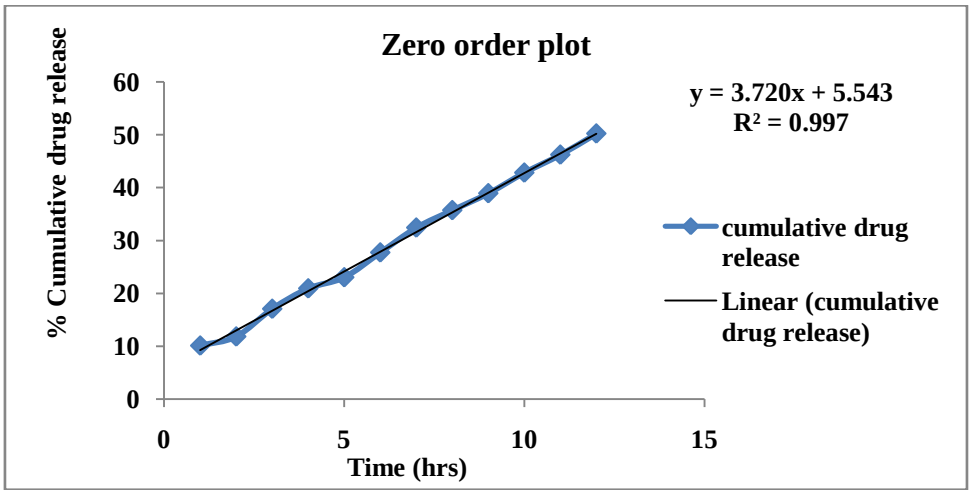


Figure 22: Zero order release plot of diclofenac sodium microspheres of optimized formulation

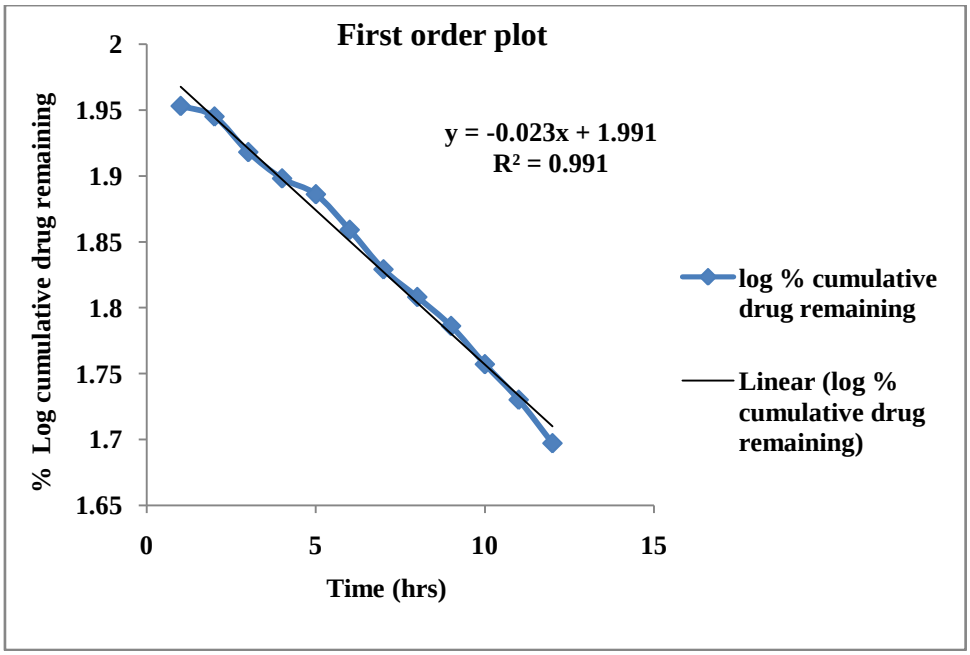


Figure 23: First order release plot of diclofenac sodium microspheres of optimized formulation

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

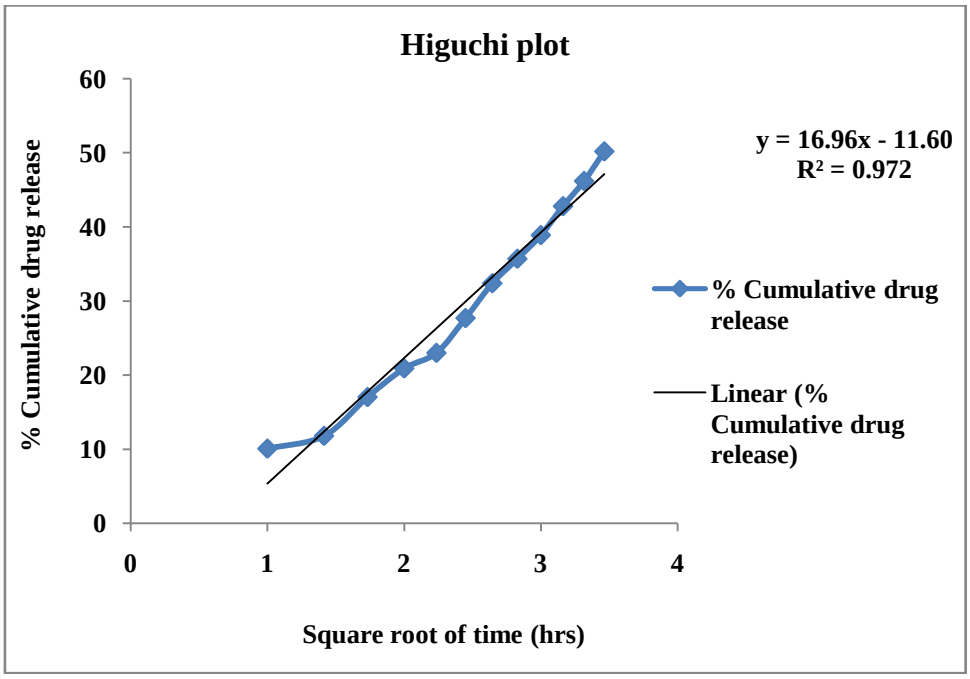


Figure 24: Higuchi's model plot of diclofenac sodium microspheres of optimized formulation

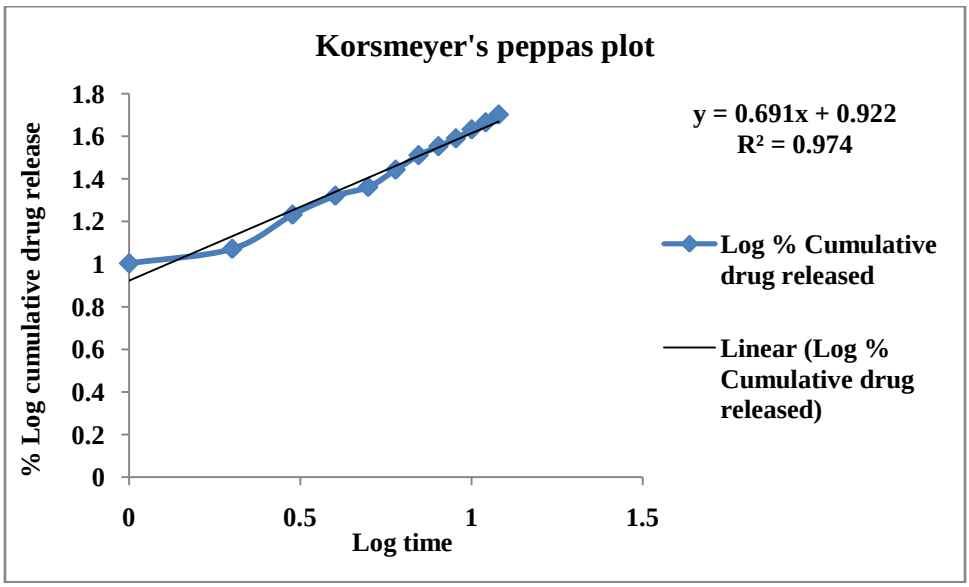


Figure 25: Korsmeyer's peppas plot of diclofenac sodium microspheres of optimized formulation

- **FT-IR of optimized formulation:** All the characteristic peaks of drug and polymers were found to be intact which indicates that there is no chemical interaction between drug and polymer.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

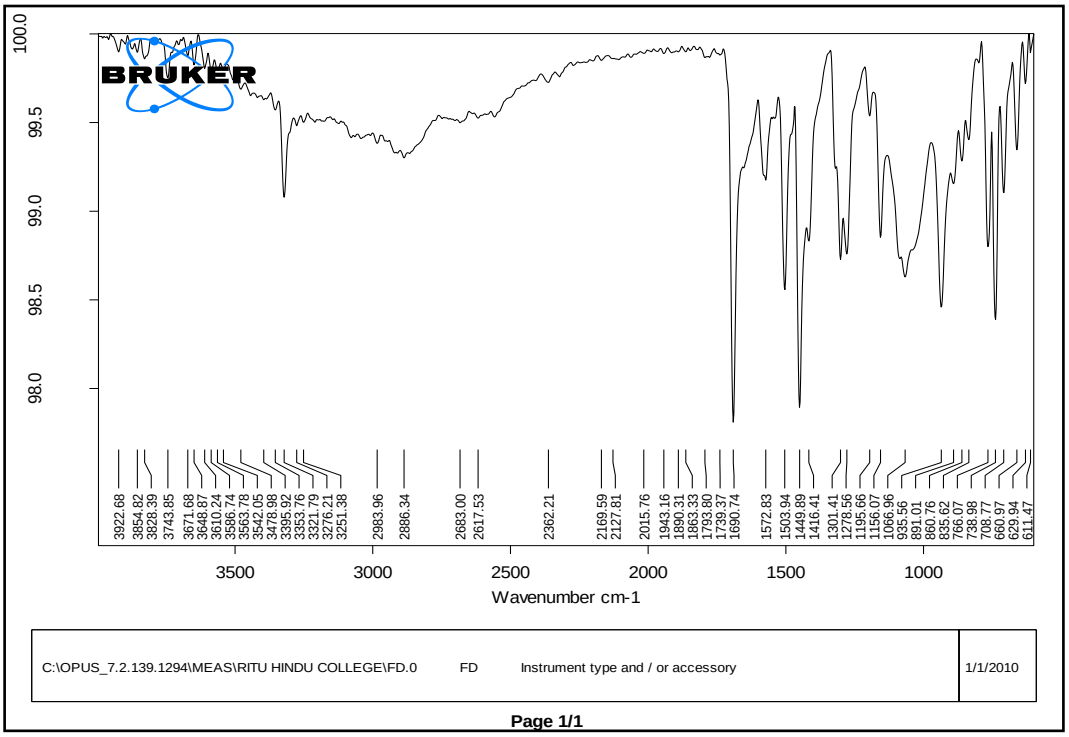


Figure 26: FT-IR spectra of optimized formulation

Table 23: Interpretation of FT-IR spectra of optimized formulation

S. No.	Obtained peaks (cm^{-1})	Standard IR peaks (cm^{-1})	Assignment
1	3353.76 broad	3310-3350	N-H stretching vibration overlapped with –OH stretching vibration
2	1503.94	1530	N-O-P vibration
3	1690.74	1580-1650	Bending vibration of N-H
4	1301.41	1180-1360	C-N
5	766.07	550-850	C-Cl
6	1195.66	1050-1250	C-O-C

- **Comparison of optimized formulation with marketed diclofenac sodium tablet**

When the optimized formulation was compared with the marketed formulation (volteran 100mg) it was found that the marketed formulation released the drug (52.04%) in 12 hrs while the release of drug up to 50.2% in 12 hrs was observed in optimized formulation which proves that the release pattern was more sustained in case of optimized formulation than the marketed formulation.

Table 24: Comparison of optimized formulation with marketed diclofenac sodium tablet

Formulation	% Cumulative drug release											
	Time in hrs											
	1	2	3	4	5	6	7	8	9	10	11	12
Optimized formulation	10.08	11.8	17.04	20.9	23	27.7	32.4	35.7	38.9	42.8	46.2	50.2
Marketed formulation	11.2	12.8	18.06	21.9	24.2	29	33.02	36.9	40.04	43.8	47.9	52.4

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

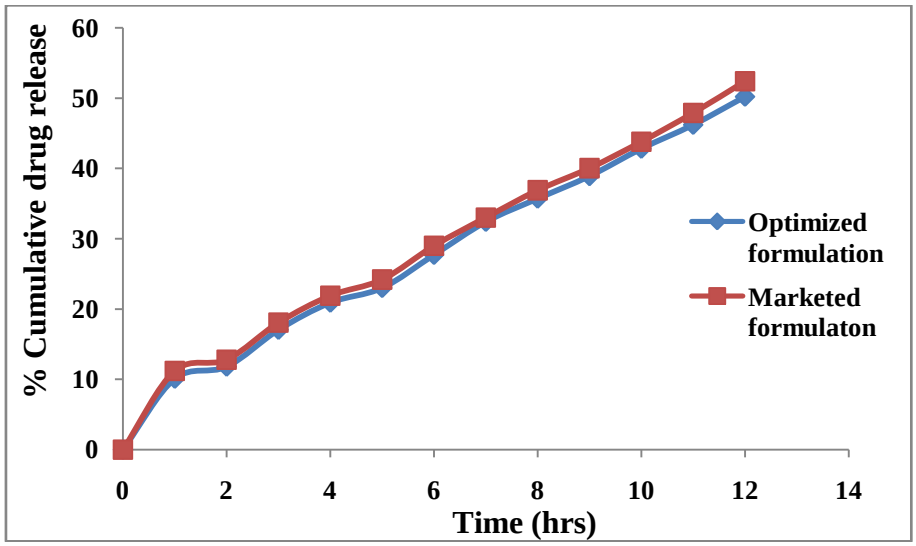


Figure 27: Comparison of in vitro dissolution profile of microspheres of diclofenac sodium during 12 hrs and marketed formulation

- **Scanning electron microscopy (SEM):** SEM of optimized formulation was carried out. The SEM of microspheres shows a hollow spherical structure with smooth surface morphology. The result is shown in the following SEM photograph.

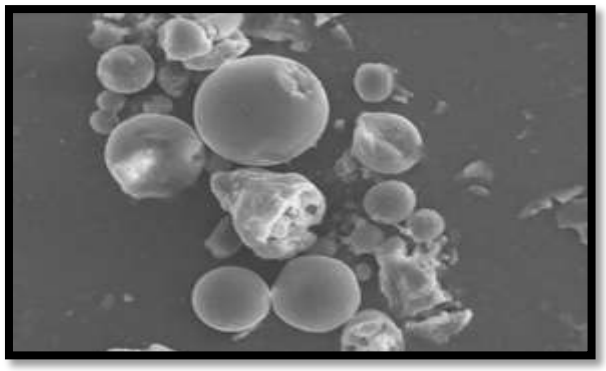


Figure 28: SEM of optimized formulation

- **Stability study:** The optimized formulation was found to be stable for period of three months, it can be observed that the formulation showed no major alteration in relation to physical appearance, particle size and entrapment efficiency.

Table 25: Stability study of optimized formulation

S. No	At the end (in days)	Physical appearance	Particle size		Entrapment efficiency	
			25±2°C, 60±5% RH	40±2°C, 70±5% RH	25±2°C, 60±5% RH	40±2°C, 70±5% RH
1	30	No change	275.7±0.47	275.6±0.1	65.1±0.09	64.84±0.16
2	60	No change	275.6±0.45	275.4±0.2	64.9±0.15	64.83±0.20
3	90	No change	275.5±0.45	275.2±0.2	64.86±0.35	64.7±0.45

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2292-2329 www.ijprajournal.com ISSN: 2249-7781

ACKNOWLEDGEMENT
All the authors have equal contribution.

CONFLICTS OF INTEREST
The authors declare no conflict of interest.

V. CONCLUSION

It may be concluded that microspheres of diclofenac sodium can be successfully prepared by ionotropic gelation technique using chitosan and tripolyphosphate as polymer and cross linker respectively. The concentration of chitosan has significant effect on particle size and entrapment efficiency. The particle size and entrapment efficiency increased with increase in chitosan concentration. The results of Box-Behnken design revealed that the concentration of chitosan, TPP and the cross-linking time significantly affect the dependent variables such as particle size and entrapment efficiency of microspheres. The evaluation test results were in acceptable ranges but the optimized formulation obtained from Box-Behnken design fulfilled maximum requisities because of better entrapment efficiency (65.1%), optimal particle size (275.7), percentage yield (84.37%) and good flow properties. In-vitro dissolution test for the optimized formulation showed 50.2% drug release in 12 hrs. Further research is necessary to establish in vitro-in vivo correlation of microspheres. Also stability study for longer duration need to be carried out. The prepared microspheres of diclofenac sodium may prove to be potential candidate for safe and effective sustained drug delivery over an extended period of time which can reduce dosing frequency and improve patient compliance.

REFERENCES

[1]. Goyal S, Agarwal G, Agarwal S, Karar PK, Oral Sustained Release Tablets: An overview with a special emphasis on matrix tablet, American journal of advanced drug delivery, 2017, 5(02), 64-76.

[2]. Rao VN et al, Oral Controlled Release Drug Delivery System: An overview, International journal of pharma and chemical research, 2015, 1(1), 6-15.

[3]. Pooja R. Alli, Pratima B. Bargaje, Nilesh S. Mhaske, Sustained release drug delivery System: A modern formulation approach, Asian journal of pharmaceutical technology& innovation, 2016, 04 (17), 108-118 .

[4]. Singh Sumita and Kunal Arora, Floating microspheres: An overview, World journal of pharmacy and pharmaceutical sciences, 2017, 6(3), 1113-1139.

[5]. Vyas, S.P, Khar, R.K, Controlled drug delivery concepts and advances, 1 Edn.,Vallabh prakashan, Delhi, 2002, 196-205.

[6]. Singh CK, Pandey Shivanshu and Mishra AC, Floating drug delivery systems: A novel approach, World journal of pharmacy and pharmaceutical sciences, 2019, 8 (1), 629-650.

[7]. Rathee Permender, Jain Manish, Garg Arun, Nanda Arun, Hooda Ashima, Gastrointestinal mucoadhesive drug delivery system: A review, Journal of pharmacy research, 2011, 4(5), 1448-1453.

[8]. Badoni A , Ojha A, Gnanarajan G, Kothiyal P, Review on gastro retentive drug delivery system, The pharma innovation, 2012, 1(8), 32-42.

[9]. Patil Prashant, Singh Santosh and Sarvanan Janardhan, Preparation and evaluation of flurbiprofen, IJPSR, 2018, 9(12), 5388-5393.

[10]. Kumar Anil, Mahajan Sourav and Bhandari Neeraj, Microspheres: A review, World journal of pharmacy and pharmaceutical sciences, 2017, 6(4), 724-740.

[11]. Sarlesh Rajput et al, A review on microspheres: methods of preparation and evaluation, World journal of pharmacy and pharmaceutical sciences, 2012, 1(1), 422-438.

[12]. JetharaSI, Patel MR and Patel AD, Sustained Release Drug Delivery Systems: A patent overview, Aperito journal of drug designing and pharmacology, 2014, 1(1), 1-14.

[13]. Ratnaparkhi MP, Gupta Jyoti P., Sustained release oral drug delivery system - An overview, International journal of pharma research & review, 2013, 2(3), 11-21.

[14]. Mandhar Piyush, Joshi Gayitri, Development of sustained release drug delivery system: A review, Asian Pac. J. Health Sci., 2015, 2(1), 179-185.

[15]. Mali RR, Goel Vaishali, Gupta Sparsh, Novel study in sustained release drug delivery system: A review, Int. J. Pharm. Med. Res., 2015, 3(2), 204-215.

[16]. Bankar AU, Bankar VH, Gaikwad PD, A review on sustained release drug delivery system, An international journal of pharmaceutical sciences, 2012, vol.3, 2049-2063.
[17]. Karna Sudhir, Chaturvedi Shashank, Agrawal Vipin, Alim Mohammad, Formulation approaches for sustained release dosage forms: A review, Asian J Pharm Clin Res, 2015, 8(5), 46-53.
[18]. Jyothi, B. Gowthami, Ch. Sireesha, S.S. Manikiran and N. Ramarao, Microsphere as a novel drug delivery – A review, World journal of pharmaceutical research, 2017, 6(6), 378-396.
[19]. Dalbanjan NS, Microsphere: A complete review, World journal of pharmacy and pharmaceutical sciences, 2018, 7(11), 786-817.
[20]. Nikam VK, Gudsoorkar VR, Hiremath SN, Dolas RT and Kashid VA, Microspheres - A novel drug delivery system: An overview, International journal of pharmaceutical and chemical sciences, 2012, 1(1), 113-128.
[21]. Lachman, L, Lieberman, H.A, Kanig, J.L, The theory and practice of industrial pharmacy., 3rd edition, Lea and Febiger, Philadelphia, 1987, 412.
[22]. Virmani Tarun and Gupta Jyoti, Pharmaceutical application of microspheres: An approach for the trearment of various diseases, IJPSR, 2017, 8 (8), 3252-3260.
[23]. Midha Kanav, Nagpal Manju and Arora Sandeep, Microspheres: A recent update. International journal of recent scientific research, 2015, 6(8), 5859-5867.
[24]. Nautiyal et al., A review: Gastroretentive drug delivery system, Indian J. Pharm. Biol. Res., 2015, 3(1), 82-92
[25]. Nikalje Anna Pratima, Shailee Tiwari, Smrutigandha Kamble, Mucoadhesive: As oral controlled gastroretentive drug delivery system, IJRPS 2012, 2(3), 32-59.
[26]. Ayre Anita, Neha D, K. G. Lalitha, Gastroretentive floating and mucoadhesive drug delivery systems-insights and current applications**,** IOSR Journal of pharmacy and biological sciences (IOSR-JPBS), 2016, 11(3) , 89-96.
[27]. Shaikh Siraj, Molvi Khurshid.I, Sayyed Nazism, Various perspectives of gastroretentive drug delivery system: A review, American journal of advanced drug delivery**,** 2013, 1(4), 443-451.
[28]. Kayak AK, Magi Rumba, Das Biswarup, Gastroretentive drug delivery systems: A review, Asian journal of pharmaceutical and clinical research, 2010, 3(1), 2-10.
[29]. Jain NK. Controlled and Novel drug delivery, CBS Publishers New Delhi, India, 4th Edition, 236- 237.
[30]. Kadam N. R. and Suvarna V, Microspheres: A brief review, Asian journal of biomedical and pharmaceutical sciences, 2015, 5(47), 13-19.
[31]. Ershad Syed et al, Preparation and evaluation of floating microspheres of ritonavir, IJRPC, 2013, 3(4), 831-834.
[32]. Madan JR, Kadam V, Bandavane S, Dua K, Formulation and evaluation of microspheres containing ropinirole hydrochloride using biodegradable polymers, Asian journal of pharmaceutics, 2013, 7, 184-188.
[33]. Priyadarshini MK et al, Formulation and evaluation of microspheres encapsulating zidovudine by ionic gelation techniques, International journal of research in pharmaceutical and nano sciences, 2014, 3(5), 461- 468.
[34]. Sigimol Joseph and CD Shaji Selvin, Formulation and evaluation of losartan microspheres, IJRPC, 2015, 5(4), 555-563.
[35]. Meshram BMS, Mishra Ashwani and Pathak Anupam, Formulation and evaluation of microsphere of rebiprazole sodium, International journal of advances in pharmaceutics, 2016, 5 (3), 76-83.
[36]. Ozdemir Nurten, Tilkan MGY, Investigation of the parameters affecting the release of flurbiprofen from chitosan microspheres**,** Braz. J. Pharm. Sci., 2017, 53(4), 1-12.
[37]. Pathange BBR, Nagoji KEV, Gaddam P, Jukanti R and Jalagam MK, Formulation and in vitro evaluation of diclofenac sodium delayed release tablets, Int. J. Chem. Sci., 2009, 7(2), 923-938.
[38]. Manjanna KM et al, Diclofenac sodium microbeads for oral sustained drug delivery, Int.J. PharmTech Res., 2009, 1(2), 317-327.
[39]. Gattani SG, Jadhav R.T, Kasture P.V, Surana S.J, Formulation and evaluation of

transdermal films of diclofenac sodium, Int.J. PharmTech Res., 2009, 1(4), 1507-1511.

[40]. Goudanavar PS, .Bagali RS, Chandrashekhara S and Patil SM, Design and characterization of diclofenac sodium microbeads by ionotropic gelation techniquel, International journal of pharma and bio sciences, 2010, 1(2), 1-10.

[41]. A.Salomy Monica, and J. Gautami, Design and evaluation of topical hydrogel formulation of diclofenac sodium for improved therapy, IJPSR, 2014, 5(5), 1973-80.

[42]. Sigimol Joseph et al, Formulation and evaluation of diclofenac sodium loaded albumin microspheres, International journal of research in pharmaceutical and nano sciences, 2014, 3(1), 6 - 11.

[43]. Goci Enkelejda, Zaloci Entela, Xhulaj Skerdilaid, Malaj Ledjan, Formulation and in vitro evaluation of diclofenac sodium gel, Int J Pharm Pharm Sci, 2014, 6(6), 259-261.

[44]. Kouchak M, Azarpanah A, Preparation and in vitro evaluation of chitosan nanoparticles containing diclofenac using the ion-gelation method, Jundishapur J Nat Pharm Prod., 2015, 10(2), 1-7.

[45]. Phaldesai Saiesh , A.R Shabaraya, Shripathy D, Leyana Soman, Kinetic modeling of drug release from topical gel, International journal of universal pharmacy and bio sciences, 2014, 3(3), 92-104.