STARCH NANOPARTICLES FOR THE CONTROLLED RELEASE OF THE ANTIDIABETIC DRUG- TOLBUTAMIDE

A dissertation submitted in partial fulfilment of the requirement for the award of the degree of

Master of Science

in

Biotechnology

Submitted by

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July 2022

DECLARATION

I hereby declare that the work presented in the dissertation entitled "**Starch nanoparticles for the controlled release of the antidiabetic drug- Tolbutamide**" in partial fulfilment of the requirement for the award of the degree of Masters of Science in Biotechnology, is an authentic record of my own work during the academic year 2021-2022, under the guidance of Dr. M. Vasundhara, Assistant professor, Department of Biotechnology, TIET, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Place: Patiala

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CERTIFICATE

This is to certify that the dissertation entitled "**Starch nanoparticles for the controlled release of the antidiabetic drug- Tolbutamide**" submitted by Ridham Bhatia in partial fulfilment of the requirement of the award of Degree of Masters of Science in Biotechnology to TIET, Patiala, is a record of student's own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university.

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ACKNOWLEDGEMENT

It is rightly said that difficult roads often lead to beautiful destination. So, in my journey on a difficult road, where I experienced several strains, a lot of people held my hand and made me travel a long way towards one of my goals.

Foremost, I would like to offer this endeavor to **Supreme Almighty God**, for the perseverance, peace of mind, strength and good health, he bestowed upon me in order to finish this research.

I want to express my gratitude towards my dad **Mr. Pawan Bhatia** and my mom **Mrs. Manju Bhatia**, whose immense blessings, sacrifice and desire made me strong enough to overcome all the hurdles not just in this research but in life as well and my loved ones who kept motivating me and supported me in all means, to pursue this undertaking.

I would like to express my special thanks to my mentor and advisor **Dr. M. Vasundhara**, Assistant Professor, Department of Biotechnology, for imparting her knowledge and expertise in this study. It was because of her presence and her scientific attitude that I was able to learn so much in this short period.

My sincere thanks to **Dr. M.S. Reddy**, Head, Department of Biotechnology. I express my regards to all the faculty members of the Department of Biotechnology for their help and moral support during my research period.

I am thankful to **Dr. Bonamali Pal**, Professor and former Head, School of Chemistry and Biochemistry for the generous allowance to utilize DLS facility and **Dr. Soumen Basu**, Associate Professor, School of Chemistry and Biochemistry for the permission to use FTIR facility in the CEEMS lab, Thapar University, Patiala.

I express my thanks to **Scanning Electron Microscopy lab**, TIET, Patiala and **Sophisticated Analytical Instruments Laboratory**, TIET, Patiala for extending SEM and X-RD facility respectively.

I am highly obliged to **Mr. Nirmalya Halder** and **Ms. Fatima**, for guiding me throughout the project. Their support allowed my study to go the extra mile and added immense value to my work.

My special thanks to **Mr. Ram Naval Yadav**, **Mr. Prabhat Bailey**, **Mr. Mohinder**, **Mr. Surinder** and **Mr. Lallan Yadav**, who were very helpful throughout all the lab work in every possible way.

I thank my fellow mates **Nidhi**, **Gurleen**, **Mansi**, **Navjot** and **Simran** for all the cooperation and help they have offered to me during my research period.

Lastly, my immense gratitude to each and every person who has contributed in my physical, mental and emotional well-being, in order to stay patient throughout my research.

Dated: 29 July, 2022

Place: Patiala

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ABSTRACT

In the era of development of novel and more powerful drugs, a major part of attention is being diverted towards method of administration of these pharmaceutically active substances. One of these approaches is the controlled delivery of drug so as to achieve the desirable administration patterns. Recently, nanoparticles are being investigated to ensure site-targeted and efficient delivery of the therapeutic agents. Moreover, efforts are being made towards preparing nanoparticles using natural polymers like PLGA, chitosan, alginates, starch, etc.

The aim of the current study, was to prepare nanoparticles for controlled delivery of tolbutamide by nanoprecipitation technique using starch as a polymer and sodium tripolyphosphate as a crosslinking agent. Starch is a natural, biodegradable and inexpensive biopolymer that has the ability to form intra and intermolecular crosslinked structures, on interaction with polyanions, like sodium tripolyphosphate (TPP).

Tolbutamide loaded starch-TPP crosslinked nanoparticles were prepared using nanoprecipitation technique in which aqueous alkaline medium and ethanol were used as a solvent and an organic non-solvent respectively. An experimental design was prepared using response surface methodology (RSM), and the given formulations were prepared and evaluated for drug entrapment efficiency and in-vitro drug release behavior. Various characterization studies like were performed using facilities such as FT-IR, SEM, DLS and X-RD for the RSM-optimized formulation in order to study the interaction between tolbutamide and starch-TPP crosslinked nanoparticles.

Tolbutamide loaded starch-TPP crosslinked nanoparticle formulation optimized using RSM, containing 5% maize starch, 88.2 mg tolbutamide and 2 g TPP, showed around 85% of in-vitro drug release in a controlled manner and had around 54.97% of drug encapsulation efficiency, poly dispersity index value of about 0.27 and a diameter around 111.2 nm. The resultant amorphous structure, suggested by X-RD graphs of starch crosslinked nanoparticles containing tolbutamide, might result in better dissolution rate which might lead to increased drug efficacy. Moreover, the preparation method that was used, turned out to have extremely low complexity, consumed lesser energy, decreased amount of solvent and did not require any addition of toxic chemicals. Hence, this method can be employed for the preparation of drug loaded starch-crosslinked nanocomposites having desirable properties. Also, RSM turns out to be a promising technique not just in order to prepare an experimental design to minimize the number of experimental runs but also to predict the optimized formulation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of Abbreviations

ABBREVIATION	DESCRIPTION
%	Percentage
w/w	Weight by weight
w/v	Weight by volume
˚C	Degree Celsius
˚θ	Degree Theta
TBM	Tolbutamide
STPP/TPP	Sodium tripolyphosphate
NPs	Nanoparticles
EE	Entrapment efficiency
SEM	Scanning electron microscopy
DLS	Dynamic light scattering
FTIR	Fourier transform infrared
X-RD	X-ray diffraction
RSM	Response surface methodology
PdI	Poly dispersity index
MS	Maize Starch
St-Unl	Unloaded starch nanoparticles
St-TBM	Tolbutamide loaded starch nanoparticles

CHAPTER 1

INTRODUCTION

Introduction

A pharmaceutical substance used to treat, cure, prevent or diagnose a disease is generally called as drug. A drug might elevate mental or physical state of a person. Drugs are usually administered from outside the organism, hence differing from intrinsic biochemicals.

Drugs are mainly administered as medicines or formulated preparations, instead of giving them in their pure form. These formulated preparations contain the pharmaceutically active ingredient (drug) along with appropriate excipients and required additives, forming simple to complex delivery systems. The route of administration significantly effects the efficacy of the drug.

According to Saket Asati et al. (2022), the pharmacokinetic parameters are affected by the route of administration which ultimately results in altered bioavailability of the administered drug.

In accordance with Genta et al. (1997), when a high dosage of drug is administered at one particular time, the same dosage needs to get repeated several hours or days later. This frequent administration of the drug might not be economical and sometimes may also result in increased toxicity leading to harmful side effects. This has diverted the attention of scientists towards delivering the drugs for a longer period of time, in a controlled fashion.

Researchers have successfully accomplished controlled delivery of drugs by incorporating the pharmaceuticals within different polymers. Not just in pharmaceutical industry, this technology has spanned diverse fields of agricultural, pesticide, food and household applications.

The incorporation of the active ingredient within the natural or synthetic non-toxic polymer, ensures controlled drug delivery by constantly releasing the drug for a longer time period from the material, in a predesigned manner. The external environment or some other external factors might disturb the cyclic release of the drug. Controlled drug delivery aims to achieve higher blood levels for a longer duration of time (Joseph Kost, 1986).

Over the past few years, the researches have proven nanoparticles as the most promising of all the drug delivery systems. The investigated nanoparticle drug delivery system ensures controlled drug release for prolonged periods of time (Sahu et al., 2013). Nanobiotechnology being a diversified research field, constitute the preparation of nanoparticles for drug delivery

systems (K.K. Jain, 2005). Nanoparticles are made up of natural or artificial polymers forming solid colloids. The size ranges from 10 to 1000 nm, specific to pharmaceutical applications (El-Rafie et al., 2011). The nanoparticles might entrap, adsorb or covalently attach the drug molecules (Armarego et al., 2013). Entrapment is a method in which the crosslinked polymer forms a coating around the small particles in order to embed or surround them within a homogenous or a heterogeneous matrix. Whereas, the drug is adsorbed on the surface of the polymer matrix through weak covalent bonds or interactions like hydrogen bonds and Van der Waals forces (Salonen et al., 2005). Nanoparticles are designed in such a way that they undergo specific target-ligand interactions and develop the ability to interact with the biological interface. This offers enormous advantages like reducing the adverse effects by guarding, aiming and delivering the drug at the site of action in the body (El-Rafie et al., 2011).

In recent approaches, the use of polymers to provide a controlled rather than just sustained release, is being primarily focused upon. These systems include the incorporation of the drug within the polymer matrix, wherein the properties of polymer-drug system become the major factor affecting the drug release thereby minimizing the environmental effects. (Joseph Kost, 1986).

Starch being a natural compound, has been investigated as a promising polymer in the preparation of nanoparticles to form the drug release formulations (Simi et al., 2007). Starch is a biodegradable, inexpensive and reproducible polymer, hence it has wide applications in the area of food, textile, chemical, papermaking and medicine industries (Song et al., 2009). Starch is most commonly used in pharmaceutical industries due to it's disintegrant and binder properties. The use of raw starch as a pharmaceutical ingredient offers some drawbacks due to some of it's properties like being poorly soluble in cold water, being highly viscous on gelatinization and the ability to retrograde. In order to enhance the functional properties of starch some modification needs to be done. Forming starch nanoparticles is highly accepted as one of the most feasible modifications which gives rise to nano-scale drug delivery systems (Ortega et al., 2010).

Starch is chemically crosslinked using a desirable crosslinking agent, to form starch-nanoparticles having a size ranging between 10 to 1000 nm. Various treatments like alkali, enzymes and acid are used to open and disperse the starch structure which results in increased hydrogen bond formation upon crosslinking. This leads to the formation of starch nanoparticles

(Hoover et al., 2010). Encapsulating a hydrophilic drug within the matrix of crosslinked starch nanoparticles offer enormous advantages like (R. Sing et al., 2009):

- Minimizing undesired drug release
- Reducing the drug-related adverse effects
- Increasing the half-life of the drug
- Enhancing the efficacy of the drug

In the present study, an attempt was made to prepare nanoparticles using starch as a polymer to ensure controlled delivery of tolbutamide. Nanoprecipitation technique, also called as solvent displacement method has been employed to prepare the tolbutamide loaded starch nanoparticles. Some modifications were made in the basic technique, which included the use of ethanol as an organic non-solvent and sodium hydroxide as aqueous alkaline solvent. This modification led to the formation of more reproducible starch nanoparticles without the consumption of large amount of solvent (Hebeish et al., 2013). The prepared starch nanoparticles were optimized using 3^2 factorial design. Further, the optimized batch was evaluated for drug entrapment efficiency and in-vitro release studies followed by various characterization studies like FTIR, X-RD, DLS, PdI and SEM to analyze the properties of tolbutamide loaded starch nanoparticles.

Objectives

1. Preparation of starch nanoparticles loaded with antidiabetic drug- tolbutamide, to treat diabetes.
2. Evaluation and characterization of tolbutamide loaded starch nanoparticles.

CHAPTER 2

REVIEW OF LITERATURE

Review of Literature

2.1 Controlled Drug Delivery

(Collet and Moreton (2002), Advantages of controlled drug delivery systems), state:

- To maintain a desired range of drug levels
- To require fewer administrations
- To optimize drug use
- To increase patient compliance

According to Brannon- Peppas et al. (1995), along with significant advantages, these systems have got potential disadvantages as well:

- Might get toxic
- Non biocompatibility of the materials used
- Surgery might be required to implant or remove the system
- Degradation of materials might give rise to undesirable by-products
- Delivery device might discomfort the patient
- Might have higher cost compared to conventional formulations

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Moghadam et al. (2022) controlled and improved the release and bioavailability of a poorly-water soluble drug, quercetin by preparing a biodegradable starch-based hydrogel nanoparticles in which starch was modified by polyethylene glycol and ferric oxide nanoparticles were prepared to enhance mechanical properties of hydrogels. The in-vitro release got increased to 56.62% from 27%.

Zhao et al. (2022) conferred controlled release of a hydrophobic drug fucoxanthin, by forming a fucoxanthin encapsulated starch nanoparticle having an entrapment efficiency of around 91% at a given concentration of 50 µg/ml.

Acevedo et al. (2018) successfully developed banana starch nanoparticles to ensure controlled release of curcumin. The drug loaded starch nanoparticles showed 80% entrapment efficiency having a mean particle size of less than 250nm.

2.2 Starch Nanoparticles

Nallasamy et al. (2022) illustrated starch as a potent drug delivery polymer by encapsulating a polyherbal drug *Triphala churna* (TC) rich in polyphenols and vitamin C, into starch biopolymers. Characterization studies revealed that starch encapsulated TC was highly stable and released the drug at a faster rate at pH 7.4. encapsulation of TC into starch nanoparticles, did not affect its antimicrobial, antibiofilm and neuroprotective activities.

Mariadoss et al. (2022) fabricate the *p*-Coumaric acid-loaded aptamer- conjugated starch nanoparticles, which showed tremendous effectiveness against triple-negative breast cancer. The drug loaded nanoparticles were found to have increased efficacy and increased cytotoxicity in MDA-MB-231 cells by regulating apoptosis. This proved starch as a potential biopolymer for drug delivery system with the encapsulation efficiency of around 80%, in case of TC.

Tao et al. (2022) successfully prepared, characterized and evaluated rice starch nanoparticles for loading capsaicin with an average particle size of 617.84nm and encapsulation efficiency of around 70.05%. different evaluation studies concluded that rice starch nanoparticles can be efficient in loading different hydrophobic drugs and a successful agent for the controlled drug delivery system.

Thomas et al. (2021) used theophylline and bovine serum albumin as model drugs to prepare starch-modified alginate nanoparticles which showed biocompatibility against L929 fibroblast cell lines. The developed nanoparticles had around 65% to 70% encapsulation efficiency, which proved them as a potential candidate for the drug delivery system.

M. Queiroz et al. (2019) incorporated chlorohexidine into starch nanoparticles by a simple, cheap and reproducible process which showed a controlled in-vitro release having an inhibiting effect on *Staphylococcus aureus* growth, as evaluated by disc-diffusion test. 10.6 g corn starch along with 6.2 g of glycerol and 20% of chlorohexidine solution was used to prepare corn-starch films as an elongated drug delivery system.

Xinge Wang et al. (2016) prepared starch nanoparticles having a mean diameter of 94.3 nm, by water in oil microemulsion method. Acid-treated granular starch was gelatinized using NaOH to form a continuous water phase, which was mixed into the oil phase containing

cyclohexane. Methylene blue and C_{16}mimBr was used as a surfactant. The drug loading and releasing properties of starch nanoparticles provided evidence of the efficiency of this method to form effective starch nanoparticles.

El-Naggar et al. (2015) successfully synthesized starch nanoparticles for the transdermal delivery of Diclofenac sodium, using 5% maize starch and gelatinizing it with 30% sodium hydroxide. Sodium tripolyphosphate was used as a crosslinking agent and TWEEN80 was used as a surfactant. 57.7mg DS and 0.5% STPP was optimized, using JMP software, to produce starch nanoparticles with a diameter of about 21.04nm with an entrapment efficiency of 91.05% and sustained drug release for nearly about 6 hours.

Ai-min Shi et al. (2015) showed the controlled release behaviour of ciprofloxacin from starch nanoparticles. Soluble starch was hydrolysed using sodium hydroxide and crosslinked with sodium meta polyphosphate by using water in oil emulsion technique which included the use of high-pressure homogenization. Two different loading methods were used to load ciprofloxacin, including coating method and adsorption method, which resulted in 40% and 7% drug loading within the nanoparticles, respectively.

Saboktakin et al. (2011) synthesized and evaluated the carboxymethyl starch-chitosan nanoparticles for the delivery of 5-aminosalicylic acid to the colon. The in-vitro release studies showed that the release of the drug from the nanoparticles was based on ion-exchange mechanism and successfully proved carboxymethyl starch-chitosan nanoparticles as a promising controlled drug delivery agent, to the colon.

Ai-min Shi et al. (2010) successfully prepared starch-based nanoparticles by combining high-pressure homogenization and miniemulsion crosslinking technique. Soluble starch was used as a polymer to form water phase by mixing it with sodium hydroxide solution containing Sodium meta polyphosphate as a crosslinking agent. Cyclohexane was used to create the oil phase, which was stirred at 5000-6000rpm using a high-pressure homogenizer. Nanoparticles were obtained by precipitation using acetic acid followed by centrifugation. The stability studies revealed that the prepared starch nanoparticles showed stability at body temperature hence, can be considered as a good carrier for in-vivo drug delivery.

2.3 Tolbutamide- Drug delivery system

Monica et al. (2022) successfully synthesized a co-amorphous material so as to increase the solubility dissolution rate of the antidiabetic drug-tolbutamide. Co-amorphous material was

prepared for tolbutamide with tromethamine. X-RD studies showed the physical stability of co-amorphous structure in dry conditions at 25℃. solubility studies revealed that the solubility of tolbutamide increased by 2.5-fold with respect to its crystalline counterpart.

Yongli et al. (2018) successfully loaded tolbutamide into polylactic-co-glycolic acid (PLGA) nanoparticles, modified with chitosan, using solvent evaporation method. The prepared nanoparticles showed a mean particle size of around 228nm. Cell viability tests were conducted using HePG2 cells, which showed that the prepared nanoparticles were non-toxic.

Imran et al. (2018) successfully improved the therapeutic efficacy of tolbutamide by incorporating into gum xanthan (GX) stabilized gold nanoparticles. The insulin secretion potentials of synthesized nanoparticles were investigated in isolated islets of mice, which showed a remarkable increase, compared to pure drug solutions. It got clear from the results that the prepared nano-carrier drug delivery systems efficiently increased the therapeutic efficacy of poorly water-soluble drugs like tolbutamide.

Pandita et al. (2017) successfully prepared self-nanoemulsifying drug delivery system for tolbutamide. The self-nanoemulsifying granules were characterized and evaluated for antidiabetic efficacy in male Wistar rats. Oleic acid was used as a lipid phase, TWEEN 20 and PG400 as optimal surfactant and co-surfactant respectively. The prepared granules had an estimate particle size of around 58.5nm. Granular formulation on comparison with pure drug suspension, showed a 1.54-fold increase in the dissolution rate of the drug. The prepared self-nanoemulsifying granules showed an overall increase in the bioavailability of tolbutamide.

Marras et al. (2015) successfully prepared interactive, resistant and versatile pectin-based hydrogel as a vehicle for oral administration of tolbutamide. Surfactants like TWEEN and Na Lauryl sulphate were used, which visibly affect the release behaviour of the drug. One, blending with agarose technique, to yield robust systems and second, freeze drying technique to generate a porous structure, were used which had an impact on drug release behaviour.

Malakar et al. (2013) used ionotropic gelation method to successfully prepare alginate beads to ensure the release of tolbutamide for longer periods of time. Potato starch was used as a polymer and calcium chloride was used as a crosslinking agent. Potato starch was added into a gel made up of sodium alginate, followed by addition of tolbutamide and then ultrasonication. The dispersion was added dropwise to calcium chloride solution to give rise to rigid beads. Response surface methodology was used to optimize the concentration of sodium alginate and

potato starch. The optimized beads exhibited 85.5% drug encapsulation efficiency and around 50.4% drug release after 8 hours.

Oviedo et al. (2008) successfully used poly vinyl alcohol (PVA) to prepare a tolbutamide loaded crosslinked hydrogel. Solvation and desolvation processes were used to physically modify PVA, thereby decreasing its crystallinity by 8%. Release studies showed 78% drug release in about 24 hours from the PVA hydrogels which differed majorly from the dissolution curves of tolbutamide tablets.

2.4 Tolbutamide- Analysis

Tolbutamide can be analysed using following techniques of analysis:

- Titrimetric Analysis
- Spectrophotometric Analysis
- Chromatographic Analysis

2.4.1 Titrimetric Analysis

2.4.1.1 Aqueous titration

El-Fatatry et al. (1979) measured the pk_a value of tolbutamide in different aqueous solvents. Tolbutamide was dissolved in suitable medium and titrated, to the pH value corresponding to it's pk_a against the standard alkali medium. Tolbutamide concentration ranging from 1 to 10 mg/ml in 60% aqueous acetone gave the accurate results.

2.4.1.2 Non-aqueous titration

Agarwal et al. (1972) dissolved tablet and pure forms of tolbutamide in tetramethyl urea. The solution was then titrated with 0.1 N lithium methoxide in methanol-benzene medium. 0.2% azo-violet in toluene was used as an indicator to visually determine the end-point.

2.4.2 Spectrophotometric Analysis

2.4.2.1 Ultraviolet analysis

Tolbutamide quantification was done using tolbutamide tablets. Abdel Hady et al. (1979) devised two methods for the quantification of tolbutamide, in which tablet excipients did not interfere.

1. Glenn's Method: concentration of tolbutamide ranging between 0.1-0.4 mg/ml was dissolved in 95% ethanol and the absorbance was measured at 200-250 nm at an interval of 4 nm. Calculated p^2 coefficient was found to be linearly related to the concentration of the tolbutamide.
2. Complex formation method: a complex was formed between tolbutamide and brilliant cresyl blue or safranine T in a ratio of 1:1. Chloroform was used to extract the complex and the absorbance of the chloroform extract was measured at 615 nm for brilliant cresyl blue and 510 nm for safranine T, against a suitable blank.

Abdel Hady et al. (1979) dissolved tolbutamide in 95% ethanol containing thiamine hydrochloride and pyridoxine hydrochloride and measured the absorbance at 274 nm and 276 nm. The difference between the absorbances was calculated and the mean recoveries were found to be 100.0 and 100.8 with a standard deviation of about 0.5%, having negligible interference by thiamine hydrochloride and pyridoxine hydrochloride.

Shibasaki et al. (1973) used citrated blood contaminated with the metabolites of tolbutamide in order to determine tolbutamide. Contaminated blood was haemolysed in phosphate buffer of pH 5. Heptane- chloroform in the ratio 4:1 was mixed with haemolysed blood and shaken for 20 minutes followed by the addition of 0.1 M sodium hydroxide solution with continuous shaking for 20 minutes. The resultant alkali phase was mixed with 3 M HCl solution in a ratio of 20:1 and the absorbance were measured at 228 nm, against a blank containing uncontaminated drug.

2.4.2.2 Spectrofluorometric Analysis

Namigohar et al. (1980) determined tolbutamide by acid hydrolysis, which comprised the reaction of acetylacetone and formaldehyde with the amide to give rise to dihydroxy pyridine. The resultant substitute was fluorometrically determined at 600 nm, with excitation at 480 nm.

2.4.2.3 Proton Magnetic Resonance Spectrometric Analysis

Al-Badr and Ibrahim (1982) determined tolbutamide in formulations and in pure forms by the means of comparison between combination of aromatic doublets of tolbutamide at 7.33-7.8 ppm with that of internal standard i.e., hexamethylenetetramine at 4.61 ppm. Recovery for pure tolbutamide and it's formulation dosage was found to be 100% ±1.5 and 99.6% ±1.4 respectively, by using DMSO as a solvent.

2.4.2.4 Mass Spectrometric Analysis

Weinkam et al. (1977) used chemical ionization mass spectrometric method to determine tolbutamide and it's metabolites from plasma and urine. Ethyl ether was used to extract tolbutamide and it's metabolites from the samples of urine and plasma, which was then treated with diazomethane. Isobutane was used as reagent gas to quantitatively measure the protonated molecular ion. Single-ion monitoring and scan mode monitoring was used to determine single component or multi-component mixtures respectively.

Sabih (1974) reported a high-resolution method of mass spectrometric analysis for the determination of methylated derivatives of tolbutamide. Dimethyl sulphate treated tolbutamide derivative was subjected to g.l.c. operated with helium as a carrier gas with a temperature programmed from 150 to 190˚C at 2˚per minute. Column effluent showed a mass spectrum at 70 eV.

Braselton et al. (1975) formed thermally stable derivatives of tolbutamide and analysed these derivatives by g.l.c.-m.s. method operated at 170˚with helium as a carrier gas, in order to determine tolbutamide and it's metabolites in human serum.

2.4.3 Chromatographic Analysis

2.4.3.1 Gas chromatographic analysis

Hartuig et al. (1980) determined methyl derivative of tolbutamide from serum using nitrogen as a gas carrier at 220˚. Hexadocosane was used as an internal standard.

Agarwal et al. (1974) determined methyl derivative of tolbutamide from plasma and urine sample using helium as a carrier gas at 150˚. Chlorpropamide was used as an internal carrier.

2.4.3.2 Liquid chromatographic analysis

Tolbutamide from it's compressed tablets was determined by Beyer et al. (1972) at 254 nm using high speed liquid chromatography column packed with 1% ethylene-propene copolymer with 0.01 M disodium hydrogen citrate containing 15% methanol as mobile phase.

2.4.3.3 High-performance liquid chromatographic analysis

Raghow and Meyer (1981) used acetonitrile as mobile phase for the quantification of tolbutamide at 254 nm, from plasma. Chlorpropamide was used as an internal standard and the results showed that the calibration curve was rectilinear for 2-80 µg/ml.

Robertson et al. (1979) used prednisone as an internal standard to quantify tolbutamide at 254 nm, from tablet dosage. A mixture of 0.06% acetic acid in ethanol: tetrahydrofuran: hexane was used as mobile phase, in the ratio 1:2:22. The results of undegraded tablets were in agreement within 0.3% with that of U.S.P.

2.5 Optimization using RSM

Kuljit kaur et al. (2018) used RSM to optimize chitosan, gelatine cross-linked by glutaraldehyde, polymer network for polymer backbone, time, pH, amount of solvent and crosslinker concentration, in order to maximize percentage swelling. RSM gave linear model as best fit with a predicted $R^2 = 0.97$, optimized conditions were, a pH of 7 with a percentage swelling of 506.8%. the results showed that hydrogels prepared using RSM is a good device to ensure the controlled release of the drug.

Bahloul et al. (2015) successfully examined the suitability of HLB-RSM approach to prepare self-emulsifying drug delivery system for fenofibrate, which results in enhanced oral bioavailability of the drug. Thermodynamic stability and zeta potential were the primary basis of evaluation of self-emulsifying drug delivery systems, on the basis of which the unstable ones were discarded and characterization studies were proceeded on the stable ones. AUC and c_{max} values justified HLB-RSM approach as an efficient method to prepare stable and promising self-emulsifying drug delivery systems.

Khushbu and Rajiv Jindal (2021) used RSM to optimize various parameters like ratio of backbones, time, amount of solvent, amount of graphene oxide and pH, prepare inclusion complexes of amlodipine besylate drug and β-Cyclodextrin to ensure sustained drug release.

CHAPTER 3

RESEARCH ENVISAGED

Research Envisaged

In order to ensure controlled release of the drug at the specific site of treatment or action, nanoparticle-based therapy has been proven as a promising medium. This nanoparticles-based drug delivery lies on the basis of choosing and forming various drug polymer combinations, in which the release kinetics of the drug and the total dosage, being variable, may be manipulated to get the desired results. Novel microencapsulation technologies include, forming various combinations by varying the molecular weight of the polymer, polymer to drug ratio etc. so as to optimize the prepared nanoparticles to provide required release profiles. Nanoparticles have seen to result in increased lifespan of the pharmaceutical agent, as well as ensures the control release of the constituents. Due to smaller size, nanoparticles have large surface area to volume ratio, which makes them as a promising carrier for controlled release of poorly water-soluble drug. (Ai-min Shi et al. 2008)

Basically, controlled release formulations are designed, keeping in mind, the duration of action of drug rather than the inherent kinetic properties of the drug molecules, which emphasizes on truly understanding the pharmacodynamic and the pharmacokinetic properties of the drug. Nanoparticulate formulation is a drug-polymer combination, hence a thorough understanding of the nature of polymer along with it's biochemical properties also play a significant role.

3.1 Selection of drug

Many drugs have lower efficacy, that might result due to their lower potential to reach to site of therapeutic action. The combination of physiochemical and biological properties of drug makes it difficult to design a controlled release system comprising that particular drug. Moreover, patients' disease state and the technological limitations adds to the difficulty factor. So, in the preparation of controlled release system, the selection of drug is made, taking into consideration a number of properties.

3.1.1 Properties of drug

3.1.1.1 Physio-chemical properties

Dosage amount: The drug should have lower therapeutic dosage.

Aqueous solubility: Should not extremely aqueous soluble.

Partition coefficient: Extreme values of partition coefficient are nonrequired.

Drug stability: Should offer extreme stability throughout the gastrointestinal tract.

Molecular size: Drugs with low molecular size are considered due to their higher diffusion coefficients, suitable for sustained release system.

3.1.1.2 Biological properties

Biological half-life: Drugs that require frequent administration, due to their shorter half-life, make a good candidate for sustained release system.

Absorption: should not undergo slow or variable absorption rate.

Distribution: Drugs having lower volume of distribution form good candidates.

Therapeutic index: drugs having a narrow therapeutic index are more a candidate for the controlled release systems, as they require precise control of the level of drug in the blood.

For the preparation of polymer-crosslinked nanoparticles, Tolbutamide has been chosen as the model drug.

3.2 Tolbutamide

It is a hypoglycemic therapeutic agent, that comes under class-I sulfonylureas, also called as insulin secretagogues. It is used to treat type-2-diabetes. It's site of action is the sulfonylurea receptor, a transmembrane receptor, present on the membrane of Beta cells of islets of Langerhans of Pancreas.

3.2.1 Mechanism of action

Tolbutamide blocks an ATP-sensitive hetero-octamer potassium channel, comprised of four subunits of Sulfonylurea Receptor 1 (SUR1) and four subunit's of Kir6.2 channel. Kir6.2 is the channel pathway that facilitates the efflux of intracellular potassium. Tolbutamide, when bind to the active site at SUR1, leads to the closure of ATP-sensitive potassium channel which ultimately ceases the efflux of intracellular potassium ions. Accumulation of potassium ions inside the cells leads to depolarization of the plasma membrane of pancreatic beta cells. Membrane depolarization further activates the voltage-sensitive transmembrane calcium channel, which then facilitates the influx of extracellular calcium ions. These calcium ions, collide with the secretory granules containing insulin and activated insulin granules fuse with the plasma membrane to result in exocytosis of the insulin outside the cell, into the blood.

3.2.2 Pharmacokinetics

It is mostly administered orally. The site of absorption is the gastrointestinal tract. It can be detected in plasma after 30-60 minutes of ingestion. It attained a peak plasma level after 3-5 hours of ingestion of a single 500mg dose. It was mainly metabolized by CYP2C9 liver enzyme, into 4-hydroxytolbutamide and 1-butyl-3p-carboxy-phenylsulfonylurea. 75-85% of an oral dose was excreted as 1-butyl-3p-carboxy-phenylsulfonylurea in urine mainly in approximately 24 hours. The plasma half-life is about 4.5 to 5.5 hours. It's duration of action is 6 to 12 hours, which is shortest of all sulfonylureas.

3.2.3 Drug description

Tolbutamide, IP(1996), is 1-butyl-3-tosylurea with a molecular weight of 270.35 and an emperical formula as $C_{12}H_{18}N_20_3S$. It's chemical structure is as follows (Synthesis of Essential Drugs, 2006):

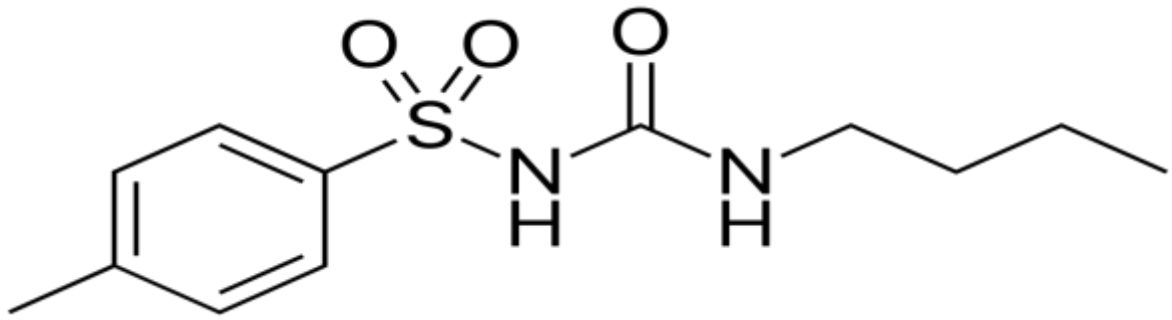


Figure 1: Chemical structure of Tolbutamide

It is an odorless, white crystalline powder, available as immediate release tablets in 500 mg to 1.5 g, administered daily as a single or in divided doses, with breakfast. It's usual strength is 500 mg, but the strength and the frequency of administration dosage depends on the severity of the patient's condition.

3.2.4 Clinical uses of Tolbutamide

Tolbutamide is efficient in treating higher levels of blood sugar, caused by type-2-diabetes (non-insulin dependent diabetes mellitus). It may also come into action when taken with other diabetic drugs.

3.2.5 Adverse effects

Tolbutamide may cause side effects like nausea, headache, stomach fullness, gaining of weight and sore throat. If the patient does not consume proper calories from the diet, or undergoes unusual exercise routine, the drug might cause a chronic hypoglycemia. Chronic lower blood sugar levels might cause dizziness, fast heartbeat, blurred vision, etc. In rare cases, it is found to cause severe allergic reactions like rash, swelling and itching of tongue or throat.

3.2.6 Precautions

Limit alcohol consumption: because while taking this medication, alcohol might react with tolbutamide and cause nausea, vomiting and dizziness.

Nursing mothers: tolbutamide is passed in human milk. Due to it's potential to cause serious side effects in infants nursing from mothers taking tolbutamide, should either discontinue nursing or discontinue uptake of the drug.

Pediatric use: in pediatric patients and adolescents less than 18 years of age, effectiveness and safety of the drug has not been established.

3.3 Selection of polymer

In order to deliver a drug inside the body and achieve a prolonged release of the drug, biodegradable polymers are much more desirable due to their potency to get degraded within the body, into biologically inert, harmless and compatible metabolites. A variety of shapes and sizes of dosage forms, for prolonged drug release, can be prepared by incorporating the drug within the biodegradable polymer, thereby formulating simple and convenient drug delivery systems.

3.3.1 Advantages of drug carriers of a biodegradable polymer

Small monomer unit's bond together to give rise to a large polymer. Biodegradable polymers are biofriendly, hence do not have the need to get eliminated necessarily. Biodegradable polymers offer a large variety of advantages like, drug delivery at specific site of action. Sustained as well as controlled rate of drug delivery or release, ensure stability of the drug within the formulation and provide a release rate which is least affected by the inherent drug properties.

3.4.2 Factors affecting polymer selection

Generally, in order to produce a commercial formulation, only the approved polyesters are used. In others cases some major factors are need to be kept into mind, like, while using a polymer which is comprised of multiple monomeric unit's, the ratio of different monomers may be manipulated to vary the polymeric properties which may affect the morphology, size, structure and intensity of drug-polymer interactions. Variations in these properties will give rise to different formulations which might differ in drug release behavior. (Kotwal and Saifee, 2007).

To achieve appropriate drug delivery system polymers like proteins, peptides, genes and oligonucleotides are mostly unsuitable due to their instability in biological environment and the immune response that body might generate against foreign molecules of such kind. Also, their lesser ability to cross biological barriers and reach the active site, might result in decreased efficacy.

In novel studies, nanoparticles are being made using amphiphilic or hydrophobic biodegradable polymers such as PLGA (poly-lactic-glycolic-acid), PEG (poly-ethylene-glycol) and PLA (poly-lactic-acid). Further studies have proven these polymeric nanoparticles as efficient drug delivery systems (Blanco and Alonso, 1997; Tobio et al. 1998; Tobio et al. 2000). But these nanoparticles have various limitations like, the preparation requires a more volumes of organic solvents, surfactants and sonication. Preparation and use of hydrophilic polymers as colloidal carriers, in place of hydrophobic ones, came out as a challenging alternative. To bring this to application, starch has come out to be a promising carrier for delivery of various therapeutic agents (Hebeish et al., 2014).

3.4 Starch

Starch is a complex carbohydrate, composed of simpler glucose monomers bound together through glycosidic linkage. It is a plant-based polymer manufactured in the green leaves and stored in the chloroplasts as granules and other storage organs like roots, seeds and tubers of plants. It is a soft, white semi-crystalline powder, which is insoluble in cold water, ethanol and most other solvents. It's chemical formula is $(C_6H_{10}O_5)_n$. the simpler linear structure of starch, called as amylose, is made up of glucose monomers bound together through α-1,4-glycodic linkage and the complex branched chain, called as amylopectin, is made up of glucose monomers bound together through α-1,6-glycosidic linkage. Amylopectin and amylose content in starch is mainly around 65-70% and 25-30% respectively. The content may vary in starches

obtained from different sources. Different types of starch are used for commercial purposes like, maize starch, potato starch, cassava starch, soluble starch, etc.

In the present study, Maize Starch has been used as a polymer.

3.4.1 Synthesis of maize starch

Industrial processing of maize starch is generally done by wet milling process. The maize seeds are obtained and checked for cobs, dust and other impurities under primary cleaning of raw material. To break the starch-protein bonds, controlled fermentation process also called as steeping process is done, in which the cleaned maize seeds are kept in steeps and are soaked in hot water for about 30-40 hours, under lactobacillus and Sulphur dioxide so as to enhance fermentation and inhibit the growth of other bacteria and fungi, respectively. To separate germ and endosperm, soft kernels are broken to loosen the hull, enhanced by the addition of water. Germs are then frequently washed followed by drying up to 4% moisture. The mill flow, after germ separation, is finely grinded and screened to remove insoluble impurities. The released starch, from endosperm, is separated from gluten and then starch refining is processed followed by starch dewatering. The processed starch may be modified depending upon the requirements of end user (ISI-Technical memorandum on production of Corn Starch).

The chemical structure of starch is as follows (Chemical properties of starch,2020):

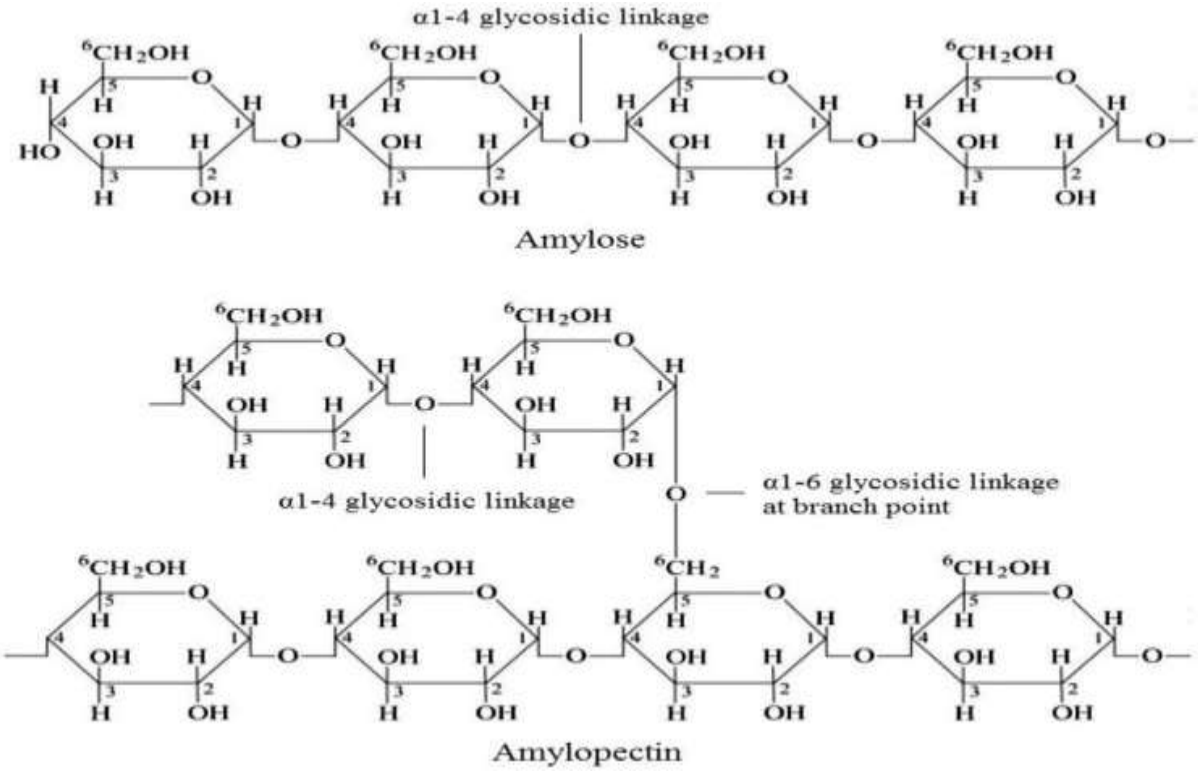


Figure 2: Chemical structure of Starch

FDA has approved corn starch as one of the safe food substances and a nutraceutical molecule.

3.4.2 Biological properties of starch (NCBI)

Biocompatible

Biodegradable to normal body constituents

Safe and non-toxic

Homeostatic and improves digestion

Nutraceutical

Anti-cancerogenic

3.4.3 Pharmaceutical properties of starch (Rowe et al. 2009)

Tablet disintegrant and glidant

Diluent, filler and bulking agent

Controlled and sustained release polymer for drugs and hormones

Binder, adherent and lubricant

Used as an alternative of metals and ceramics in Bone tissue engineering

Adjuncts to ORS and acts as a plasma volume expander

3.5.4 Pharmacokinetics

In the present study, maize starch undergoes alkaline gelatinization followed by freeze drying to form partially indigestible starch particles. According to Achour et al. (1997), the partially indigestible maize starch undergoes a slow intestinal digestion and continues to ferment in the colon for up to 10-13 hours after it's ingestion, in healthy human beings.

3.5 Crosslinking agent

Sodium tripolyphosphate (STPP)

Tripolyphosphate (TPP) also called as Sodium triphosphate; Triphosphoric acid, pentasodium salt; Pentasodium triphosphate; Pentasodium tripolyphosphate is a well-established and extensively researched crosslinker. It's empirical formula is $(Na_5O_{10}P_3)_n$ with a molecular weight of about 367.864 g/mol. The chemical structure of STPP is as follows (Sugih et al. 2019):

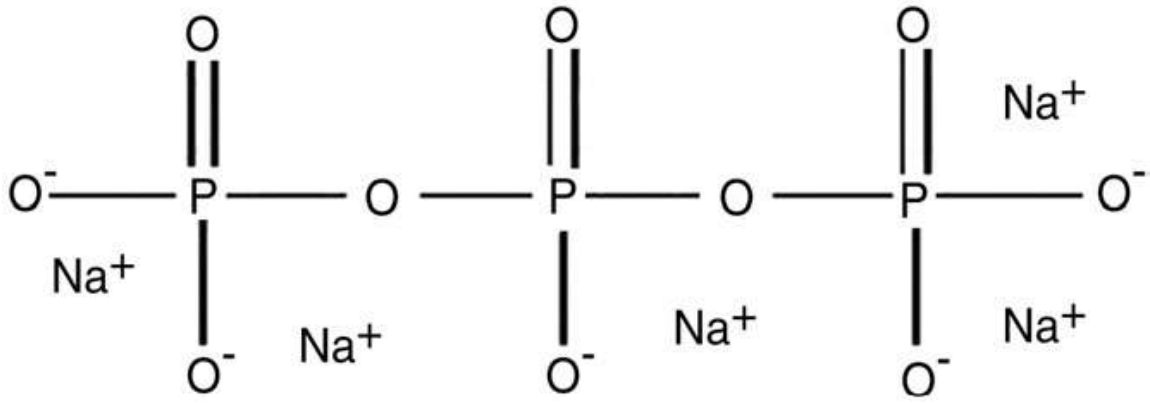


Figure 3: Chemical structure of STPP

One molecule of TPP has five bonding sites. TPP reacts with starch to give rise to di-starch phosphate. Multiple functional groups of TPP reacts with negatively charged hydroxyl groups of starch to yield inter and intra ester linkages through the formation of phosphodiester bonds. (El-Naggar et al. 2015).

Crosslinking starch with TPP may provide desired stability properties of the granules by forming new covalent bonds (Hebeish et al., 2014).

3.5.1 Preparation

Disodium phosphate (Na_2HPO_4) and monosodium phosphate (NaH_2PO_4) mixture is heated under controlled conditions to give rise to industrial preparation of Sodium tripolyphosphate.

$$2\ Na_2HPO_4 + NaH_2PO_4 \Longrightarrow Na_5P_30_{10} + 2H_20$$

3.5.2 Uses

TPP is listed as "generally recognized as safe" by Food and Drug Administration (USA). TPP has got following applications:

- Preservative for meats, poultry and sea foods
- Additive in toothpaste, detergents, toilet cleaners and surface cleaners
- Builder in soaps and detergents, tends to improve their cleansing ability
- Cleaner in industrial cleaning processes and ceramics manufacture
- TPP also performs significant chemical functions like:
- Sequesters hardness of water
- Enhances functional effectiveness of surfactants
- Enhances dirt emulsification and grease hydrolysis
- Dissolves dispersed dirt particles
- Shows effective pH buffering capacity

CHAPTER 4

EXPERIMENTAL WORK

Experimental Work

Numerous chemicals and instruments used in the preparation and characterization of crosslinked nanoparticles are listed in table 1.

Table 1: Experimental materials and equipments.

Experimental material	**Equipments**
• Native Maize starch (extra pure, LOBA Chemie) • Tolbutamide (Sigma-Aldrich) • Sodium tripolyphosphate (analytical grade 90-95%, Sigma-Aldrich) • Absolute ethyl alcohol (analytical grade 99.9%) • Acetone (analytical grade 90-95%) • Methanol (analytical grade 99.9%) • Sodium hydroxide flakes (purity 97%, LOBA Chemie) • Buffer (pH 1.2) • Buffer (pH 6.8) • Distilled water	• Weighing balance (Sartorius) • Homogenizer (Remi) • UV-Vis Spectrophotometer (UV-1900, Shimadzu) • Shaking incubator (Thermo Scientific) • Centrifuge (Thermo Scientific) • Lyophillizer (Thermo Electron Co.) • Refrigerator (-80℃) (Brunswick) • Scanning Electron Microscope (Zeiss) • Fourier Transform Infrared Spectrophotometer (Shimadzu) • X-ray Diffractometer (Panalytical) • DLS Zetasizer (Malvern) • Disposable syringes • Measuring cylinders (100ml, 500ml, 1000ml) • Micropipettes (20µl, 200µl, 1ml) • Glass pipette (25ml) • Glass beakers (100ml, 200ml, 250ml) • Falcons (50ml) • Open mouth flasks (250ml) • Test tubes (25ml, 50ml) • Glass vials (500 µl)

4.2 Preparation of standard curves of Tolbutamide

Concentration of Tolbutamide in the solution was estimated by UV-Vis Spectrophotometer by reading the instrument at 228nm. Tolbutamide standard curves were prepared in distilled water, buffer solution of pH 1.2 and buffer solution of pH 6.8.

4.2.1 Preparation of buffer (pH 1.2)

50 ml of 0.2 M Potassium Chloride solution was added to a 250ml volumetric flask, followed by the addition of 85 ml of 0.2M Hydrochloric acid solution and made up the volume to 200 ml using distilled water.

4.2.2 Preparation of buffer (pH 6.8)

Dissolved 2.7744g of potassium dihydrogen phosphate in 80ml in distilled water in a 250ml flask and dissolved 7.0168g of disodium hydrogen phosphate in another 250ml flask containing 80ml distilled water. Mixed both the solutions to adjust the pH to the desired pH. Made up to 200 ml, using distilled water.

4.2.3 Preparation of standard stock and working solutions

10mg accurately weighed drug was dissolved in 10ml methanol to prepare 1mg/ml stock solution. Diluted the solution with methanol to produce a 0.001% (w/v) solution. 6 µl to 48 µl were pipetted out from the standard working methanol solution and volume was made up to 3ml by adding distilled water to obtain the dilutions containing the desired concentration of Tolbutamide ranging from 2 µg/ml to 16 µg/ml. the absorbance of different dilutions was measured at 228nm against suitable blank using stable beam spectrophotometer.

Similarly, standard stock and working solutions were prepared for buffer (pH 1.2) and buffer (pH 6.8).

Table2: Standard curve data of tolbutamide in distilled water

S. No.	Concentration (µg/ml)	Absorbance (228 nm)
1	2	0.1603
2	4	0.2907
3	6	0.3869
4	8	0.4782
5	10	0.6243
6	12	0.7241
7	14	0.8509
8	16	1.0017

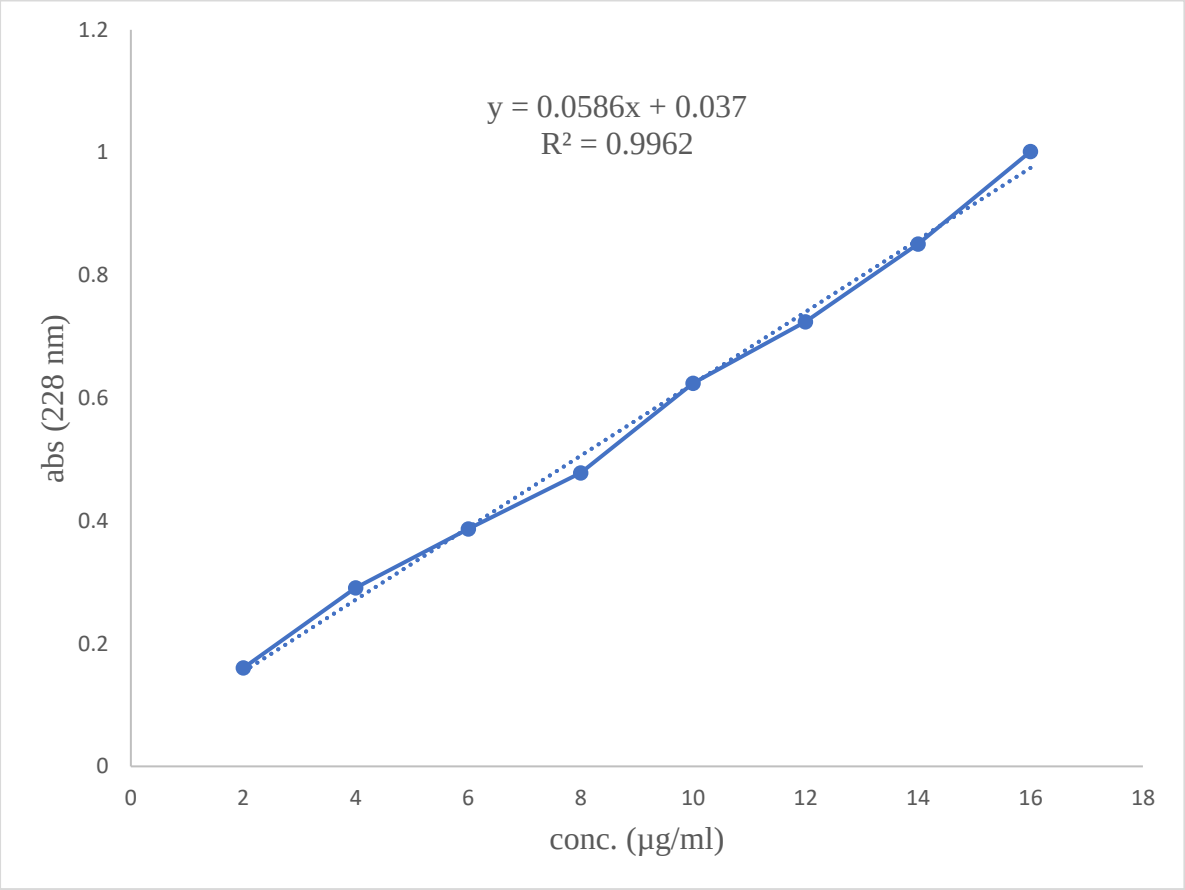


Figure 4: Standard curve of tolbutamide in distilled water

Table 3: Standard curve data of tolbutamide in buffer (pH 1.2)

S. No.	Concentration (µg/ml)	Absorbance (228 nm)
1	2	0.1072
2	4	0.3513
3	6	0.5413
4	8	0.687
5	10	0.8688
6	12	1.0662
7	14	1.2751
8	16	1.4943

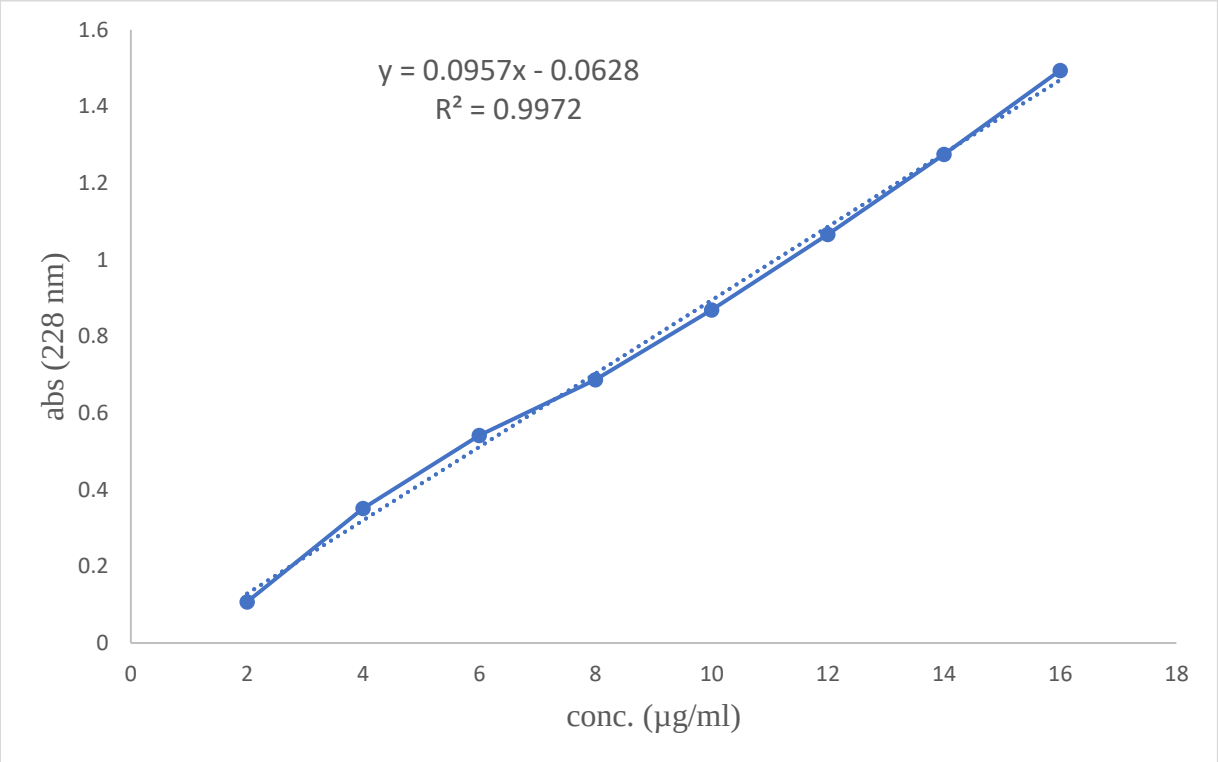


Figure 5: Standard curve of tolbutamide in buffer (pH 1.2)

Table 4: Standard curve data of tolbutamide in buffer (pH 6.8)

S. No.	Concentration (µg/ml)	Absorbance (228 nm)
1	2	0.1533
2	4	0.3711
3	6	0.5515
4	8	0.6956
5	10	0.8677
6	12	1.028
7	14	1.1875
8	16	1.3791

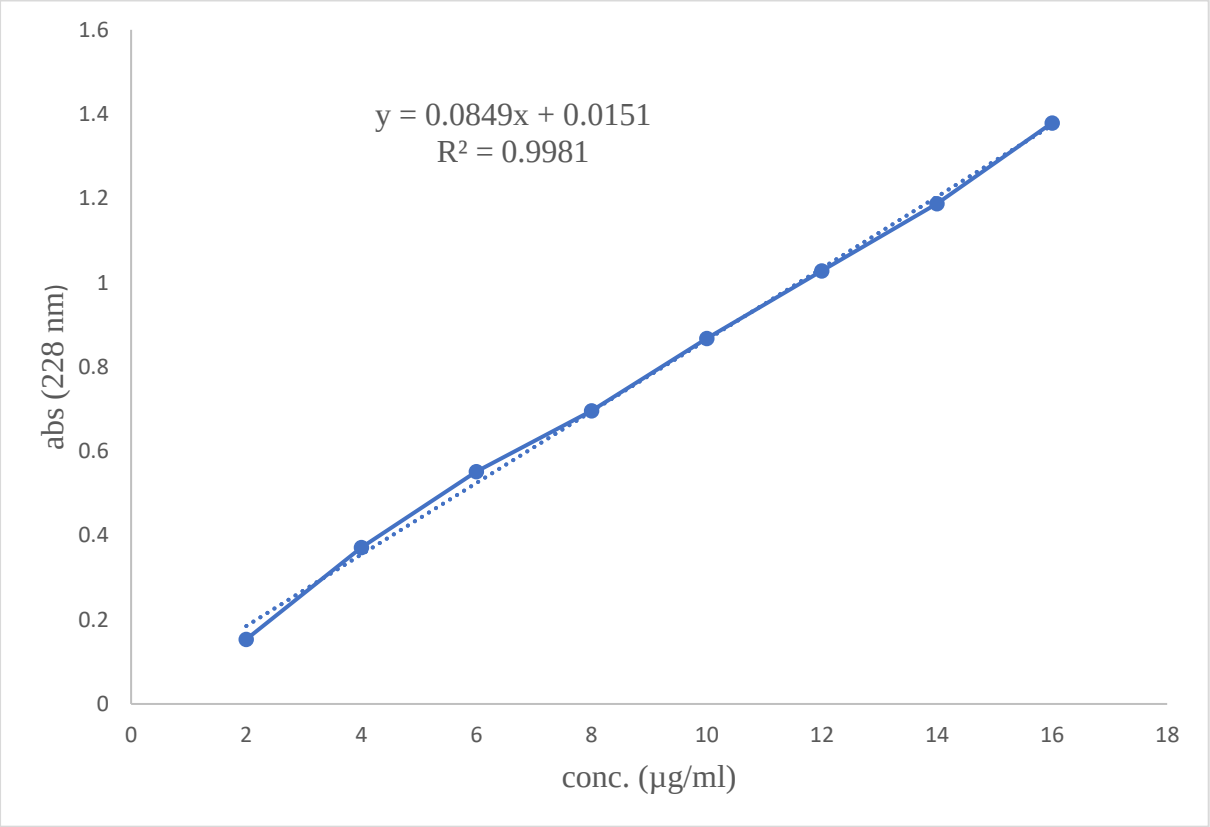


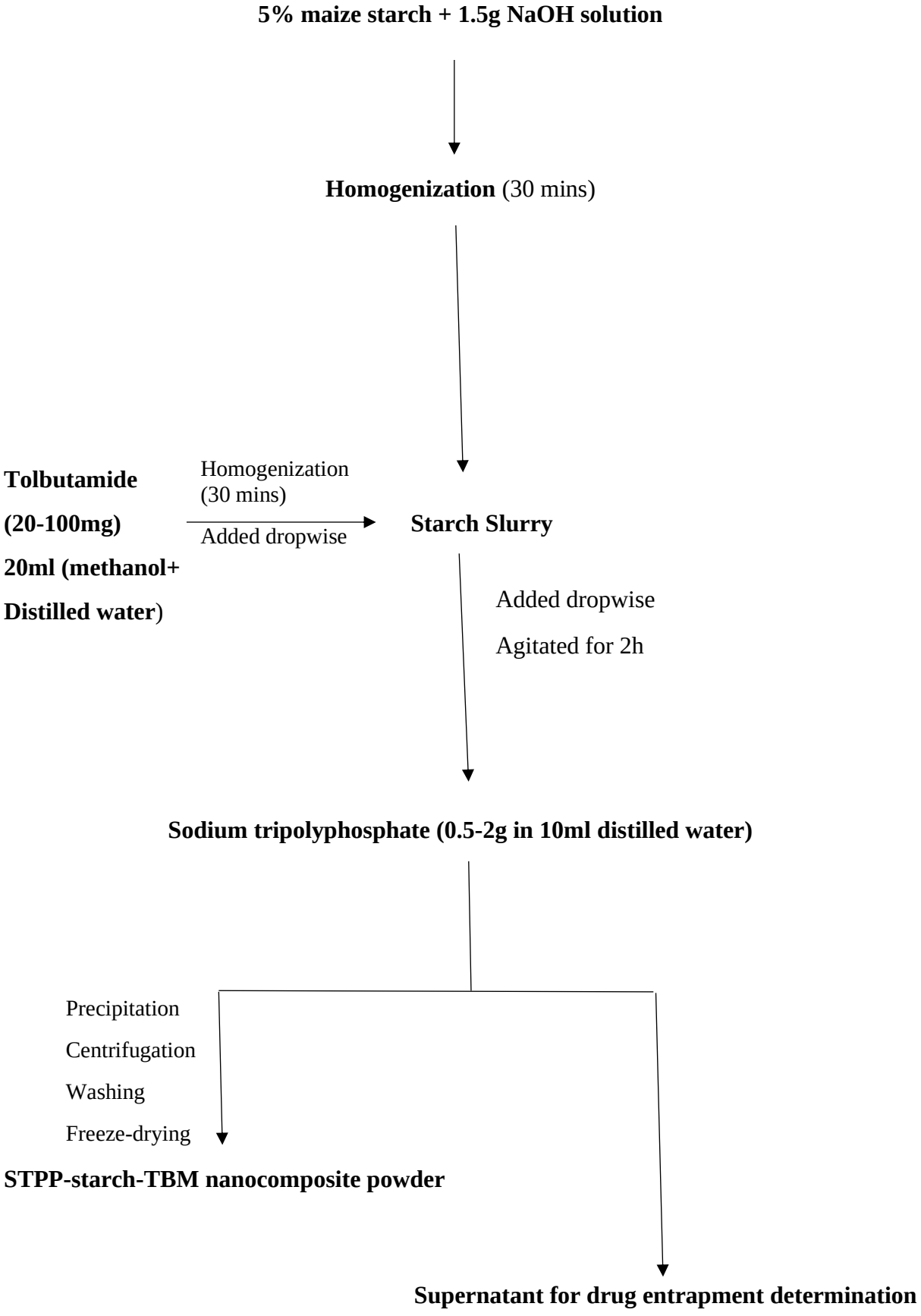
Figure 6: Standard curve of tolbutamide in buffer (pH 6.8)

4.3 Preparation of starch nanoparticles

Starch nanoparticles were prepared by nanoprecipitation technique, also called as solvent displacement method.

In this method (El-Naggar et al., 2015) native maize starch (5%) was gelatinized using sodium hydroxide (30%; with respect to weight of starch) in 70ml distilled water followed by homogenization using homogenizer (Remi, India), at high speed at room temperature, for 30 minutes. Different amount of tolbutamide (ranging 20mg to 100mg) was dissolved in 8ml acetone followed by addition of distilled water to make up the volume up to 20ml. this solution was then added dropwise, using a disposable syringe, into the dispersed starch solution under high homogenization speed for 30 minutes. Varying amount of TPP (ranging 0.5g to 2g), was dissolved in 10 ml distilled water (keeping in mind the total volume of reaction mixture is 100ml), using a vortex (Spinix, USA). The dispersed starch solution was added dropwise, using another disposable syringe, into the TPP solution and kept stand at room temperature for 2 hours, under constant agitation. Tolbutamide encapsulated starch nanoparticles were precipitated using 100ml absolute ethanol. The nanocomposite powder was then centrifuged (UV-1900, Thermo Scientific, USA) at 10,000 rpm for 30 minutes, followed by washing twice with 80/20 absolute ethanol/water to remove unreacted compounds and then final washing was done using absolute ethanol. The supernatant was separated and examined for the loss in tolbutamide. The nanocomposite mass was placed in a glass vial and kept at -80℃ for 12-15 hours in an ultra-freezer (Brunswick Scientific, USA) and then freeze-dried using a Lyophillizer (Thermo Electron Co., USA).

4.3.1 Experimental Design



4.4 Optimization using 3^2 factorial design

In order to prepare and optimize the best batches and evaluate them for better entrapment efficiency and desirable release behavior, the amount of tolbutamide (X1= 20-100mg) and amount of TPP as crosslinker (X2=05-2g) were considered as independent variables, varied at two levels low level (-1), intermediate (0) and high level (+1), for which drug encapsulation efficiency was taken as a response factor. Design Expert 13 Software (Stat-Ease Inc. USA) was used to design all the trial formulations. This was done in order to reduce the number of trial runs, and to optimize the independent variables so as to get maximum response.

Following were the trial formulations designed by design expert 13, using Response Surface Methodology (RSM):

Table 5: Formulations of tolbutamide loaded crosslinked starch-TPP nanoparticles at varying drug and TPP concentrations.

S. No.	**RUN**	**Drug Concentration (mg) (X1)**	**TPP Concentration (g) (X2)**
1	R1	60	1.25
2	R2	60	2
3	R3	60	0.5
4	R4	31.7157	0.71967
5	R5	20	1.25
6	R6	31.7157	1.78033
7	R7	100	1.25
8	R8	88.2843	1.78033
9	R9	88.2843	0.71967

4.5 Characterization of Tolbutamide loaded crosslinked starch-TPP nanoparticles

1. Percentage Yield
2. Entrapment efficiency
3. Equilibrium swelling studies
4. In-vitro release studies
5. FTIR spectroscopy
6. X-ray diffraction study
7. Hydrodynamic size and particle size distribution measurements
8. Scanning Electron Microscopy

4.5.1 Percentage yield

The prepared starch nanoparticles were collected, freeze dried and weighed. The percentage yield was calculated as

$$\% \text{ Yield} = \frac{\text{weight of dried nanoparticles recovered}}{\text{Weight of starch + weight of TPP}} \times 100$$

Table 6: Percentage yield of different batches of starch-TPP nanoparticles

Batch no.	**Percentage yield (%)**
R1	80.9024
R2	88.39
R3	68.6
R4	71.84
R5	81.54
R6	81.95
R7	82.352
R8	85.69
R9	74.11

4.5.2 Entrapment efficiency

At the time of nanoparticle preparation, the nanoparticle suspension was centrifuged at 10,000 rpm for 30 minutes and the supernatant solution was separated. 1ml of supernatant was diluted for 10 ml solution with distilled water and the absorbance was measured using UV-Vis. spectrophotometer (UV 1900, Shimadzu Corp., Japan) at 228 nm, against a suitable blank. Entrapment efficiency was calculated by subtracting the amount of drug lost in the supernatant from the initial amount of drug taken.

Entrapment efficiency (%) = (drug given - drug loss)/ drug given x 100

Table 7: Entrapment efficiency of TBM loaded starch-TPP nanoparticles

Batch no.	**Encapsulation efficiency (%)**
R1	44.76
R2	45.36
R3	33.35
R4	32.36
R5	33.28
R6	34.91
R7	66.17
R8	54.92
R9	49.76

4.5.3 Equilibrium Swelling studies

Swelling properties of TBM loaded starch-TPP nanoparticles were determined in buffer (pH 1.2 and pH 6.8). 100mg of nanoparticles were immersed in 35ml of buffer solution in a 250ml flask and kept in a shaking incubator at 37℃ till equilibrium was achieved and no further changes in size was observed. Swollen nanoparticles were filtered and blotted in order to remove the excess amount of water adhered to the surface, and weighed on a weighing balance. Percentage swelling of nanoparticles at equilibrium was calculated as follows:

$$\%E_{sw} = (W_e - W_o)/ W_o \times 100$$

Where;

E_{sw} = nanoparticles swelling at equilibrium (%)

W_o = initial weight of nanoparticles

W_e = weight of nanoparticle at equilibrium

Table 8: Percentage swelling of different batches of nanoparticles

Batch no.	**E_{sw} in buffer (pH 1.2) (%)**	**E_{sw} in buffer (pH 6.8) (%)**
R1	79.88	110
R2	49.63	123.6
R3	34.7	96.31
R4	81.36	129.6
R5	87.9	277.6
R6	58.7	191.1
R7	45.6	79.1
R8	58.3	185.5
R9	51.63	82.38

(a) (b)

Figure 7: (a) nanocomposites (b) nanocomposites after 6 hours in pH 6.8 (swelling study)

4.5.4 In-vitro release studies

100 mg of drug encapsulated starch nanoparticles were immersed in 35 ml of buffer (pH 1.2), in a 250 ml volumetric flask, for each batch of prepared nanoparticles. The flasks were kept in shaking incubator at 37˚C at 120 rpm. After two hours the nanoparticles were filtered and placed into 35 ml of buffer (pH 6.8) and incubated at 37˚C, for another 5 hours. Starting from time 0 hour, 1ml sample was withdrawn and same amount was replaced with fresh buffer, up to next 7 hours. The withdrawal amount at each desired interval was diluted and evaluated for the amount of drug released at that interval. After the completion of 7 hours another 1 ml was withdrawn after 24 hours, and same procedure was repeated to evaluate the amount of drug release after 24 hours. Drug release profiles for each batch is given below:

Table 9: Drug release profile of batch R1

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	12.72
3	2	17.66
4	3	26.6
5	4	36.7
6	5	46.5
7	6	58.2
8	7	61.5
9	24	63.6

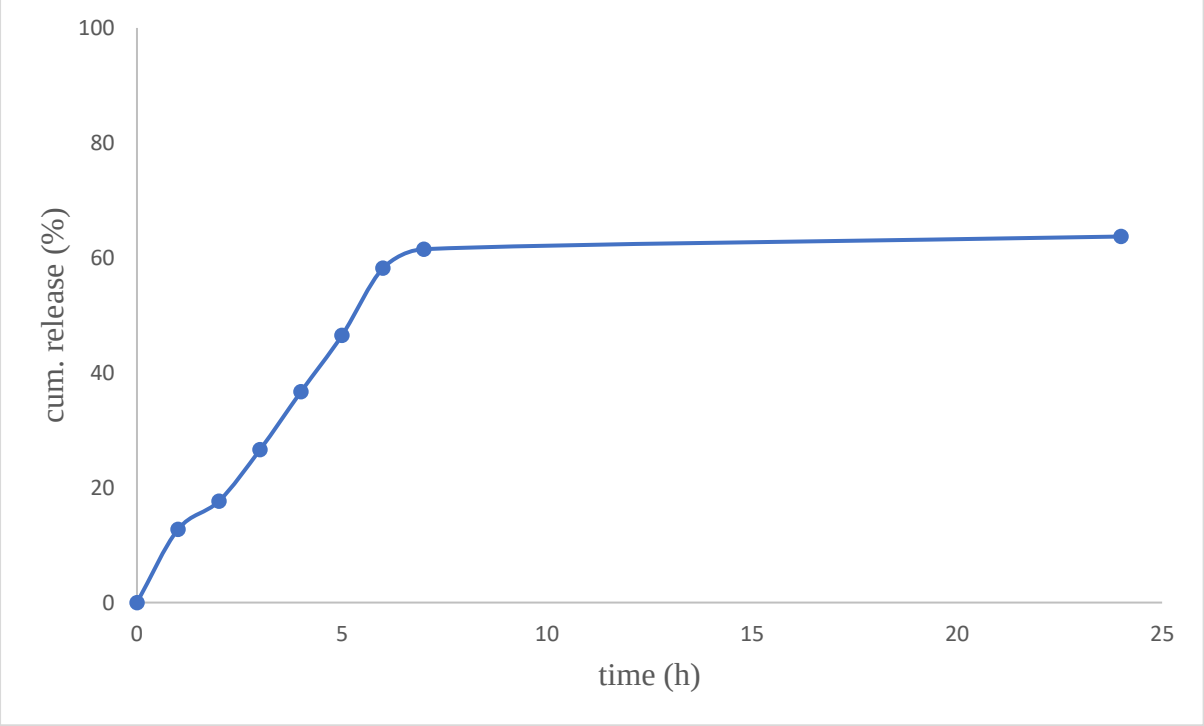


Figure 8: Drug release curve of batch R1

Table 10: release profile of batch R2

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	1.62
3	2	11.90
4	3	19.29
5	4	46.68
6	5	56.96
7	6	62.71
8	7	71.14
9	24	74.64

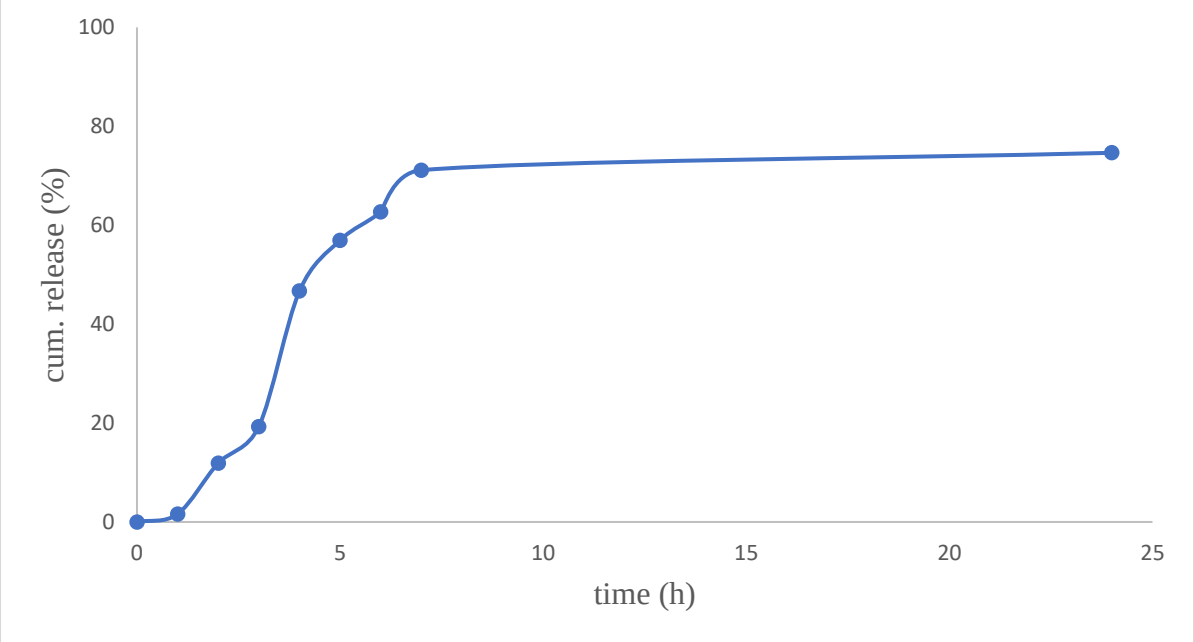


Figure 9: Drug release curve of batch R2

Table 11: Drug release profile of batch R3

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	0.0
3	2	0.0
4	3	5.813
5	4	24.14
6	5	46.05
7	6	52.13
8	7	57.8
9	24	58.95

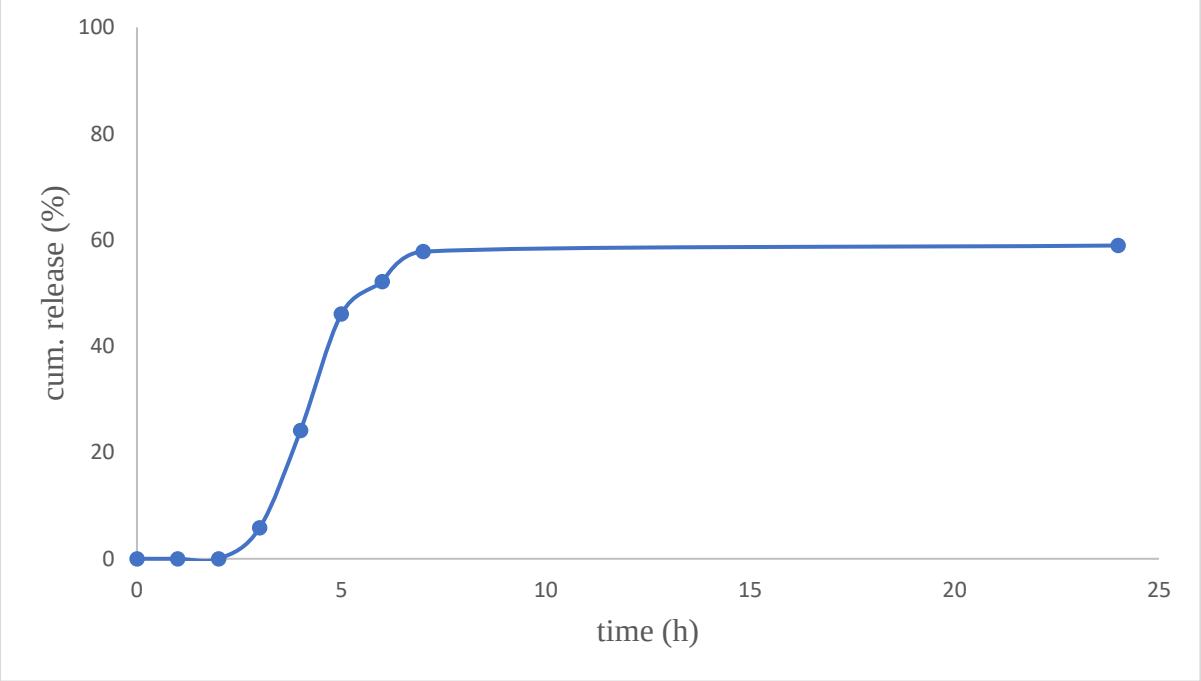


Figure 10: Drug release curve of batch R

Table 12: Drug release profile of batch R4

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	14.72
3	2	16.27
4	3	17.01
5	4	21.81
6	5	44.51
7	6	64.41
8	7	72.36
9	24	74.74

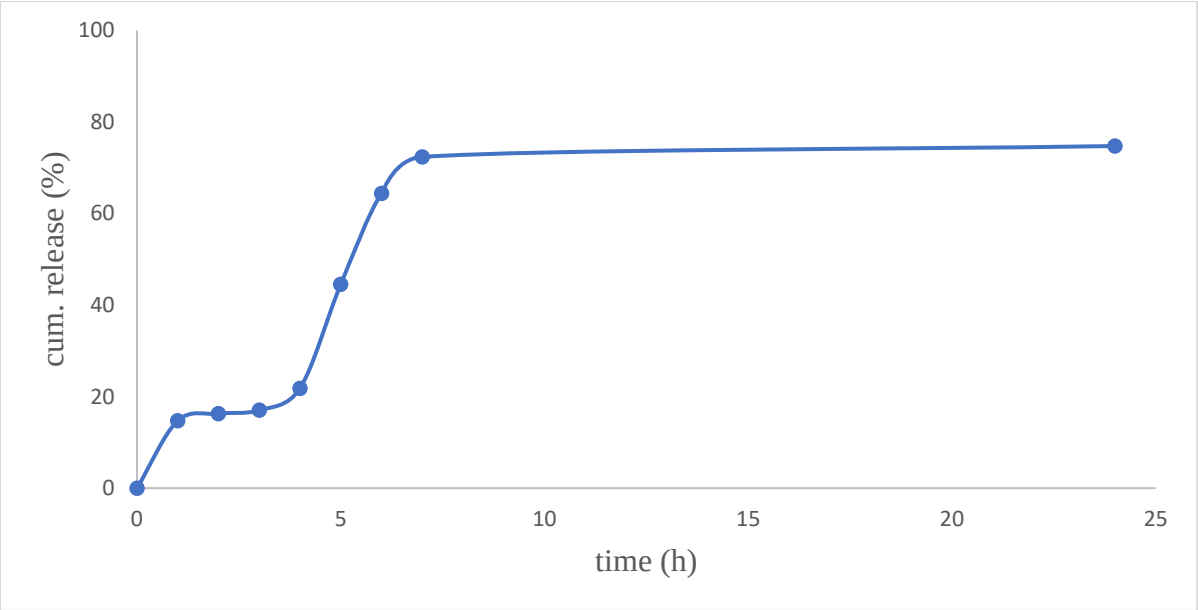


Figure 11: Drug release curve of batch R4

Table 13: Drug release profile of batch R5

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	18.48
3	2	41.91
4	3	85.13
5	4	99.68
6	5	99.68
7	6	99.68
8	7	99.68
9	24	99.68

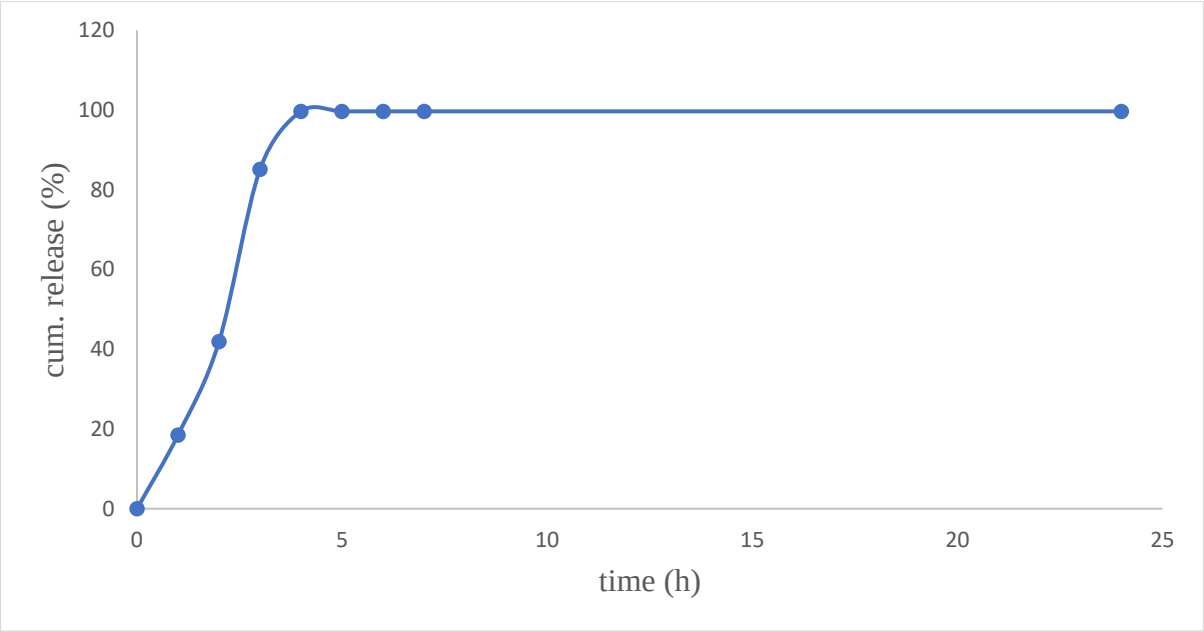


Figure 12: Drug release curve of batch R5

Table 14: Drug release profile of batch R6

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	15.51
3	2	26.24
4	3	48.94
5	4	62.06
6	5	81.8
7	6	98.36
8	7	98.36
9	24	98.36

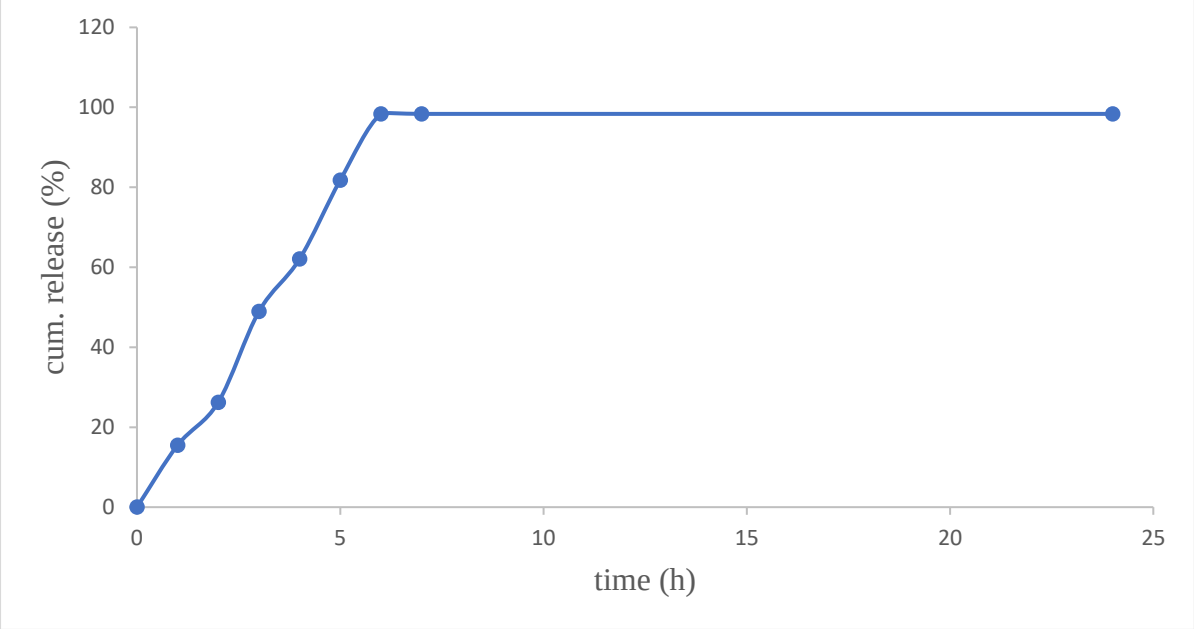


Figure 13: Drug release curve of batch R6

Table 15: Drug release profile of batch R7

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	1.55
3	2	1.94
4	3	3.6
5	4	18.13
6	5	22.64
7	6	27.14
8	7	28.75
9	24	30.18

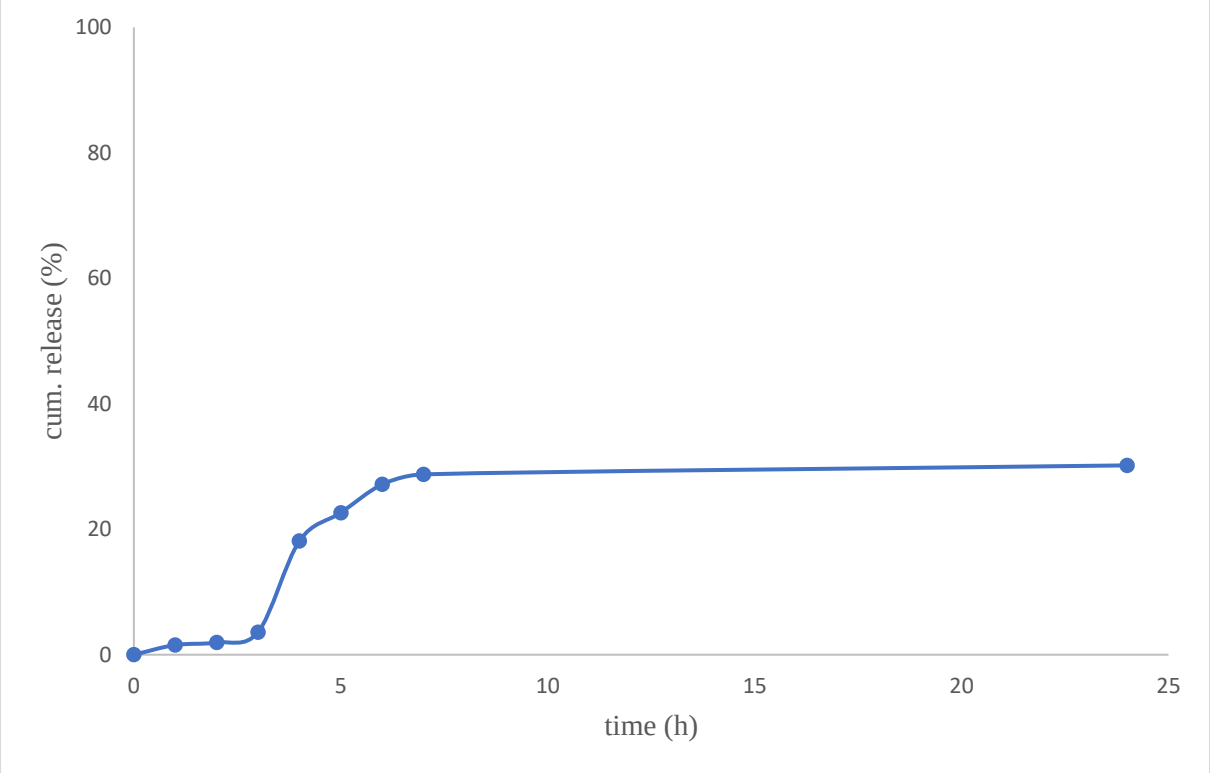


Figure 14: Drug release curve of batch R7

Table 16: Drug release profile of batch R8

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	6.43
3	2	12.62
4	3	21.2
5	4	45.66
6	5	64.70
7	6	78.48
8	7	85.44
9	24	88.65

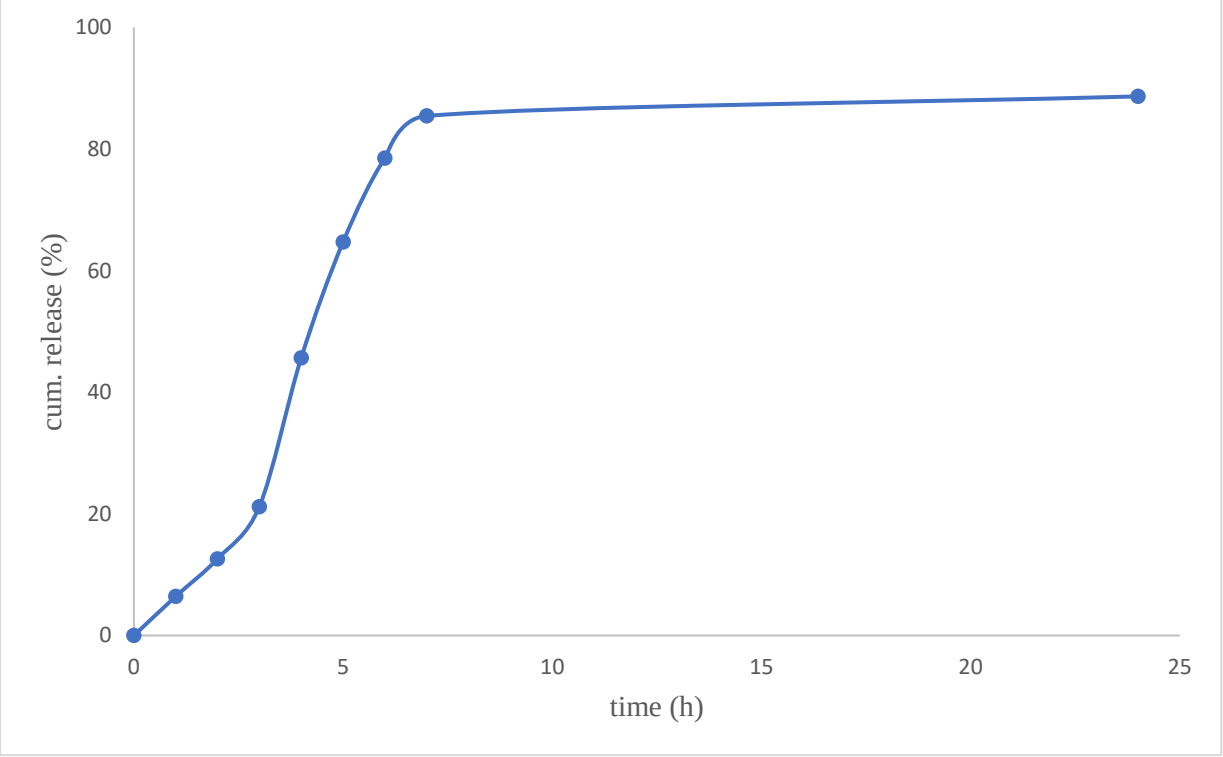


Figure 15: Drug release curve of batch R8

Table 17: Drug release profile of batch R9

S. No.	Time (h)	Cumulative release (%)
1	0	0
2	1	2.7
3	2	4.3
4	3	8.4
5	4	18.52
6	5	26.715
7	6	30.6
8	7	34.7
9	24	38.9

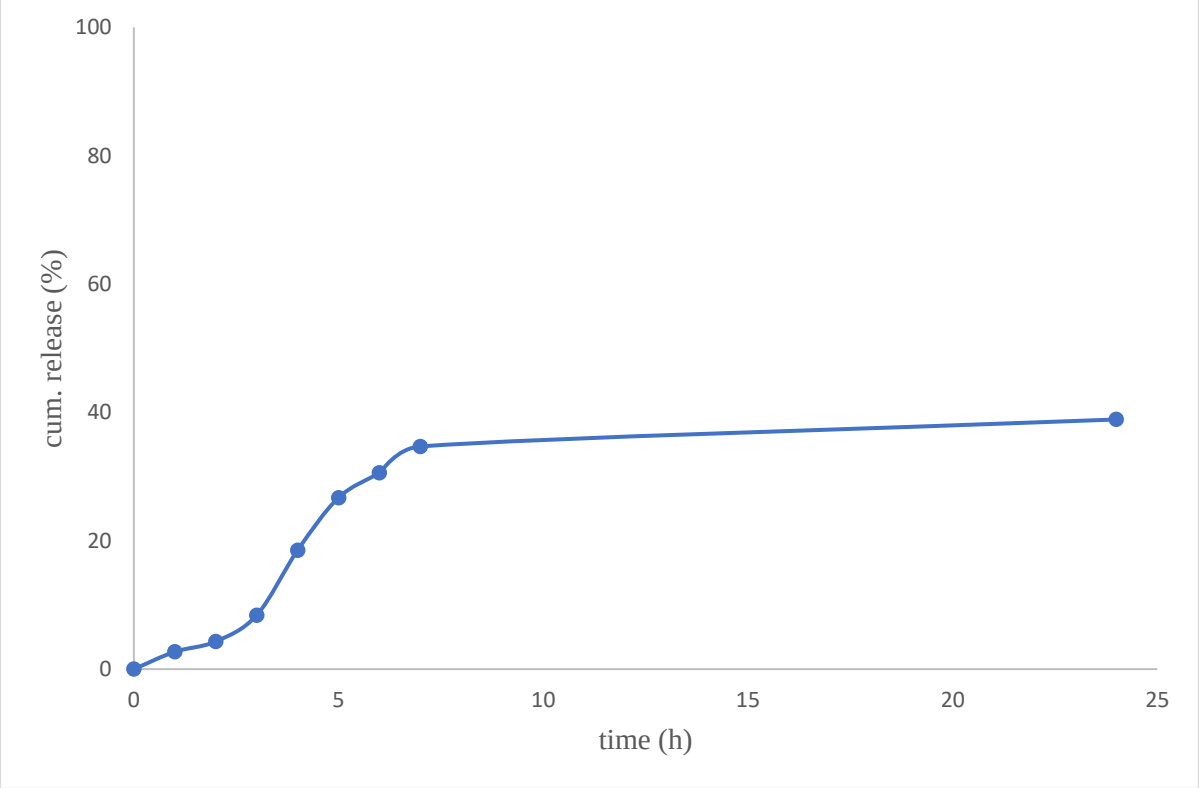


Figure 16: Drug release curve of batch R9

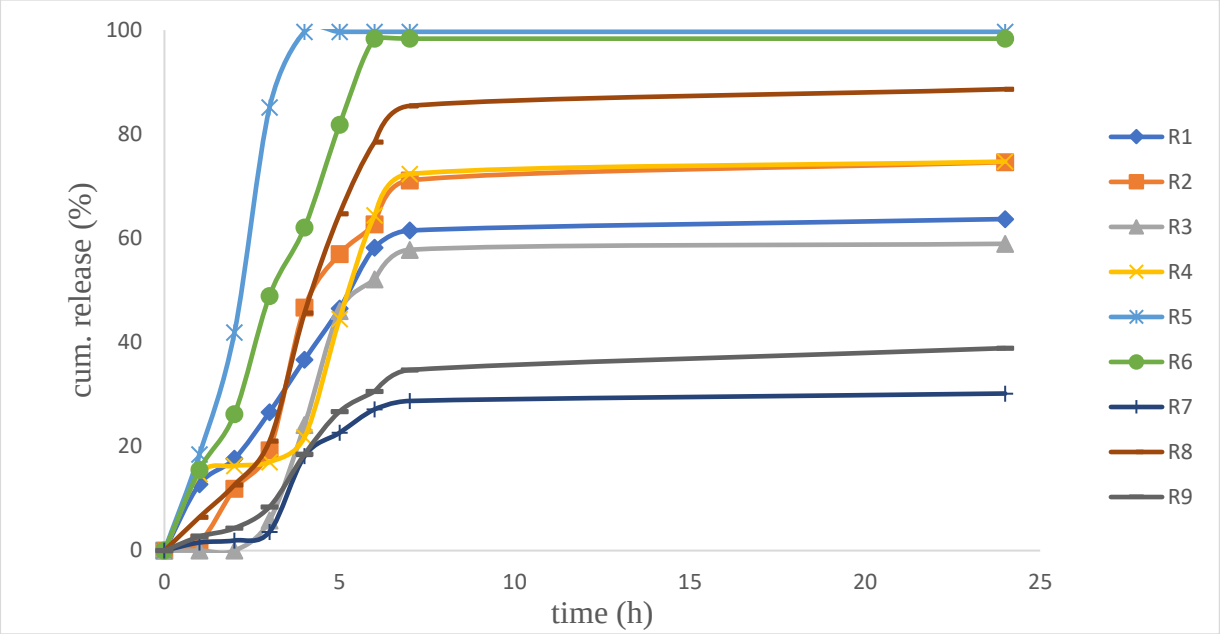
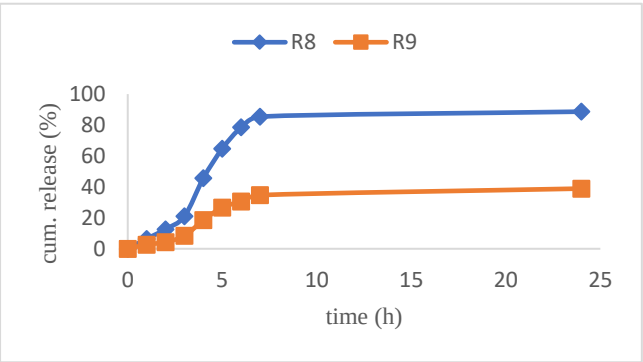
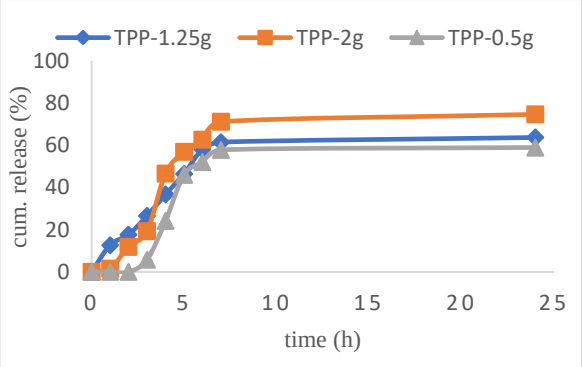


Figure 17: Drug release curves of batches R1 to R9



(a)



(b)

Figure 18: Drug release behavior of nanoparticles at varying TPP concentrations (a) TBM= 88 mg and (b) TBM= 60mg

4.5.5 Fourier transform infrared (FT-IR) Spectroscopy

In order to ensure the possibility of chemical bond interaction between the drug and the polymer, the FTIR spectroscopy, using FTIR spectrophotometer (Shimadzu Corp., Japan), was done for the pure drug, native maize starch (unpolymerized), unloaded starch-TPP crosslinked nanoparticles and TBM loaded starch-TPP

crosslinked nanoparticles. A thin film of freeze-dried nanoparticles was transferred on the sample platform of the spectrophotometer and slowly rotated the knob to bring above the solid sample and screwed down gently to fixate the sample. Scanning spectra was evaluated in wavelength region between 4000-400 cm^{-1}, at a resolution of 2 cm^{-1} and 1 cm/s scanning speed.

4.5.6 X-ray diffraction (XRD) study

In order to ensure the crystallinity state of pure tolbutamide and tolbutamide loaded in starch crosslinked nanoparticles, X-RD study was carried out using X-ray diffractometer, Panalytic, UK). The pure drug and nanocomposite powders were mounted on aluminum stages with glass bottoms. The surface was flattened using a clean glass slide and ensured that there is no cavity left. The X-RD spectra was measured for each sample, from 10 degrees to 50 degrees 2θ with a step increment of 0.02 2θ degrees and 1s dwell time at each step.

4.5.7 Hydrodynamic size and particle size distribution measurements

The particle size and distribution of the TBM loaded starch nanoparticles and unloaded starch nanoparticles were recorded using dynamic light scattering (Nano-ZS, Malvern, UK) technique. The unloaded starch-TPP crosslinked nanoparticulate powder and the TBM loaded nanocomposite powder were diluted using distilled water to a dilution of 1mg/ml and analyzed using a Zetasizer. The scattering angle was 90 degrees at 25˚C. The reported PDI was the particle size distribution, whose value lies in the range 0-1. The values closer to 0.5 represent homogenous size distribution and the values close to 1 represent heterogenous size distribution.

4.5.8 Physical appearance

Each batch from R1-R9 was evaluated for its morphology. All the batches had white colored nanocomposites. The nanoparticles from the batches R1, R2, R5 and R6 appeared as a white powdered substance, slightly sticky in nature. In case of R3 and R4, the nanoparticles appeared to have slightly fine and more round shape compared to R1, R2, R5 and R6. Nanoparticles from batches R8 and R9 appeared to have proper particle like spherical morphology. But, in case of R7, mass like structure was found. This might be possible due to presence of high amounts of both drug as well as TPP or may be because of improper formation or lyophilization of the batch. Following figures depict the physical appearance of each batch. Figure 19 (h) depicts the physical appearance of nanoparticles formed from optimized batch.

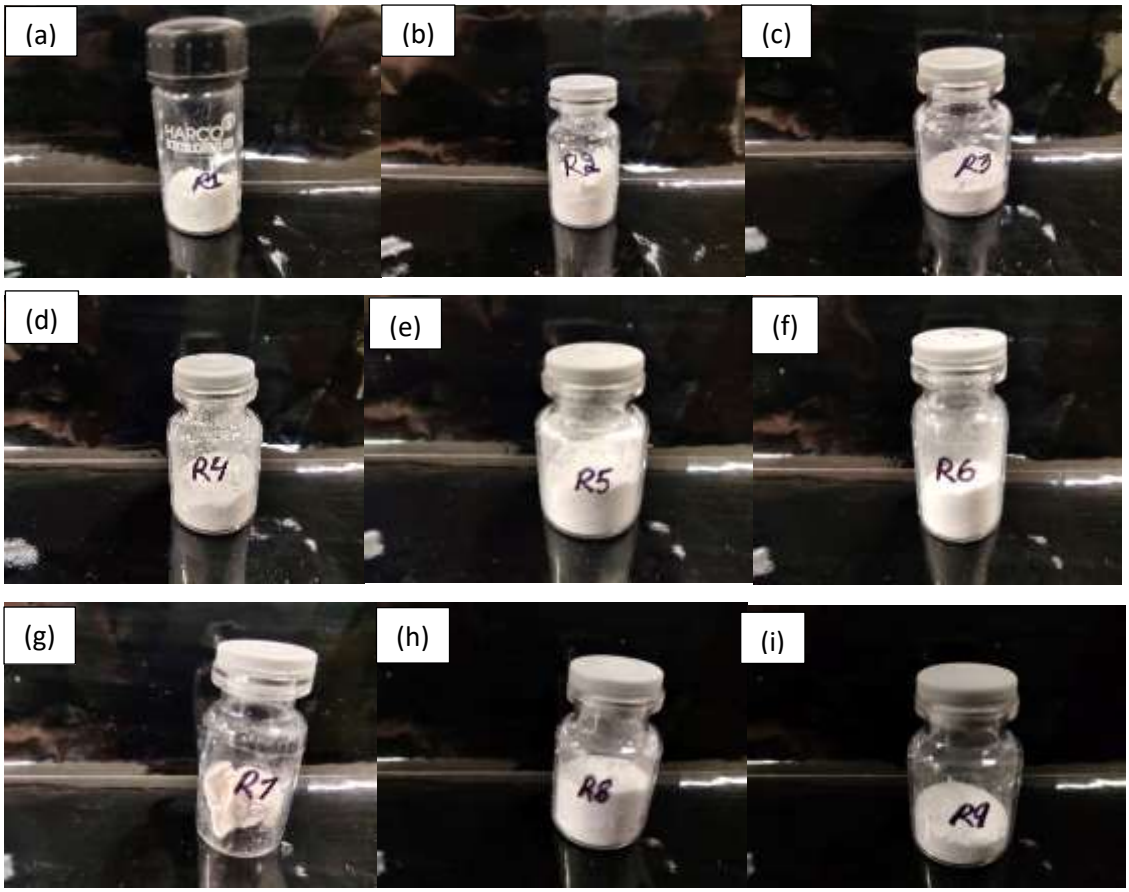


Figure 19: Physical appearance; (a) R1, (b) R2, (c) R3, (d) R4, (e) R5, (f) R6, (g) R7, (h) R8 and (i) R9

4.5.9 Morphology through scanning electron microscopy

The surface morphology of optimized batch was evaluated using Scanning Electron microscope (Sigma 500, Zeiss, Germany). The Scanning electron microscope was operated at 5.00 KV, with a magnification of 50.00 KX. Absolute ethanol was used to compact the loose nanocomposite powder, which was then mounted on a stub made up of aluminium, using a double-side adhesive carbon tape. The sample was then, coated with gold and sputtered for a few minutes. The stub was placed and the sample was analysed under the scanning electron microscope.

CHAPTER 5

RESULTS & DISCUSSION

Results & Discussion

6.1 Optimization using 3^2 factorial design

In order to study all the factors and their possible combinations, in a process, by running minimum experimental runs, factorial design is used. It analyses all the independent variables' influence and their interactions with one another. In this case, Design Expert13 software (Stat-ease Inc., USA) proposed 9 trial runs for two independent variables; the amount of drug (X1) and the amount of TPP (X2) as crosslinker, which were varied for three levels; low (-1), intermediate (0) and high (+1) and Drug entrapment efficiency was considered as the response factor (dependent variable). The evaluated response, drug entrapment efficiency, with respect to independent variables, for all the trial runs were presented in table 6. Model equations were got by fitting all the response values in the 3^2 factorial design. A quadratic equation including each main and interaction factor for the response parameter as best-fitting mathematical model was suggested by Design Expert 13 software. This was based on the comparison of various statistical parameters, like predicted R^2, adjusted R^2, correlation coefficient (R^2) and predicted residual sum of squares (PRESS).

The model equation for DEE (%) as response parameter was:

Entrapment efficiency = +8.23848 +0.063645 (X1) +32.11794 (X2) – 0.006500 (X1) (X2) +0.002739 $(X1)^2$ -10.64578 $(X2)^2$ [R^2 = 0.9480, F-value = 25.53, $p<0.05$]

The summary statistics for best-fitting model is presented in table 18:

Table 18: Fit statistics summary

Std. Dev.	**2.95**		R^2	**0.9480**
Mean	44.45		**Adjusted R^2**	0.9109
C.V. %	6.64		**Predicted R^2**	0.8016
			Adeq Precision	17.0622

The ANOVA results, table 19, indicated that the model was significant for the response parameter.

Table 19: ANOVA statistics

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1112.85	5	222.57	25.53	0.0002	significant
A-drug conc.	944.46	1	944.46	108.33	< 0.0001	
B-TPP conc.	58.83	1	58.83	6.75	0.0355	
AB	0.0380	1	0.0380	0.0044	0.9492	
A^2	33.39	1	33.39	3.83	0.0912	
B^2	62.36	1	62.36	7.15	0.0318	
Residual	61.03	7	8.72			
Lack of Fit	24.78	3	8.26	0.9114	0.5108	not significant
Pure Error	36.25	4	9.06			
Cor Total	1173.88	12				

Response surface methodology was further used to evaluate the effect of independent variables on the response parameter. It is majorly used in the optimization (i.e., maximizing the response and either minimizing, maximizing or keeping the factors within a satisfactory range) and development of drug delivery systems. The three-dimensional response surface graph and the two-dimensional contour plot gives main and interaction effects of independent variables and visual representation of response values, respectively. (Figures 20 and 21)

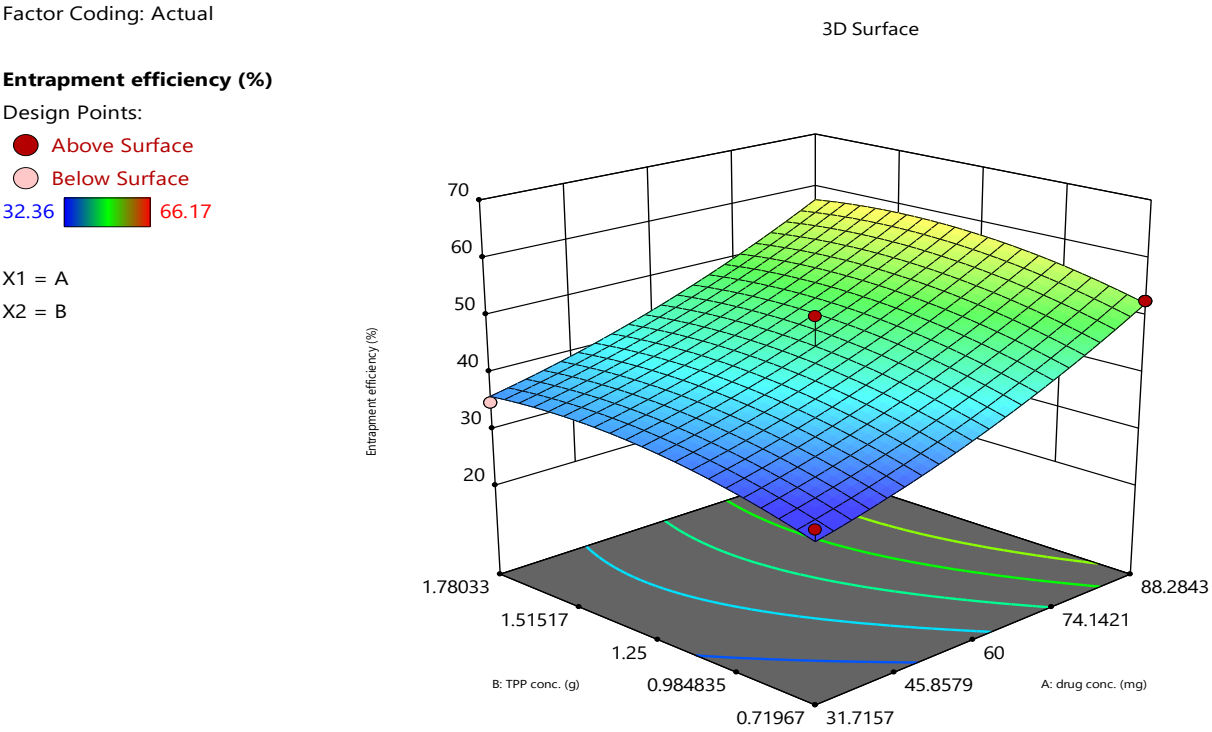


Figure 20: Effect of tolbutamide and TPP amount on the drug entrapment efficiency (%) presented by response surface plot

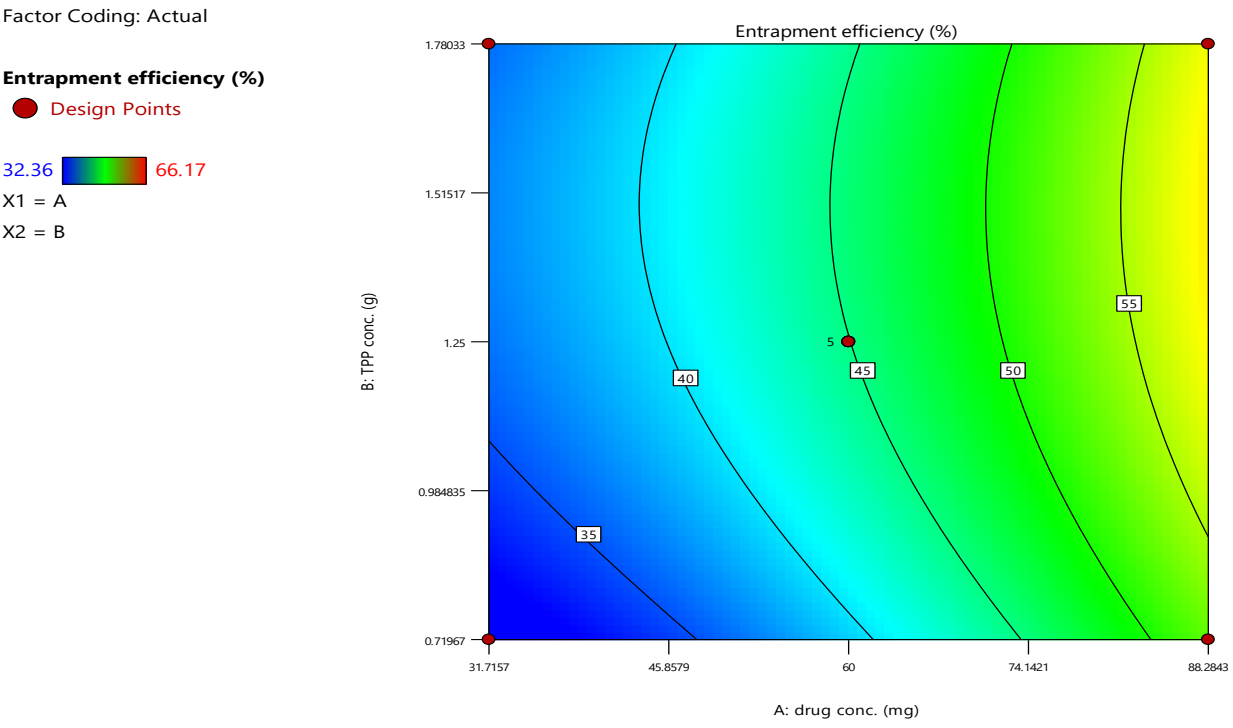


Figure 21: Effect of tolbutamide and TPP amount on the drug entrapment efficiency (%) presented by contour plot

Three-dimensional plot indicated that entrapment efficiency increases with increase in both the amount of TPP as well as the amount of drug.

Both the factors collectively influence the entrapment efficiency, as can be seen, from figure 20, highest entrapment efficiency can be obtained where the value of amount of TPP and tolbutamide is highest.

As the contour plot indicated, majority of the two-dimensional plots were non-linear, indicating a non-linear relationship between both the independent variables i.e., amount of tolbutamide (X1) and the amount of TPP (X2).

A numerical optimization technique was used to maximize the amount of TPP and tolbutamide to get maximum value of entrapment efficiency. The numerical analysis proposed 5 solutions and the final batch was made for the solution having desirability =1.0., in order to evaluate the optimization ability of the design used. The optimized starch nanoparticles showed an entrapment efficiency (%) = 54.92±3.44%, indicating the suggested model from 3^2 factorial design was well fitted. Malakar et al. (2013) used RSM (Design Expert 8 Software) to optimize tolbutamide loaded potato starch-blended alginate beads. The optimized batch having 100 mg tolbutamide, 425 mg potato starch and 500 mg sodium alginate showed around 85.57% of drug encapsulation efficiency.

Table 20: Confirmation of optimization ability

Analysis	Predicted Mean	Predicted Median	Observed	Std Dev	n	SE Pred	95% PI low	Data Mean	95% PI high
Entrapment efficiency	57.6185	57.6185	54.92	2.95267	4	2.76197	51.0875	56.9975	64.1496

6.2 Entrapment efficiency

The similar method used for the preparation of starch-TPP crosslinked nanoparticles for Diclofenac sodium, the resultant entrapment efficiency was reported to be in the range of 73.32% to 91.94%, where the optimized batch showed an entrapment of around 95.01% (El-Naggar et al.,2015). The drug entrapment efficiency (%) of all the starch crosslinked nanoparticles ranged from 32.36 to 66.17% (w/w). The maximum entrapment efficiency was found in batch R7 having amount of drug = 100mg and TPP amount = 1.25g. The minimum

amount of entrapment was found in batch R4 containing tolbutamide amount = 31.7 mg and TPP amount = 0.7 g (table 7). Relatively lower affinity of tolbutamide for the starch nanoparticles matrix, is responsible for low values of entrapment efficiency. Most probable reason for poor entrapment efficiency might be associated with drug's solubility and ionization. More amount of TPP, provides more space to incorporate the drug. For constant amount of drug, it is observed that, increasing the concentration of TPP increases the entrapment efficiency of the drug within the crosslinked starch nanoparticles. The variation in the results as reported by El-Naggar et al. (2015), might be due to the selection of a completely different drug.

6.3 Swelling behavior

Swelling studies were performed on each trial formulation prepared, from R1-R9 (table 8). The swelling behavior of nanoparticles were observed both in buffer pH 1.2 and pH 6.8. the percentage swelling was observed to be lower in buffer pH 1.2 as compared to pH 6.8. the maximum swelling was found to be 87.9% and 277.6% in pH 1.2 and pH 6.8 respectively, for the batch R5 (table 8). The percentage swelling in pH 1.2 and pH 6.8 was in the range 34.7%-84.9% and 79.1%-277.6% respectively. In comparison to alginate beads of tolbutamide, made up using potato starch as a polymer, the swelling behavior of optimized batch was reported to be around 800% in pH 6.8 and around 100%-120% in pH 1.2 (Malakar et al.,2013). The results of swelling studies indicated that the nanoparticles might show a slight swelling in the stomach pH followed by slight erosion and dissolution of the matrix, ultimately releasing smaller amounts of drug in the stomach. Larger values of percentage swelling in pH 6.8 (table8) indicated a major swelling and slow erosion of matrix so as to release higher amounts of drug.

6.4 In-vitro release studies

In-vitro release studies might depict the bioavailability of the drug from different formulations. In-vitro release studies were performed for each batch in buffer pH 1.2 for initial 2 hours and the in-buffer pH 6.8 for the next 5 hours. All the formulations showed a similar release profile except R5 and R6 formulations (figure 17). In R5 and R6, rapid drug release was observed. Approximately more than 50% of the entrapped drug is observed to get released from the nanoparticles, before 2 hours i.e., in the acidic period. This might be due to the fact, that TPP concentration in R5 and R6 was 1.25 g and 1.78 g respectively, which attributes to higher amount of TPP. El-Naggar et al. (2015) made it clear that more the amount of TPP, denser the network of crosslinked starch becomes, due to which the free volume available to the

penetrating drug gets decreased and TBM gets adsorbed to the surface of the matrix. Also, in these formulations the amount of TBM used is also less i.e., R5 consists 20mg TBM and R6 consists 31.7 mg of TBM. The solubility and ionization of TBM might be responsible for lesser affinity for such low amounts, towards the crosslinked matrix. Hence, due to higher TBM adsorption in case of R5 and R6, greater number of molecules might have diffused out of the material and passed into release medium, thereby showing a rapid release in lesser period of time. All the other formulations showed a fast initial release phase, followed by more gradual and slower release in the second phase up to 6 hours due to the diffusion of TBM from the channels or pore of the matrix and erosion and degradation of the matrix. Within first 30 mins, the burst release was observed, that might occur due to desorption of the TBM bound or adsorbed to the surface of the crosslinked matrix of nanoparticles. The release of TBM showed a greater rate and extent from R8 (figure 15), containing 88.2 mg TBM and 1.78 g TPP, about 85.44% release, compared to other formulations. The increased rate of release of TBM for prolonged period of time, from R8 might be possible due to the fact that TPP being a low molecular weight compound, crosslinks with the hydroxyl groups of the starch, at two of its terminals and starch being an ultrahigh molecular weight molecule, forms a network that contains wide pore sizes, thereby possessing a greater capacity to imbibe TBM molecules and results in an increased TBM release. This also satisfied the result of release behavior in figure 18, where TBM release increases with an increase in the concentration of TPP. For similar drug concentrations; (figure 18), in case of R4 and R6 containing lower and higher amount of TPP respectively, the controlled release of TBM for prolonged period in R4 might be due to the fact that lesser amount of TPP sufficiently entraps or holds enough amount of drug in the network, ensuring lesser surface adsorption of TBM, thereby releasing TBM gradually for prolonged time period due to matrix erosion and degradation. Least extent of TBM release was observed from R7, which might be due to undesirable morphology and instability of the formulation. This might have occurred due to either inappropriate formation or improper lyophilization of the formulation. From the results, (figure 17) it gets clear that the lesser release percentage of TBM is due to the formation of more compact matrix around the TBM, by crosslinked starch nanoparticle. The results were in accordance with the results reported in literature for the release of tolbutamide from potato starch blended alginate beads, in which the drug release was in the range of 50-75% (Malakar et al., 2013). This signifies the sustained release behavior of nanoparticles, for prolonged periods of time as shown in the release curves of R1, R2, R3, R4, R8 and R9.

6.5 Fourier Transform Infrared spectroscopy

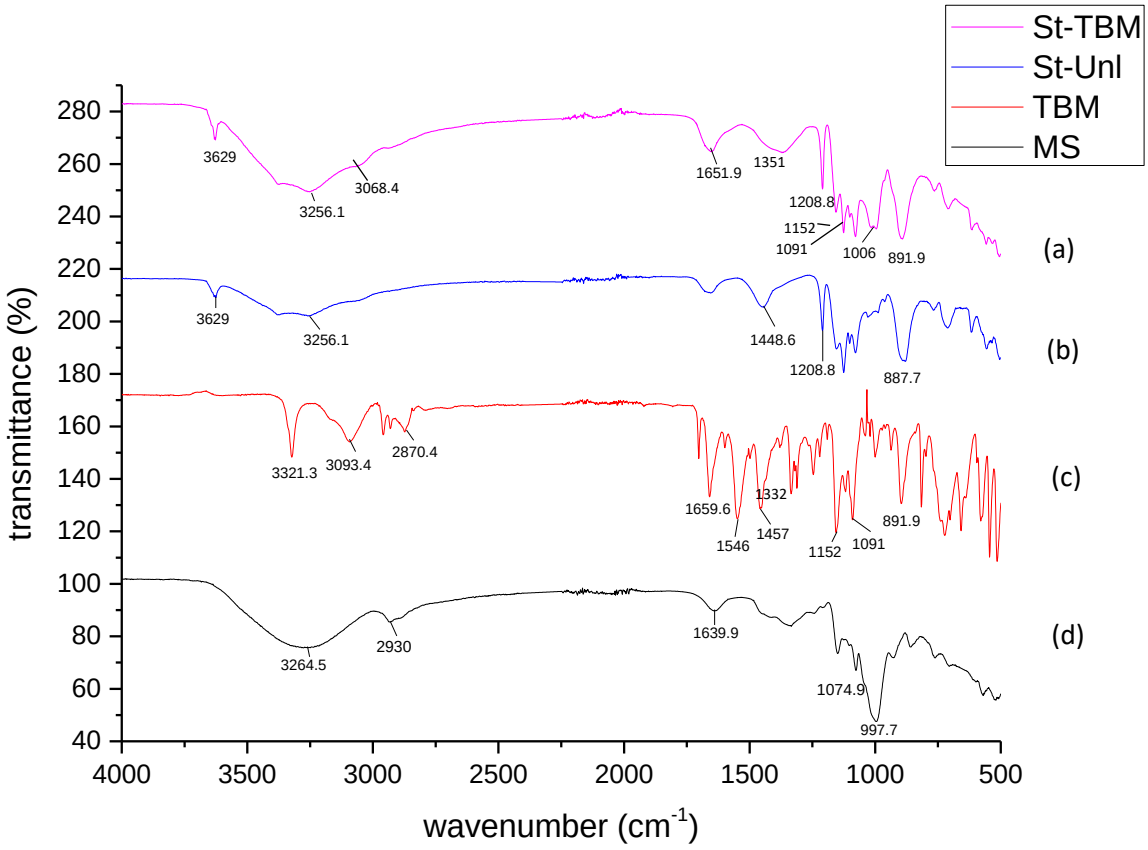


Figure 22: FTIR curves of (a) tolbutamide loaded starch nanoparticles (St-TBM), (b) unloaded starch nanoparticles (St-Unl), (c) pure tolbutamide (TBM) and (d) maize starch (MS)

The FTIR curve of pure tolbutamide [figure 22 (c)] showed principal peaks at 3321.3 cm^{-1} (due to N-H stretching of urea group), 3093.4 and 2870.4 cm^{-1} (due to C-H stretching of aromatic and alkane groups respectively), 1659.6 (due to C=O stretching of urea group), 1332-1350 (due to S=O bending of sulphonamide group), 1250-1020 (due to C-N bending of urea group). FTIR spectra of maize starch [figure 22 (d)] showed broad peak at 3264.5 (due to O-H stretching of hydroxyl group), 2930 (due to C-H stretching), 1639.9 (due to C=O stretching), 1074.9 (due to C-O-C bending), which were quite close to the values reported in literature by Keraliya et al. (2010). FTIR spectra of unloaded starch nanoparticles (b) and TBM loaded starch nanoparticles (a), showed a slight shift in the peak due to O-H stretching at 3256.1. this might be due to gelatinization treatment with NaOH. The observed difference in the primary peaks of (a) and (b) might be due to the presence of TBM in (a). FTIR spectra of TBM loaded starch nanoparticles showed a characteristic peak at 1659.6 (due to C=O stretching of urea group), as seen in FTIR spectra of TBM (c), broad peak at 1351 (due to S=O bending of sulphonamide group), characteristic peaks at 1152, 1091 and 891.9 similar to the peaks in FTIR

spectra of TBM (c). Overall, tolbutamide loaded starch nanoparticles showed significant characters of tolbutamide in FTIR spectrum, implying that there was no interaction between tolbutamide and maize starch as the polymer.

6.6 X-ray diffraction study

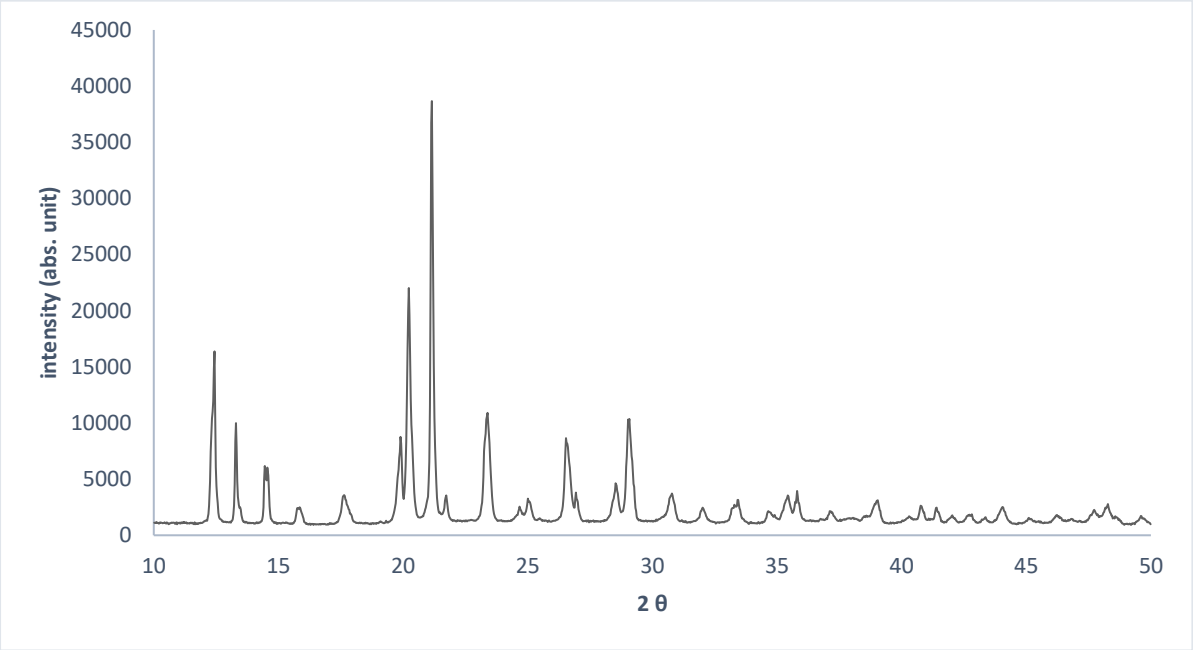


Figure 23 (a): XRD curve of Tolbutamide

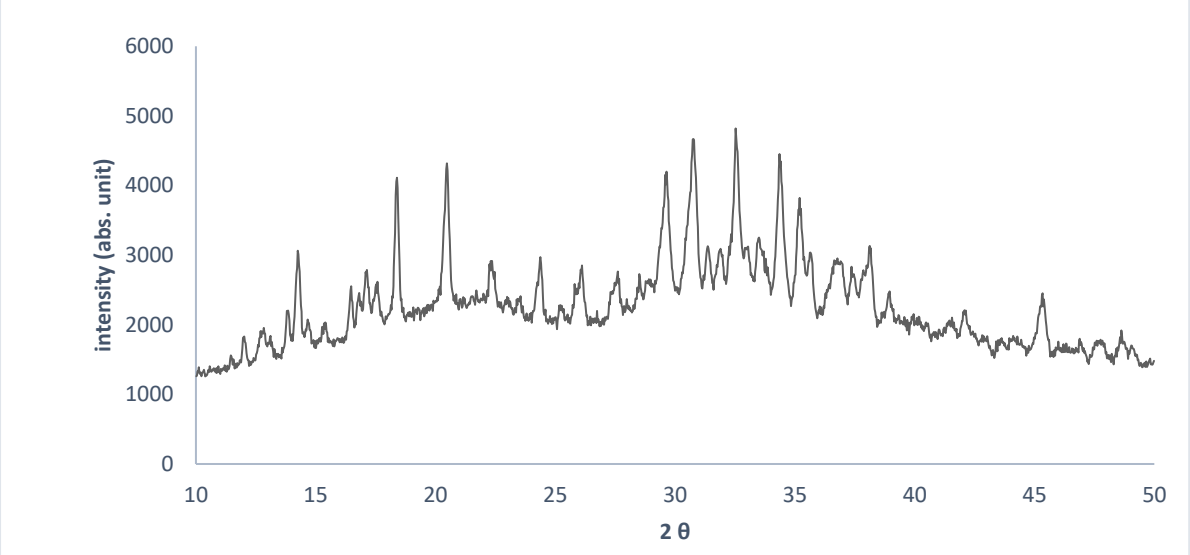


Figure 23 (b): XRD curve of Tolbutamide loaded starch nanoparticles.

The X-ray diffraction study was conducted for pure tolbutamide and the batch optimized by using RSM, i.e., R8. The crystalline nature of the tolbutamide can be depicted by figure 23 (a) having characteristic XRD patterns with peaks at about 2θ = 12.43, 13.3, 19.8, 20.2, 21.12,

23.41, 26.5, 29.1 indicating crystalline structure of tolbutamide. The results were in agreement with the crystallinity of tolbutamide, reported in literature by Thirunahari et al. (2010). After tolbutamide was loaded into starch nanoparticles, typical diffraction peaks were found at 2θ = 14.29, 18.42, 20.49, 22.38, 29.6, 30.7, 32.5, 34.38, 35.35, indicating the presence of tolbutamide in the starch crosslinked nanoparticles in the amorphous phase, mainly due to weakening of diffraction peaks. The slight shift observed in the diffraction peaks of TBM might be because of the electrostatic interaction between tolbutamide and crosslinked starch, instead of chemical reaction between the both.

6.7 Morphology through Scanning electron microscopy

Scanning electron microscopy was done for unloaded starch nanoparticles and optimized batch of tolbutamide loaded starch nanoparticles. SEM monographs were taken at 5.00 KV and 50.00 KX.

As shown in figure 24 (a), the unloaded starch nanoparticles have regular spherical particle-like morphology. After loading the drug within the starch nanoparticles, the morphology of tolbutamide loaded starch nanoparticles is as seen in figure 24 (b). SEM monographs of tolbutamide loaded starch nanoparticles, depict somewhat irregular cubical morphology of the particles. The most probable reason for this difference in morphology of nanoparticles after loading tolbutamide, might be due to the fact that tolbutamide has needle-like crystalline morphology. SEM monographs of pure tolbutamide are reported in literature, which depict it's needle-like structure (Thirunahari et al., 2010).

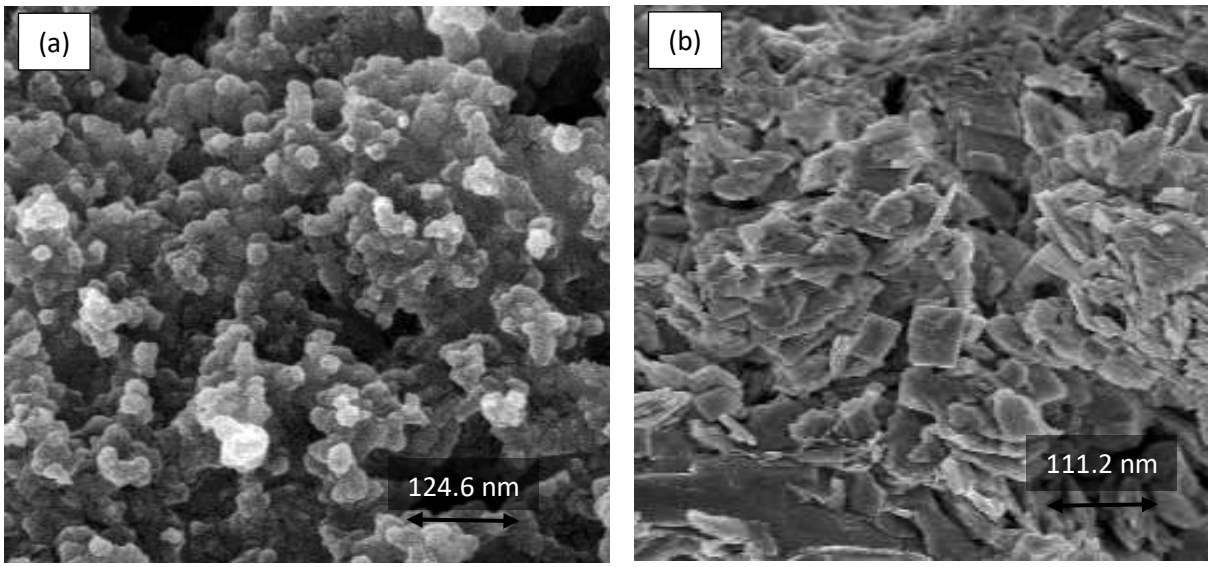


Figure 24: SEM images of (a) unloaded starch nanoparticles, (b) tolbutamide loaded starch nanoparticles

6.8 Hydrodynamic size and Particle size distribution

Hydrodynamic size and particle size distribution measurements were taken using dynamic light scattering technique. The DLS study was performed for batch optimized using RSM. The DLS results of batch R8 depicted the average size of the tolbutamide loaded starch nanoparticles to be around 111.2 nm and a PdI of around 0.278. The resultant low PdI value indicates homogenity within the size distribution of the particles (figure 25).

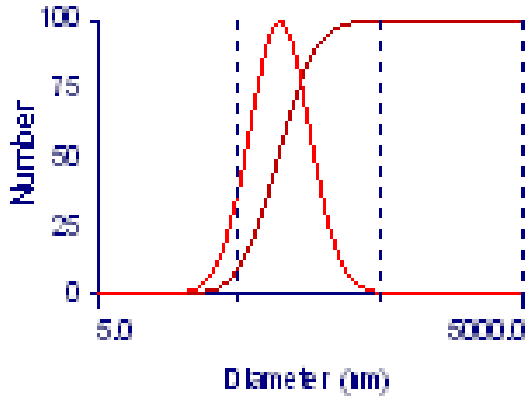


Figure 25: Particle size distribution by number of batch R8

CHAPTER 6
CONCLUSIONS

Conclusions

Starch nanoparticles have proven to be effective drug delivery carriers as they offer enormous advantages. Starch being a natural, biocompatible and biodegradable polymer, can be considered as a safe and effective material for drug delivery systems, to ensure prolonged drug release. The current study aims at the development, optimization and characterization of a nanoparticulate drug delivery system in order to ensure controlled release of tolbutamide.

Starch nanoparticles prepared by using 5% native maize starch, loaded with different concentrations of tolbutamide, were crosslinked using varying TPP concentrations. A 3^2 factorial design was used to optimize the formulation, that results in high entrapment efficiency. The formulated nanoparticles were evaluated for percentage swelling, drug entrapment efficiency and in-vitro drug release behavior. The optimized batch was found to have 54.92% of drug entrapment efficiency (%) and showed around 85.44% release at a controlled rate, after 5 hours from the starting time of evaluation. Various characterization studies were performed, for the optimized formulation, including SEM, X-RD, FTIR and DLS.

Analysis of the resultant characterization studies revealed the possible interactions between tolbutamide and crosslinked starch nanoparticles. The crystalline structure of tolbutamide completely changes to amorphous on interaction with crosslinked starch nanoparticles. As reported in literature, amorphous structure results in increased dissolution of the drug thereby increasing it's efficacy. Experimental design allowed to optimize a formulation resulting in 54.92% drug entrapment efficiency (%), controlled drug release behavior of 85.44%, particle size of 111.2 nm and 0.27 polydispersity index, which might result in better dissolution rate ultimately resulting in increased bioavailability and efficacy of the drug.

The current study involves successful formulation of a highly effective, safe and convenient means of drug delivery, using starch as a polymer for controlled delivery of a hydrophobic drug, tolbutamide, which is being reported for the first time.

CHAPTER 7

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