FORMALDEHYDE CROSS-LINKED HYDROGEL BEADS: A NEW MATRIX FOR SUSTAINED RELEASE OF DEXIBUPROFEN

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IN

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By

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CERTIFICATE

This is to Certify that the dissertation entitled **"FORMALDEHYDE CROSS-LINKED HYDROGEL BEADS: A NEW MATRIX FOR SUSTAINED RELEASE OF DEXIBUPROFEN"** submitted by **Mr.ABDULKADAR M [Register Number: 26102201**] for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli under my guidance and direct supervision.

Place : Tiruchirappalli
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Date :

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(ABDUL KADAR M)

CONTENTS

LIST OF TABLES

LIST OF FIGURE

ABBREVIATIONS

NVP	N-vinyl-2-pyrrolidone
VAc	Vinyl acetate
MAA	Methacrylic acid
PEGA	Polyethylene glycol acrylate
PEGMA	Polyethylene glycol methacrylate
PEGDA	Polyethylene glycol diacrylate
PEGDMA	Polyethylene glycol dimethacrylate
G	Guluronic acid
M	Mannuronic acid
DD	Deacetylation
Mw	Molecular weight
HPMC	Hydroxy Propyl Methyl Cellulose
RA	Rheumatoid Arthritis
MRI	Magnetic Resonance Imaging
RF	Rheumatoid Factor
ESR	Erythrocyte Sedimentation Rate
DNA	Deoxyribonucleic acid
AA	Acrylic Acid
HEMA	Hydroxy Ethyl Methacrylate
HPMA	N-(2-hydroxypropyl) methacrylate
ANA	Antinuclear antibody
SLE	Systemic lupus erythematosus
TNF	Tumer Necrosis Factor
ACPA	Against citrullinated peptides
HLA	Humen leukocyte antigen

HPLC	High Performance Liquid Chromatography
$KHCO_3$	Potassium bicarbonate
RH	Relative humidity
HPMC	Hydroxy propyl methyl cellulose
$CaCl_2$	Calcium chloride
SCMC	Sodium Carboxy Methyl Cellulose
TPP	Tri poly phosphate
SEM	Scanning electron microscopy
AUC	Area under the curve
IV	Intra venous
ED_{50}	Effective dose
FTIR	Fourier Transform Infrared
SR	Sustained release
ICH	International conference on harmonization
PG	Prostaglandins
PG I_2	Prostacyclin
COX	Cyclooxegenase
NSAID	Non-steroidal antiinflammatory
GFR	Glomerular filtration rate
LD	Lethal dose
UV	Ultra violet light
KBr	Potassium bromide

SUSTAINED DRUG DELIVERY SYSTEM [1, 2, 3, 4]

Oral drug release system is the most popular route, which is due in part to the ease of administration and to the fact that gastrointestinal physiology offers more flexibility in dosage form design than most other routes. With the concomitant recognition of the therapeutic advantages, greater attention has been focused on the development of controlled or extended release drug delivery systems. The first sustained release tablets were made by Howard Press in New Jersey in the early 1950's.

The first tablets released under his process patent were called 'Nitroglyn' and made under license by Key Corp. in Florida. The plethora of oral controlled release products in the market place, for example in 1998, the U.S Food and Drug Administration (FDA) approved 90 oral controlled release products. From 1998 to 2003 an additional of 29 new drug applications that used controlled release technologies and 12 of them were based on matrix systems were approved.

Sustained release, sustained action, prolonged action, controlled release, extended action, timed release, depot and repository dosage forms are terms used to identify drug delivery systems that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose.

Sustained release formulation is an important program for new drug research and development to meet several unmet clinical needs. There are several reasons for attractiveness of these dosage forms: Provides increased bioavailability of drug product, Reduction in the frequency of administration to prolong duration of effective blood levels, reduces the fluctuation of peak, concentration and side effects and possibly improves the specific distribution of the drug.

If one were to develop an ideal drug delivery system, two pre-requisites would be required:

Firstly single dose for the duration of treatment whether for days or weeks as with infection, diabetes, arthritis or hypertension etc.

Secondly it should deliver the active entity directly to the site of action minimizing the side effects. In general, the goal of sustained release dosage form is to maintain therapeutic blood levels of drug for extended period; this is usually accomplished by attempting to obtain zero-order release kinetics from the dosage form.

Difference between conventional and sustained release dosage form

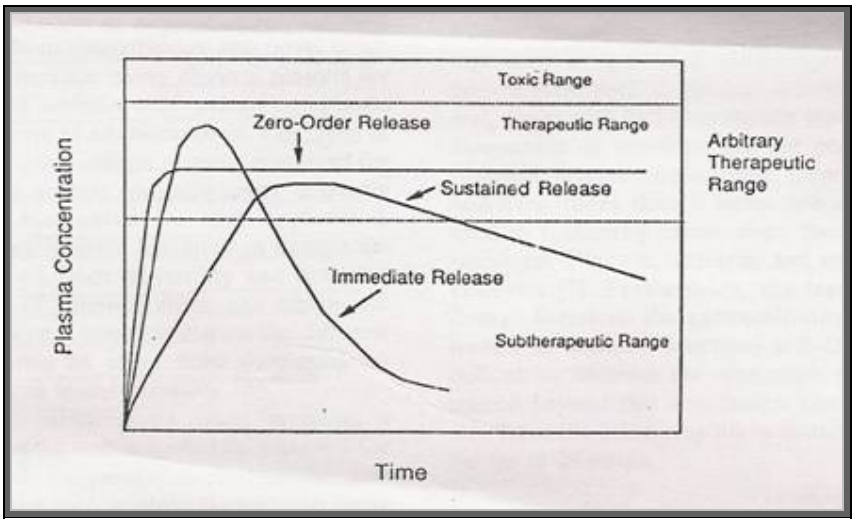


Figure.1

There are certain considerations for the preparation of extended release formulations:

- If the active compound has a long half-life, it is sustained on its own.
- The pharmacological activity is not directly related to its blood levels,
- The absorption of the drug involves an active transport and
- The active compound has very short half-life then it would require a large amount of drug to maintain a prolonged effective dose. The above factors need serious review prior to design.

Sustained drug delivery is used to describe a pharmaceutical dosage form formulated to retard the release of a therapeutic agent so that its appearance in systematic circulation is delayed and/or prolonged and its plasma profile is sustained in duration. The onset of its pharmacological action is often delayed and duration of its therapeutic effect is sustained.

Zero-order release constitutes drug release from the dosage form that is independent of the amount of the drug in the delivery system (i.e., a constant release rate). Sustained-release systems generally do not attain this type of release and usually try to mimic zero-order release by providing drug in a slow first-order fashion. (i.e., Concentration-dependent)

Controlled release products are formulated to release active ingredient gradually and predictably over long period. These formulations potentially provide for greater effectiveness in the treatment of chronic conditions through more consistent delivery of the medication, reduced side effects, greater convenience, and higher levels of patient compliance due to a simplified dosage schedule, compared with those of immediate-release drugs.

RATIONALE OF SUSTAINED DRUG DELIVERY

The main objective to formulate an active pharmaceutical ingredient in an extended or modified drug delivery system is related to its pharmacokinetics. An appropriate formulation can make the absorption, distribution, metabolism and elimination (ADME) profile of a drug much more favourable. This change of the ADME can have a profound impact on many aspects of the clinical use of the drug from patient compliance and convenience to its very efficacy, tolerance and safety parameters.

ADME	»	Original pKa profile
Extended drug delivery	»	New pKa profile (Convenience, tolerance, efficacy and safety)

Sustained release can be achieved by,

- Incorporating the drug in a carrier system
- Altering the structure of the drug at the molecular level
- Controlling the input of the drug into the bio-environment to ensure a programmed and desirable bio-distribution.

The primary objectives are to ensure safety and to improve efficacy of drugs as well as patient compliance. This is achieved by better control of plasma drug levels and less frequent dosing.

ADVANTAGES AND DISADVANTAGES OF ORAL SUSTAINED RELEASE DELIVERY SYSTEM

ADVANTAGES

- The extended release formulations may maintain therapeutic concentrations.
- The use of extended release formulations avoids the high blood concentration.
- Extended release formulations have the potential to improve the patient compliance.
- Reduce the toxicity by slowing drug absorption.
- Increase the stability by protecting the drug from hydrolysis or other degradative changes in gastrointestinal tract.
- Minimize the local and systemic side effects.
- Improvement in treatment efficacy.
- Minimize drug accumulation with chronic dosing.
- Usage of less total drug.
- Improve the bioavailability of some drugs.
- Improve the ability to provide special effects.
  Ex: Morning relief of arthritis through bed time dosing.

DISADVANTAGES

- High cost of preparation.
- The release rates are affected by various factors such as food and the rate of transit through the gut.
- Some differences in the release rate from one dose to another dose but these have been minimized by modern formulations.
- Extended release formulation contains a higher drug load and thus any loss of integrity of the release characteristics of the dosage form.
- The larger size of extended release products may cause difficulties in ingestion or transit through gut.
- Sometimes the target tissue will be exposed to constant amount of drug over extended period results in drug tolerance.

MULTIPARTICULATE DRUG DELIVERY SYSTEMS[5]

The market for drug delivery system has come a long way and will continue to grow at an impressive rate. Today's drug delivery technologies enable the incorporation of drug molecules into a new delivery system, thus providing numerous therapeutic and commercial advantages. Multiparticulate drug delivery systems provide several advantages including greater flexibility and adaptability of microparticulate dosage forms which gives clinicians and those engaged in product development powerful new tools to optimize therapy. Therefore, such systems are growing rapidly in popularity.

DESIGN OF MULTIPARTICULATE DRUG SYSTEMS[5]

The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behaviour due to unit to unit variation, change in gastro-luminal pH and enzyme population.

A generally accepted view is that multiparticulate systems perform better *in-vivo* than single unit systems, as they spread out throughout the length of the intestine causing less irritation, enjoy a slower transit through the colon and give a more reproducible drug release. Coated multiparticulates, often referred to as 'pellets' or 'beads', commonly form the basis for a wide range of modified-release dosage forms. The benefits of multiparticulates are as follows:

1. Minimizing irritant effects- whole, non-disintegrating tablets can potentially lodge in restrictions within the gastrointestinal tract, causing the release of drug to be localized and thus cause mucosal damage should the drug possess irritant effects. This potentially harmful effect can be minimized with multiparticulates. Since their small size reduces the likelihood of such entrapment, while the drug concentration is spread out over a large number of discrete particles.

2. Reducing the consequences of imperfect coatings - film coatings, deposited using a spray technique, can potentially possess imperfections (such as pores) that could compromise the performance of modified-release dosage forms. Traditional coated tablets can be problematic in this regard since the whole dose could potentially be released quite rapidly if such imperfections (in the coating) exist. With multiparticulates, such a risk is greatly reduced since an imperfect coating on one or two pellets (out of 50-200 that constitute a single dosage unit) is likely to have little effect in terms of lack of benefit, or even harm, to the patient.

3. Capitalizing on small size (typically 0.5-2.0 mm) - gastrointestinal transit times can be somewhat erratic for non-disintegrating tablets (especially as a result of food effects); particles smaller than about 2.0 mm can pass through the constricted pyloric sphincter even during the gastric phase of the digestion process and distribute themselves more readily throughout the distal part of the gastrointestinal tract.

4. Reducing the impact of poor coating uniformity - film-coating processes are incapable of ensuring that every entity (tablet, pellet, etc.) within a batch of product will receive exactly the same amount of coating. With a tablet, the complete dose of drug is contained in that dosage unit, so any tablet-to-tablet variation in the amount of coating applied can result in variable drug release from dosage unit to dosage unit.

5. The types of process used for coating multiparticulate are renowned for achieving better uniformity of distribution of the coating material. A further advantage relates to the possible problem of 'dose dumping' resulting from a defect in a film coat. With multiparticulate, because the total dosage unit is made up of a large number of discretely coated particles, a defect in one pellet will only 'dump' a small fraction, say $1/200^{th}$, of the total unit dosage. This is likely to have no pharmacological consequences. In contrast, a defect in a coated monolithic tablet could release 24 hours worth of drug into a patient in just a few minutes.

Although multi-unit systems are more complicated to formulate and manufacture, they provide many advantages over single-unit modified release systems.

a) Their physicochemical characteristics remain unaltered for long periods allowing long term storage.

b) They protect encapsulated drug from enzymatic or pH-dependent degradation.

c) They are suitable for industrial production.

d) They can be formulated to provide constant drug concentration in the blood or to target drugs to specific cells or organs.

e) They can also be formulated to treat local diseases that require a sustained concentration of the drug at a particular site. e.g: gingival, subgingival, periodontal and adjacent tissues in the treatment of pathologies such as gingivitis and periodontitis. Besides ease of local administration, they do not have to be removed after the treatment period.

f) These systems are less dependent on gastric emptying rate, showing less subject variability in gastrointestinal transit time and dietary state. There is a lower tendency for local irritation as the drug is more widely distributed in the GI tract. In addition, because each unit functions as an individual modified release system, failure of a few units is less dangerous than the failure of a single-unit system, and reduces the possibility of dose dumping. The flexibility of programming defined position and release rates, into (potentially) each individual unit, provides considerable clinical advantages in delivering complex release profiles.

HYDROGEL BEADS[6]

Hydrogels are polymeric networks that absorb large quantities of water while remaining insoluble in aqueous solutions due to chemical or physical cross-linking of individual polymer chains.

Hydrogel Beads are highly cross linked hydrophilic spherical particles ranging from 0.05- 2.00 mm. It consists of natural or synthetic polymer in which the active pharmaceutical ingredients are cross liked with cross-linking agent to form a network of matrix.

It have been widely used of their advantageous properties such as non-toxicity, biocompatibility, biodegradability ability to modify the properties of aqueous environment, capacity to thicken, emulsify, stabilize, encapsulate, swell and to form gels, films.

They are three dimensional hydrophilic polymeric networks which absorb and retain 10–20% (an arbitrary lower limit) up to thousands of time of water or biological fluid than their dry weights. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. To avoid this dissolution/ degradation, cross-linking is introduced within the hydrogels.

They have been considered to be useful in medical applications. Many structural factors (e.g. charge, concentration and pKa of the ionisable group, degree of ionization, crosslink density and hydrophilicity) influence the degree of swelling of ionic polymers. In addition, properties of the swelling medium (e.g. pH, ionic strength and the counter ion and its valences) affect the swelling characteristics. In recent years, much attention has been given on the development of hydrogels from natural, biodegradable and biocompatible polymeric materials.

POLYMER FOR DRUG DELIVERY

The use of biodegradable polymeric carriers for the drug delivery systems has gained a wide interest, mainly for their biocompatibility and among the microparticulate systems, microspheres show a special importance for providing sustained action. Hydrophobic polymeric networks such as poly (lactic acid) (PLA) or poly (lactide-co-glycolide) (PLGA) which have limited water-absorption capabilities, while hydrophilic hydrogels exhibit many unique physicochemical properties that make them advantageous for biomedical applications including drug delivery. For example, hydrogels are excellent candidates for encapsulating bio-macromolecules including proteins and DNA due to their lack of hydrophobic interactions which can denature these fragile species.

In addition, compared to commonly used hydrophobic polymers such as PLGA, the conditions for fabricating hydrogels are relatively mild. Gel formation usually proceeds at ambient temperature and organic solvents are rarely required. *In-situ* gelation with cell and drug encapsulation capabilities further distinguishes hydrogels from the other hydrophobic polymers.

Hydrogels can be prepared from natural or synthetic polymers. Although hydrogels made from natural polymers may not provide sufficient mechanical properties and may contain pathogens or evoke immune/ inflammatory responses, they do offer several advantageous properties such as inherent biocompatibility, biodegradability, and biologically recognizable moieties that support cellular activities. Synthetic hydrogels, on the other hand, do not possess these inherent bioactive properties. Fortunately, synthetic polymers usually have well-defined structures that can be modified to yield tailorable degradability and functionality.

CHARACTERISTICS OF IDEAL POLYMER SYSTEM[41]

1. It should be chemically inert and compatible with the environment.
2. It should be non-toxic.
3. It should be easy and inexpensive to fabricate.
4. It should be good mechanical strength.
5. It should have acceptable shelf life.

NATURAL POLYMERS AND SYNTHETIC MONOMERS USED IN HYDROGEL FABRICATION

Natural polymer

1. Chitosan
2. Alginate
3. Fibrin
4. Collagen
5. Gelatin
6. Hyaluronic acid
7. Acrylic acid (AA)
8. Dextran

Synthetic monomer:

1. Hydroxyethyl methacrylate (HEMA)
2. N-(2-hydroxypropyl) methacrylate (HPMA)
3. N-vinyl-2-pyrrolidone (NVP)
4. Vinyl acetate (VAc)
5. Methacrylic acid (MAA)
6. Polyethylene glycol acrylate/methacrylate (PEGA/PEGMA)
7. Polyethylene glycol diacrylate/dimethacrylate (PEGDA/PEGDMA)

SODIUM ALGINATE[42]

Alginate is a water-soluble linear polysaccharide extracted from brown seaweed and is composed of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues. The structure of alginate shows of mannuronic and guluronic acid residues and the binding between these residues in alginate. Because of the particular shapes of themonomers and theirmodes of linkage in the polymer, the geometries of the G-block regions, M-block regions, and alternating regions are substantially different. Specifically, the G-blocks are buckled while the M-blocks have a shape referred to as an extended ribbon. If two G-block regions are aligned side by side, a diamond shaped hole results.

Structure of alginates shown that polymer has a chain of two guluronic acid (G) monomers and two mannuronic acid (M) monomers, with (1–4) linkages.

Structure of alginate

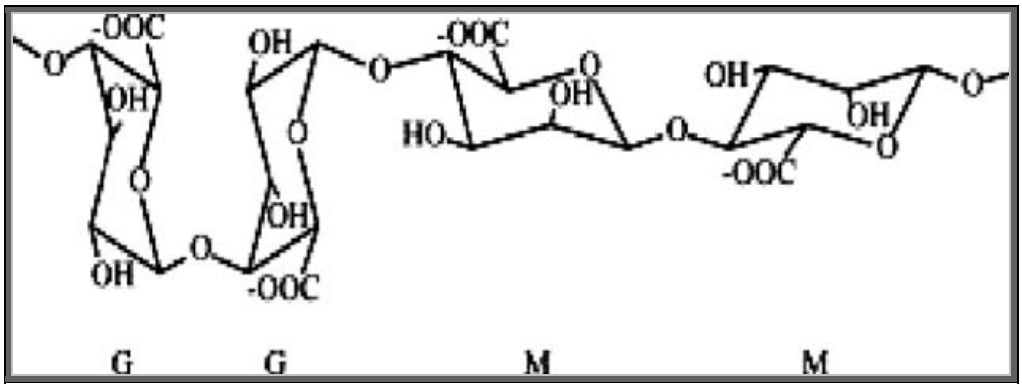


Figure.2

This hole has dimensions that are ideal for the cooperative binding of calcium ions as in case of calcium ion cross-linking. The homopolymeric regions of β-D-mannuronic acid blocks and α-L-guluronic acid blocks are interdispersed with regions of alternating structure (β-D-mannuronic acid–α-L-guluronic acid blocks). The composition and extent of the sequences and the molecular weight determine the physical properties of the alginates.

Alginates (poly-saccharides) are known to be haemocompatible and do not accumulate in any organs of the human body. It has been reported that sodium alginate can be cross linked with formaldehyde. The chemical reaction was between hydroxyl groups of sodium alginate and formaldehyde. Because of its skeletal component of the algae, it has the nice property of being strong and yet flexible. Alginic acid can be either water soluble or insoluble depending on the type of the associated salt.

Alginates are established among the most versatile biopolymers, used in a wide range of applications. The conventional use of alginate as excipient in drug products generally depends on the thickening, gel-forming and stabilizing properties. At low pH hydration of alginic acid leads to the formation of a high-viscosity"acid gel". Alginate is also easily gelled in the presence of water after cross-linking with formaldehyde. Dried beads re-swell, creating a diffusion barrier decreasing the migration of small molecules.

Alginate is currently widely used in food, pharmaceutical. The properties of alginate utilized in these products are thickening, stabilizing, gel-forming, and film-forming. Alginate polymers isolated from different alginate sources vary in properties. Different algae, or for that matter different part of the same algae, yield alginate of different monomer composition and arrangement.

The polymers with hydroxyl group require drastic conditions (low pH, high temperature, etc) in order to establish cross-linking with aldehydes, whereas amine containing polymers can be cross-linked with the same reagent under mild conditions where Schiff bases are formed.

CHITOSAN [42]

Chitosan is a co polymer of D-glucosamine and N-acetyl glucosamine. Chitosan is a linear co polymer polysaccharide consisting of β (1–4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units in Figure 3. The structure of chitosan is very

similar to that of cellulose (made up of β (1–4)-linked D-glucose units), in which there are hydroxyl groups at C2 positions of the glucose rings. Chitosan is poly [β-(1–4)-2-amino-2-deoxy-D-glucopyranose].

The term chitosan is used to describe a series of polymers of different degrees of deacetylation (DD), defined in terms of the percentage of primary amino groups in the polymer backbone, and average molecular weights (Mw). The DD of typical commercial chitosan is usually between 70% and 95%, and the Mw between 10 and 1000 kDa. The properties, biodegradability and biological role of chitosan is frequently dependent on the relative proportions of N-acetyl-D-glucosamine and D-glucosamine residues.

Structure of chitosan

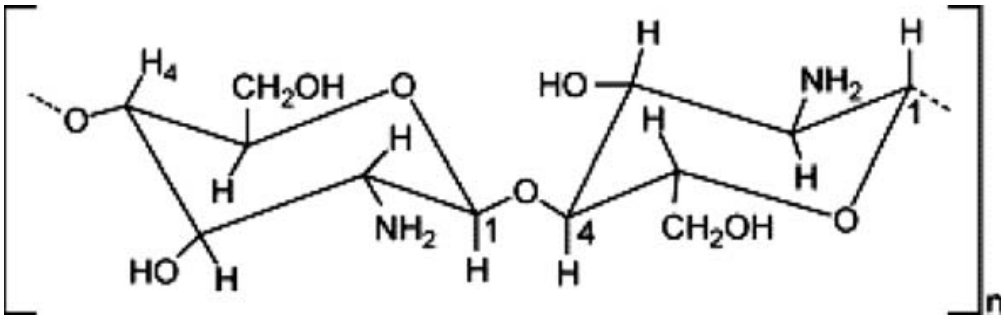
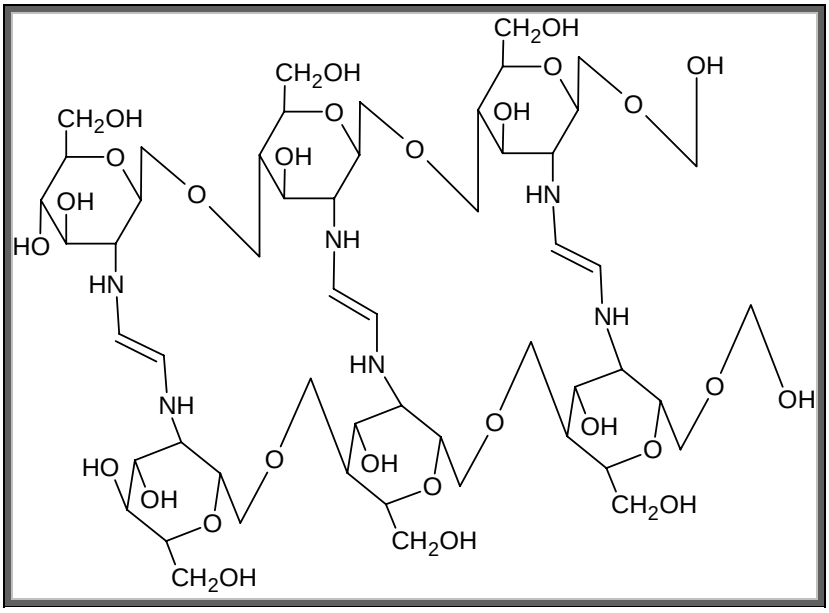


Figure.3

Alginate has the property of shrinking in low pH and getting dissolved in higher pH, whereas chitosan dissolves in low pH and is insoluble in higher pH ranges. In view of these limitations encountered in pure alginate and chitosan bead systems, the concept of alginate– chitosan complexes gained acceptance. The easy solubility of chitosan in low pH is prevented by the alginate network since alginate is insoluble in low pH conditions. The possible dissolution of alginate at higher pH is prevented by the chitosan which is stable at higher pH ranges.

Mechanism of cross-linking of Chitosan with formaldehyde



Among other biodegradable polymers, chitosan, a linear polysaccharide has been found to be a good chemical entity for synthesizing hydrogels because of its greater cross linking ability due to the presence of amino ($–NH_2$) group. The biodegradability, biocompatibility and other unique properties of chitosan have been used in a variety of areas such as medicine, pharmaceutics, tissue engineering, antimicrobial agents as well as biomedical applications.

GELATIN[42]

Gelatin is a fibrous protein that is produced from collagen and forms an aqueous gel. Gelatin gels are widely used in various fields of application, such as photography, pharmaceuticals, cosmetics and the food industry, because of the remarkable mechanical properties of gels and the natural biological origins of gelatin. In addition, gelatin is the most important wall material in the production of pharmaceutical micro-capsules that contain an active agent.

MECHANISM OF CROSS LINKING

Although Ca^{2+} alginate beads can be prepared by simple and mild procedures, this method has a major limitation and that is the drug loss during bead preparation, by leaching through the pores in the beads. Therefore many modifications of alginate have been tested for the drug delivery purposes. Cross-linking of alginate with aldehyde has been done successfully.

Sodium alginate alone or gelatin has been cross-linked with aldehydes and their microparticles and beads have been prepared for various applications. Cross-linked alginate has more capacity to retain the entrapped drugs and also it shows a more controlled release profile of entrapped drugs.

It has been proposed that aldehyde groups can produce cross-linkage between alginate molecules through formation of bonds between two hydroxyl groups.

The polymer having amino and/or hydroxyl group has ability to cross-linked with formaldehyde to form network structure. Alginate, gelatin and chitosan are having the cross-linking functional group to cross-linked with aldehyde. The common reaction is given below.

Figure.4 Mechanism of cross linking

MECHANISMS OF DRUG RELEASE FROM HYDROGEL BEADS[13,30]

Hydrogel have a unique combination of characteristics that make them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amounts of water. Therefore, the molecule release mechanisms from hydrogels are very different from hydrophobic polymers. Both simple and sophisticated models have been previously developed to predict the release of an active agent from a hydrogel device as a function of time. These models are based on the rate limiting step for controlled release and are therefore categorized as follows:

1. Diffusion-controlled
2. Swelling-controlled
3. Chemically-controlled.

1. Diffusion-controlled

Diffusion-controlled is the most widely applicable mechanism for describing drug release from hydrogels. Fick's law of diffusion with either constant or variable diffusion coefficients is commonly used in modeling diffusion-controlled release. Drug diffusivities are generally determined empirically or estimated a priori using free volume, hydrodynamic, or obstruction-based theories.

2. Swelling-controlled

Swelling-controlled release occurs when diffusion of drug is faster than hydrogel swelling. The modeling of this mechanism usually involves moving boundary conditions where molecules are released at the interface of rubbery and glassy phases of swollen hydrogels. The release of many small molecule drugs from hydroxypropyl methylcellulose (HPMC) hydrogel tablets is commonly modeled using this mechanism. For example, Methocel® matrices, a combination of methylcellulose and HPMC, from Dow Chemical Company are commercially available for preparing swelling-controlled drug delivery formulations exhibiting a broad range of delivery timescales.

3. Chemically-controlled

Chemically-controlled release is used to describe molecule release determined by reactions occurring within a delivery matrix. The most common reactions that occur within hydrogel delivery systems are cleavage of polymer chains via hydrolytic or enzymatic degradation or reversible or irreversible reactions occurring between the polymer network and releasable drug. Under certain conditions the surface or bulk erosion of hydrogels will control the rate of drug release. Alternatively, if drug-binding moieties are incorporated in the hydrogels, the binding equilibrium may determine the drug release rate.

Chemically-controlled release can be further categorized according to the type of chemical reaction occurring during drug release. Generally, the liberation of encapsulated or tethered drugs can occur through the degradation of pendant chains or during surface erosion or bulk-degradation of the polymer backbone. A more thorough discussion of these mechanisms can be seen in a later section of this review as well as in several other excellent reviews.

RHEUMATOID ARTHRITIS [8,9,55,26]

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. Rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium, pleura and sclera, and also nodular lesions, most common in subcutaneous tissue. Although the cause of rheumatoid arthritis is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and RA is considered a systemic autoimmune disease

Difference between Normal joint and joint affected by Rheumatoid Arthritis

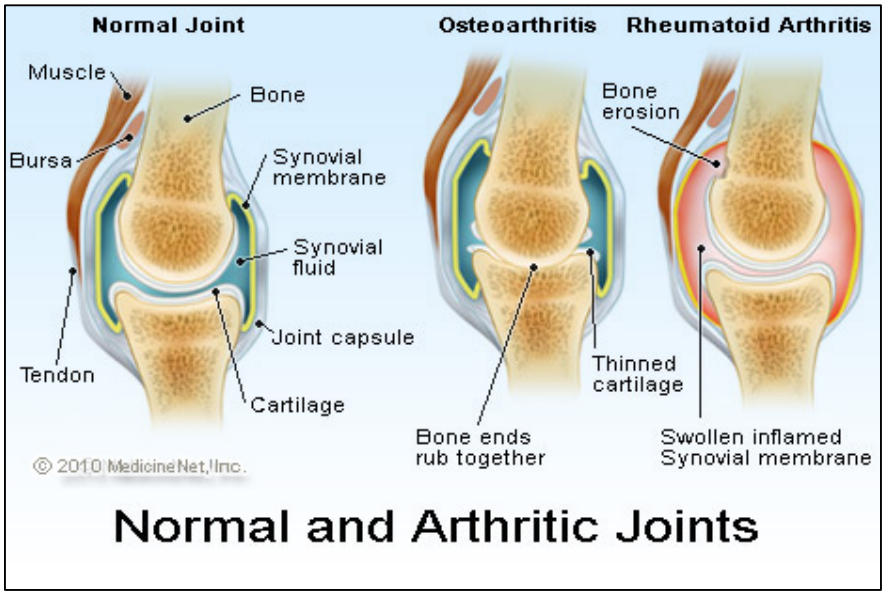


Figure.5

SIGNS AND SYMPTOMS

While rheumatoid arthritis primarily affects joints, problems involving other organs of the body are known to occur. Extra-articular ("outside the joints") manifestations other than anemia (which is very common) are clinically evident in about 15–25% of individuals with rheumatoid arthritis. It can be difficult to determine whether disease manifestations are directly caused by the rheumatoid process itself, or from side effects of the medications commonly used to treat it – for example, lung fibrosis from methotrexate or osteoporosis from corticosteroids

- Joints -With time RA nearly always affects multiple joints (it is a polyarthritis), most commonly small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved. With time RA nearly always affects multiple joints (it is a polyarthritis), most commonly small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved.

- Skin- The rheumatoid nodule, which is often subcutaneous, is the cutaneous feature most characteristic of rheumatoid arthritis. The initial pathologic process in nodule formation is unknown but may be essentially the same as the synovitis, since similar structural features occur in both. The nodule has a central area of fibrinoid necrosis that may be fissured and which corresponds to the fibrin-rich necrotic material found in and around an affected synovial space. Surrounding the necrosis is a layer of palisading macrophages and fibroblasts, corresponding to the intimal layer in synovium and a cuff of connective tissue containing clusters of lymphocytes and plasma cells, corresponding to the subintimal zone in synovitis

Lungs

Fibrosis of the lungs is a recognized response to rheumatoid disease. It is also a rare but well recognized consequence of therapy (for example with methotrexate and leflunomide). Caplan's syndrome describes lung nodules in individuals with rheumatoid arthritis and additional exposure to coal dust. Pleural effusions are also associated with rheumatoid arthritis. Another complication of RA is Rheumatoid Lung Disease. It is estimated that about one quarter of Americans with RA develop Rheumatoid Lung Disease.

Kidneys

Renal amyloidosis can occur as a consequence of chronic inflammation. Rheumatoid arthritis may affect the kidney glomerulus directly through a vasculopathy or a mesangial infiltrate but this is less well documented.

Heart and Blood Vessels

- atherosclerosis
- risk of myocardial infarction (heart attack)
- stroke

- pericarditis
- endocarditis
- left ventricular failure
- valvulitis
- fibrosis

DIAGNOSIS

Imaging

X-rays of the hands and feet are generally performed in people with a polyarthritis. In rheumatoid arthritis, there may be no changes in the early stages of the disease, or the x-ray may demonstrate juxta-articular osteopenia, soft tissue swelling and loss of joint space. As the disease advances, there may be bony erosions and subluxation. X-rays of other joints may be taken if symptoms of pain or swelling occur in those joints.Other medical imaging techniques such as magnetic resonance imaging (MRI) and ultrasound are also used in rheumatoid arthritis.

Blood Test

When RA is clinically suspected, immunological studies are required, such as testing for the presence of rheumatoid factor (RF, a non-specific antibody). A negative RF does not rule out RA. The arthritis is called seronegative. This is the case in about 15% of patients. During the first year of illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time. RF is also seen in other illnesses, for example Hepatitis C, chronic infections and in approximately 10% of the healthy population, therefore the test is not very specific.

Also, several other blood tests are usually done to allow for other causes of arthritis, such as lupus erythematosus. The erythrocyte sedimentation rate (ESR), C-reactive protein, full blood count, renal function, liver enzymes and other immunological tests (e.g., antinuclear antibody/ANA) are all performed at this stage.

DIFFERENTIAL DIAGNOSIS

Several other medical conditions can resemble RA, and usually need to be distinguished from it at the time of diagnosis

- Crystal induced arthritis (gout, and pseudogout) – usually involves particular joints (knee, heels) and can be distinguished with aspiration of joint fluid if in doubt. Redness (RA doesn't have redness at the joints), a-symetric distibution of affected joints, pain occurs at night and the starting pain is less than an hour with gout.
- Osteoarthritis – distinguished with X-rays of the affected joints and blood tests, age (mostly older patients), starting pain less than an hour, a-symetric distribution of affected joints and pain worsens when using joint for longer periods.
- Systemic lupus erythematosus (SLE) – distinguished by specific clinical symptoms and blood tests (antibodies against double-stranded DNA)
- One of the several types of psoriatic arthritis resembles RA – nail changes and skin symptoms distinguish between them
- Lyme disease causes erosive arthritis and may closely resemble RA – it may be distinguished by blood test in endemic areas
- Reactive arthritis (previously Reiter's disease) – asymmetrically involves heel, sacroiliac joints, and large joints of the leg. It is usually associated with urethritis, conjunctivitis, iritis, painless buccal ulcers, and keratoderma blennorrhagica.
- Ankylosing spondylitis – this involves the spine and is usually diagnosed in males, although a RA-like symmetrical small-joint polyarthritis may occur in the context of this condition.
- Hepatitis C – RA-like symmetrical small-joint polyarthritis may occur in the context of this condition. Hepatitis C may also induce Rheumatoid Factor auto-antibodies

PATHOPHYSIOLOGY

Rheumatoid arthritis is best considered a clinical syndrome spanning several disease subsets. These different subsets entail several inflammatory cascades which all lead towards a final common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present.

Inflammation

One key inflammatory cascade includes overproduction and over expression of TNF. This pathway drives both synovial inflammation and joint destruction. TNF overproduction has several causes, including interactions between T and B lymphocytes, synovial-like fibroblasts, and macrophages. This process leads to overproduction of many cytokines such as interleukin, which also drives persistent inflammation and joint destruction. Overproduction of other proinflammatory cytokines (eg, interleukin-1) differs from the process for interleukin 6 in that production is either less marked or is specific to one or more disease subsets, as best shown by the effects of interleukin-1 blockade in subforms of juvenile idiopathic arthritis or adult-onset Still's disease.

Synovial cells and cartilage cells

The dominant local cell populations in joints affected by rheumatoid arthritis are synovial and cartilage cells. Synovial cells can be divided into fibroblast-like and macrophage-like synoviocytes. Overproduction of proinflammatory cytokines is believed to be led predominantly by macrophage-like synoviocytes. Fibroblast-like synoviocytes show abnormal behavior in rheumatoid arthritis. In experimental models, co-implantation of fibroblast-like synoviocytes with cartilage leads to fibroblasts invading cartilage, behaviour that correlates with joint destruction. Considerable information has accumulated about joint destruction and the role of osteoclast activation as a key process leading to bone erosion. This association is proven because specific inhibition of osteoclast activation can reduce joint destruction yet not affect joint inflammation. We are unclear about whether arthritis starts as a primary problem in the bone and

subsequently moves to the joint, or the other way around. One argument for rheumatoid arthritis starting in the joint is the observation that fibroblast-like synoviocytes showing altered behaviour can spread between joints, suggesting how polyarthritis might develop.

Regulation of immune inflammation depends on balances between the number and strength of different cell types. Control of arthritogenic immunoresponses has been studied in mice in which the specific antigen is known. Infusion of low numbers of T cells with specific characteristics ameliorates arthritis in a rodent model of the disease, showing T cells can be protective. Ongoing research should translate these experimental findings into clinical practice.

Autoantibodies

Rheumatoid factor is the classic autoantibody in rheumatoid arthritis. IgM and IgA rheumatoid factors are key pathogenic markers directed against the Fc fragment of IgG. Additional (and increasingly important) types of antibodies are those directed against citrullinated peptides (ACPA). Although most, but not all, ACPA-positive patients are also positive for rheumatoid factor, ACPA seem more specific and sensitive for diagnosis and seem to be better predictors of poor prognostic features such as progressive joint destruction.

Ongoing research aims to identify antibody specificities relevant for different patients' subsets and disease stages. 50–80% of individuals with rheumatoid arthritis have rheumatoid factor, ACPA, or both. Composition of the antibody response varies over time, with limited specificities in early rheumatoid arthritis and a mature response, in which more epitopes are recognised and more isotypes used in late disease.

Evidence from animal models and in-vivo data suggest that ACPA are pathogenic on the basis of induction of arthritis in rodent models and because immunological responses are present in ACPA-positive patients in a citrulline-specific manner. Findings of clinical studies show that patients with rheumatoid arthritis and both rheumatoid factor and ACPA (autoantibody-positive disease) differ from individuals with so-called autoantibody-negative disease.

For example, histologically, people with ACPA-positive disease have more lymphocytes in synovial tissue, whereas those with ACPA-negative rheumatoid arthritis have more fibrosis and increased thickness of the synovial lining layer. ACPA-positive disease is associated with increased joint damage and low remission rates.

Genetics

50% of risk of developing rheumatoid arthritis is attributable to genetic factors. Much progress has been made in identification of genetic regions tagged by structural variation (single nucleotide polymorphisms); more than 30 genetic regions are associated with rheumatoid arthritis.

CLASSIFICATION AND DIAGNOSIS

Early classification criteria were designed to distinguish established rheumatoid arthritis from other types of established joint diseases. They ensured researchers studied homogeneous patients' groups, particularly in clinical trials.

CLASSIFICATION OF EARLY ARTHRITIS

The American College of Rheumatology (ACR) 1987 criteria are limited by poor sensitivity and specificity for classification of patients with early inflammatory arthritis as having rheumatoid arthritis. They fail to identify individuals with very early arthritis who subsequently develop rheumatoid arthritis.

Effective treatment in early arthritis averts or delays patients fulfilling these 1987 criteria, and two criteria-erosive joint damage and extra-articular disease are late changes prevented by modern treatment. Prediction models have been developed from prospective observational studies of treated patients with early arthritis. These models are designed to forecast outcomes in individuals with early arthritis who do not currently meet the 1987 criteria.

Several factors can establish whether patients are likely to develop rheumatoid arthritis. In the presence of inflammatory arthritis, evidence of systemic inflammation shown by high acute-phase reactants and prolonged

morning stiffness and autoantibodies in serum, particularly ACPA and rheumatoid factor increases the likelihood of individuals having rheumatoid arthritis.

EPIDEMIOLOGY

Frequency

Findings of population-based studies show rheumatoid arthritis affects 0·5–1·0% of adults in developed countries. The disease is three times more frequent in women than men. Prevalence rises with age and is highest in women older than 65 years, suggesting hormonal factors could have a pathogenic role. Estimates of the frequency of rheumatoid arthritis vary depending on the methods used to ascertain its presence. Incidence ranges from 5 to 50 per 100 000 adults in developed countries and increases with age.

Prevalence of rheumatoid arthritis varies geographically. The disease is common in northern Europe and North America compared with parts of the developing world, such as rural West Africa. These variations are indicative of different genetic risks and environmental exposures. Some evidence suggests incidence of rheumatoid arthritis might be declining, with onset happening later in life.

Environmental risk factors

Smoking is the dominant environmental risk factor and doubles risk of developing rheumatoid arthritis. Its effect is restricted to patients with ACPA-positive disease. Although pathogenetically very important, on a population level, the risk is too low to be clinically relevant. Other potential environmental risk factors include alcohol intake, coffee intake, vitamin D status, oral contraceptive use, and low socioeconomic status, although supporting evidence for these other factors is weak.

LITERATURE REVIEW

Dilipkumar Pal et al., 2012[10] developed multiple-unit alginate based floating system for delivery o cloxacillin. Bead size ranged from 1.73 ± 0.04 to 1.97 ± 0.08 mm. Increasing bead size was found to be increasing the sodium alginate concentration. Liquid paraffin entrapped calcium alginate beads containing cloxacillin was observed with increasing of polymer content and decreasing of liquid content in the beads. The diffusion mechanism of drug release demonstrate both diffusion controlled as well as swelling controlled from beads.

Mradul R. Gupta et al., 2010[11] reviewed alginate/chitosan particulate system for Diclofenac sodium release by ionic gelation technique (Ca^{2+} and Al^{3+}). The release of sodium diclofenac was prevented at acidic pH, while it was complete in a few minutes when pH was raised up to 6.4 and 7.2. The alginate/chitosan ratio and the nature of the jellifying cation controlled the release rate of the drug.

Santanu Chakraborty *et al*., 2010[12] formulated micro-particulate drug delivery of poorly water soluble drug Aceclofenac using alginate-carbopol as a rate controlling polymer was able to restrict the drug release in stomach. Formulation was able to release drug upto 12 hour and follow the anomalous non-fickian diffusion mechanism.

Miqin Zhang et al., 2010[13] reviewed that application of chitosan in hydrogel for controlled and localized drug delivery. Method of drug loading into hydrogel such as direct addition of drug, incorporation of separate release system, covalent attachment and method of cross linking and formulation of hydrogel beads were given. Importantly, how the unique cationic properties of chitosan offer greater latitude in the types of hydrogels that can be formed and the mechanisms by which they fragment and degrade in the body.

Girhepunje K M *et al.*, 2010[14] prepared microbead formulations of celecoxib inclusion complex using sodium alginate and Eudragit FS 30-D as a carrier for colonic administration to extend the retention of the drug in order to treat colorectal cancer. Coated celecoxib microbeads (1:1ratio) showed cytotoxicity against HT-29 cells. DNA Fragmentation study confirms the better anti cancer activity of celecoxib microbeads against human colorectal adenocarcinoma cell line HT-29. Hence the formulations can be effectively tested for its anticancer activity.

Patel H K *et al.*, 2010[15] prepared and evaluated the colon-specific alginate beads of 5-Fluorouracil for the treatment of colon cancer. The core beads were coated with Eudragit S-100 to prevent drug release in the stomach and provide controlled dissolution of enteric coat in the small intestine and maximum drug release in the colon. No significant release was observed at acidic pH, however, when it reached the pH where Eudragit S-100 starts to dissolve, drug release was observed. Also, release of drug was found to be higher in presence of rat caecal content.

Senthil kumaran K *et al.*, 2010[16] formulated mosapride-controlled release beads were prepared with the help of the ionotropic gelation method, using sodium alginate containing $KHCO_3$ as the gas-forming agent. The percentage of mosapride drug entrapment efficiency ranged from 97.4± 0.08 to 99.1 ± 0.04%. The percentage of mosapride content from the beads was determined by high performance liquid chromatography (HPLC) and ranged from 97.9 ± 0.08 to 99.6% ± 0.01. The *in-vitro* release study was carried out for 14 hours and the formulate beads were kept for stability studies for 90 days at 40°C / 75% RH.

Badarinath A V *et al.*, 2010[17] formulated Flurbiprofen microbeads by varying the alginate, $CaCl_2$ and HPMC concentrations. Prepared beads were evaluated for granulometric studies, micromeretic, scanning electron microscopy, drug entrapment efficiency and in-vitro dissolution studies etc. The prepared beads were free flowing and white in color. The drug loaded beads showed 83.6 – 98.2

% drug entrapment, which was found to increase with increase in sodium alginate concentration. Scanning electron microscopy revealed that the beads were spherical and rough in structure. *In-vitro* drug release study of these microbeads indicated controlled release for flurbiprofen 84.54 – 97.74 % release at the end of 10 hour.

Baljit Singh *et al* 2010[18] reported that Gastroretentive floating sterculia–alginate beads for use in antiulcer drug delivery by using aldehyde as cross-linking agent.

C. Kumaresan 2010[19] reported that Dexibuprofen is the superior non steroidal anti-inflammatory agents for development of pharmaceuticals. The racemic mixture of S (+) Ibuprofen and R (-) Ibuprofen being used, but the biological activity is mainly due to S (+) Ibuprofen. The development of dosage forms with ibuprofen involves major problem to achieve bioavailability and stability. Enantiomers have same physical and chemical properties. But differ in three-dimensional spatial arrangement. This leads to difference in pharmacodynamics and pharmacokinetics. The differences often depend on whether the center of asymmetry of the drug is in close proximity to the points of attachment to the protein. S (+) Ibuprofen is over 100-fold more potent an inhibitor of cyclooxygenase- I than R-Ibuprofen.

Alam M I *et al*., 2010[20] prepared various formulations of mucoadhesive beads of gliclazide using two different natural polymers, alginate and ispaghula, in various stoichiometric proportions with $CaCl_2$ as a source of counter ions and they were characterized for entrapment efficiency, particle size, surface morphology, and swelling index. The kinetics of drug release and their mucoadhesive nature in vitro using goat intestinal mucosa was also investigated at various physiological pH conditions. The effective mucoadhesion property with sustained release profile was observed from optimized mucoadhesive beads consisting of alginate and ispaghula husk (1:1) and polymer (2:1) with 5–10% w/v counter ions ($CaCl_2$). These formulations showed optimum mucoadhesion behavior having more than

70% w/v of drug entrapment and particle sizes of 896.7±0.8 and 920.6±1.2µm, respectively.

Sridhar B K *et al.*, 2010[21] prepared alginate and chitosan beads of theophylline by ionotropic gelation method followed by enteric coating with Eudragit S100.All formulations were evaluated for particle size, encapsulation efficiency, swellability and *in vitro* drug release. The drug release from coated beads depends on coat weights applied and pH of dissolution media. Mechanism of drug release was found to be swelling and erosion-dependent. The studies showed that formulated alginate and chitosan beads can be used effectively for the delivery of drug to colon and a coat weight of 20% weight gain was sufficient to impart an excellent gastro resistant property to the beads for effective release of drug at higher pH values.

Manjanna K M *et al.*, 2009[22] developed sustained release dexibuprofen microbeads using sodium alginate as the hydrophilic carrier in combination with Guar gum, Chitosan and pectin as coated polymers to modify the drug release. Microbeads prepared only with sodium alginate show faster drug release. To overcome such inconveniences, polymer-coated alginate microbeads and/or appropriately interpenetrating polymer network structures formed with alginate and other macromolecules were developed. Microbeads coated with chitosan and guar gum aqueous polymer dispersion shows optimum level of sustained release and exhibited zero-order kinetics followed by super case-II transport.

Sanap Gajanan S *et al.*, 2009[23] formulated mucoadhesive beads of glipizide employing sodium alginate, carbopol 974P and Sodium Carboxy Methyl Cellulose(SCMC). The prepared formulations were evaluated for physical parameter, mucoadhesive properties, *in-vitro* wash-off test, *in-vitro* drug release and *in-vivo* test.

Sachan N K *et al.*, 2009[24] prepared hydrogel microbeads of metformin by ionotropic gelation method using the blends of pre-gelatinized *Bora rice* along with sodium alginate as mucoadhesive backbone. The drug entrapment efficiency was initially very low for the metformin hydrochloride because it is being highly water soluble, diffuses out to calcium chloride solution at the time of encapsulation or during curring. This was improved considerably by some changes in encapsulation process as the drug was thoroughly mixed with pre-gelatinized starch gel to get it to penetrate to the hollow hilum of rice granule that hinders diffusion.

Patel V A *et al* ., 2009[25] developed the chitosan beads containing methotrexate by using ionotropic gelation method by dropping methotrexate containing solution of positively charged chitosan into tri poly phosphate solution. It was observed that beads containing higher proportion of span 80 showed faster release and the beads with the higher proportion of TPP showed delayed release. Drug release kinetics from these formulations corresponds best to the first order kinetics.

Manjanna K M *et al.*, 2009[26] formulated diclofenac microbeads by using sodium alginate alone and combination with coating polymers like HPMC, chitosan, pectin and calcium chloride used as cross linker. The drug entrapment efficiency increased progressively with increasing concentration of both sodium alginate and coating polymer resulting in the formation of larger microbeads entrapping greater amounts of the drug. Increase in concentration of sodium alginate tends to make particle more spherical. Increase in concentration of calcium chloride significantly affects the mean diameter but no appreciable change in morphology and release behavior.

Srinatha A *et al.*, 2008[27] studied chitosan beads loaded with ciproflaxin hydrochloride were fabricated by ionic cross linking with sodium tri poly phosphate. The beads showed excellent water retention property. Drug release was higher in acidic medium (pH 1.2) vis- a vis intestinal medium (pH 7.4) ciproflaxin hydrochloride release increased with an increasing concentration of ciproflaxin and decrease in the proportion of chitosan. Drug release followed both first order and Higuchi's root time kinetics showing Non- Fickian release mechanism.

[24]**Frances Stops *et al*., 2008**[28] developed Floating calcium alginate beads for the delivery of Riboflavin with citric acid. SEM confirmed spherical structure of beads. Citric acid increase rate of drug release from alginate beads was slowest in acidic medium pH 1.2 and quickest in more alkaline medium. The release of Riboflavin from the calcium alginate beads complete within 6 hours. Furthermore modifications are required to alter the rate of drug release from floating calcium beads.

Sanjay K. Jain *et al* 2007[37] developed multiparticulate system of chitosan hydrogel beads by aldehyde a cross linker for colon targeted delivery of satranidazol chitosan hydrogel beads coated with Eudragit S100 having size upto 1.95±0.05mm. The drug released after 24 hours was found to be 97.67% ± 1.25%. Degradation of the chitosan hydrogel beads in the presence of extracellular enzymes indicate the potential of the microparticulate system to serve as a carrier to deliver the molecules specifically to the colon.

[18]**Andrew T. Metters et al., 2006**[30] reported hydrogel have been a vital role in the biomedical field with increasing efforts devoted to sustained release will continue in future due to need of sustained action of drug. Further more advanced release system would be developed such as affinity hydrogel microparticle system and in situ forming gel. Proper network design and accurate mathematical modeling are keys to tuning the drug release rates.

Emilia Abraham T *et al.*, 2006[31] reported that Chitosan–alginate complexes as a pH sensitive hydrogel have been studied for the development of oral delivery. Alginate has the property of shrinking in low pH and getting dissolved in higher pH, whereas chitosan dissolves in low pH and is insoluble in higher pH ranges. Complexation of chitosan with alginate reduces the porosity of alginate beads and decreases the leakage of the encapsulated drugs.

Almeida A.J. *et al,* 2004[32] reported alginate-gelatin beads cross linked by aldehyde (formaldehyde or glutaraldehyde) as cross-linking agent to formulate a new matrix for controlled release of pindolol. All formulations were evaluated for particle size, encapsulation efficiency, gelling rate, swelling rate and *in-vitro* drug release studies. Gelling rate indicates the speed and degree of polymer reaction on the formation of reticulated structure. Swelling studies indicates speed and easiness of a liquid to penetrate the alginate-gelatin matrix. Also,cross-linking agent delays drug release from beads. Polymer such as Eudragit (RD100, RL30D, RS30D), Carbopol or Ethylcellulose, did not improve the matrix ability to prolong drug release.

Tejraj M. Aminabhavi et al., 2004[33] reported chitosan bead containing clozapin microparticle with glutaraldehyde as cross linker showed high entrapment efficacy of upto 98.97% and release upto 12 hours. *In-vivo* Albino rate absortion of drug was delayed from microparticles. Since AUC was higher when compared to neat clozapin. *In-vivo* studies indicated the absorption pattern of clozapin of from microparticle were in a controlled manner.

Mansoor Amiji et al., 2003[34] reported that physical, chemical and biological properties make chitosan a good candidate for the development of Novel gastrointestinal drug delivery system such as stomach specific drug delivery, intestinal drug delivery and colon specific drug delivery. Drug targeting with chitosan was illustrated by in vivo animal model revealed that chitosan is a good candidate for gastrointestinal drug delivery.

Bonabello *et al.*, 2003[35] studied the anti-inflammatory and analgesic effect of Dexibuprofen and ibuprofen in rodents. From the animal studies in rats, S(+) ibuprofen isomer was found to be more potent than the racemic formulation in analgesic and anti-inflammatory activities. From IV dose, both drug (Dexibuprofen and Ibuprofen) showed significant analgesic effect. But ED_{50} of dexibuprofen was half of effective dose of Ibuprofen. From peroral administration, the ED_{50} of Dexibuprofen was less when compared to ED_{50} of Ibuprofen. Showed that dexibuprofen have better analgesic and anti-inflammatory effect than racemic ibuprofen.

Gonzalez-Rodriguez M.L *et al.*, 2002[36] reported Alginate/chitosan particulate systems for sodium Diclofenac release with Al^{3+} and Ca^{2+}.The release from the beads was examined as a function of pH of the dissolution medium indicated that drug release was prevented in acidic pH. Alginate microspheres containing diclofenac start to release the drug after the pH of the environment increases above 7. Alginate/chitosan ratio and the nature of the jellifying cation allow a control of the release rate of the drug.

Mitsutoshi Nakajima et al 2002[50] developed a new technique for gelatin (Type B) microbeads with narrow size distribution by using microchannel emulsification for W/O and O/W emulsion.

Tejraj M. Aminabhavi *et al.*, 2000[38] reported Glutaraldehyde cross-linked sodium alginate beads containing liquid pesticide for soil application. The absence of chemical interactions between active ingredients and polymer as well as cross-linking agent was confirmed by FTIR spectral measurements. The SEM data indicated that the structure of the walls of the beads are smooth and nonporous. From 'n' value, indicating that diffusion deviate slightly from Fickian transport.

John T. Fell *et al.*, 2000[39] formulated alginate beads by varying the alginate using $CaCl_2$ as a counter ion. Prepared beads were evaluated. Drug release studies showed that beads prepared with the drug in solution provided some sustained release characteristics and these could be improved by the addition of amylose. Scanning electron microscopy revealed that the beads were almost spherical in shape. *In-vitro* drug release study of these beads indicated sustained release for amoxycillin.

Tejraj M. Aminabhavi et al., 1999[40] designed sodium alginate containing diclofenac sodium by the precipitation of alcohol followed by glutaraldehyde as a cross linker in acidic medium. Entrapment efficacy depends upon the preparation. SEM indicates non-porous surface of beads. The particle size was not varied either by increasing exposure time to cross link or by increasing percentage loading of active ingredient. The beads exposed to long time to glutaraldehyde required long time to dry when compared to beads exposed to lesser time to glutaraldehyde.

Fernandez-Hervas M.J *et al.*, 1998[37] developed diclofenac beads from sodium alginate, chitosan and /or Eudragit L30D by $CaCl_2$ as cross-linking agent for the delivery of practically insoluble drug diclofenac salt into the small intestine. High entrapment efficacy was obtained with chitosan having the range of 91.26% to 95.99%. Release depends upon the pH.SEM shows Spherical nature of beads after drying in room temperature. At low pH, the non swelling should reduce the matrix permeability and limit the drug diffusion.

Kinam Park *et al.*, 1993[41] reported biodegradable hydrogels have been exploited in the controlled drug delivery area due to various advantages. Biodegradable hydrogel can possess other properties like bioadhesive, pH-sensitive, temperature-sensitive or other environmental sensitive properties. These properties have been used to design the sustained release drug delivery.

AIM AND OBJECTIVE

The aim of the present study is to formulate and evaluate the sustained release of cross-linked hydrogel dexibuprofen beads by using formaldehyde as a cross linking agent with different ratio of polymer like sodium alginate, chitosan and gelatin.

Dexibuprofen [S (+)-ibuprofen], pharmacologically active enantiomer of racemic ibuprofen, is a non-steroidal substance with anti-inflammatory and analgesic effects. That is absorbed primarily from the small intestine. But it undergoes a significant first pass metabolism which may reduce the systemic bioavailability of Dexibuprofen. After metabolic transformation in the liver, the pharmacologically inactive metabolites are completely excreted, mainly by the kidneys (90%), but also in the bile. The elimination half- life is 1.8-3.5 hours.

Oral ingestion is the traditionally preferred route of drug administration, providing a convenient method of effectively achieving both local and systemic effect. In conventional oral drug delivery systems, there is very little control over release of the drug. The effective concentration can be achieved by intermittent administration of excessive doses, which, in most situations, often results in constantly changing, unpredictable, and often sub- or supra-therapeutic plasma concentrations leading to marked side effects.

An ideal oral drug delivery system should steadily deliver a measurable and reproducible amount of drug to the site over a prolonged period. Sustained release (SR) delivery systems provide a uniform concentration/amount of the drug at the absorption site and thus, after absorption, allow maintenance of plasma concentrations within a therapeutic range, which minimizes side effects and also reduces the frequency of administration.SR products are formulations that release active drug compounds into the body gradually and predictably over a long period and that can be taken once a day. Typically, these products provide numerous benefits compared with immediate release drugs, including greater effectiveness in the treatment of chronic conditions, reduced side effects, greater convenience, and higher levels of patient compliance due to a simplified dosing schedule.

PLAN OF WORK

- Collection of drug and Polymer
- Preformulation studies
    a. Description
    b. Solubility
    c. Identification of the drug
        - UV absorption maxima
        - FT-IR Studies
    d. Drug-Excipient Compatibility studies by FTIR
- Formulation of Hydrogel Beads
- Evaluation parameters
        - Gelling rate
        - Particle size by Optical Microscopy
        - Shape and Surface morphology by Scanning Electron Microscopy
        - Drug Content Analysis and Percentage Drug Entrapment
        - *In-vitro* release profile
        - Swelling studies
- Release kinetics
- Stability testing
- Results and discussion
- Conclusion

DRUG PROFILE[20, 42, 59, 60]

Drug Name	:	Dexibuprofen
IUPAC Name	:	(2S)-2-[4-(2-methylpropyl) phenyl] propanoic acid
Molecular Structure	:	

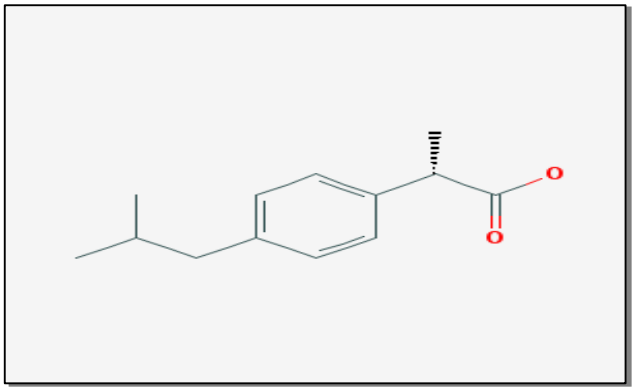


Figure.6

Molecular formula	:	$C_{13}H_{18}O_2$
Molecular weight	:	206.280 g/mol
Metabolism	:	Liver
Mode of action	:	Inhibition of cyclooxygenase
Elimination Half Life	:	1.8-3.5hours
Protein binding	:	About 99%
t_{max}	:	About 2 hour
Dose	:	1200mg/day (Maximum)
Excretion	:	Mainly by renal
Use	:	Arthritis
Storage	:	Store at 25ºC or below

MECHANISM OF ACTION[8]

Major mechanism of Dexibuprofen is inhibition of PG genenation. Prostaglandins, prostacyclin(PG I_2) and thrombaxane A_2 are produced from arachidonic acid by the enzyme cyclooxegenase. Which exists in a constitutive (COX-1) and an inducible (COX-2) isoforms; former servers physiological 'house keeping' funtion, while the latter,normally present in minute quantities, is induced by cytokines and other signal molecules at the site of inflammation. Which cause the generation of PGs locally which mediate many of the inflammatory changes.

Mechanism of action

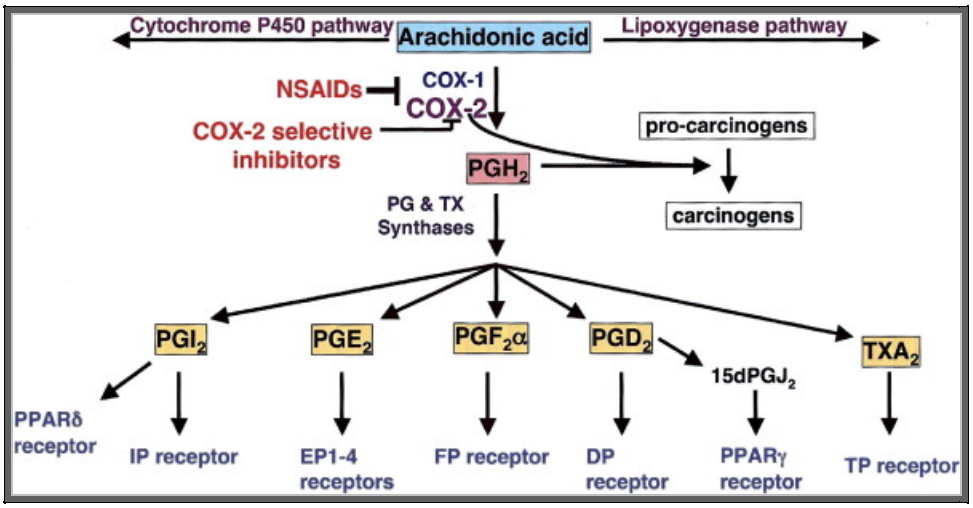


Figure.7

Therapeutic Indications

- Symptomatic treatment for the relief of pain and inflammation associated with osteoarthritis.
- Acute symptomatic treatment of pain during menstrual bleeding (primary dysmenorrhoea).
- Symptomatic treatment of other forms of mild to moderate pain, such as muscular-skeletal pain or dental pain Contraindications
- Patients previously sensitive to dexibuprofen, to any other NSAID

- Patients in whom substances with a similar action (e.g. aspirin or other NSAIDs) precipitate attacks of asthma, bronchospasm, acute rhinitis, or cause nasal polyps, urticaria or angioneurotic oedema.
- Patients with active or suspected gastrointestinal ulcer or history of recurrent Gastrointestinal ulcer.
- Patients with active Crohn's disease or active ulcerative colitis.
- Patients with severe heart failure.
- Patients with severe renal dysfunction (GFR < 30ml/min).
- Patients with severely impaired hepatic function.
- Patients with haemorrhagic diathesis and other coagulation disorders, or patients receiving anticoagulant therapy.
- Patients who have gastrointestinal bleeding or other active bleedings or bleeding disorders.

Drug interaction

In general, NSAIDs should be used with caution with other drugs that can increase the risk of gastrointestinal ulceration or gastrointestinal bleeding or renal impairment.

- Acetylsalicylic acid
- Antihypertensives
- Cyclosporin, tacrolimus
- Corticosteroids
- Digoxin
- Methotrexate
- Phenytoin
- Thiazides, thiazide-related substances, loop diuretics and potassium-sparing diuretics.

Posology

For individual dosage film-coated tablets with 200, 300 and 400 mg dexibuprofen are available. The recommended dosage is 600 to 900 mg dexibuprofen daily, divided in up to three single doses. For the treatment of mild to moderate pain, initially single doses of 200 mg dexibuprofen and daily doses of 600 mg dexibuprofen are recommended.

The maximum single dose is 400 mg dexibuprofen. The dose may be temporarily increased up to 1200 mg dexibuprofen per day in patients with acute conditions or exacerbations. The maximum daily dose is 1200 mg. For dysmenorrhoea a daily dose of 600 to 900 mg dexibuprofen, divided in up to three single doses, is recommended. The maximum single dose is 300 mg, the maximum daily dose is 900 mg.

Dexibuprofen has not been studied in children and adolescents (< 18 years). Safety and efficacy have not been established and therefore it is not recommended in these age groups. In general NSAIDs (non-steroidal anti-inflammatory drugs) are preferably taken with food to reduce gastrointestinal irritation, particularly during chronic use. However, a later onset of action in some patients may be anticipated when the tablets are taken with or directly after a meal.

Precautions

Precautions are taken for symptoms or history of gastro-intestinal disease, asthma, impaired hepatic, cardiac or renal function. NSAID may mask infections or temporarily inhibit platelet aggregation. In late pregnancy, as with other NSAIDs, it should be voided as it may cause premature closure of ductus arteriosus. Dexibuprofen should be used with caution in nursing mothers.

Special Warnings and Precautions

- Care is recommended in conditions that predispose patients to the gastrointestinal adverse effects of NSAIDs such as dexibuprofen, including existing gastrointestinal disorders, previous gastric or duodenal ulcer, ulcerative colitis, Crohn's disease and alcoholism.
- Gastrointestinal bleeding or ulceration/perforation have in general more serious consequences in the elderly. They can occur at any time during treatment with or without warning symptoms or a previous history of serious gastrointestinal events.
- In the treatment of patients with heart failure, hypertension, renal or hepatic disease, especially during concomitant diuretic treatment, the risk of fluid retention and a deterioration in renal function must be taken into account. If used in these patients, the dose of dexibuprofen should be kept as low as possible and renal function should be regularly monitored.
- Caution is required in patients suffering from, or with a previous history of bronchial asthma since NSAIDs can cause bronchospasm in such patients.
- In common with other NSAIDs dexibuprofen may reversibly inhibit platelet aggregation and function and prolong bleeding time. Caution should be exercised when dexibuprofen is given concurrently with oral anticoagulants.

Pregnancy

Inhibition of prostaglandin synthesis may adversely affect the pregnancy and/or the embryo/fetal development, and as the consequences of inhibiting the synthesis of prostaglandins are not fully known, dexibuprofen, like other drugs of this class, should only be administered in the first 5 months of pregnancy if clearly needed, in the lowest effective dose and as short as possible. During the third trimester of pregnancy, all prostaglandin synthesis inhibitors may expose the fetus to

- Cardiopulmonary toxicity
- Renal dysfunction
- Possible prolongation of bleeding time,
- Inhibition of uterine contractions resulting in delayed or prolonged labour.

The use of dexibuprofen, as with any drug substance known to inhibit cyclooxygenase / prostaglandin synthesis is not recommended in women attempting to conceive.

Lactation

Ibuprofen is slightly excreted in human milk. Breast-feeding is possible with dexibuprofen if dosage is low and the treatment period is short.

Concomitant use not recommended:

- Anticoagulants
- Methotrexate
- Lithium
- Other NSAIDs and Salicylates

EXCIPIENTS PROFILE[4]

SODIUM ALGINATE

Sodium alginate consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid.

Empirical formula: $(C_6H_70_6Na)n$

Chemical structure:

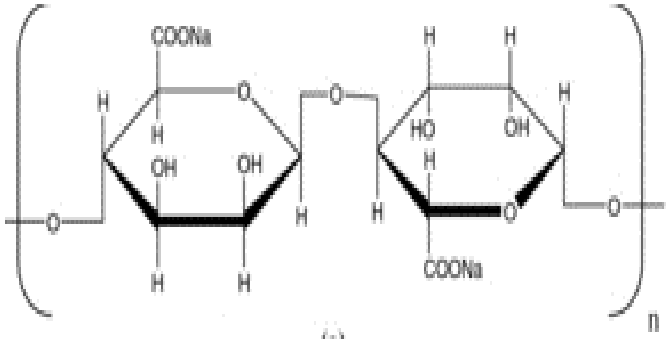


Figure.8

Solubility

It is slowly soluble in water and forming a viscous colloidal solution. It is insoluble in alcohol & hydro alcoholic solution. It is insoluble in other organic solvents and aqueous acidic solutions in which the pH of the resulting solution is less than 3.0.

Viscosity

- 1% w/v aqueous solution should be 20-400 cps at 20°C

pH : ~ 7.2

Description

It occurs as odorless and tasteless, white to pale yellowish-brown colored powder.

Safety

Sodium alginate is regarded as a non-toxic and non-irritant material.

Incompatibility

It is incompatible with crystal violet, acridine derivatives, phenyl mercuric nitrate and acetate, ethanol, calcium salts in concentration greater than 5% and heavy metals.

Application

Used as a stabilizer in emulsions as a suspending agent and tablet binder. sodium alginate has been used in combination with an H_2-receptor antagonist in the management of gastro esophageal reflux. It is also used as a haemostatic agent in surgical dressing.

GELATINE

Nonproprietary Names:

BP	:	Gelatin
JP	:	Gelatin
PhEur	:	Gelatina
USPNF	:	Gelatin

Synonyms

Byco, Cryogel, Gelatine, Instagel, Solugel.

Emperical formula

Gelatin is a generic term for a mixture of purified protein fractions obtained either by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of animal collagen.

Gelatin may also be a mixture of both types. The protein fractions consist almost entirely of amino acids joined together by amide linkages to form linear polymers, varying in molecular weight from 15 000–250 000.

Solubility

Practically it is insoluble in acetone, chloroform, ethanol (95%), ether and methanol. But, soluble in glycerin, acids and alkalis, although strong acids or alkalis cause precipitation. In water, gelatin swells and softens, gradually absorbing between five and 10 times its own weight of water. Gelatin is soluble in hot water, forming a jelly, or gel, on cooling to 35–40°C. At temperatures >40°C, the system exists as a sol. This gel–sol system is heat-reversible, the melting temperature being slightly higher than the setting point; the melting point can be varied by the addition of glycerin.

Viscosity (dynamic)

- ✓ 4.3–4.7 cps for a 6.67% w/v aqueous solution at 60°C
- ✓ 18.5–20.5 cps for a 12.5% w/v aqueous solution at 60°C

Stability and Storage Conditions

Dry gelatin is stable in air. Aqueous gelatin solutions are also stable for long periods if stored under cool, sterile conditions. At temperatures above about 50°C, aqueous gelatin solutions may undergo slow depolymerization and a reduction in gel strength may occur on resetting. Depolymerization becomes more rapid at temperatures above 65°C, and gel strength may be reduced by half when a solution is heated at 80°C for 1 hour. The bulk material should be stored in an airtight container in a cool, dry place.

Safety

Gelatin is widely used in a variety of pharmaceutical formulations including oral and parenteral products. In general, when used in oral formulations gelatin may be regarded as a nontoxic and nonirritant material.

Bloom Strength

Various grades of gelatin are commercially available that differ in particle size, molecular weight, and other properties. Grading is usually by gel strength, expressed as ‘Bloom strength’, which is the weight in grams that, when applied under controlled conditions to a plunger 12.7 mm in diameter, will produce a

depression exactly 4 mm deep in a matured gel containing 6.66% w/w of gelatin in water.

Acidity/alkalinity: (for a 1% w/v aqueous solution at 25°C)

- pH = 3.8–6.0 (type A)
- pH = 5.0–7.4 (type B)

Density

- 1.325 g/cm3 for type A
- 1.283 g/cm3 for type B

Isoelectric point

- 7–9 for type A
- 4.7–5.3 for type B

Moisture content

- 9–11%

Functional Category

Coating agent, film-former, gelling agent, suspending agent, tablet binder and viscosity increasing agent.

Applications in Pharmaceutical Formulation or Technology

Gelatin is widely used in a variety of pharmaceutical formulations, including its use as a biodegradable matrix material in an implantable delivery system, although it is most frequently used to form either hard or soft gelatin capsules.

CHITOSAN

Nonproprietary Names

- BP : Chitosan hydrochloride
- PhEur : Chitosani hydrochloridum

Synonyms

- 2-Amino-2-deoxy-(1,4)-ß-D-glucopyranan;
- Deacetylated chitin;
- Deacetylchitin
- ß-1,4-poly-D-glucosamine
- poly-D-glucosamine
- poly-(1,4-ß-D-glucopyranosamine)

Chemical Name

- Poly-ß-(1,4)-2-Amino-2-deoxy-D-glucose.

Empirical Formula and Molecular Weight

Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerization and it is therefore not easily defined in terms of its exact chemical composition. A clear nomenclature with respect to the different degrees of *N*-deacetylation between chitin and chitosan has not been defined and as such chitosan is not one chemical entity but varies in composition depending on the manufacturer.

In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80–85%. Chitosan is commercially available in several types and grades that vary in molecular weight by 10,000–10,00,000 and vary in degree of deacetylation and viscosity.

Structural formula

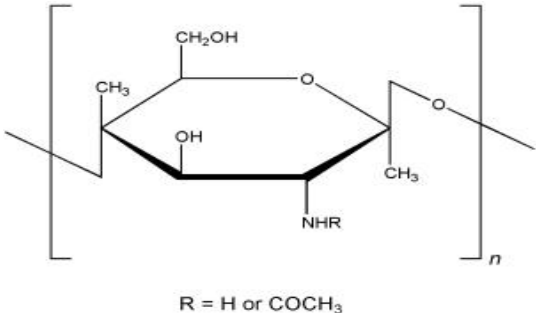


Figure.9

Description

Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look 'cotton like'.

Typical properties

Chitosan is a cationic polyamine with a high charge density at pH <6.5; and so adheres to negatively charged surfaces and chelates metal ions. It is a linear polyelectrolyte with reactive hydroxyl and amino groups (available for chemical reaction and salt formation). The properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. The presence of a number of amino groups allows chitosan to react chemically with anionic systems, which results in alteration of physicochemical characteristics of such combinations.

Acidity/alkalinity:

- pH = 4.0–6.0 (1% w/v aqueous solution)

Density

- 1.35–1.40 g/cm^3

Glass transition temperature

- 203°C

Moisture content

Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Particle size distribution

- <30μm

Solubility

Sparingly soluble in water; practically insoluble in ethanol(95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulfuric acids).

Viscosity (dynamic)

A wide range of viscosity types is commercially available. Owing to its high molecular weight and linear, unbranched structure, chitosan is an excellent viscosity-enhancing agent in an acidic environment. It acts as a pseudo-plastic material, exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solutions increases with increasing chitosan concentration, decreasing temperature, and increasing degree of deacetylation.

Stability and Storage Conditions

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. The PhEur 2005 specifies that chitosan should be stored at a temperature of 2–8°C.

Incompatibilities

- Chitosan is incompatible with strong oxidizing agents.

Safety

Chitosan is being investigated widely for use as an excipients in oral and other pharmaceutical formulations. It is also used in cosmetics. Chitosan is generally regarded as a nontoxic and nonirritant material. It is biocompatible with both healthy and infected skin. Chitosan has been shown to be biodegradable.

LD_{50} (mouse, oral) : >16 g/kg

Regulatory Status

Chitosan is registered as a food supplement in some countries

Functional Category

Coating agent, disintegrant, film-forming agent, mucoadhesive, tablet binder, viscosity increasing agent.

Applications in Pharmaceutical Formulation or Technology

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications, use as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems, and use for gene delivery.

Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets, and coatings for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spraydrying, coacervation, direct compression and conventional granulation processes.

MATERIALS AND METHODS

LIST OF MATERIALS

List of Materials Used

S.NO	MATERIALS USED	MANUFACTURER
1.	Dexibuprofen	Shasun Pharmaceuticals Ltd., Puduchery.
2.	Sodium alginate	Otto kemi, Mumbai.
3.	Gelatin	Kemphasol, Mumbai.
4.	Chitosan	Kemphasol, Mumbai.
5.	Sodium hydroxide	Alba cheme, Chennai.
6.	Potassium dihydrogen Phosphate	Alba cheme, Chennai.

Table.1

LIST OF EQUIPMENTS

List of Equipments Used

S.No	EQUIPMENTS	MANUFACTURER
1.	UV-Spectrophotometer	Shimadzu 1700, Japan.
2.	FT-IR	Perkin Elmer, USA.
3.	Electronic Balance	Sartorius, Germany.
4.	pH Meter	Elico L1120.Hyderabed, AP, India.
5.	Magnetic Stirrer	Remi, Gujrat.
6.	Environment Stability Testing Chamber	Heco Environment Chamber, Germany.
7.	SEM	JSM-5610LV, Joel Ltd, Tokyo, Japan.

Table.2

PREFORMULATION STUDIES

Preformulation test is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be man produced.

The following preformulation studies are carried out:

- Description
- Solubility
- Finding the absorption maxima
- Standard curve
- Infra red spectroscopy studies (compatibility studies)

Description

About 1g of sample is taken in a dry petridish and the sample is observed for compliance against the specification.

Solubility Studies[5]

The spontaneous interaction of two or more substance to form a homogeneous molecular dispersion is called as solubility. A semi quantitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute or vice versa. After each addition, the system vigorously shaken and examined visually for any undisclosed solute particles. The solubility was expressed in turns of ratio of solute and solvent.

The approximate solubility's of substances are indicated by the descriptive terms in the accompanying table. The results were given in table 6.

Solubility profile I.P.1996

DESCRIPTIVE TERM	PARTS OF SOLVENT REQUIRED FOR 1 PART OF SOLUTE.
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10000
Practically Insoluble or insoluble	Greater than or equal to 10000

Table.3

Identification of Dexibuprofen

Finding the absorption maxima (λ_{max})

The absorption maxima were found for drug identification. Ultraviolet Visible spectrophotometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the Ultraviolet/visible region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption.

The drug solutions (µg/ml concentration) in 0.1N HCl was taken in a standard cuvette and scanned in the range of 200-400 nm in UV-spectrophotometer. It exhibits absorption maxima at 222.0nm. Similarly, the procedure can be repeated for phosphate buffer pH 7.4. Maximum absorption was seen at 222.5nm. The results were given in figure 12 and 13.

Standard Curve

The drug solutions (10, 20, 30, 40, 50 and 60µg/ml in 0.1N HCl) were taken in a standard cuvette and scanned in the range of 200-400nm by using UV-Spectrophotometer. The absorbance of each sample was measured at 222.0nm. The standard curve is plotted against absorbance and concentration of the sample.

Similarly, the procedure can be repeated for phosphate buffer pH 7.4. The results were given in table 7, 8 and figure 14.

Fourier Transform Infrared (FTIR) spectral analysis

FT-IR is used to identify the functional groups in the molecule. The drug is mixed with KBr and a small thin pellet is formed. Each KBr pellet was scanned at 4 m/s at a resolution of 2 cm over a wave number region of 400 to 4000 cm^{-1}. The characteristic peaks were recorded. The results were given in table 9 to 15 and figure 15 to 21.

Drug- Excipients Compatibility studies by FT-IR

Fourier infrared spectroscopy (FT-IR) analysis was performed for the pure drug, polymer individually and then formulation and FT-IR was taken to find out that there is no interaction between drug and polymers.

FORMULATION OF BEADS [32, 40]

Composition of drug/polymer suspensions

Each formulation contain 100mg of Dexibuprofen

Formulation	Sodium alginate (%w/v)	Gelatin (%w/v)	Chitosan (%w/v)
SA_1	1	-	-
SA_2	2	-	-
SA_3	3	-	-
SA_4	4	-	-
$SAC_{0.5}$	3	0.5	-
SAC_1	3	1	-
$SAC_{1.5}$	3	1.5	-
$SAG_{0.5}$	3	-	0.5
SAG_1	3	-	1
$SAG_{1.5}$	3	-	1.5

Table.4

SA-Sodium alginate; SAC-Sodium alginate with Chitosan;

SAG-Sodium alginate with Gelatin; 0.5,1,1.5,2,3,4 indicate the percentage.

Composition of dispersing medium for 100ml

- ✓ 3ml of methanol
- ✓ 1ml of formaldehyde
- ✓ 8ml of HCl
- ✓ Distilled water upto 100 ml

Formulation of Dexibuprofen Hydrogel beads by Ionotropic gelation method using Formaldehyde as cross-linking agent

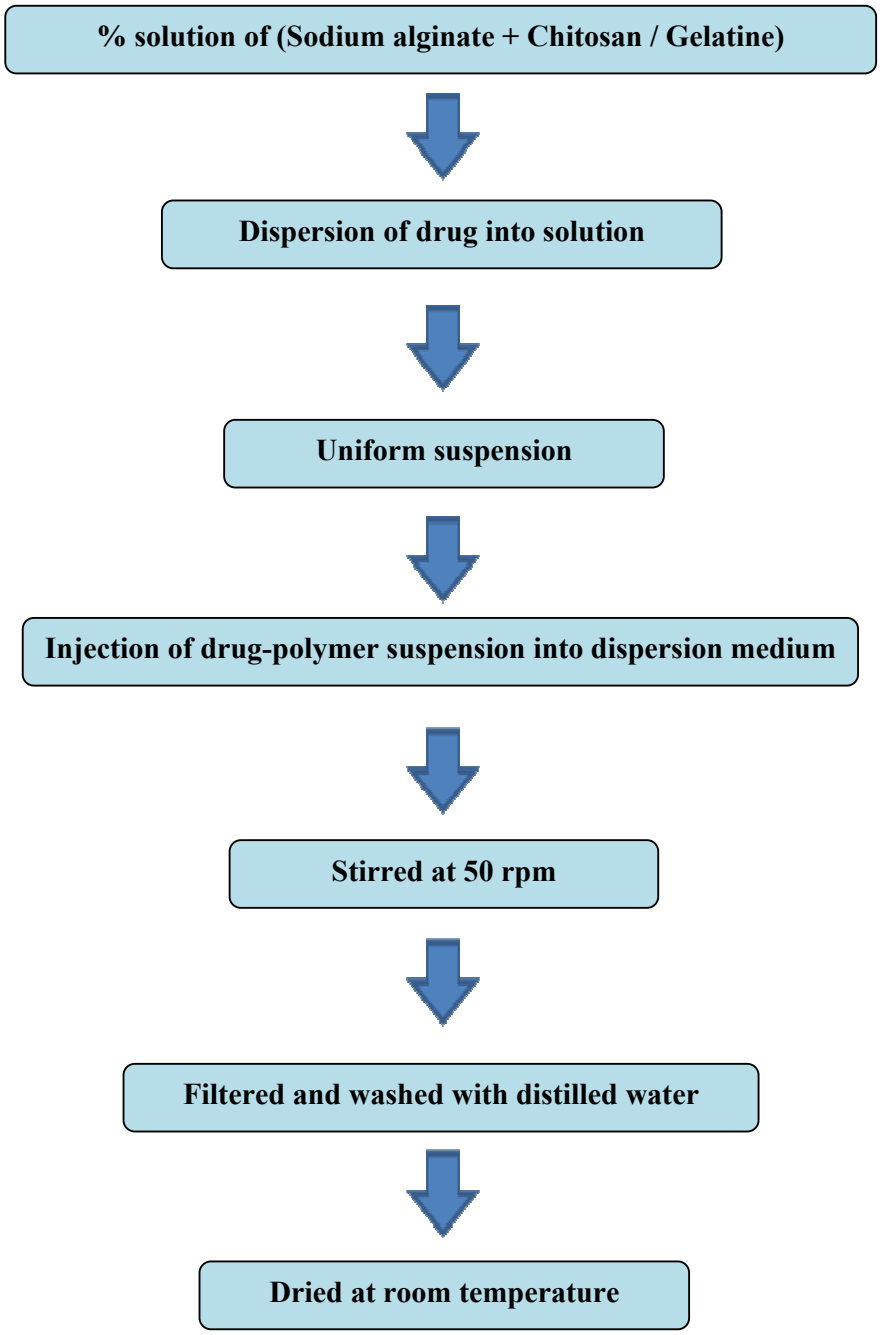


Figure.10

Wet Hydrogel Bead

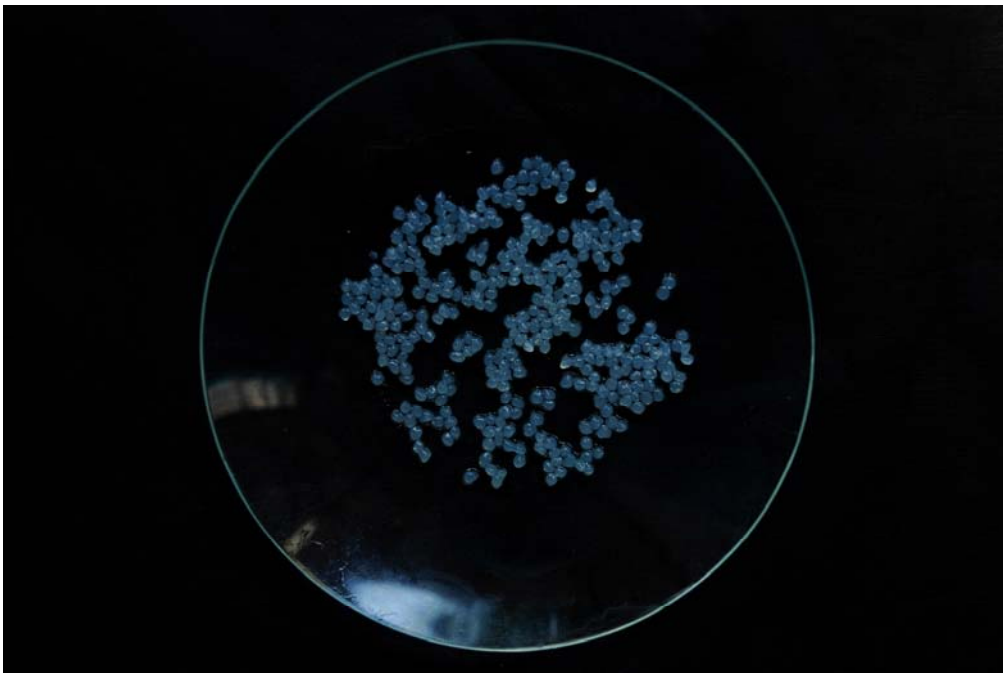


Figure.11

EVALUATION OF BEAD

Particle Size Measurement[61]

Determination of particle size by optical microscopy

A few numbers of beads were transferred on the glass slide and was focused in microscope. By using eyepiece micrometer, the diameter of beads is determined. The results were given in table 15 and figure 30.

Standardization of eyepiece micrometer

6 division of the eyepiece micrometer scale coincide with 8 division of stage micrometer.

One division of stage micrometer =10 µm

$$\text{Hence one division of eyepiece micrometer} = \frac{\text{No .of division of stage micrometer}}{\text{No. of division of eyepiece micrometer}} \times 10$$

Scanning electron microscopy[29]

The purpose of the Scanning Electron Microscopy study was to obtain a topographical characterization of beads. Scanning electron micrographs were obtained by scanning the surface of samples with high energy beam of electrons. The beads were mounted on brass stubs using double-sided adhesive tape. Scanning electron microscopy photographs were taken with a scanning electron microscope (JSM-5610LV, Joel Ltd, Tokyo, Japan) at the required magnification at room temperature. The working distance was maintained, and the acceleration voltage used was 15 kV, with the secondary electron image as a detector. The results were given in figure 33.

Gelling rate[32]

The gelling rate is performed during particle formation. After dropping suspension, which contains drug and polymer, into the dispersion medium contain cross-linking agent, beads were collected at appropriate time intervals. Beads are weighed after removal of moisture from the surface with filter paper. The results were given in table 16 to 18 and figure 33 to 35. Results are expressed in % weight loss.

$$\textbf{\% Weight Loss} = \frac{\textbf{Weight after removal of moisture}}{\textbf{Initial Weight of beads}} \times \textbf{100 \%}$$

Determination of Drug Content and Entrapment Efficiency[18, 32]

Drug content and the Entrapment efficiency are important parameters. During cross-linking polymer and/or polymer-polymer, there is possibility of leaching of drug into dispersion medium. The entrapment efficiency (%) of hydrogel beads was determined spectrophotometrically at a wavelength of maximum absorbance (λ_{max}=222.5nm) using a spectrophotometer (Shimadzu 1700, Japan) by measuring amount of drug left in the solution during the synthesis of beads. The results were given in table 19 and figure 36. The entrapment efficiency was calculated as:

$$\text{Entrapment efficiency (\%)} = \frac{[C_1 - C_2]}{C_1} \times 100\%$$

Where, C_1 is the known concentration of Dexibuprofen in polymer solution that is amount Dexibuprofen taken for formulation and C_2 is the concentration of Dexibuprofen in dispersion medium.

***In vitro* release profile[35]**

The dissolution studies were carried out using USB baskets (Type I) apparatus at 50rpm and 37±0.5°C. The beads were filled into colorless hard gelatin capsules and placed in basket separately. The dissolution medium was 0.1 N HCl having pH 1.2 as simulated gastric fluid (SGF) for the first 2 hour, followed by phosphate buffer pH 7.4 as simulated intestinal fluid (SIF) for the next 10 hour.

5 ml samples were withdrawn at specific time intervals and replaced immediately with an equal volume of fresh medium. Samples were assayed by UV-spectrophotometry, using a spectrophotometer (Shimadzu 1700, Japan).From the absorbance values, the cumulative percent release were determined. The results were given in table 20 and figure 27.

Dissolution Study

Apparatus	:	USP Type I
Speed	:	50rpm
Temperature	:	37±0.5°C
Medium	:	0.1N HCl for 2 hour followed by Phosphate buffer pH 7.4 upto hour

Swelling studies[18,32]

Known weight of the beads were taken and immersed in excess of Phosphate buffer pH 7.4 for definite time interval at 37 °C and then beads were removed and weighed immediately. The difference in weight gave the amount of water uptake by the beads after definite time intervals (30 min). The results were given in table 21 to 24 and figure 39 to 42. The percentage swelling (**Ps**) of the beads was calculated as:

$$\text{Percentage Swelling (Ps)} = \frac{W_s - W_d}{W_d} \times 100$$

Where, W_s is the weight of swollen bead.

W_d is the weight of dry bead.

KINETICS OF DRUG RELEASE[10,12]

Analysis of release profiles

The rate and mechanism of release of both drugs from the prepared matrix beads were analyzed by fitting the dissolution data into the zero-order equation. A plot of Cumulative % drug release versus time would be linear if the drug release follows zero order. (ie. Concentration independent release)

Zero order equation

$$\mathbf{Q = k_0 t}$$

Where, Q is the amount of drug released at time t

$\mathbf{k_0}$ is the release rate constant,

First order equation

A plot of log of % remaining drug versus time would be linear, if the drug release follows first order (ie. Concentration dependent release)

$$ln\ (100\text{-}Q) = ln\,100 - K_1 t$$

Where, k_1 is the release rate constant.

Higuchi's equation

$$Q = K_2 t^{1/2}$$

Where, k_2 is the diffusion rate constant.

Peppas equation

The mechanism of swelling and drug release has been discussed in detailed in our earlier study. Swelling of the polymers and the drug release profile from the polymer have been classified into three types of diffusion mechanisms on the basis of relative rate of diffusion of water into polymer matrix and rate of polymer chain relaxation. In case of water uptake, the weight gain, Ms, is described by following equation.

$$Ms = Kt^n$$

where, n is the release exponent indicative of the mechanism of release,

Ms is weight gain,

t is the release time,

k is the kinetic constant.

Fickian diffusion

Normal Fickian diffusion is characterized by $n = 0.5$, while Case II diffusion by $n = 1.0$. A value of n between 0.5 and 1.0 indicates a mixture of Fickian and Case II diffusion, which is usually called non-Fickian or anomalous diffusion. The above power law expression could be used for the evaluation of drug release from swellable systems.

In this case, **Mt/M∞** replace **Ms** in above equation to give equation.

$$M_t/M_\infty = Kt^n$$

where **Mt/M∞** is the fractional release of drug in time **t**, '**k**' is the constant characteristic of the drug–polymer system, and '**n**' is the diffusion exponent characteristic of the release mechanism. **Mt** and **M∞** is drug released at time't' and at equilibrium respectively. The results were given in table 25 and figure43.

ACCELERATED STABILITY STUDIES[16,43,44]

Stability

Stability is officially defined as the time lapse during which the drug product retains the same property and characteristics that it possessed at the time of manufacture. This process begins at early development phases.

Definition

Stability of a pharmaceutical preparation can be defined as "the capability of a particular formulation (dosage form or drug product) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life.

Instability in modern formulation is often undetectable only after considerable storage period under normal conditions. To assess the stability of a formulated product, it is usual to expose it to high stress conditions to enhance deterioration and therefore the time required for testing is reduced.

Common high stress factors are temperature and humidity. This will eliminate unsatisfactory formulation.

Purpose of stability testing

- To study of drug decomposition kinetics
- To develop stable dosage form
- To establish the shelf life or expiration date for commercially available drug product.
- To ensure the efficacy, safety and quality of active drug substance and dosage forms.

Stability Conditions Chart

S.No	Study	Storage Condition	Minimum Period
1.	Long-term	25°C±2°C 60%±5%	12 Months
2.	Intermediate	30°C±2°C 60%±5%	6 Months
3.	Accelerated	40°C±2°C 75%±5%	6 Months

Table.5

Procedure for Accelerated Stability Studies:

Selected batches were placed in a high density polyethylene container along with wrapper. They are kept in stability chamber maintained at 40ºc and 75% RH. The stability studies were carried out for a period of three months. The beads were tested and checked at regular intervals for changes in percentage of drug content. The results were given in table 26.

Results and discussion

Preformulation studies

Description : White, Odourless powder

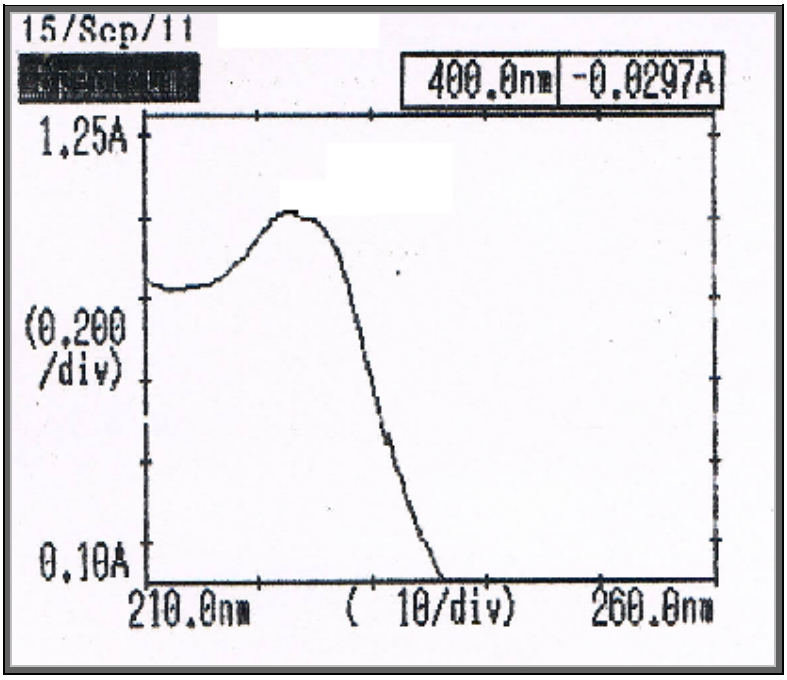
Solubility :

Solubility Profile of Dexibuprofen

S. NO	SOLVENT	SOLUBILITY
1.	Chloroform	Very soluble
2.	Water	Slightly Soluble
3.	Alcohol	Soluble
4.	Ether	Very soluble

Table.6

Identification of Dexibuprofen in 0.1N HCl



Identification of λ_{max} for Dexibuprofen in 0.1N HCl

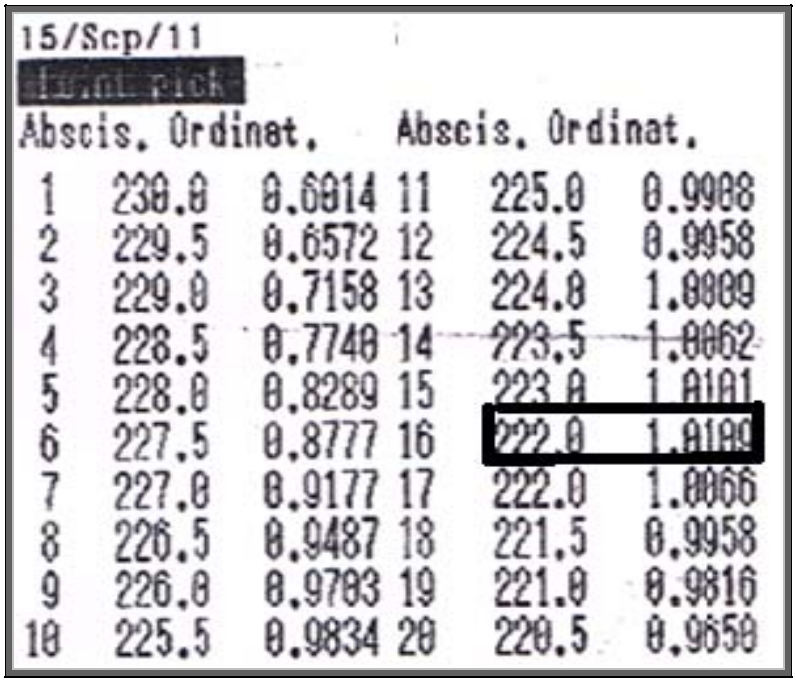
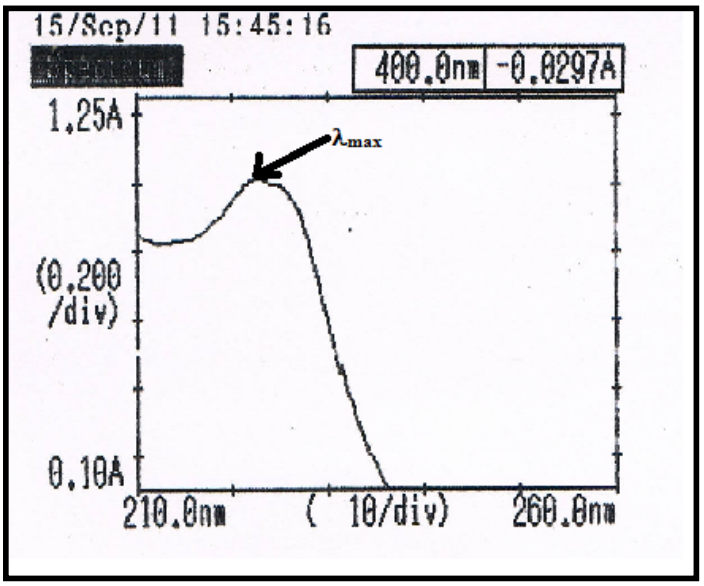


Figure.12

It exhibits absorption maxima at 222.0nm

Identification of Dexibuprofen in Phosphate buffer pH 7.4



Identification of λ_{max} for Dexibuprofen in phosphate buffer pH 7.4

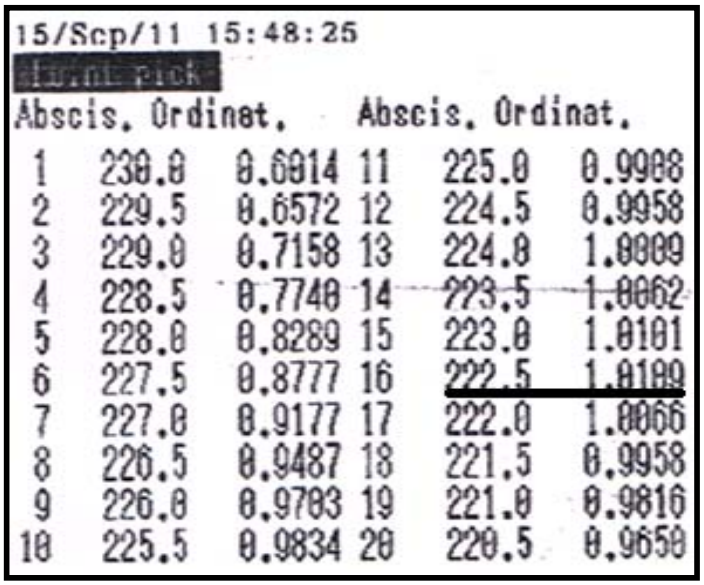


Figure.13

It exhibits absorption maxima at 222.5nm.

Standard Curve

Standard curve in 0.1N HCl

S.No	Concentration (µg/ml)	Absorbance at 222.0 nm
1.	0	0
2.	10	0.0546
3.	20	0.1120
4.	30	0.1498
5.	40	0.1880
6.	50	0.2487
7.	60	0.2958

Table.7

a=0.0249, b=0.0041, r=0.996

Standard curve in 0.1N HCl

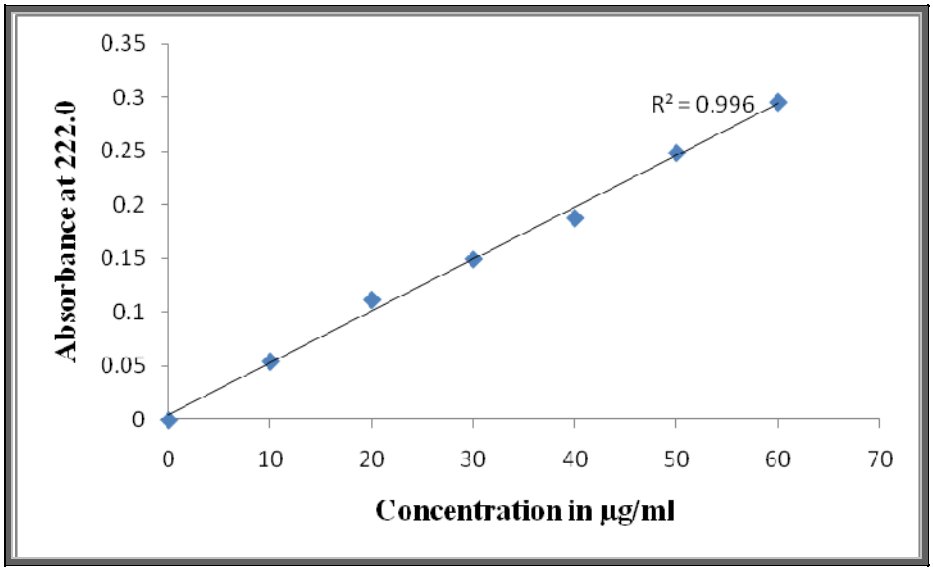


Figure 14

The Standard curve has good regression coefficient(r=0.996) and it shows the linearity.

Standard curve in phosphate buffer pH7.4

S.No	Concentration (µg/ml)	Absorbance at 222.5 nm
1.	0	0
2.	10	0.0564
3.	20	0.1040
4.	30	0.1504
5.	40	0.1996
6.	50	0.2467
7.	60	0.2926

Table.8

a=0.0247, b=0.0045, r=0.9990

Standard curve in phosphate buffer pH7.4

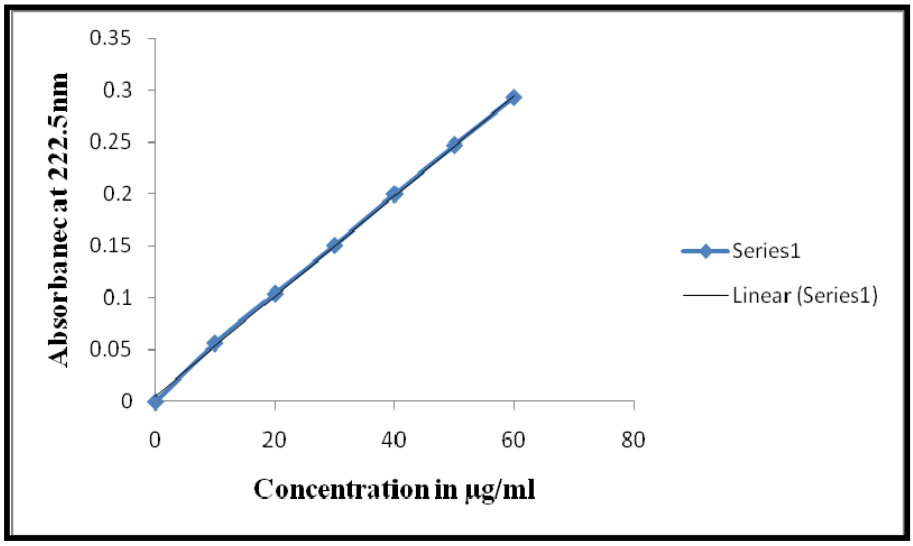


Figure.15

The Standard curve has good regression coefficient(r=0.9990) and it shows the linearity.

FTIR

FTIR spectra of Dexibuprofen

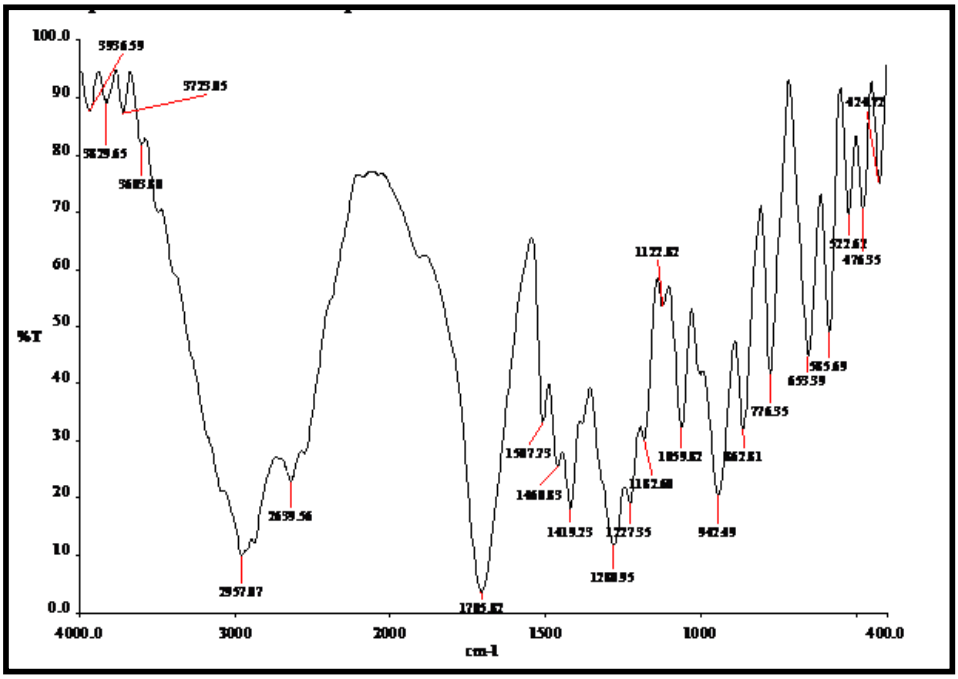


Figure.16

FT-IR spectral assignments for the pure drug Dexibuprofen

Wave number in cm^{-1}	Assignment
2658.56	Aliphatic C-H Stretching
1122.52	C-O Stretching
1152.68	C=O Stretching
653.59,585.69,522.62	C-H out of plane bending

Table.9

FTIR spectra of Sodium Alginate

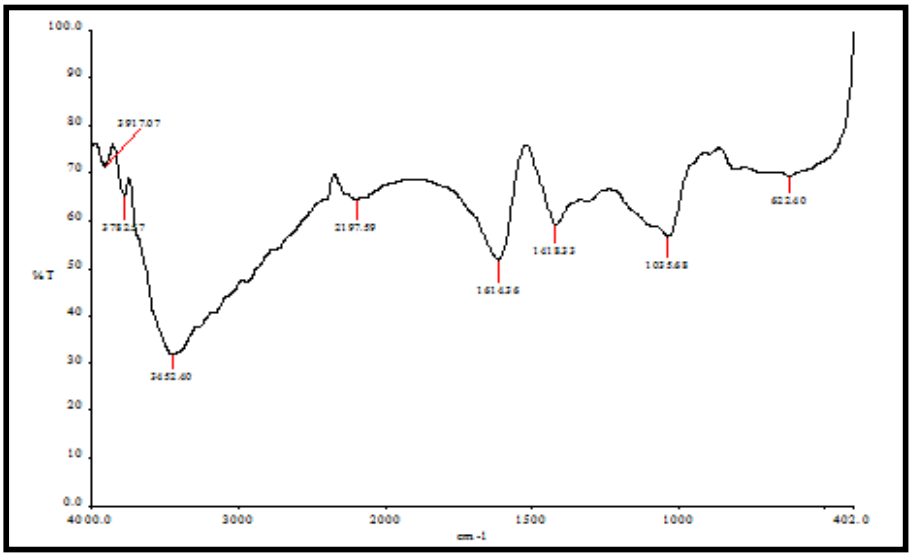


Figure.17

FTIR spectral assignments for Sodium Alginate

Wave number in cm^{-1}	Assignment
3405.03	O-H Stretching
2137.75	C-H Stretching
1097.58	C=O Stretching
1033.05	C-O Stretching
816.95	C-H out of plane bending

Table.10

FTIR spectra of Chitosan

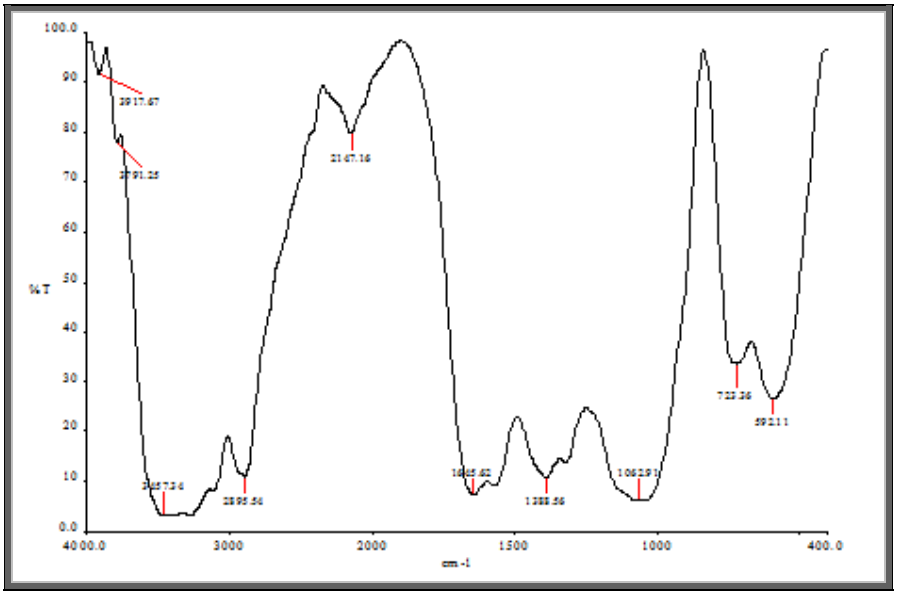


Figure.18

FT-IR spectral assignments for Chitosan

Wave number in cm^{-1}	**Assignment**
2348.64	Aliphatic C-H stretching
3415.62,3341.75	Aliphatic O-H stretching
2935.36	N-H stretching
1415.34	O-H deformation

Table.11

FTIR spectra of Gelatin

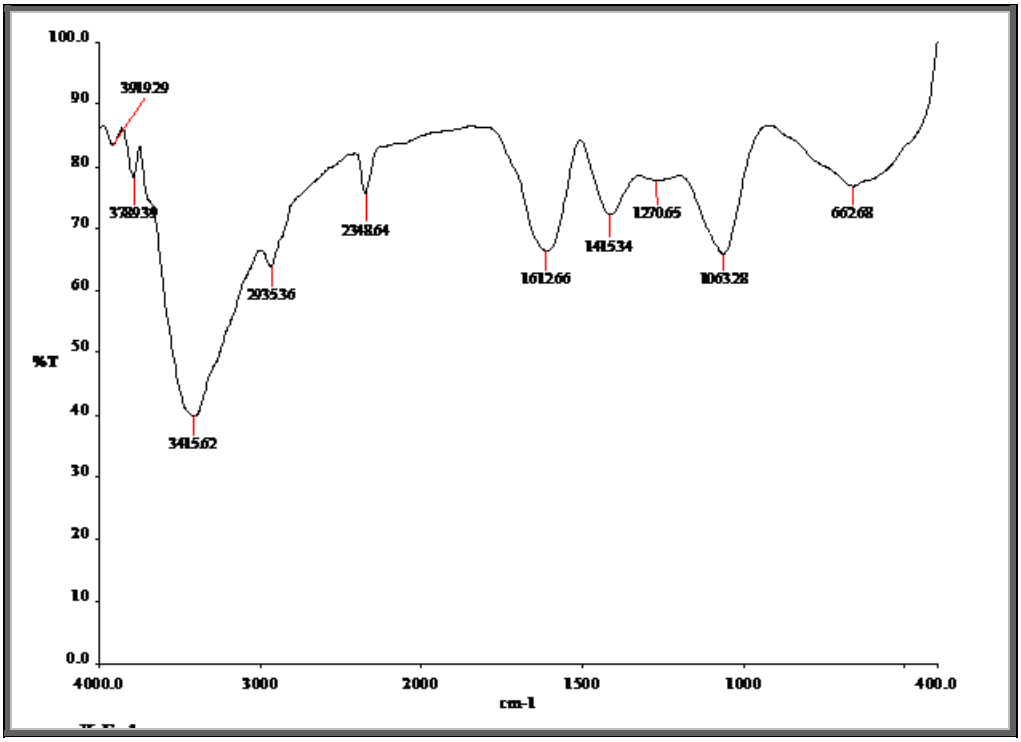


Figure.19

FT-IR spectral assignments for Gelatin

Wave number in cm^{-1}	Assignment
2935.36	N-H Stretching
2348.64	Aliphatic C-H Stretching
662.68	C-H out of plan bending

Table.12

FTIR Spectra of Sodium Alginate beads

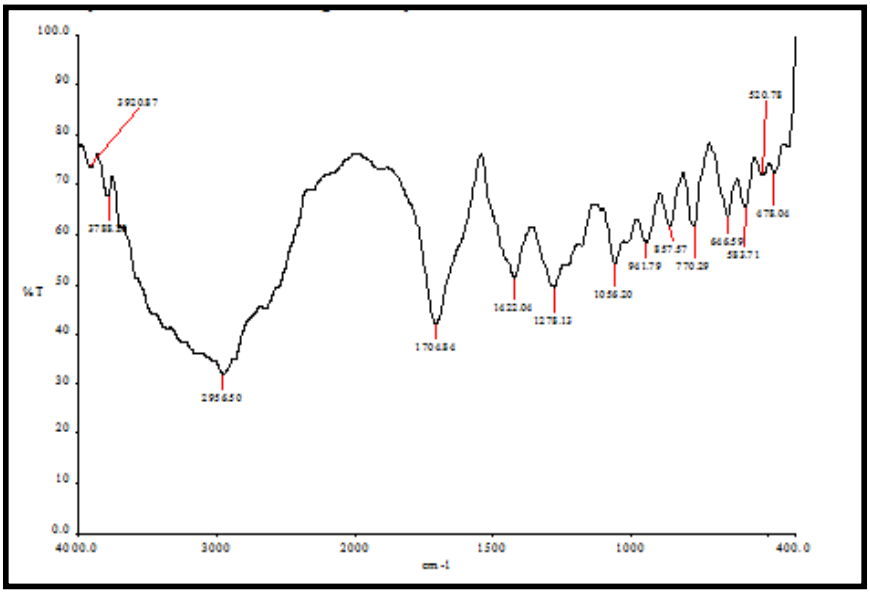


Figure.20

FT-IR spectral assignments for Sodium Alginate beads

Wave number in cm^{-1}	Assignment
3563.87	O-H Stretching
2650.87	C-H Stretching
1058.20	C=O Stretching
686.65,770.28	C-H out of plan bending

Table.13

FTIR Spectra of Sodium Alginate-Chitosan beads

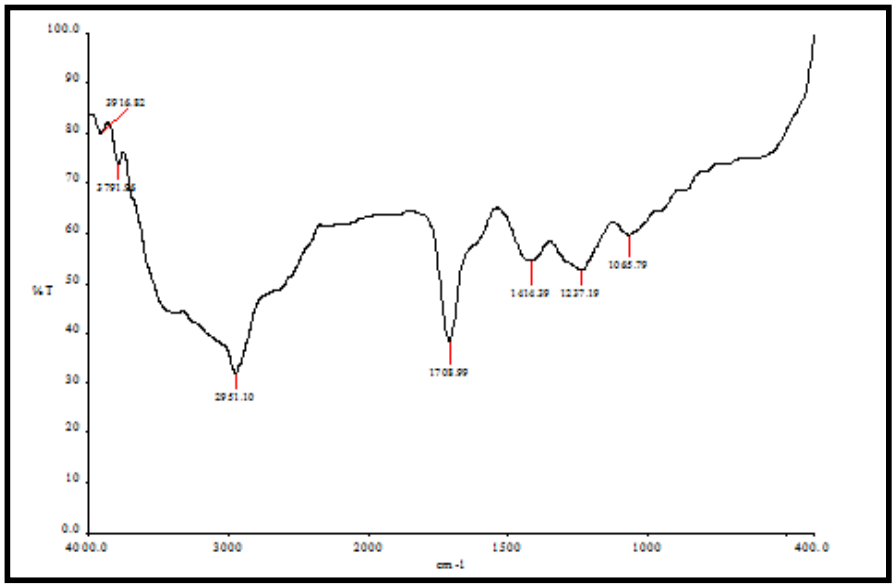


Figure.21

FT-IR spectral assignments for Sodium Alginate-Chitosan beads

Wave number in cm^{-1}	Assignment
3791.25	Aliphatic O-H stretching
2951.10	N-H stretching

Table.14

FTIR Spectra of Sodium Alginate-Gelatin beads

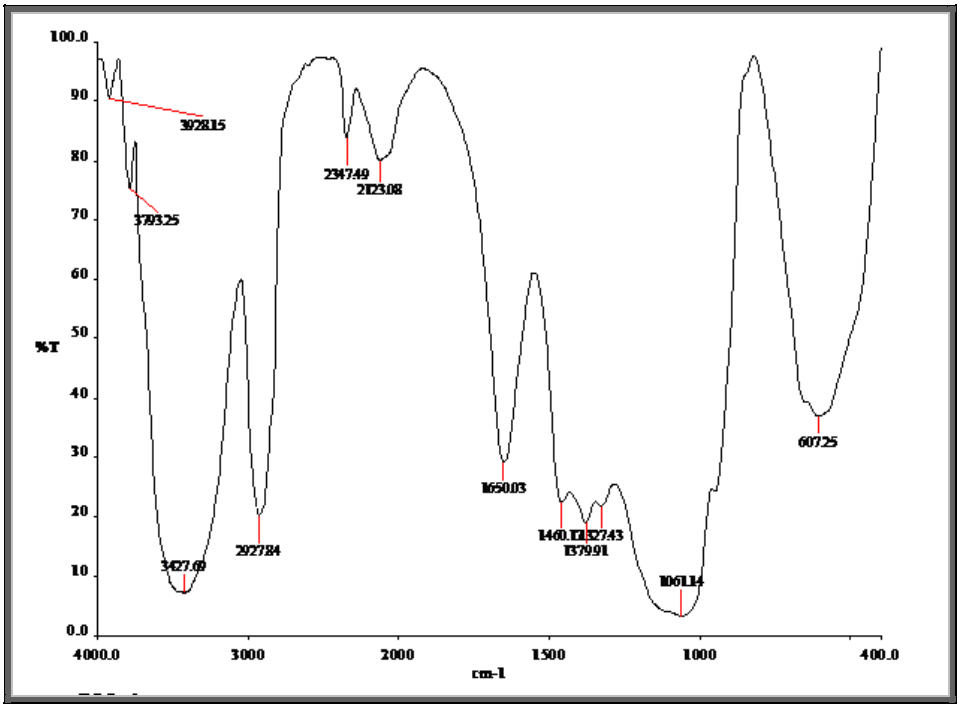


Figure.22

FT-IR spectral assignments for Sodium Alginate-Gelatin beads

Wave number in cm^{-1}	Assignment
1379.91	C-O-O Stretching
2347.49	Aliphatic C-H Stretching
2927.84	N-H Stretching

Table.15

Determination of Particle size by microscopic method

The prepared formulations are taken for the microscopic analysis. It reveals that the prepared beads are in the size range of 1.55mm to 1.94mm.

Particle size analysis

S.No.	Formulation	Mean diameter in mm[a]
1	SA1	1.62 ±0.02
2	SA2	1.75 ±0.03
3	SA3	1.88 ±0.02
4	SA4	1.94 ±0.01
5	SAC0.5	1.59 ±0.04
6	SAC1	1.83 ±0.03
7	SAC1.5	1.69 ±0.02
8	SAG0.5	1.79 ±0.03
9	SAG1	1.55 ±0.03
10	SAG1.5	1.91 ±0.02

[a] Mean ± S.D.; n=3

Table.16

Comparative Particle size analysis

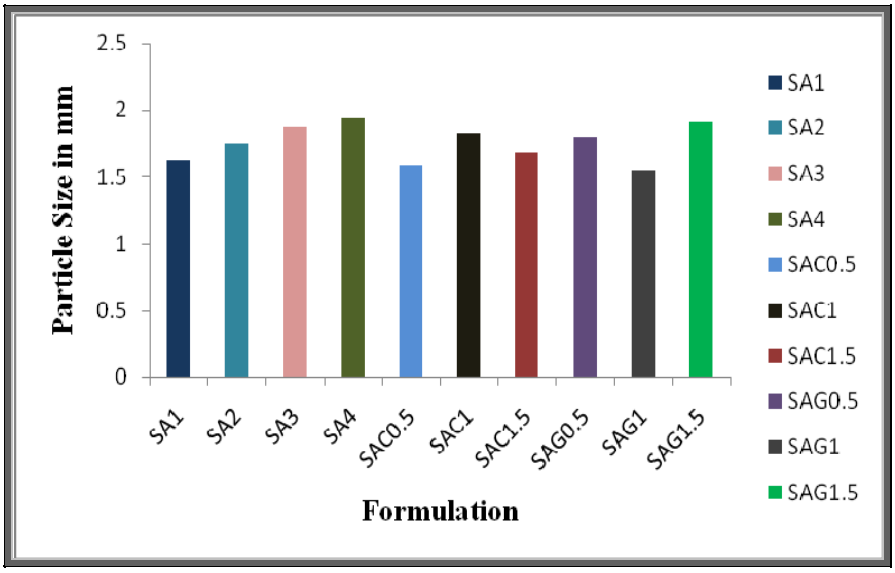


Figure.23

SEM PHOTOMICROGRAPH OF ALGINTE-CHITOSAN BEAD

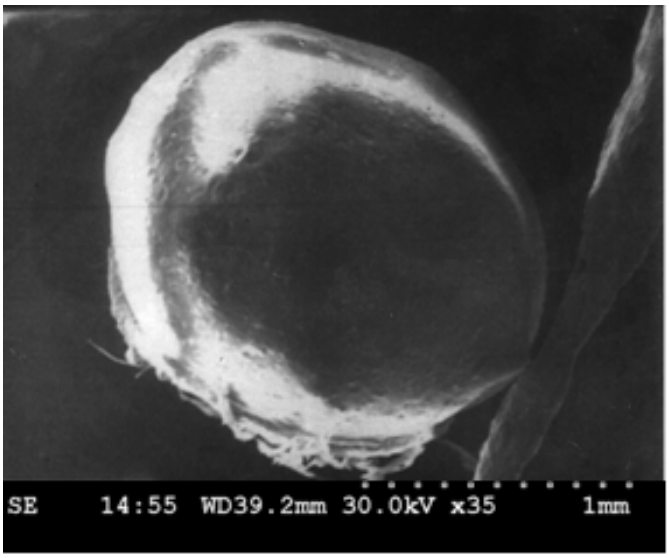


Figure.24

Gelling rate for formulation SA1 to SA4

Time in Minute	Percentage weight loss[a] (%)			
	SA1	SA2	SA3	SA4
0	100	100	100	100
5	80.42±1.4	68.59±1.2	55.94±0.9	55.83±0.9
10	68.28±1.2	53.74±0.9	44.94±0.7	43.82±0.7
15	56.84±0.9	46.73±0.8	42.93±0.6	41.83±0.6
20	48.95±0.7	43.25±0.7	41.64±0.6	40.72±0.6
30	42.10±0.6	42.93±0.6	41.94±0.6	40.38±0.6
45	42.82±0.6	42.94±0.6	41.92±0.6	40.73±0.6

[a] Mean ± S.D.; n=3

Table.17

Comparative Gelling rate profile for formulation SA1 to SA4

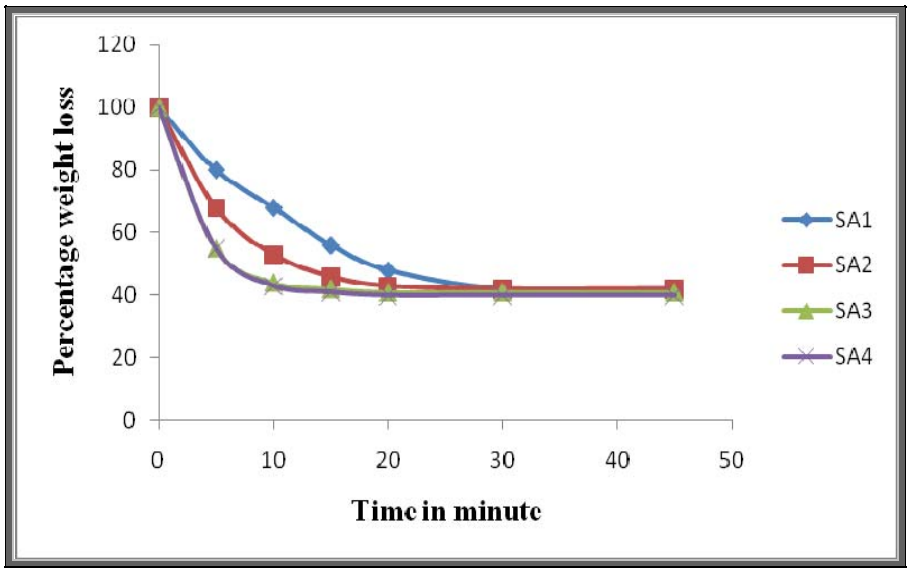


Figure.25

Gelling rate for formulation SAC0.5 to SAC1.5

Time in Minute	Percentage weight loss[a] (%)		
	SAC0.5	SAC1.0	SAC1.5
0	100	100	100
5	81.36±1.4	78.60±1.0	80.93±1.4
10	61.03±0.9	58.83±0.9	60.03±0.9
15	51.29±0.7	50.65±0.8	52.67±0.9
20	43.94±0.6	46.29±0.7	45.98±0.7
30	41.38±0.6	41.94±0.6	43.97±0.6
45	41.56±0.6	41.56±0.6	41.54±0.6

[a] Mean ± S.D.; n=3

Table.18

Comparative Gelling rate profile of formulation SAC0.5 to SAC1.5

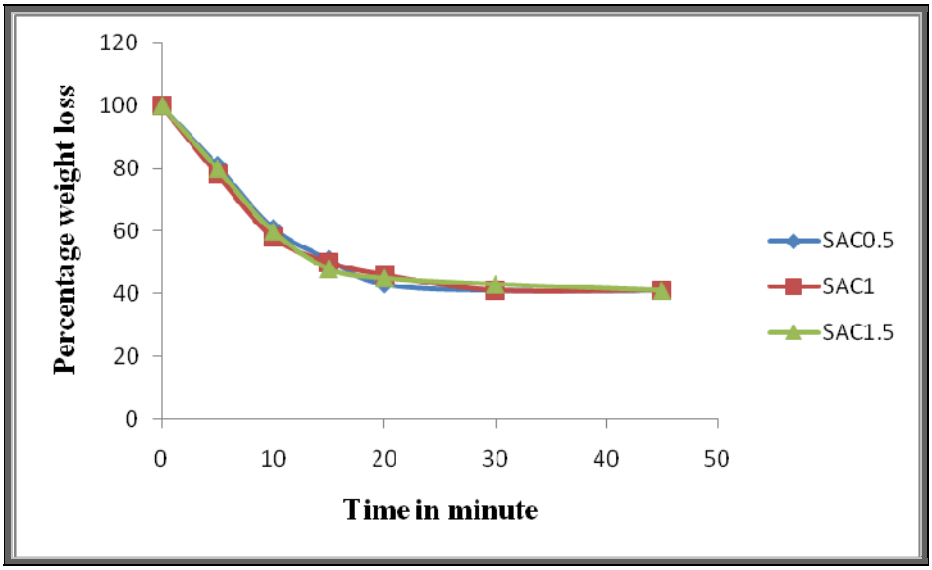


Figure.26

Gelling rate of formulation SAG0.5 to SAG1.5

Time in Minute	Percentage weight loss[a] (%)		
	SAG0.5	SAG1.0	SAG1.5
0	100	100	100
5	68.83±1.27	72.63±1.34	70.73±1.25
10	50.65±0.73	48.63±0.81	45.36±0.74
15	43.75±0.78	42.56±0.74	40.38±0.71
20	40.38±0.72	41.74±0.76	40.74±0.70
30	38.12±0.67	38.18±0.63	38.59±0.66
45	38.41±0.61	38.23±0.60	37.93±0.58

[a] Mean ± S.D.; n=3

Table.19

Comparative Gelling rate profile of SAG0.5 to SAG1.5

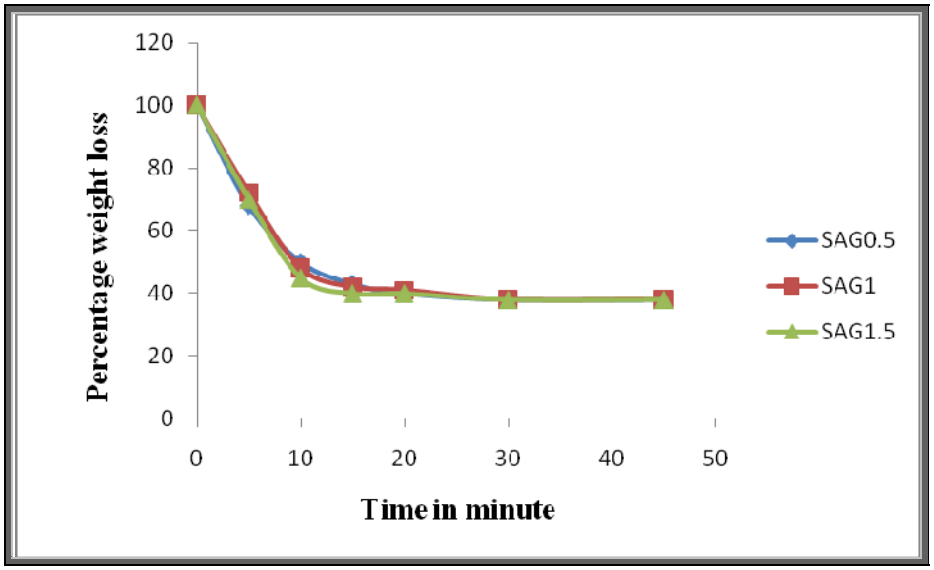


Figure.27

Determination of Entrapment Efficacy

S.No.	Formulation	% Entrapment Efficacy[a]
1.	SA1	63.45±1.23
2.	SA2	68.20±0.94
3.	SA3	72.10±0.87
4.	SA4	67.02±0.34
5.	SAC0.5	76.64±0.93
6.	SAC1	79.73±0.74
7.	SAC1.5	82.42±1.09
8.	SAG0.5	69.92±0.89
9.	SAG1	69.37±0.92
10.	SAG1.5	68.82±0.79

[a] Mean ± S.D.; n=3

Table.20

Comparative Entrapment efficiency for all formulation

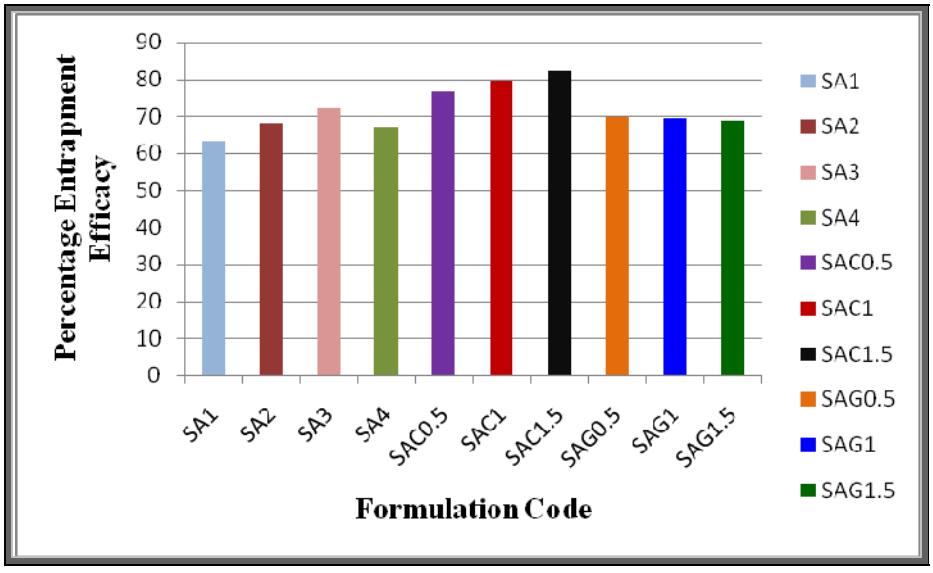


Figure.28

Dissolution Study

Comparative *In-vitro* drug release profile for all formulation

Time in hour	[a] CUMULATIVE PERCENTAGE DRUG RELEASE (%)									
	SA1	SA2	SA3	SA4	SAC0.5	SAC1	SAC1.5	SAG0.5	SAG1	SAG1.5
0	0	0	0	0	0	0	0	0	0	0
0.5	1.98±0.04	1.72±0.03	1.37±0.37	1.63±0.03	1.12±0.12	1.13±0.94	1.19±0.38	1.72±0.63	1.02±0.04	1.72±0.51
1	2.92±0.06	2.56±0.13	2.83±0.74	2.83±0.82	2.10±0.09	2.32±0.62	2.65±1.03	2.82±0.30	2.03±0.82	2.82±0.64
2	4.28±0.02	3.28±0.30	3.20±0.63	3.29±0.63	4.23±0.03	4.12±0.47	4.25±0.94	3.82±.83	5.43±0.25	3.74±0.37
3	27.38± 0.74	29.96±1.03	22.74±0.94	30.83±0.35	11.38±0.34	12.65±0.38	13.93±0.72	10.29±0.93	10.93±0.56	10.29±0.72
4	43.03± 1.23	45.93±1.12	50.84±0.47	52.63±0.26	20.35±0.73	18.85±0.36	19.75±0.93	16.98±0.71	16.63±0.48	14.98±0.74
5	66.85± 0.95	69.06±1.02	61.83±0.64	62.84±0.83	25.63±0.46	23.75±0.27	26.69±0.74	19.02±1.02	21.93±0.82	19.02±0.01
6	80.03± 0.93	82.72±0.94	70.38±0.73	71.82±0.83	28.25±0.72	29.45±0.36	31.74±1.82	22.82±0.35	24.83±0.45	20.84±0.83
7			74.73±	75.83±0.93	34.63±0.93	35.86±0.98	38.63±0.73	25.73±0.62	27.73±0.92	23.93±0.83
8					36.74±0.37	38.97±1.02	42.94±0.92	27.83±0.26	30.37±0.27	24.37±0.82
9					42.28±0.95	44.69±0.27	48.69±0.45	30.92±0.62	32.82±0.34	26.39±0.45
10					44.93±0.28	48.83±0.46	52.83±0.82	31.24±0.09	33.98±0.27	29.03±0.71
11					45.99±0.38	52.63±0.28	56.63±0.48	32.02±0.12	34.37±0.83	30.39±0.93
12					48.48±0.82	54.48±0.37	61.48±0.73	33.01±0.86	35.48±0.35	31.03±0.57

[a] Mean ± S.D.; n=3

Table 21

Comparative *in-vitro* release profile for all formulation

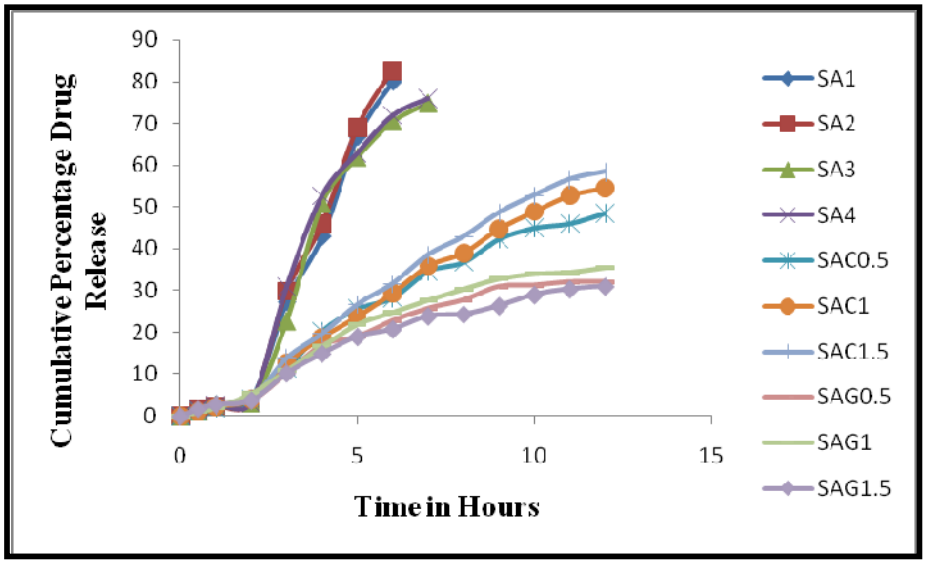


Figure.29

Comparative Swelling studies for SA1 to SA4

Time in minute	Percentage swelling [a]			
	SA1	SA2	SA3	SA4
0	0	0	0	0
10	42.03±0.89	40.37±0.38	38.03±1.31	41.93±0.93
20	80.94±0.85	82.82±0.74	78.02±0.83	80.93±0.94
30	107.03±0.85	106.03±0.38	110.93±0.74	112.92±0.48
45	130.83±0.95	128.94±0.84	136.83±0.93	133.93±0.84
60	140.72±0.38	142.93±0.83	148.82±1.30	148.27±0.84
90	151.92±0.38	152.83±1.02	154.94±0.83	155.88±0.84

[a] Mean ± S.D.; n=3

Table.22

Comparative Swelling studies for SA1 to SA4

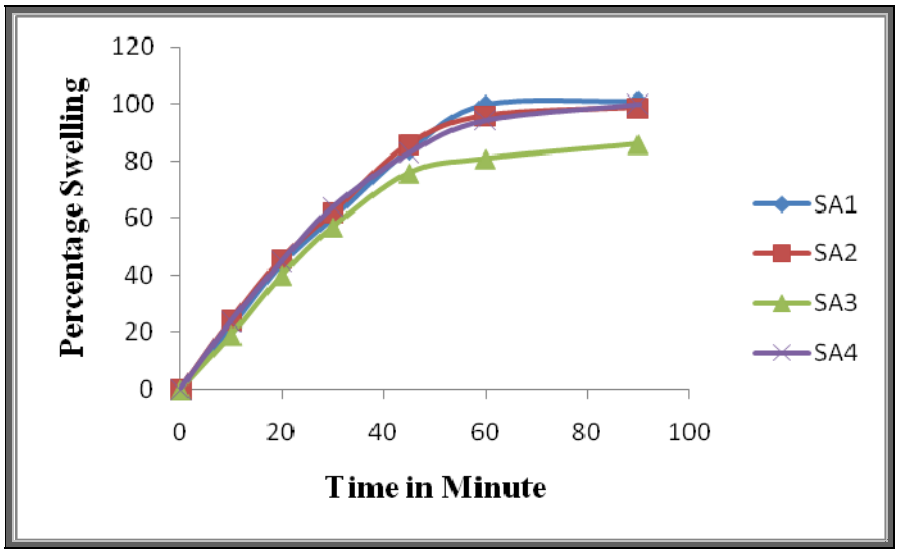


Figure.30

Swelling studies for SAC0.5 to SAC1.5

Time in minute	Percentage swelling [a] (%)		
	SAC0.5	SAC1.0	SAC1.5
0	0	0	0
10	10.94 ±0.36	12.64 ±0.93	**11.84 ±0.83**
20	22.78 ±0.27	21.34 ±0.27	**23.93 ±0.37**
30	34.67 ±0.89	33.12 ±0.84	**34.83 ±1.02**
45	47.36 ±0.77	48.83 ±0.37	**49.63 ±0.83**
60	51.27 ±0.95	56.49 ±0.85	**53.93 ±0.47**
90	53.83 ±1.04	57.78 ±0.94	**55.83 ±0.38**

[a] Mean ± S.D.; n=3

Table.23

Comparative Swelling studies for SAC0.5 to SAC1.5

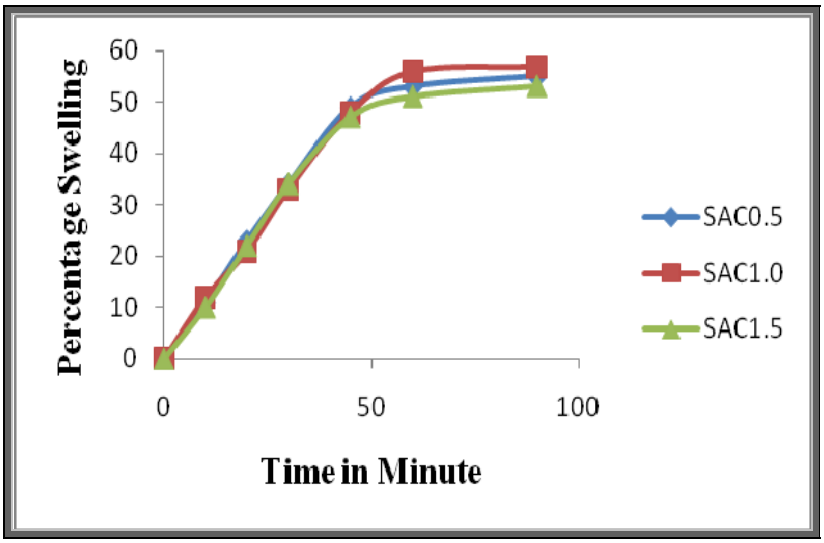


Figure.31

Swelling studies for SAG0.5 to SAG1.5

Time in minute	Percentage swelling [a]		
	SAG0.5	SAG1.0	SAG1.5
0	0	0	0
10	20.26 ± 1.02	18.34 ± 0.74	19.73 ± 0.84
20	30.93 ± 0.98	29.63 ± 0.59	28.74 ± 0.74
30	48.73 ± 0.82	46.27 ± 0.60	45.83 ± 0.78
45	60.68 ± 0.92	62.68 ± 0.58	60.67 ± 0.84
60	65.91 ± 0.53	66.76 ± 0.84	65.83 ± 1.93
90	68.94 ± 0.57	69.35 ± 0.83	69.84 ± 0.56

[a] Mean ± S.D.; n=3

Table.24

Comparative Swelling studies for SAG0.5 to SAG1.5

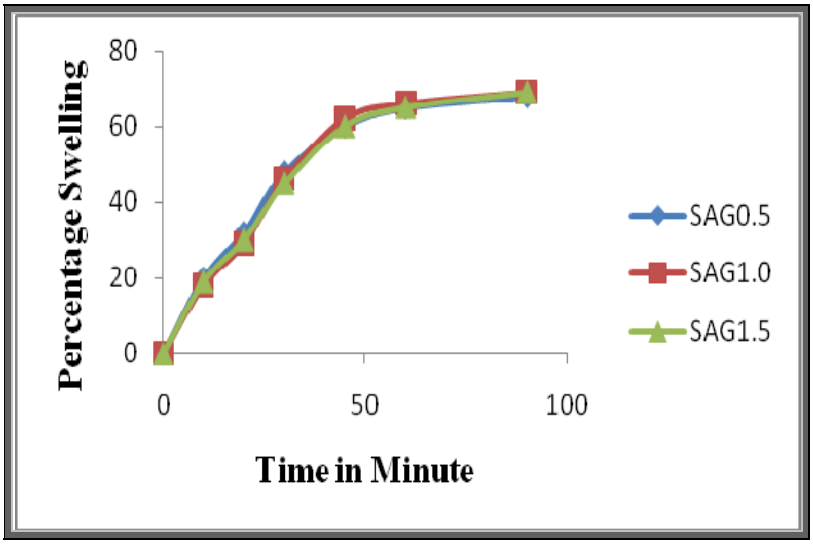


Figure.32

Comparative Swelling studies for all formulation

Time in minute	Percentage Swelling [a]									
	SA1	**SA2**	**SA3**	**SA4**	**SAC0.5**	**SAC1.0**	**SAG1.5**	**SAG0.5**	**SAG1.0**	**SAG1.5**
0	0	0	0	0	0	0	0	0	0	0
10	42.03 ±0.89	40.37± 0.38	38.03±1.31	41.93±0.93	10.94 ±0.36	12.64 ±0.93	11.84 ±0.83	20.26 ± 1.02	18.34 ± 0.74	19.73 ± 0.84
20	80.94 ±0.85	82.82± 0.74	78.02±0.83	80.93±0.94	22.78 ±0.27	21.34 ±0.27	23.93 ±0.37	30.93 ± 0.98	29.63 ± 0.59	28.74 ± 0.74
30	107.03 ±0.85	106.03± 0.38	110.93±0.74	112.92±0.48	34.67 ±0.89	33.12 ±0.84	34.83 ±1.02	48.73 ± 0.82	46.27 ± 0.60	45.83 ± 0.78
45	130.83 ±0.95	128.94± 0.84	136.83±0.93	133.93±0.84	47.36 ±0.77	48.83 ±0.37	49.63 ±0.83	60.68 ± 0.92	62.68 ± 0.58	60.67 ± 0.84
60	140.72 ±0.38	142.93± 0.83	148.82±1.30	148.27±0.84	51.27 ±0.95	56.49 ±0.85	53.93 ±0.47	65.91 ± 0.53	66.76 ± 0.84	65.83 ± 1.93
90	151.92 ±0.38	152.83± 1.02	154.94±0.83	155.88±0.84	53.83 ±1.04	57.78 ±0.94	55.83 ±0.38	68.94 ± 0.57	69.35 ± 0.83	69.84 ± 0.56

Table.25 [a] Mean ± S.D.; n=3

Comparative Percentage Swelling for all Formulation

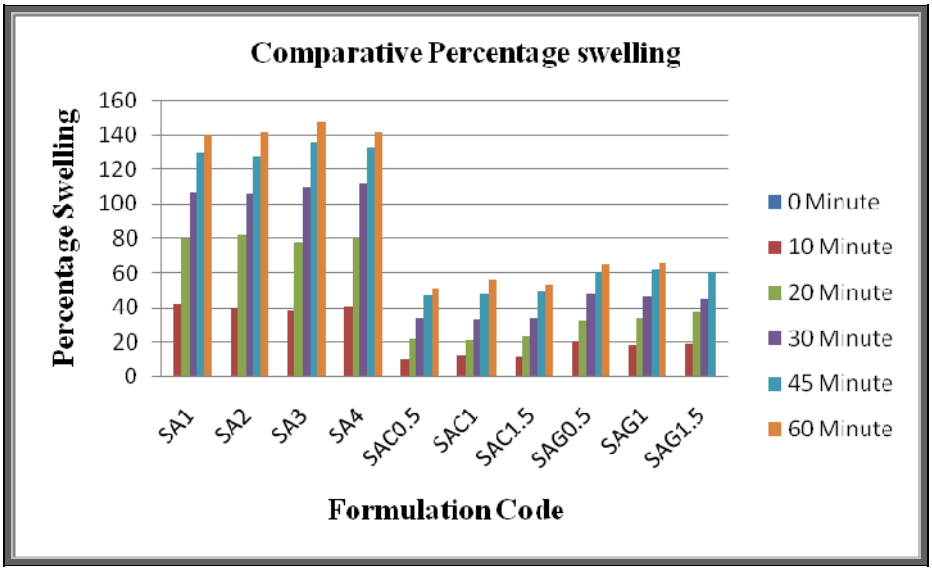


Figure 33

Kinetics of drug release

Results of curve fitting of the *in-vitro* dexibuprofen release data from different hydrogel beads in simulated intestinal fluid.

Formulation Code	Correlation coefficient (r^2)				'n'- Release Exponent
	Zero order [r]	First order [r]	Higuchi [r]	Korsmeyer-Peppas [r]	
SAC0.5	0.929	0.912	0.933	0.836	0.723
SAC1.0	0.956	0.908	0.911	0.838	0.823
SAC1.5	0.994	0.931	0.955	0.895	0.961
SAG0.5	0.974	0.913	0.935	0.919	0.689
SAG1.0	0.905	0.929	0.947	0.728	0.801
SAG1.5	0.975	0.956	0.956	0.886	0.799

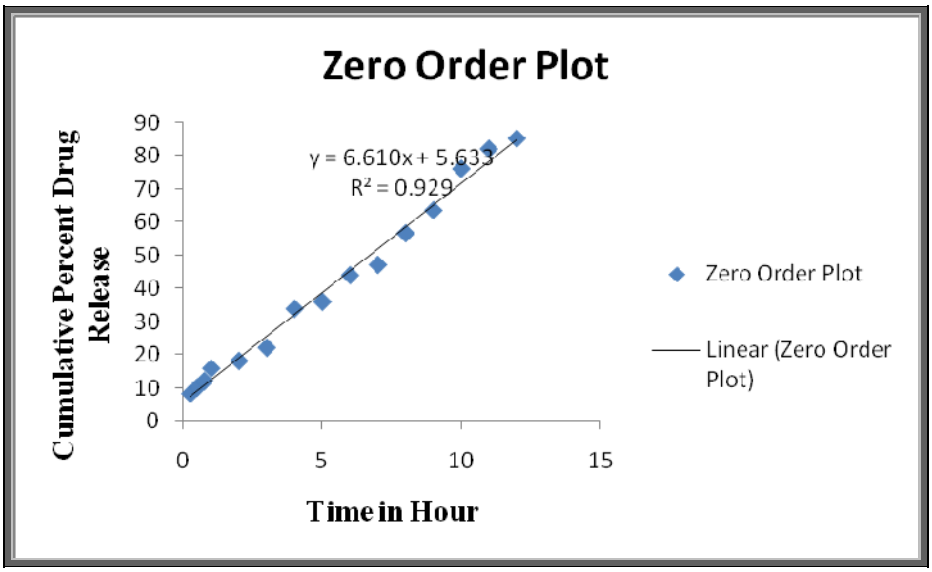
Table.26

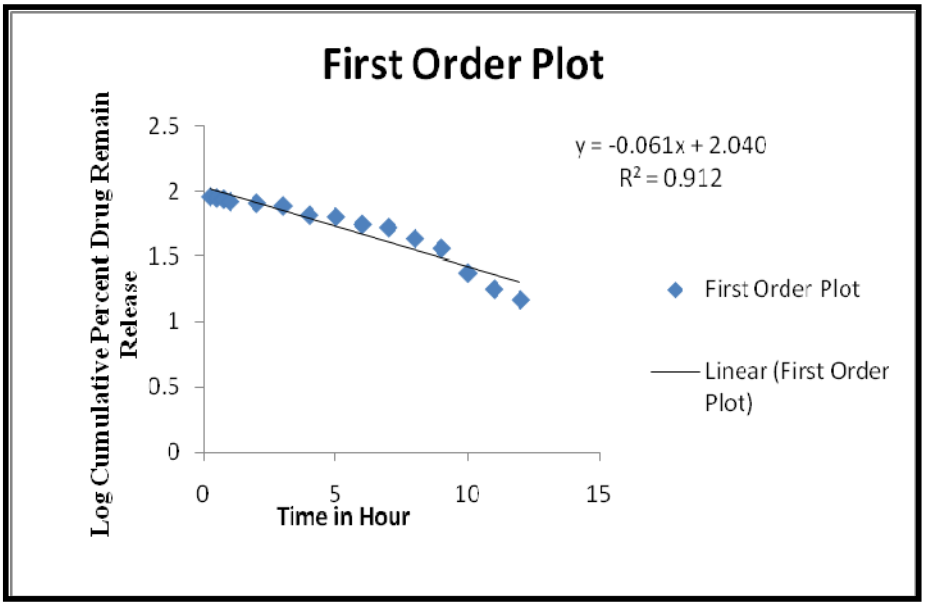
n = Diffusion exponent related to mechanism of drug release, according to equation $Mt/M\infty = K t^n$,

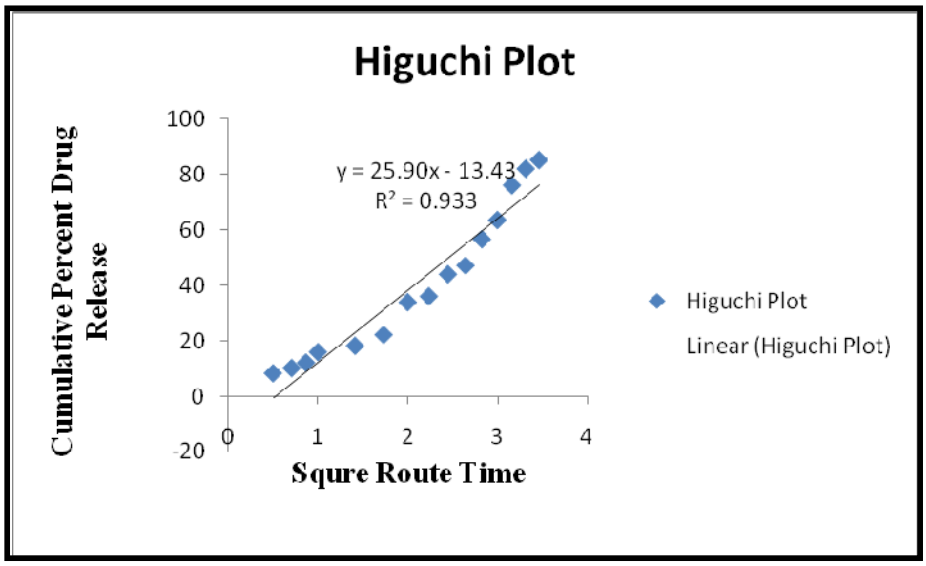
r = Correlation coefficient

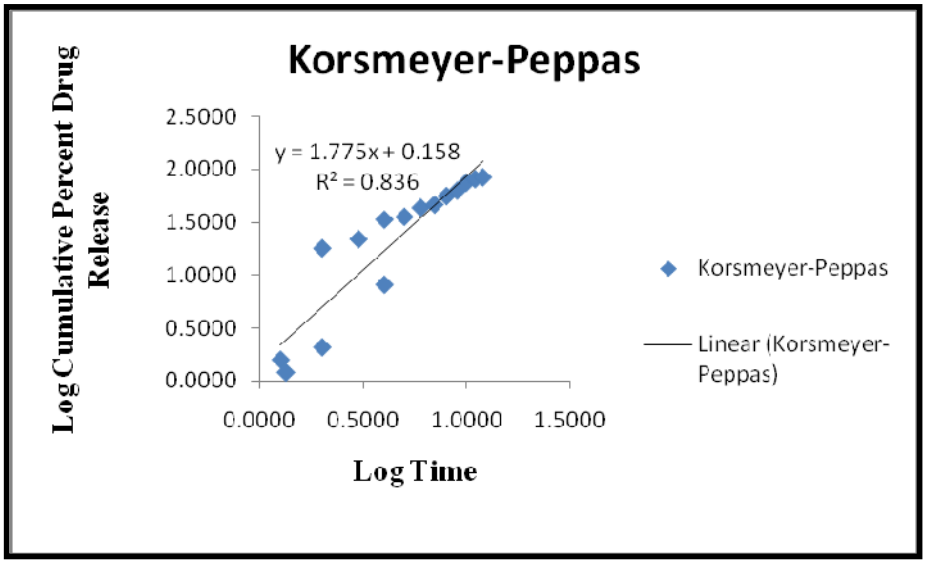
Figure.34

Release Kinetics for Formulation SAC0.5

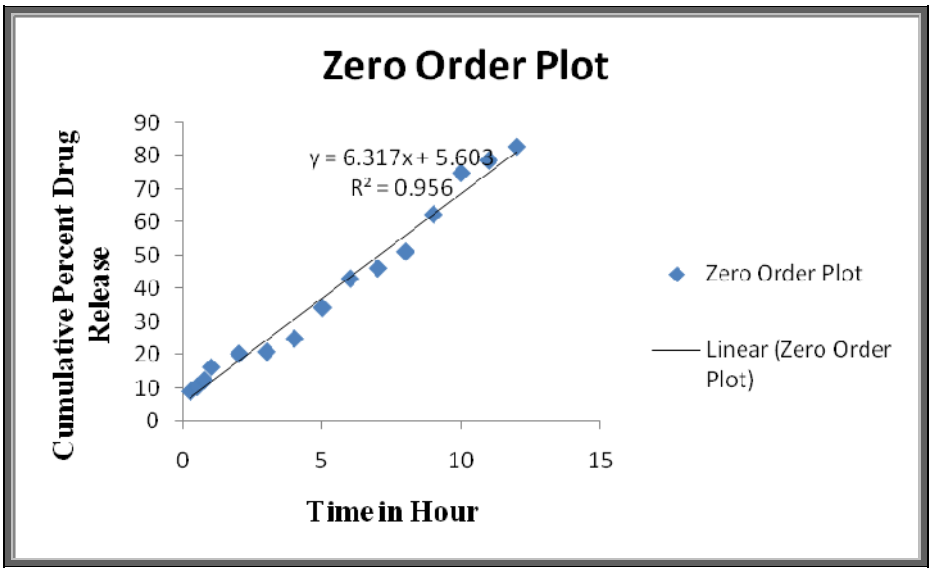


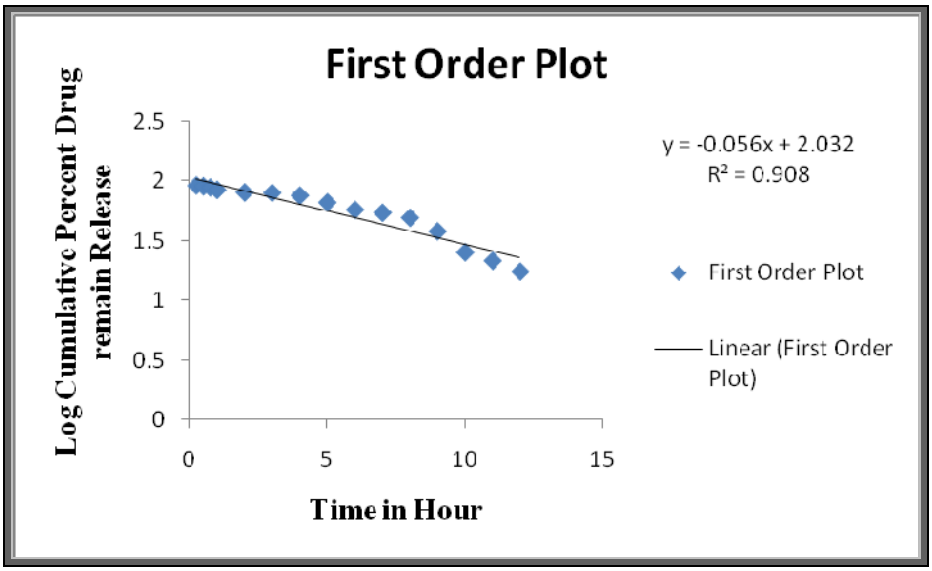


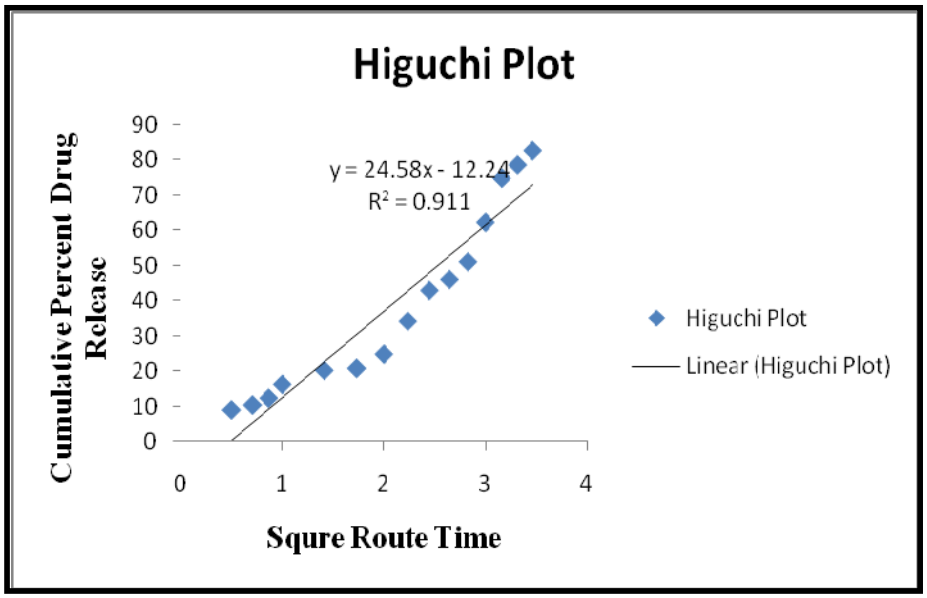

Higuchi Plot
Cumulative Percent Drug Release
y = 25.90x - 13.43
R² = 0.933
100
80
60
40
20
0
-20
0
1
2
3
4
Squre Route Time
Higuchi Plot
Linear (Higuchi Plot)

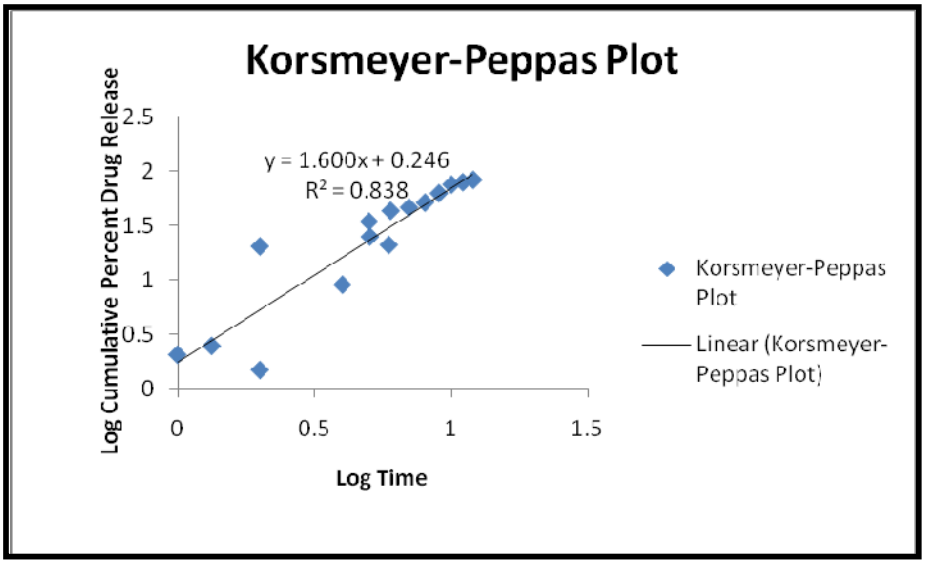

Korsmeyer-Peppas
Log Cumulative Percent Drug Release
y = 1.775x + 0.158
R² = 0.836
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
0.0000
0.5000
1.0000
1.5000
Log Time
Korsmeyer-Peppas
Linear (Korsmeyer-Peppas)

Release Kinetics for Formulation SAC1.0

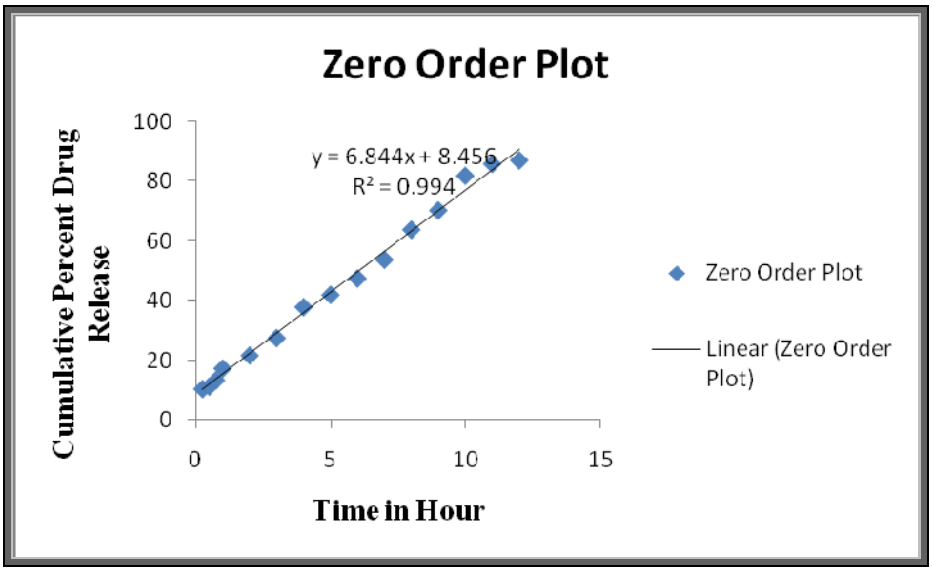


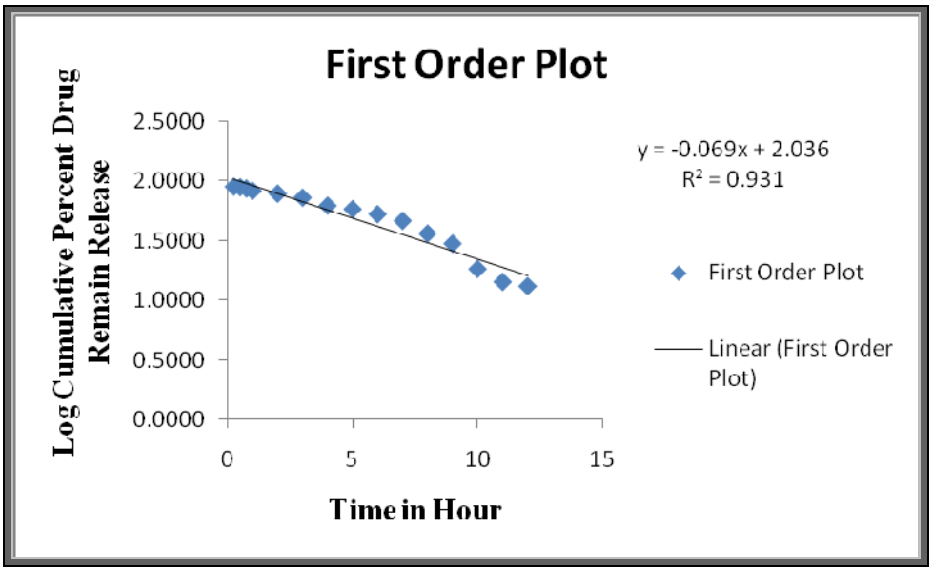


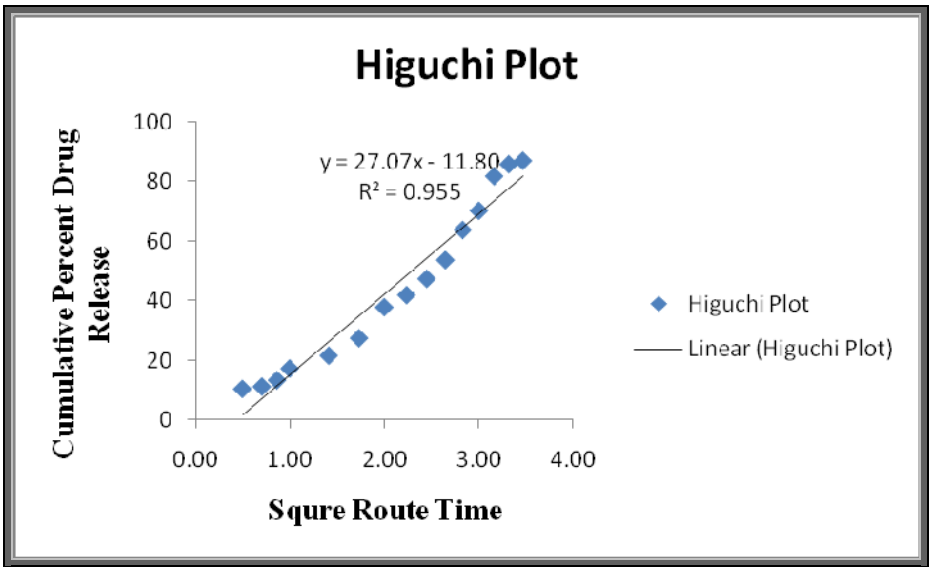

Higuchi Plot
Cumulative Percent Drug Release
y = 24.58x - 12.24
R² = 0.911
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
Squre Route Time
Higuchi Plot
Linear (Higuchi Plot)

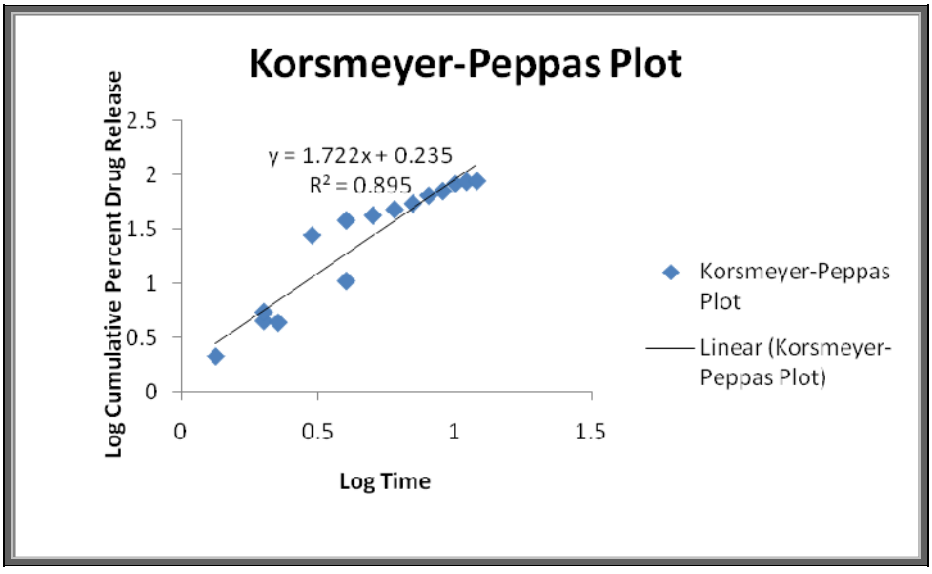

Korsmeyer-Peppas Plot
Log Cumulative Percent Drug Release
y = 1.600x + 0.246
R² = 0.838
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
Log Time
Korsmeyer-Peppas Plot
Linear (Korsmeyer-Peppas Plot)

Release Kinetics for Formulation SAC1.5

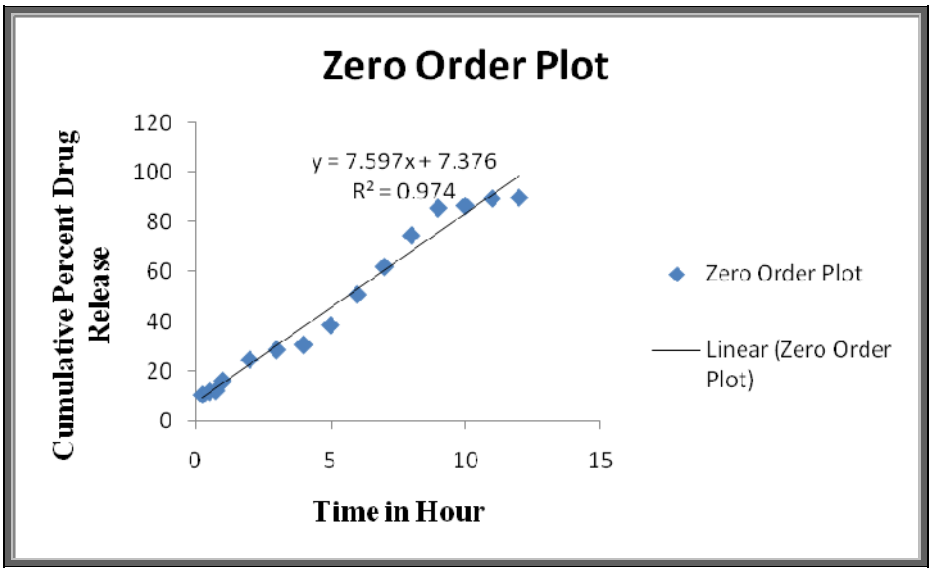


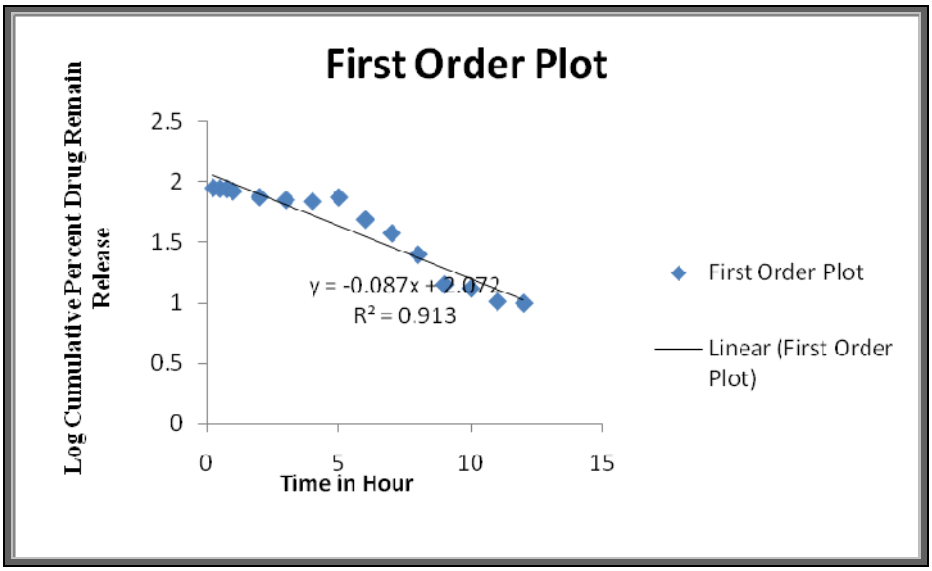


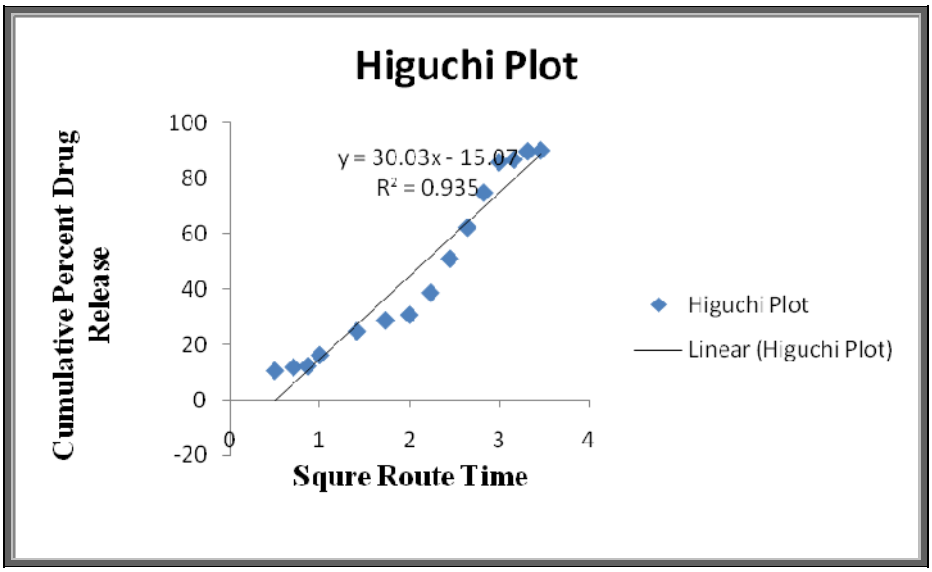

Higuchi Plot
y = 27.07x - 11.80
R² = 0.955
Cumulative Percent Drug Release
100
80
60
40
20
0
0.00
1.00
2.00
3.00
4.00
Squre Route Time
Higuchi Plot
Linear (Higuchi Plot)

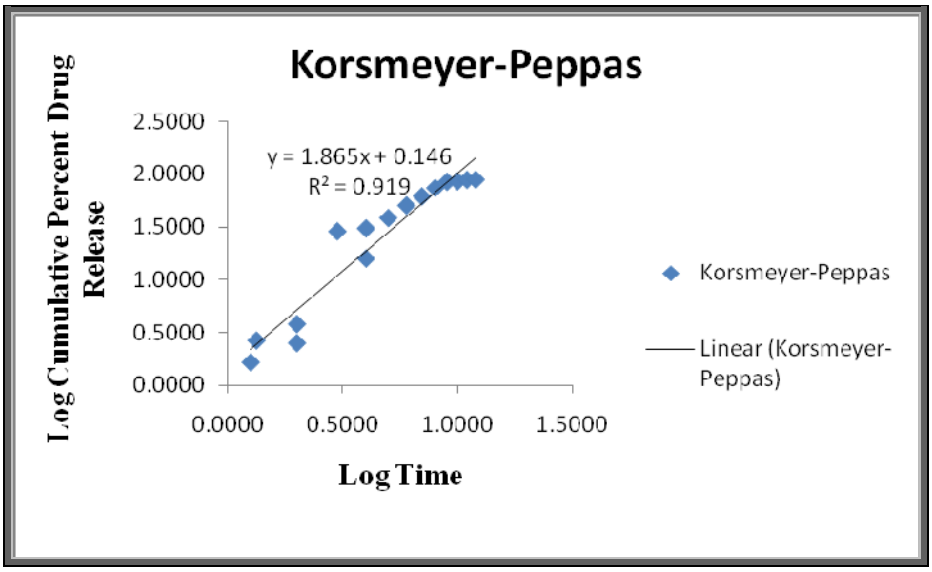

Korsmeyer-Peppas Plot
y = 1.722x + 0.235
R² = 0.895
Log Cumulative Percent Drug Release
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
Log Time
Korsmeyer-Peppas Plot
Linear (Korsmeyer-Peppas Plot)

Release Kinetics for Formulation SAG0.5

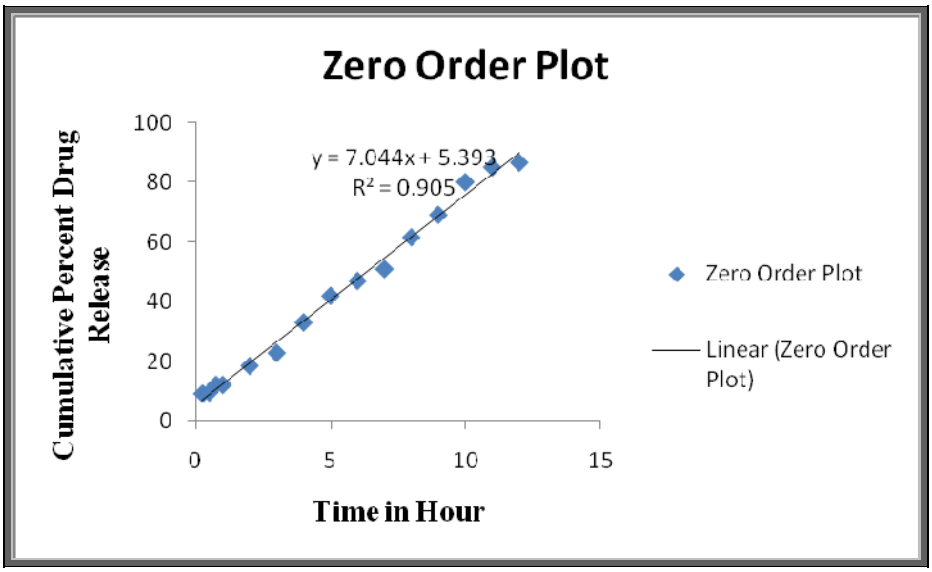


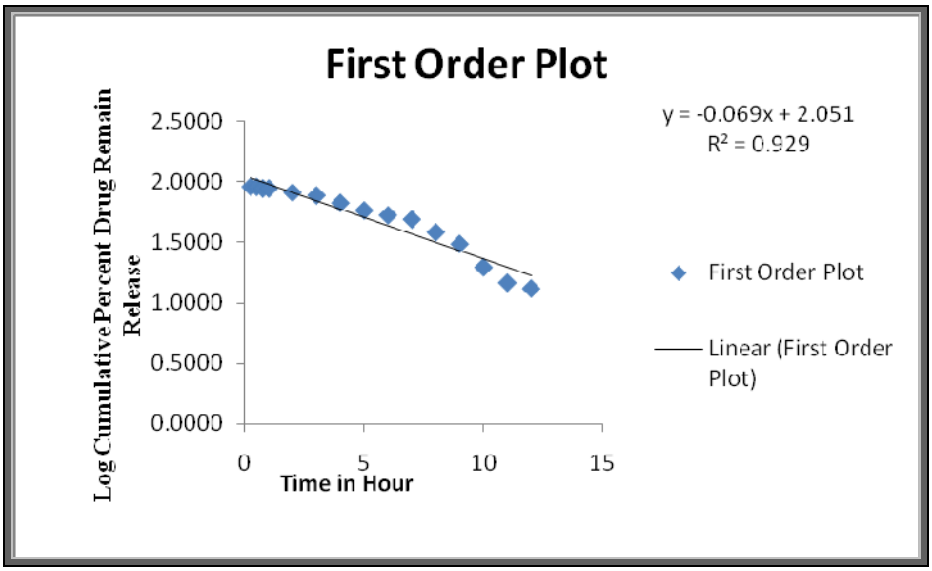


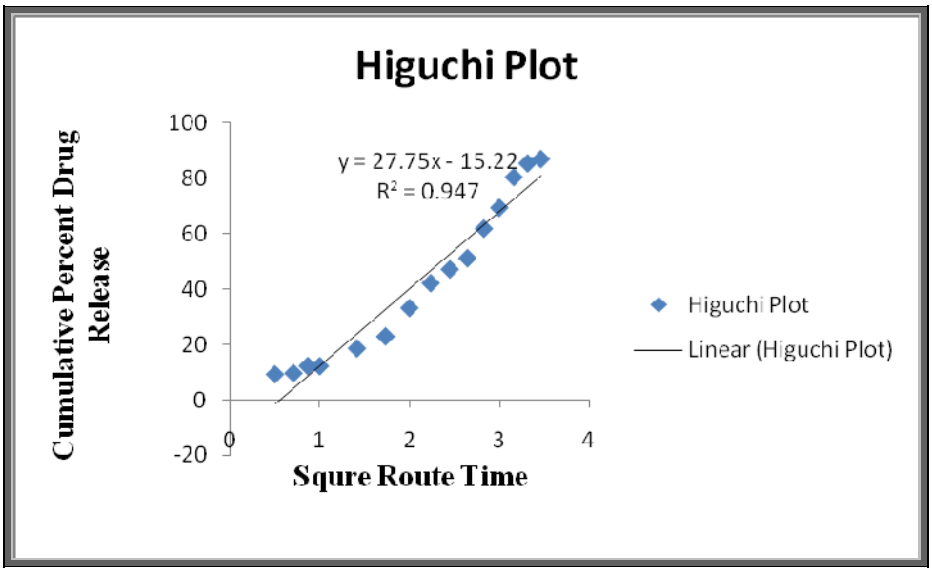

Higuchi Plot
Cumulative Percent Drug Release
y = 30.03x - 15.07
R² = 0.935
100
80
60
40
20
0
-20
0
1
2
3
4
Squre Route Time
Higuchi Plot
Linear (Higuchi Plot)

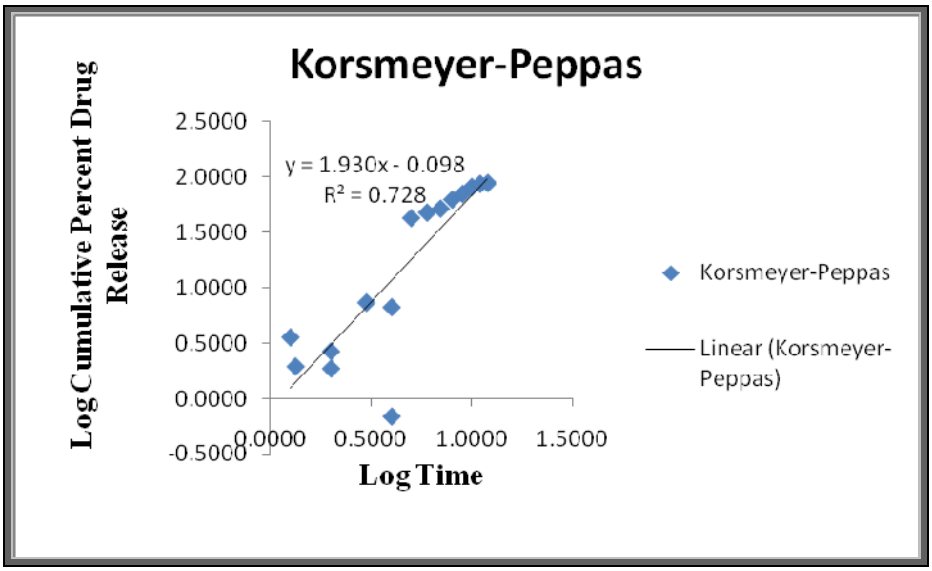

Korsmeyer-Peppas
Log Cumulative Percent Drug Release
y = 1.865x + 0.146
R² = 0.919
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
0.0000
0.5000
1.0000
1.5000
Log Time
Korsmeyer-Peppas
Linear (Korsmeyer-Peppas)

Release Kinetics for Formulation SAG1.0

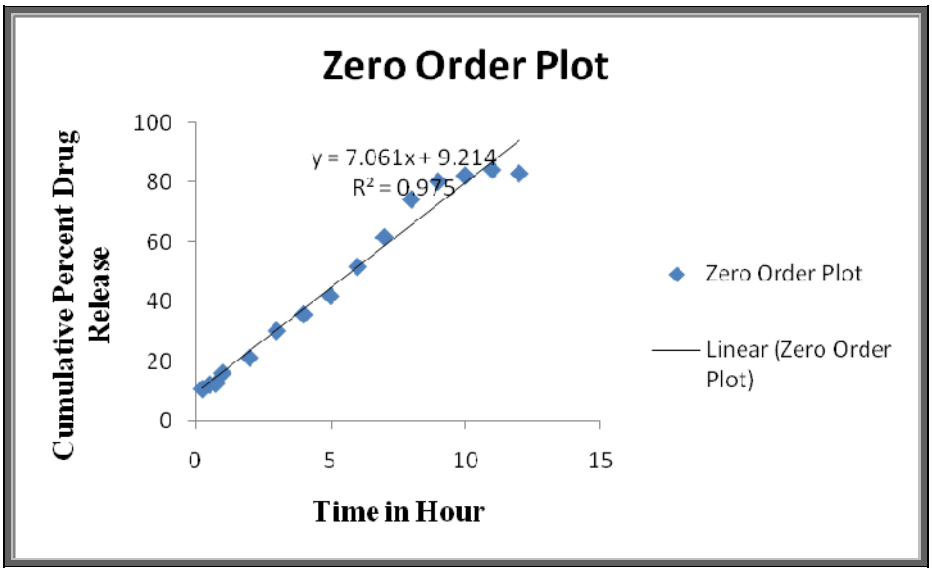


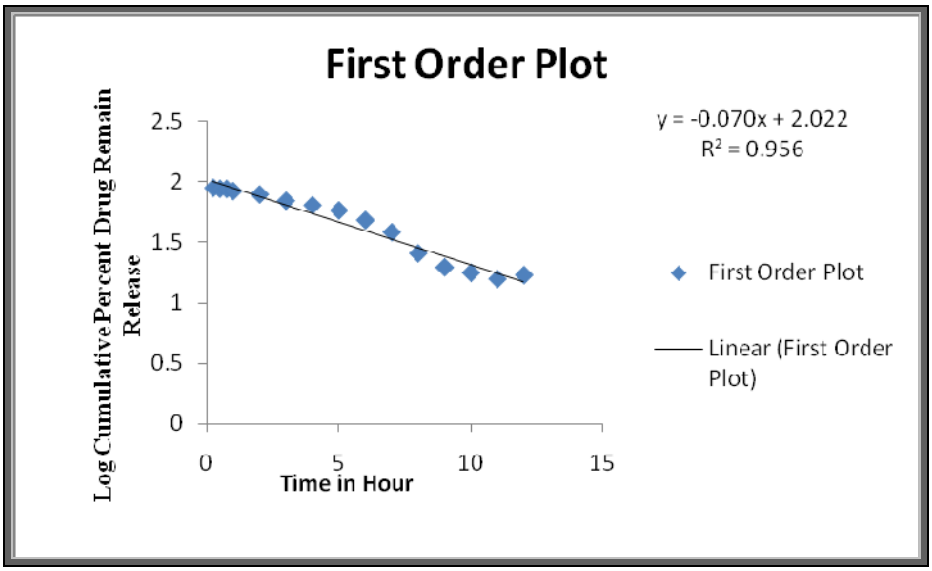


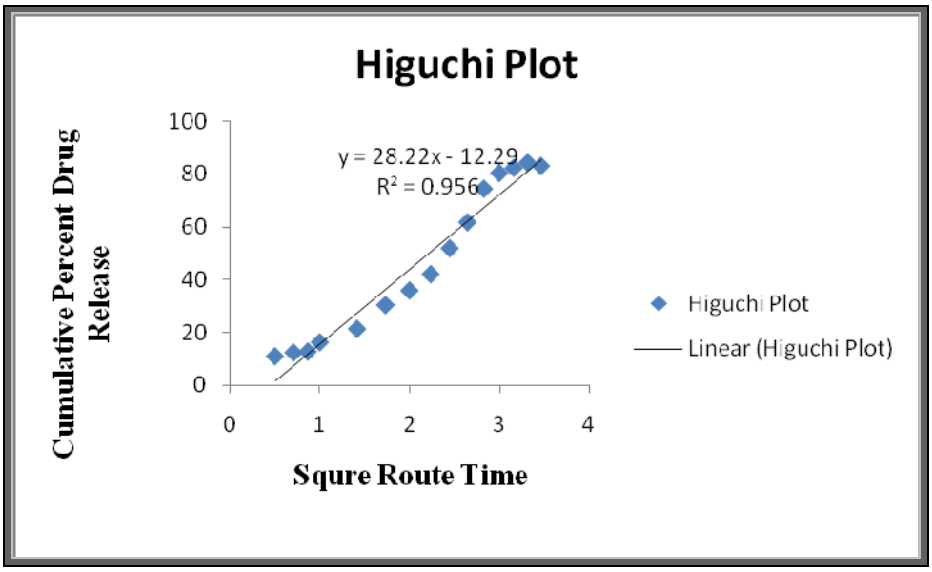

Higuchi Plot
Cumulative Percent Drug Release
y = 27.75x - 15.22
R² = 0.947
100
80
60
40
20
0
-20
0
1
2
3
4
Squre Route Time
Higuchi Plot
Linear (Higuchi Plot)

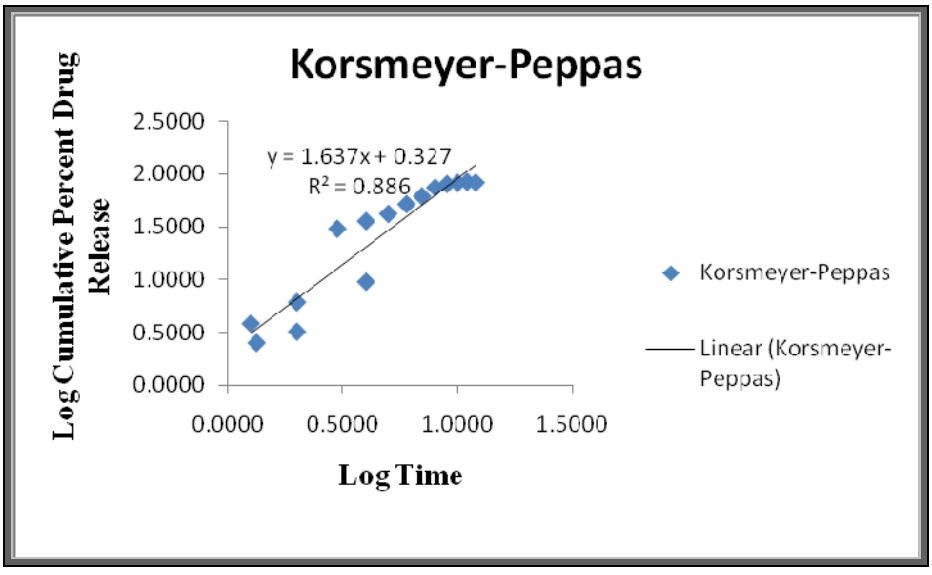

Korsmeyer-Peppas
Log Cumulative Percent Drug Release
y = 1.930x - 0.098
R² = 0.728
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
-0.5000
0.0000
0.5000
1.0000
1.5000
Log Time
Korsmeyer-Peppas
Linear (Korsmeyer-Peppas)

Release Kinetics for Formulation SAG1.5






Higuchi Plot
Cumulative Percent Drug Release
y = 28.22x - 12.29
R² = 0.956
100
80
60
40
20
0
0
1
2
3
4
Squre Route Time
Higuchi Plot
Linear (Higuchi Plot)


Korsmeyer-Peppas
Log Cumulative Percent Drug Release
y = 1.637x + 0.327
R² = 0.886
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
0.0000
0.5000
1.0000
1.5000
Log Time
Korsmeyer-Peppas
Linear (Korsmeyer-Peppas)

Stability Study

S.No.	Formulation	Before storage	Stored at 40°C ± 2°C and 75% ± 5% RH		
			First month	Second month	Third month
1.	SAC1.5	75.42%	73.02%	72.40%	70.10%

Table.27

Discussion

The efforts of many researchers have been concerned to solve problems of NSAID that are mainly at the gastric level are well known, following oral administration, through a variety of techniques of protection of the gastric mucosa or alternatively to prevent the NSAID release in this gastric region.

In the present work, with the potential utility of polymer, such as sodium alginate, chitosan and gelatin were investigated in controlling the release of dexibuprofen in simulated intestinal environment. Since among the multiparticulate systems, hydrogel beads have a special interest as carriers for NSAID, mainly to extend the duration of the dosage form, aimed to investigate possible applicability of sodium alginate hydrogel beads with Gelatin and Chitosan as drug release modifiers to get sustained release system.

Prepared beads containing dexibuprofen by ionotropic gelation method were examined the effects of various factors (concentration of sodium alginate, concentration of polymer ratio [Chitosan and/or gelatin], curing time i.e., Gelling rate and swelling studies).

The beads were prepared by dropping a mixture of colloidal copolymer suspension containing dexibuprofen into the methanol containing formaldehyde dispersion medium, in which formaldehyde acted as a cross-linking agent. The droplets instantaneously form a gel like bead due to cross-linking of formaldehyde with the polymer. Formulations were given in table 4.

The reaction between polymer and formaldehyde to form hydrogel beads were utilized for development of new matrix system containing dexibuprofen. In particular, the N-H group, this would be the most probable to react with an aldehyde. The beads were collected, filtered, washed to remove the impurities and dried at room temperature.

The IR spectra of the drug and polymer were compared with the spectra of the pure drug and individual polymers in which no shifting of peaks was significantly found, indicating the stability of the drug during encapsulation process.

In an attempt to determine the minimum necessary exposure time (curing time), the extent of ionotropic gelation was indirectly assessed through the weight variation corresponding to water loss during matrix formation. Determination of gelling rate allows the evaluation of speed and degree of polymer reaction on the formation of reticulated structure as show in Table 17, 18, 19 and Figure 25, 26, 27. The reaction between the polymer and formaldehyde is fairly rapid being completed at the end of 30 minute. Total amount of water expelled out was same for all formulations studied (approximately 50% of initial weight of wet bead) suggesting that a 30-min curing period is adequate to obtain cross-linked alginate beads.

The mean particle size of drug loaded beads were analysed by Optical microscopy. Drug incorporation leads to increase in particle size, and so dexibuprofen containing beads have a mean size range from 1.59 to 1.94mm. From the SEM analysis for SAC1.5, size was found to be 1.6mm.

The physical parameter like shape, size and surface morphology were analysed by SEM (Scanning Electron Microscopy) for formulation SAC0.5, SAC1.0 and SAC1.5. During particle drying, the beads were spherical in shape, which is confirmed by SEM in figure 24 for SAC1.5. Microscopy analysis shows that beads prepared with formaldehyde shows spherical in shape. The inclusion of polymer in matrix creates beads with smooth surface. In addition, no particle aggregation was found in the formulation, owing to cross-linking reaction.

Encapsulation efficacy was performed for all formulation. Results were given in table 20 and figure 28. Among them, formulation SAC1.5 shows maximum entrapment efficacy [82.42%]. The results indicated that drug content was more in SAC1.5.

All formulations were studied for dissolution test for a period of 12 hours. Comparative studies of drug from all prepared formulation were tabulated in Table 21. From the comparative profile, Beads with sodium alginate alone (SA1 to SA4) does not extent drug release much more when compared to bead with chitosan and gelatin polymer. Beads with chitosan and gelatin polymer extend the drug release over the period of time. Chitosan start dissolve only in acidic medium. But alginate starts at alkaline medium. These two polymer combination extend drug release when compared to gelatin combination. When a cross-linking agent was added to polymer, thus promoting bonding between polymer chains, the release behavior was different, suggesting a change in the matrix structure with new characteristics. Among the 6 formulation (SAC0.5, SAC1.0, SAC1.5, SAG0.5, SAG1.0, SAG1.5), maximum percentage of 61.48% were obtained from the formulation SAC1.5 during 12 hours. Dexibuprofen release was significantly sustained in phosphate buffer pH 7.4 for the formulation of SAC1.5.

Swelling behaviour of beads indicates the speed and easiness of a liquid to penetrate the hydrogel matrix, as a necessary to retard the drug release from the matrix. These studies indicate swelling of beads during dissolution process. As show in Table 22 to 25 and Figure 30 to 33, percentage swelling of bead(around 150%) with sodium alginate alone(SA1 to SA4) increases much more than bead with chitosan and gelatin. Controlled swelling indicates the sustained release of drug from the formulation SAC0.5 to SAC1.5 and SAG0.5 to SAG1.5. Among the formulation SAC0.5, SAC1.0, SAC1.5, SAG0.5, SAG1.0, SAG1.5, beads with chitosan shows controlled swelling.

The in vitro drug release data of beads, obtained by ionotropic gelation technique with chitosan and gelatin containing dexibuprofen, were evaluated kinetically using various mathematical models like Zero order, First order, Higuchi, and Korsmeyer-Peppas model. The results of the curve fitting of these above-mentioned mathematical models are given in Table 26 and Figure 34.

When respective correlation coefficients of these beads in simulated intestinal fluid were compared, the dexibuprofen release from these beads was found to follow Zero order (r^2 = 0.973–0.993) over a period of 12 h. The value of release exponent (n) determined from in vitro drug release data of various dexibuprofen loaded cross-linked hydrogel beads ranged from 0.689 to 0.961 in simulated intestinal fluid, indicating anomalous (non-Fickian) diffusion mechanism for drug release. The anomalous diffusion mechanism of drug release demonstrates both diffusion controlled, and swelling controlled drug release from cross-linked hydrogel beads containing dexibuprofen.

A stability study of the formulation (SAC1.5) was carried out for three month as per standard procedure. During this period the formulation showed without significant change in drug content.

Conclusion:

Based on the literature survey, dexibuprofen was selected for the research work. The results herein presented indicate that a new dexibuprofen-loaded cross-linked hydrogel beads [SAC1.5] were obtained with high encapsulation efficiency and good sustained release over 12 hours presenting promising characteristics as compared to conventional calcium alginate beads from previous studies.

The use of polymer combination successfully delays dexibuprofen release, which can be tailored to produce a suitable single dose administration per day.

Physicochemical characterization demonstrates the formation of a new hydrogel matrix shows that dexibuprofen does not interfere with the new matrix hydrogel formation process.

Therefore, matrix modification by an appropriate method, such as suitable cross-linking reaction, is crucial in the controlled release of dexibuprofen from hydrogel beads.

The formulation SAC1.5 has achieved the objective of sustained cross-linked hydrogel beads.

The ionotropic gelation method for the preparation of these cross-linked hydrogel beads containing dexibuprofen system for oral delivery was found to be simple, reproducible, easily controllable, economical and consistent process.

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