ISOLATION AND CHARACTERIZATION OF STARCH FROM ASSAM SOFT RICE (KUMOL CHAWL) AND PREPARATION, EVALUATION OF CURCUMIN LOADED STARCH NANOPARTICLES

A Thesis is submitted to

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY,
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In the Partial Fulfillment of the Requirement for the
Award of the Degree of

MASTER OF PHARMACY (M.PHARM)
In
PHARMACEUTICS

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Declaration by the candidate

I hereby declare that the Dissertation report "ISOLATION AND CHARACTERIZATION OF STARCH FROM ASSAM SOFT RICE (KUMOL CHAWL) AND PREPARATION, EVALUATION OF CURCUMIN LOADED STARCH NANOPARTICLES" is a bonafide and genuine Research work carried out by me under the guidance of of **Dr. Bhupen Kalita**, Assistant Professor, Department of Pharmaceutics, **Girijananda Chowdhury Institute of Pharmaceutical Science, Hatkhowapara, Azara, Guwahati-17.**. The work is original and has not been submitted in part or in any form to any other University for the award of any Degree or diploma or Fellowship.

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I wish her all success in life.

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Aim:

Isolation and Characterization of Starch from Assam Soft Rice (Kumol Chawl) and Preparation, Evaluation of Curcumin Loaded Starch Nanoparticles.

Objectives:

To meet the above aim the following objectives are undertaken:

1. Isolation of starch from Assam soft rice (Kumol Chawl)
2. Characterization of isolated starch
3. Preparation of Curcumin loaded starch nanoparticles
4. Evaluation of Curcumin loaded nanoparticles

Method:

Starch was isolated from Assam soft Rice or kumol chawl by using the alkali deproteination extraction method and the extracted starches were characterized for physico-chemical properties including particle size, melting point, pH, viscosity, moisture content, swelling index, water absorption index, gelatinization temperatures, and for the phytochemical test. Curcumin was loaded onto starch nanoparticles by using *in situ* nanoprecipitation method and water-in-oil micro emulsion system. Curcumin loaded starch nanoparticles were evaluated for effects of formulation parameters such as types of the reaction medium, types of surfactant, surfactant concentrations, oil/ethanol ratios, loading time on the particle size, and loading efficiency of the curcumin loaded starch nanoparticles and in-vitro release study.

Result:

Extracted starch particles were found to have particle size 514 nm with PDI 1, melting point more than 2200C, pH 7-7.75, viscosity 1.070 cps, moisture content 0.23%, swelling index 150 %, water absorption index more than 200%, and gelatinization temperature 750C. Nanoparticles loaded with curcumin show improved solubility in an aqueous solution compared to free Curcumin. The particle size of prepared nanoparticles was found to be 242nm with PDI0.55 and the maximum percentage of drug loading efficacy was found to be 58.33%. . In vitro release studies showed sustained release of drug till 32 hr. Fourier transfer infrared spectroscopic performed on a 1:1 physical mixture of Curcumin and starch indicated that there were no major interactions between the drug and excipients.

Conclusion:

The Assam soft rice (kumol Chawl) starch was observed to enhanced the solubility of curcumin in aqueous media and Curcumin loaded Starch nanoparticles formed showed retardant release property. Thus, the study concludes that starch from Assam soft rice i. e. Assam kumol chawl can be explored in the future as a potential excipient for sustained release of drug in the drug delivery system.

Key words: Assam soft rice or Kumol Chawl Starch, physicochemical properties, Nanoparticles, in-vitro release.

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List of Abbreviation

Sl No.	Abbreviations	
1.	pH	Hydrogen ion concentration
2.	℃	Degree Celsius
3.	hr/hrs	Hour/Hours
4.	min	Minute
5.	g	Gram
6.	mg	milligram
7.	kg	Kilogram
8.	ml	Milliliter
9.	µl	Micro liter
10.	nm	nanometer
11.	mm	Millimeter
12.	cm2	Centimeter square
13.	cm-1	Per Centimeter
14.	g/kg	Gram per Kilogram
15.	g/ml	Gram per milliliter
16.	g/cm3	Gram per cubic centimeter
17.	µg/ml	Microgram per milliliter
18.	mg/ml	Milligram per milliliter
19.	cps	Centipoises
20.	CO2	Carbon dioxide
21.	UV	Ultraviolet
22.	DNA	Dioxyribo nucleic acid
23.	kv	kilovolt
24.	S. I.	Swelling index
25.	WAI	Water absorption index

CHAPTER 1- INTRODUCTION

1.1. Starch

Starch is a powder or polymer which is produce by plants or animals naturally. It works as an energy store for the both plants and animals. The starch present in the animal is known as glycogen. Actually starch is the main storage polysaccharides deposited in the seeds, tubers, roots, stem pith of plants which occurs as tiny granules of size ranging from 1 to 100 microns and shape of the granules are plant species specific [1]. In the recent years, solid waste environmental pollution has increased tremendously because of the use of synthetic polymers. This is due to the fact that synthetic polymers such as polyethylene (PE) and polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) persist for the long duration after their removal. The environmental friendly polymers are classified into two groups according to the raw materials: degradable synthetic polymers and renewable natural polymers [2]. Some renewable natural polymers are starch, cellulose, soya and chitosan. Starch has been getting much more attention since 1970s [3]. Starch is the most attractive material among these renewable natural polymers; due to its low cost, availability and capability of high production from renewable resources [4-6]. Starch is renewable from carbon dioxide (CO2), water, sunlight by photosynthesis in plants [7].In the field of pharmaceutical starch is becoming more valuable substance because of its many advantages, such as it is versatile, and in expensive polysaccharide which has received great attention in drug delivery applications as they are hydrophilic, biodegradable, and biocompatible with tissue and cells [8]. In pharmacy starches are used as binding agent, diluents, disintegrating agent etc. and with its advantageous nature they are now used in the field of nano drug delivery system. Starches are extracted from different sources. Conventional sources of starch include sources like cereal corps and

legume seeds, tuber crops, and some root tubers [9-12]. Due to unbelievable increasing demand for starch some nonconventional starch resources have been investigated in recent Years. Testing and experiments are going on to characterize the property of the starches extracted from unconventional sources or from new different sources. Normally starch from maize, potato, wheat, rice, sorghum is used in large amount in the field of pharmacy..

1.1.2 Chemical Structure and Properties of Starch

Starch is the reserve carbohydrate in plants tubes and seed endosperm plants [7] and occurs as granules in the cell in plastids, separated from the cytoplasm. The largest source of starch is corn and rice. Starch is a white soft amorphous powder and without sweetness. It is insoluble in water, alcohol and ether and it is non reducing carbohydrate [13].Starch is a mixture of two glucans, amylose and amylopectin. Most starches contain 10-20% water soluble amylose and 80-90 % water insoluble amylopectin depending on the source [14]. Waxy or glutinous starch from contains little or no amylase, while non glutinous or non waxy starch contain amylose in greater abundance than amylopectin [15]. In addition to these glucans, small amounts of proteins and lipids are also present in starch [16].

Amylose is straight chain polysaccharides in which α-D-glucose units are joined 1-4. Chain lengths vary from 250 to 350 glucose units, and the long molecules appear to be coiled in α helix. Amylose is soluble in water (Wallace *et al.,* 1981) but forms hydrated micelles. In such micelles the long chain is twisted into a helical coil. This structure is responsible for the blue color produced by iodine with starch [17]. The structure of amylase contributes to the gelling characteristics of cooked and cooled starches. The molecular structure of amylopectin is presented in Fig. 1.

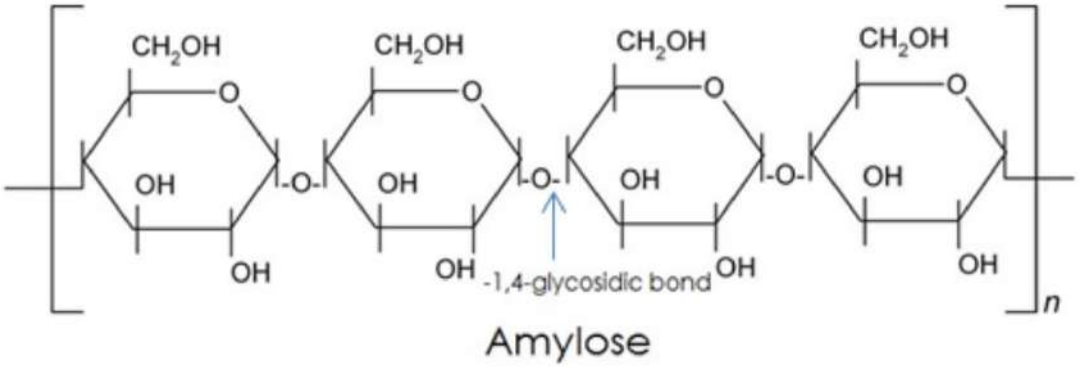

Fig 1: Chemical structure of Amylose [69].

Amylopectin also has a backbone of α- (1→4) linkages but, in addition, the molecule is branched through α- (1→6) linkages to the extent 4-5 percent. The length of the linear unit in amylopectin is about 20-25 percent glucose units. Amylopectin is responsible for the thickened properties of starch preparations but it does not contribute to gel formation [17]. The partial structure of amylopectin is presented in Fig 2.

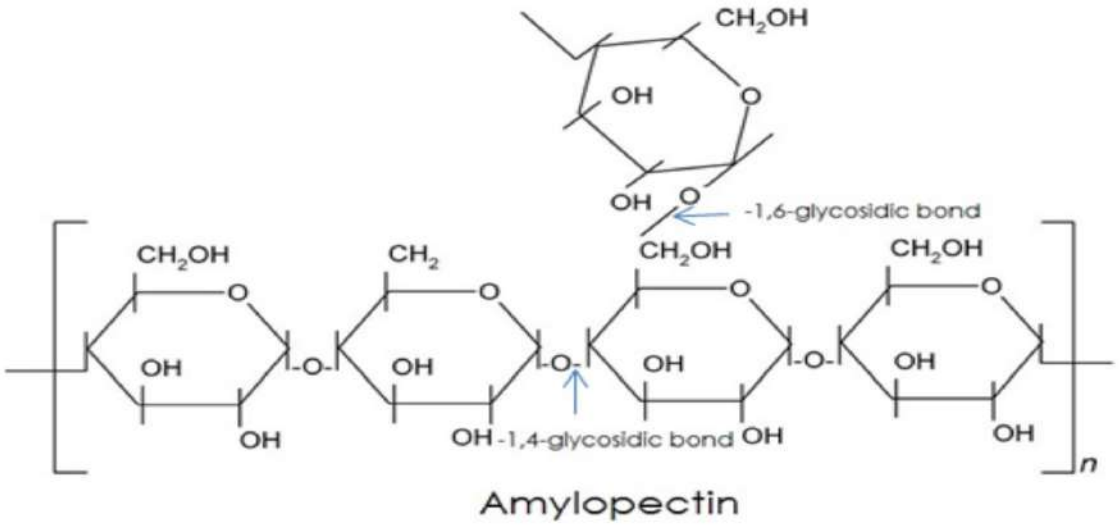

Fig 2: Chemical structure of Amylopectin [69]

1.1.3. Conventional Sources of Starch

Industrially, starch is obtained from cereal grains such as wheat and maize (corn), or from tubers, such as potatoes, tapioca, and arrowroot, or from the pith of the sago plant. By far, the larger part of the starch is obtained from maize, wheat, potato, and tapioca. Starches from the different sources have different properties that affect their functionality (table1) and, therefore, it is in their end-of-use. In India only starches from maize and tapioca are produced in a significant amount [1]. Starch is the polysaccharide which is the second most important renewable resource in terms of availability after cellulose; worldwide production of starch is more than 50 million ton per year [19]. Starch is present in almost all the tissue such as leaves, roots, tubers, seeds, stems, flowers, etc. of green plants however there are some plants which are grown commercially for the starch which includes cereal such as wheat, corn, sorghum, and rice, tuber mainly potato, root like tapioca and arrowroot, stem of sago, and legume crops mainly pea. Worldwide production of starch is depending on the use of cereals as the raw materials. Corn or maize is the main corp which supplies around 80% of the global starch market conquering the title of world largest industry situated in U.S.[18].

Different procedures are used to extract starch from different sources primarily determined by the source of the starch and its intended usage. In addition to the commonly employed wet milling method, the use of several solutions and the enzymatic treatment method is used for protein removal and recovery of starch. A lower degree of starch degradation is associated with the enzymatic treatment method, while, the addition of several alkalis like sodium hydroxide and sodium dodecyl-sulfate in the steeping medium increases the starch

damage. Ultimately, each approach has benefits and drawbacks; therefore, it is critical to select an accurate method with high starch yield and purity.

i. Corn or maize starch

Corn or maize was 1st grown in Central Mexico and they have been cultivated for more than 5000 to 7000 years. Modern varieties of corn are different from the earlier one or the first corn which were produce through mutation, hybridization, and random and conscious selection. Among the corn varieties existing today like popcorn, sweet corn, dent corn, flint corn, and flour, dent corn is mostly used in production of starch because of their availability at law price, their storability and their high starch content (70-73% per weight). In 1884 commercial productions of maize started and a small plant was established in Jersey City, N.J., U.S.A., and in Columbus, Ohio, U.S.A. A larger plant was built in Oswego, N.Y., U.S.A. in 1888 and since then the manufacturing technology of starch from maize was steadily improved representing the dominant raw material for starch manufacture. U.S.A. is the largest producer of maize starch worldwide hence most of the development took place here. There are different kinds of process for the manufacturing of starch from maize but most commonly used and most preferable process is wet milling which has been used since then.

ii. Sorghum starch

Another major cereal corp is sorghum (Sorghum bicolor) which is also known as Milo. It was probably cultivated 5000-7000 years ago in eastern Africa. It is a major cereal crop contains 71.2% of starch and same as corn in general composition but sometimes it is

considered as inferior than corn starch in case of food, feed and industrial uses. In comparison to corn, sorghum can be effectively grown in more arid region and it can be milled in similar process. Sorghum starch has many properties which are same as corn starch and extraction process of sorghum starch is similar with the extraction process of corn starch i.e. wet milling.

iii. Wheat starch

Wheat cultivation was probably started in the Middle East and it has been grown by man for 8000-9000 years. In 1500s Commercial production of wheat starch Started in England .Wheat is mainly harvested by mechanical means, starch is isolated by using several processes such as the Martin process, the Batter process, the Fesca process, the ammonia process, the acid process, wet wheat milling, whole wheat fractionation, the Rasio process, or the Hydroclone process. The first two of these processes are use more often. Wheat has 60%-70% starch on a whole seed basis. In 1835 the martin process was used first and uses wheat flour as its raw material. Starch extracted from the wheat is the co product of vital gluten (the main protein of wheat) present in the wheat. Due to change in the agriculture market of Europe in seventies wheat emerged as significant sources of starch in Europe. Terrified made the import of maize and hard wheat (containing 17% gluten) too expensive so to make European soft wheat (containing 8-9% gluten) and to fortified with vital gluten, the demand for availability of vital gluten was increased which leads to the increased availability of the wheat starch in the Europe and made Europe largest producer of vital gluten as well as wheat starch in the world.

iv. Rice starch

Rice *(Orziya sativa)* has been used in the Asian country since ancient time. It is the main food of Asian countries. There is different variety of rice available so depending upon the variety of rice the quality of rice starch changes according to the character or properties of the rice. Rice is a small grain consisting of an edible grain (caryopsis). The outer fibrous layer of rice grain is covered with starchy endosperm and the germ. The outer layer that is endosperm contains mainly the starch granules around 60-65% and protein bodies. For isolation of starch generally rice powder (after dehulled and blending) are treated with 0.1-0.5% sodium hydroxide for 24-48 hrs to remove protein part. The rice starch slurry after removing protein part are further isolated by centrifugation washing and dried. Generally rice starch is used in a little amount because of their high cost of production in comparison to other commercial starches.

v. Potato starch

Potato starch is extracted from the tubers of *Solanum tuberosum*, which was first cultivated around ad 200 in Peru. And it is mainly produced in Europe especially in the Netherlands and Germany [1, 19]. Potato starch manufacture is regulated by subsidy system and around 25% of starches produced in Europe are produces from potato. About 1.7 million ton of potato starch spread over several Europeans countries which are offered by regulation to the subsidiaries. Potato starch is isolated from cull potatoes, surplus potatoes, and waste streams from potato processing. However, there are special cultivators developed for starch production. The tubers generally contains 65–80% starch. A large amount of potato starch is used to compress commercial soup, and pre-gelatinized starch is useful in instant

pudding. Other use of starch contains pie fillings, sweets, chewing gums, and extrusion cooking etc.

vi. Tapioca starch

Tapioca starch is produced from the large tuberous root of cassava plat (*Manihot utilissima, Manihot esculenta)* grows in many equatorial regions. Cassava roots are may be sweet or bitter depending upon the presence of hydrogen cyanide per kilogram of fresh root. Sweet root vanities are used for food purposes while bitter varieties are used for industrial uses and in both cases hydrogen cyanide level is lowered to acceptable ranges during processing. In some Asian countries a commercial product of tapioca starch is available which is known as 'sago' and this product has no relation with the original sago starch. Sago produced from tapioca contains 24% of starch and this starch contains 0.5-1.00 mm diameter long starch pearls which are made from the extracted tapioca starch cake. Tapioca starch is mainly produced in southern Asia, Brazil, and India and in India 50,000 million ton of the tapioca starch produced is converted in to "sago" pearls [1]. In India most of this starch is produced in Salem district of Tamil Nadu. Manufacturing process of potato and tapioca starch is almost similar though there is difference in the details.

vii. Sago starch

Sago starch is derived from the stem of palms (principally *Metroxylon spp., Arenga spp.,* and *Maurilia spp)* which are eight or more years old. In regard to sago production, currently Malaysia is the third largest sago producer in the world after Indonesia

and Papua New Guinea which combined produce approximately 94.6 % of the world production. Indonesia, the biggest producer of sago starch in the world, produces 585,093 tons per year [24]. One palm trunk can yield 90–180 kg of sago starch; the granules of sago starch are large containing 20-60 mm diameter. Sago starch is used in foodstuffs or in textile sizing and adhesives. They are produced manually in home and commercially production of this starch is almost same as household method though they used machines in some steps.

viii. Arrowroot starch

Arrowroot starch is produced from the square root of a tropical perennial plant *Maranta arundinacea*. The roots are harvested after 6 to 12 months of cultivation, and able to contain more than 20% of the starch, the majority of which has been extracted in the same manner as the tapioca starch. The difference in processing between the two roots is that arrowroot requires more washing than cassava. This starch of the arrowroot is mainly produce in China, Brazil, India and Saint Vincent in the West Indies. It is a thickening agent used to add texture and structure in cooking and baking application.

ix. Pea starch

Pea starch is extracted from the genus *Pisum sativum*. It is produced by all over the world with many other names such as field pea, garden pea, green pea, yellow pea, smooth pea, wrinkled peas, etc. Canada is the world's largest producer by producing 25% of the total pea and it exports 40% of the total number of peas. France produces approximately 17% of the total pea starch production. Pea starch is the cheap source of starch as compared to the

corn, wheat, potato starch as they are by product of protein extraction. Due to its poor functional properties their use in food is limited but they are used effectively in industrial application. Starch extraction from green peas is very difficult due to the presence of a non-soluble flocculent proteins, and fine fibers. Pea starch is isolated with the aid of water-based methods, as well as the dry methods (pin milling and air classification) and most commonly used commercial method of pea starch isolation is air classification.

1.1.4. Non-Conventional Sources of Starch

Starch derived from different sources exhibits different physicochemical properties. And, these physicochemical properties determine the application of starch in a different field. So, there is a growing interest for novel and unique starch sources [25]. New botanical materials are gaining momentum as unconventional sources of starch, raising their commercial importance and, as a result, providing a higher demand for starch with novel and unique characteristics to replace traditional starch sources. With the development of food and other industries, these non-conventional sources of starch have been investigated deeply in recent years. Some of the unconventional starch sources are shown in Table 2. Because of their high level of starch, mango, jackfruit, litchi, longan, and loquat fruits starch has been explored as a source for starch production among the starches acquired from fruits. Guo K. [26] in their study, isolated starch from kernels of mango, jackfruit, litchi, longan, and loquat fruits and investigated the structural and functional properties (Table 2). Availability of 64, 56, 53, 59, and 71 % starch, respectively, in the five fruit kernels, indicates that they are good sources of starch. The starches were spherical, elliptical, and irregularly shaped with varying sizes containing approximately 25% amylose. Mango, jackfruit, and longan starches displayed A-

type crystallinity whereas litchi and loquat starches showed C-type crystallinity. Significant differences were also observed in enthalpy, gelatinization temperature, viscosity, crystalline lamellar intensity, and susceptibility to enzyme hydrolysis [26].

Banana starch has been suggested as a promising source of starch, because of its high content of starch, approximately 69.5% and 22.6% in dry flesh and peel, respectively. The starch had irregular and oval-shaped granules with eccentric hila. Both flesh and peel exhibited B-type crystallinity with similar lamellar structures and relative crystallinity. Banana starch is also renowned for being a good source of indigestible carbohydrates. For native starch, the contents of rapidly digestible starch, slowly digestible starch, and resistant starch of flesh and peel were 1.7 %, 4.3 %, 94.1 %, and 1.4 %, 3.4 %, 95.2 %, respectively, and 73.0 %, 5.1 %, 21.9 %, and 72.3 %, 4.5 %, 23.2 %, respectively for gelatinized starch [28]. Banana starch isolation typically generates 70% starch with 94% purity. However, as starch purity is linked to the development of the fruit, it is more closely linked to the degree of ripeness than to the process of starch extraction [29]. Huang J. [27] isolated starch from the rhizomes of *Curcuma longa (C. longa*), *Canna edulis (C. edulis*), *Canna indica (C. indica)*, and *Lilium lancifolium* (*L. lancifolium*) bulb and compared the structural properties (Table 3). They observed variations in the structural and functional properties of the isolated starch. The isolated starches were different in morphology and size. *C. edulis* starch with the largest granule size had the highest breakdown viscosity, pasting peak, and swelling power. While *C. longa* starch with the smallest granule size possessed very high resistance to hydrolysis and digestion. A high content of amylopectin long branch chain and low content of amylopectin short branch chain was observed in *C. longa*.. The

L. lancifolium starch possesses low resistance to gelatinization, digestion, and hydrolysis. X-ray diffraction pattern revealed the B type crystallinity of all the isolated starches [27]. The non-conventional sources of starch have been less utilized. So, it's critical to make use of these underutilized starch sources for a variety of food and non-food uses. Also, it will be beneficial to improve the economic worth of underutilized starch sources by isolating starch from non- conventional sources. Because starch has a longer shelf life, post-harvest losses are avoided, and these starches can be used to generate a variety of innovative products [30].

1.1.5. Application of Starch

Starch is having the internal properties that are appropriate for pharmaceutical use. It is also used in a wide range of specialty drug delivery applications, such as delivering of complex molecules and targeting specific areas of the body. While there are several official native starch carriers with different identities, new sources are available that will continue to grow with the political, economic, and scientific interest in starch and starch-based products. The size of the starch granules on the functional application is important for starches. Taking for example the rice starch grain is among the smallest in size which make it a desirable candidate to be used in both medicated powders and cosmetic powders for topical use. Due to the small size of starch, it can be used as an absorbent of oil from the skin; hence it is suitable as excipients in dry shampoo and also as a lubricant in some diagnostic and surgical materials. Starch or starch-based as excipients have been revealed to offer several advantages in drug formulation and production in terms of the product safety lower in cost, and quality of product. The starch has been used as binder,

diluents, disintegrant, lubricant and glidant in granules, capsules, and in tablet formulation [32].

i. Diluents

As a diluent, the starch provides mass to solid formulations that contain a small dose of the active ingredient and are added to become a fundamental part of the formulations like granules, tablets, and capsules. When large quantity of diluents are added the properties of the product like granule cohesion, compaction properties, flow, and drug release. They have been utilized for the planning of normalized pulverizes of colorants and strong medications, and to work with blending and taking care of. Local starches are insoluble diluents and have certain attractive characteristic properties like the shortfall of hazardous collaborations with most normal APIs and excipients, the shortfall of physiological and pharmacological exercises just as reliable physicochemical and utilitarian properties. At the point when utilized in high focus starch will upgrade the separation and communication with water without meddling with the inborn solvency of the API in the medium. Then again, its helpless compaction capacity doesn't settle on it a famous decision when direct pressure activity is to be thought of, this is ordinarily a result of its low flexibility, high humidity affectability, and its inclination to adhere to the punches and dies, covering or capping, and low rigidity. Notwithstanding, compaction capacity and the stream can be improved by the wet granulation of the powder blend. In spite of the fact that local starch is modest, it's anything but diluents that will rely upon such factors as the relative focus, plan strategy and the properties of the APIs, and other excipients that will be utilized [32].

ii. Disintegrant

The most popularly used disintegrating agent in the formulation of tablets and capsules of branded and generic medicine is starch. Since starch is a hydrophilic substance which absorb water and leads to swelling and hence break the tablet into smaller fragments for drug release. For the most part, a good disintegrant should be compelling at low fixations to stay away from or decrease its effect on different properties of the tablet such as hardness, friability, and compaction capacity. The properties of the API and different subordinates are likewise compelling in the proficiency of starch as a disintegrant. As a disintegrant, local starch is utilized inside the scope of 3–25% w/w of the granules' or tablets' weight, an ordinary fixation is 15% w/w. During the formulation of granule, ideal disintegrant action is obtained when a part of the starch is used for the mixture of granules as endo-disintegrant while the other half is joined straightforwardly into the dried granules as exo-disintegrant. When the concentration of starch is below the optimum concentration, then there will be difficulties in the entry of water in the granules for it to disintegrate [33].

iii. Binder

Starch is dispersed in water and when heat is applied to it, starch gel is formed which can be used in tablets and capsules as a binding agent to the other excipients holding them together to form granules by using the wet granulation technique. The major contributions of wet granulation using starch as binder is by providing ingredient homogeneity, compaction enhancing, powder density and flow enhancement, dust reduction, drug release

adjusting, and provides required tablets and granules form. Binder plays an important role in the formulation of chewable tablets, as starch contains amylose and amylopectin that increase the cohesiveness and provide a good bond with the other excipients with a concentration of 5-10 % [34]. From all the known approved starches, corn starch is the most commonly used in the formulation technology of granules and tablets but new studies have shown that the novel or modified starches are potential binders and may possibly be used as a replacement for corn starch[32].

iv. Glidant

Starch a hydrophilic glidant can be used in the conventional capsules and tablets formulation with a concentration of 2-10 % w/w to reduce the inter-particulate abrasion and provides a better powder and granules flow. Different types of starch like Maize starch are mainly used as a glidant in capsules and tablets formulation, and starch from yam, cassava, and fonio have shown to be a potential glidants in the formulation of tablets. The flow rate, angle of repose, and flow factor are the properties of starch as a glidant that is evaluated in pharmaceutical powders and granules. Particle size of starch as a glidant is a significant parameter that controls the efficacy of glidant; that is the smaller the glidant particle size, the more efficient is the glidant properties [35].

v. Lubricant

Lubricants are excipients that are added typically in little amounts to powders and granules during tablets and capsules formulation to decrease the interfacial friction between the die walls and the tablet's surface needed in ejection, prevent sticking of the granules and

tablets to the punch, also prevent sticking of powders in the dosator (a type of capsule filling machine) and tamping pins while filling the capsules. The concentration of starch is 2-10 % of the granules and powder weight. When starch is to be used as a lubricant in tablets and capsules formulation, enough trials need to be done to acquire an optimized concentration as starch can cause alteration in the flow and compaction of the mixed powder. Maize starch BP is commonly used as a lubricant, having dual mechanism like increasing the powder flow, prevents sticking of the tablets and granules to the die walls and punches [36].

1.1.6 Novel application

As a novel application starch has been used as a drug carrier for controlled drug delivery. As starch is composed of amylose and amylopectin, it can be used as a coating or film forming polymer which makes it compatible for target delivery and control the release of drug from the system due to its mucoadhesive properties. Studies on maize starch has been evaluated as an effective film coat for tablets which retards or slow down the dissolution rate and confer controlled drug release and the starch coating can also be used in the matrix system and nanoparticles to carry the drug to the specific sites like the lungs, colon, and to target cancer cells [37,38]. To improve the starch in film-forming, it can be combined with other polymers such as chitosan, sodium alginate, and PVP.
The use of starch as a coating polymer has made it to be used in delivering of drugs through microspheres system. The properties for starch as microspheres in the effect of cross-linking are the particle size increase with the increase in the time of cross-linking which will increase the concentration of drug loading, the particles swelling ratio was a

function of the type of cross linker and not of the cross linking time. Drug release from starch microspheres depend on the other cross-linking agent also, because when starch is cross-linked with epichlorhydrine release the drug within 30 min. but when link with gluteraldehyde or formaldehyde the drug release rate gets decreased [39] The cross-linking of starch can also be used in the targeting drug delivery system of protein drug matrix that is used in the targeting of drug to the colon. This is done by double modification of cross-linking the pregelatinized maize starch along with POCl3 which will decrease the enzymatic degradation and can be used as a potential drug carrier to target the colon. The resistant starch shows similar release of drug and it is envisaged to be resistant to the amylase enzyme in the intestinal wall, without the formed nanoparticles fast release of drug is shown when compared to the conventional or native starch. When the branch is reduced, it implies an idyllic precursor for directing ligand conjugation in design of oral colon-specific nano-particulate drug carrier [40]

The starch nanoparticles can be prepared by different methods like cross-linking microemulsion, ultra-sonication, nanoprecipitation, acid hydrolysis, recrystallization, and enzymolysis [40]. Starch-based nanoparticles have been utilized for the transdermal drug delivery of the medications like testosterone, flufenamic acid and caffeine. The skin penetration information for the three medications recommends that starch nanoparticles have the potential for transdermal medication delivery applications. Exemplification and delivery properties of these nanoparticles were considered, showing high epitome effectiveness for these three tried medications (testosterone, flufenamic acid, and caffeine); likewise, a near straight delivery profile was noticed for hydrophobic medications with an invalid starting burst impact [42].

Table 1: Proximate physicochemical and functional property of starch extracted from conventional Sources.

Property	Corn starch	Sorghum starch	Wheat starch	Rice starch	Potato starch	Tapioca starch	Sago starch	Arrowroot starch	Pea starch
Morphology	Polyhedral, A-type crystanility	Oval or semispherical, A type crystanility	Spherical, A type or B type crystanility	Polyhedral C-Type crystanility	Oval, B-type crystanility	Small round, C-type crystanility	Oval, C-type crystanility	Irregular or predominant oval shape C-type crystanility	Oval, C-type crystanility
Starch Content (per weight)	70-73%	71.2 %	60-70%	60-65%	65-80%	15-33%	>80%	>99%	53.61-57.23 %
Amylose Content	24-28%	25.5 %	24-28%	<5% to >24%	20-23%	17-20%	21.4-30.0%	>40%	35-39%
Granule mean diameter in µm	2-32, 13.5 Avg*	5.5-30, 15.5 Avg*	2-45, 20 Avg*	2-20, 5 Avg*	10-100, 46 Avg*	5-35, 13 Avg*	10-15,	29-126, 57 Avg*	5-90, 30 Avg*
Gelatinization Temperature (^{0}c)	62-72	68-78	51-60	68-78	60-65	67-70	69-71	63.94	62
Moisture Content	12%	25%	13%	14%	18%	10-13%	3-14%	12-13%	14%
Swelling Temperature (^{0}c)	64	60	55	72	63	60-90	18-22	60	60
Reference	[56]	[57]	[56]	[56]	[56]	[58, 59]	[60]	[61]	[56]

Avg*- Average

Table 2: Proximate physicochemical and functional property of starch extracted from Mango, Jackfruit, Litchi, L0ngan, loquat, banana.

Property	Mango starch	jackfruit starch	Litchi starch	Longan starch	Loquat starch	Banana starch
Granule Shape	elliptical, spherical or dome-shaped, A-type crystal linity	irregular, truncated, spherical, A-type crystal linity	Round to oval, C-type crys tallinity	oval or irregular, polygonal, A-type crysta llinity	irregular, truncated, spherical, C-type crystal linity	rod shape, irregular oval, B-type crystall inity
Starch Content (per weight)	64%	56%	40.7%	59%	71%	81.71-89.62%
Amylose Content	32.14%	24.1-26.4%	24.1-26.4%	24.1-26.4%	10.53-45.69%	23.10-32.05%
Granule size mean diameter in µm	7.98-36.48, 15 Avg	7-11%, 10 Avg	3-10, 6.5 Avg	<20	29.05-43.66Avg	21-24
Gelatinization Temperature (^{0}c)	77.9-88.8	84.2-92.0	73.3-82.6	71.9-83.5	59.4-75.9	62.08-87.99
Moisture Content	9.95%	6.28-9.02%	0.48-1.62%	72.3-83%	7.36-8.29%	9.53-11.8%
Swelling Temperature (^{0}c)	80-95	85	60-90	70-85	65	85-95
Reference	[62]	[63]	[64]	[65]	[66]	[67,68]

Avg*- Average

Table 3: Proximate physicochemical and functional property of starch extracted from *Curcuma longa* (*C. longa*), *Canna edulis* (*C. edulis*), *Canna indica* (*C. indica*), and *Lilium lancifolium* (*L. lancifolium*).

Property	*C. longa* starch	*C. indica* starch	*C. edulis* starch	*Lilium lancifolium* starch
Starch Morphology	flaky triangular shape, B-type crystallinity	elliptical shape with different sizes, B-type crystallinity	rounded and oval-shaped, B-type crystallinity	Triangular, elliptical or nearly round, B-type crystallinity
Starch Content (per weight)	45.24-48.48%	24%	70-80%	69.07%
Amylose Content	29%	25.0%	25.2%	28.17%
Granule size, mean diameter in µm	18.6	31.8	41.4	24.4
Gelatinization temperature (^{0}c)	12.1-72.9	15.0-60.6	13.9-60.0	8.8-60.2
Moisture Content	15.02%	10.08%	18.17-27%	-
Swelling Temperature (^{0}c)	75-95	65-80	65-85	65-70
Reference	[27]	[27]	[27]	[27]

Avg*- Average

1.2 Kumol rice or Kumol Chawl

Assam is a place with variety of indigenous germplasm occupying 2.58 million hectare area. Among the different classes of rice available, glutinous / waxy rice is an important class, in the sense of being glue-like or sticky. The waxy rice of Assam has been classified in two groups viz., Bora (glutinous) and Chokuwa (Semi-glutinous) based on amylose content [45]. Depending upon the presence of amylose and amylopectin they are differentiate into glutinous and semi glutinous rice. Normally rice with high amylose contain are more glutinous than the rice with more amylopectin and low amylose content. These two classes of rice varieties are very closely associated with the Assamese culture and are invariably grown by the farmers even though these are known for their relatively low yield potential. These groups of rice are considered to have tremendous commercial value. Chokuwa rice has special significance in its use in various delicious preparations in Assam. It is used in daily breakfast and also in social as well as religious ceremonies. Most of the chokuwa rice varieties do not become sticky on cooking but become soft in consistency. Hence, they are also locally known as 'Kumol Chawl' or soft rice [45]. "Soft rice (kumol chawl)" are called as "Magical Rice" of Assam as they are prepared by soaking the rice either in cold or hot water for a brief period of time and then consumed with sugar or molasses, milk or curd and even with salts and oils and pickles without cooking due to which they are also called as "Zero Fuel Rice". Thus this class of rice is metaphorically termed as "magical rice" as it becomes ready to use just by soaking with no fuel requirement and it has a great demand in the domestic as well as foreign market. The multiplicities of uses make the glutinous rice very popular among farmers. In spite of the advent of modern high yielding rice varieties, this soft rice being mostly landraces are

highly valuable and possess traits that are most preferred by farmers. Thus, it assumes as a very high commercial importance as this can be utilized for preparation of instant rice, which could be supplied to the soldiers camping at remote and inaccessible places, factory workers, tea garden laborers etc. This is also popular for its peach rice, puffed rice (Akhoi), beaten rice (Chira) and several palatable dishes [45]. All this varieties of rice has different amount chemical compositions leading to different physicochemical, functional characteristic. The mean value of the chokuwa group for crude fibre content was significantly higher, while the crude protein was lower than both bora and non-glutinous rice .Amylose content of the chokuwa rice was much higher compared to bora varieties, while it was lower than non-glutinous rice. On the other hand amylopectin content of the chokuwa rice was lower than the bora rice but higher than non-glutinous rice.

Table-4: Mean Value Of Biochemical Character Of Chokuwa Rice (Kumol Chaol).

Biochemical Characters (%)	Mean Value Of Biochemical Character Of Chokuwa Rice (Kumol Chaol)	Reference
Crude protein	**8.71-10**	**[44,45, 70]**
Crude fat	**1.5-2.60**	**[45,70]**
Crude fibre	**0.3-1.73**	**[44,45,70]**
Starch	**79.52**	**[70]**
Amylose	**16.86**	**[70]**

Amylopectin	**83.14**	**[70]**
Ash	**0.6-1.03**	**[44,45, 70]**
Phosphorus	**0.29**	**[70]**
Calcium	**0.036**	**[70]**
Iron	**2.67**	**[70]**

1.3 Nanoparticles

Nanoparticles are to be defined as a particulate dispersion or solid particles with a size range of 10-1000 nm. Nanoparticles can be divided into main two groups: nanospheres and nanocapsules. Nanospheres are considered as matrix particles whose entire mass are solid whereas Nanospheres and nanocapsules are generally spherical but non-spherical shape can be encountered [46]. Polymeric nanoparticles plays a vital role in drug delivery as they generally increase the stability of any volatile pharmaceutical agents and that they are easily and cheaply fabricated in large quantities by a multitude of methods [47] for example nanoparticles are being developed to assist the transportation of chemotherapy drugs directly to cancerous growths, as well as to deliver drugs to areas of arteries that are damaged in order to fight cardiovascular disease. From among the polymers, biodegradable polymers like starch has received great attention in drug delivery applications as they are hydrophilic, biodegradable, versatile, inexpensive and biocompatible with tissue and cells. The major aim in designing nanoparticles as a delivery system are to achieve control particle size, surface properties and to release pharmacologically active agents to achieve

the site-specific action of the drug at the therapeutically optimal rate and dose regimen [48].

1.3.1 Nano carriers

Nanocarriers are colloidal drug carrier systems having submicron particle size typically <500 nm. Nanocarriers have been extensively investigated in the past few decades as they showed great promise in the area of drug delivery. Nanocarriers, owing to their high surface area to volume ratio, have the ability to alter basic properties and bioactivity of drugs. Improved pharmacokinetics and biodistribution, decreased toxicities, improved solubility and stability, controlled release and site-specific delivery of therapeutic agents are some of the features that nanocarriers can incorporate in drug delivery systems [49]. Moreover, the physiochemical properties of nanocarriers can be tuned by altering their compositions (organic, inorganic or hybrid), sizes (small or large), shapes (sphere, rod or cube) and surface properties (surface charge, functional groups, PEGylation or other coating, attachment of targeting moieties)[50]. The overall goal of utilizing nanocarriers in drug delivery is to treat a disease effectively with minimum side effects. Chemotherapeutics when delivered via conventional drug delivery systems presents a number of unique problems, including poor specificity, high toxicity and induction of drug resistance [51]. These obstacles decrease the therapeutic value of many anticancer drugs. Nanocarrier-based platforms have enabled effective delivery of anticancer drugs into the tumors by exploiting the pathophysiology of tumor microenvironment, thereby significantly improving the therapeutic outcomes for oncological conditions [52]. Furthermore, the over expressed receptors on tumor cells surface have also been targeted with nanocarriers platforms decorated with targeting ligands. A number of nanocarrier-

based products have been approved for the treatment of various tumors, and many others are in different phases of clinical trials.

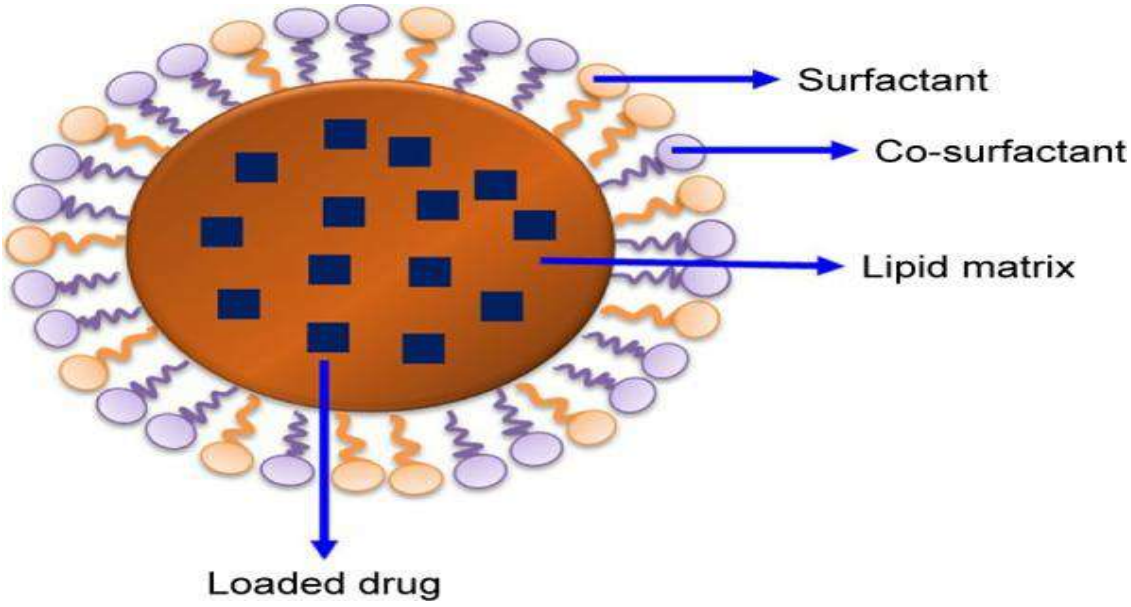

Fig.3: structure of drug loaded nano carrier or nanoparticles. [53]

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CHAPTER 2- DRUG PROFILE

2.1. Drug Profile: Curcumin

Curcumin is a natural polyphenol found in the rhizomes of turmeric (*Curcuma longa*) and exhibited antioxidant, anti-inflammatory, bantiproliferative, anti-invasive, and antiangiogenic activities [1, 2]. More recently, curcumin has shown to have antitumor effects in many cancer cell lines [3, 4] and the clinical effects of curcumin are being studied in human clinical trials and animal models on various conditions and numerous myelomas [8-10]. Curcumin, a polyphenolic natural product, exhibits therapeutic activity against a number of diseases, attributed mainly to its chemical structure and unique physical, chemical, and biological properties. It is a diferuloyl methane molecule (1, 7-bis (4-hydroxy-3- methoxyphenyl)-1, 6-heptadiene-3, 5-Dione)] containing two ferulic acid residues joined by a methylene bridge. It has three important functionalities: an aromatic o-methoxy phenolic group, alpha, beta-unsaturated beta-diketo moiety and a seven carbon linker. Extensive research in the last two decades has provided evidence for the role of these different functional groups in its crucial biological activities. A few highlights of chemical structural features associated with the biological activity of Curcumin are: The o-methoxyphenol group and methylenic hydrogen are responsible for the antioxidant activity of Curcumin, and Curcumin donates an electron/ hydrogen atom to reactive oxygen species. Curcumin interacts with a number of biomolecules through non-covalent and covalent binding. The hydrogen bonding and hydrophobicity of Curcumin, arising from the aromatic and tautomeric structures along with the flexibility of the linker group are responsible for the non-covalent interactions. The alpha, beta-unsaturated beta-diketone moiety covalently interacts with protein thiols, through Michael reaction. The beta-diketo group forms chelates with transition metals, thereby reducing the metal

induced toxicity and some of the metal complexes exhibit improved antioxidant activity as enzyme mimics. New analogues with improved activity are being developed with modifications on specific functional groups of Curcumin [5].

2.1.1 Chemical Structure

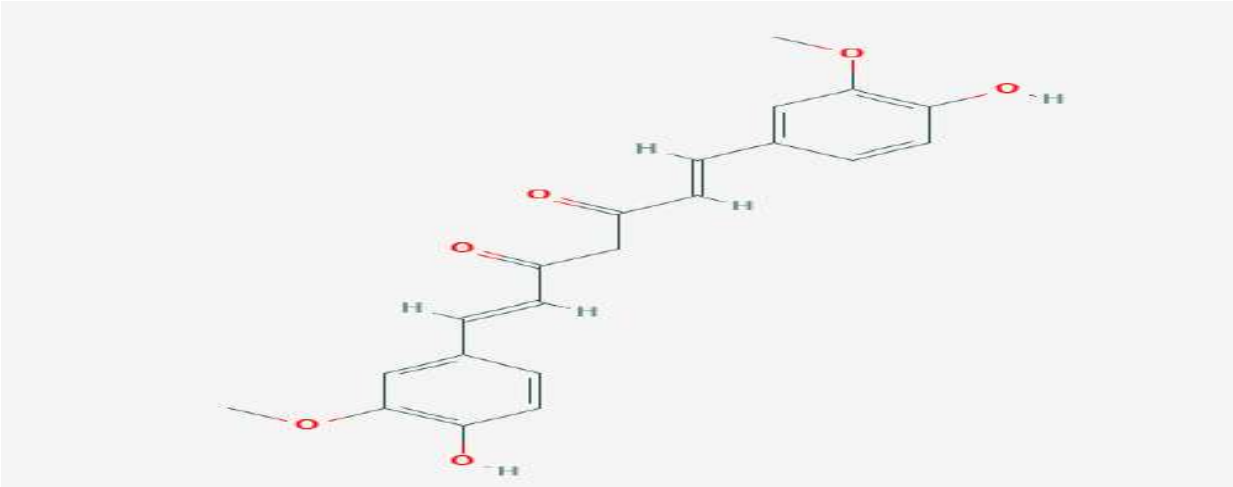

Fig.4 Chemical structure of Curcumin [6]

2.1.2 IUPAC Name (National Center for Biotechnology Information)

- (*1E*, *6E*)-1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5-Dione

2.1.3 Molecular Formula (National Center for Biotechnology Information)

- $C_{21}H_{20}O_6$

2.1.4 Synonyms (National Center for Biotechnology Information 2021)

- Curcumin
- Diferuloylmethane
- Turmeric Yellow
- Yellow, Turmeric

2.1.5 Molecular weight (National Center for Biotechnology Information 2021)

- 368.4g/mol

2.1.6 Physical Description

- Solid Curcumin appears as orange-yellow needles or Orange-yellow crystalline powder

2.1.7 Color/Form/odor

- Orange-yellow crystal powder or Orange-yellow needles; gives brownish-red color with alkali; light-yellow color with acids.
- Spicy, fresh odor reminiscent of sweet orange & ginger; slightly pungent, bitter flavor. Slightly fluorescent.
- Specific gravity: 0.9348 at 15 °c

2.1.8 Melting point (National Center for Biotechnology Information 2021)

- 179 °C-183 °C

2.1.9 Solubility (National Center for Biotechnology Information 2021)

- Slightly soluble in hot water
- Insoluble in cold water
- Insoluble in water
- Insoluble in ether; soluble in alcohol, glacial acetic acid
- Very soluble in ethanol, acetic acid

2.1.10 Density (National Center for Biotechnology Information 2021)

- 0.9348 at 59 °F

2.1.11 Vapor Pressure (National Center for Biotechnology Information 2021)

- 3.08X10-12 mm Hg at 25 °C (est)

2.1.12 Henrys Law Constant (National Center for Biotechnology Information 2021)

- Henry's Law constant = 7.04X10-22 atm-cu m/mol at 25 °C (est)

2.1.13 Stability/Shelf Life (National Center for Biotechnology Information 2021)

- Stable under recommended storage conditions. Orange-yellow turmeric will hold shade better than lemon-yellow when exposed directly or indirectly to sun light.

2.1.14 Decomposition (National Center for Biotechnology Information 2021)

- Hazardous decomposition products formed under fire conditions. Carbon oxides.

2.2 Drug and Medication Information

2.2.1 Drug Indication

- No approved therapeutic indications[7]
- Turmeric is a popular herb derived from the roots of the plant *Curcuma longa* found mostly in India and Southern Asia. Turmeric has an intense yellow color and distinct taste and is used as a dye as well as a spice in the preparation of curry. Turmeric and its purified extract curcumin are also used medically for their purported anti-inflammatory and antioxidant effects to treat digestive complaints including ingestion, diarrhea and liver diseases. Turmeric and curcumin have been associated with a low rate of transient serum enzyme elevations during therapy and while having a long history of safety, turmeric products have recently been implicated in over a dozen instances of clinically apparent acute liver injury. [7]

2.2.2 Drug Classes (National Center for Biotechnology Information 2021)

- Herbal and Dietary Supplement

2.3 Pharmacology

- Curcumin is a phytopolylphenol pigment isolated from the plant Curcuma longa, commonly known as turmeric, with a variety of pharmacologic properties. Curcumin blocks the formation of reactive-oxygen species, possesses anti-inflammatory properties as a result of inhibition of cyclooxygenases (COX) and other enzymes involved in inflammation; and disrupts cell signal transduction by various mechanisms including inhibition of protein kinase C. These effects may play a role in the agent's observed antineoplastic properties, which include inhibition of tumor cell proliferation and suppression of chemically induced carcinogenesis and tumor growth [7].

2.3.1 Pharmacological Classification (National Center for Biotechnology Information 2021)

- **Anti-Inflammatory Agents, Non-Steroidal**

  Anti-inflammatory agents those are non-steroidal in nature. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic, and platelet-inhibitory actions; other mechanisms may contribute to their anti-inflammatory effects.

- **Antineoplastic Agents**

  Substances that inhibit or prevent the proliferation of neoplasm.

- **Coloring Agents**

Chemicals and substances that impart color including soluble dyes and insoluble pigments. They are used in inks; paints; and as indicators and reagents.

- **Enzyme Inhibitors**

  Compounds or agents that combine with an enzyme in such a manner as to prevent the normal substrate-enzyme combination and the catalytic reaction.

2.4 Absorption, Distribution and Excretion

2.4.1 Absorption

- Curcumin displays poor absorption into the gastrointestinal tract. In a rat study, oral administration of a single dose of 2 g of curcumin resulted in a plasma concentration of less than 5 μg/ml, which indicates poor absorption from the gut[7].

2.4.2 Route of Elimination

- Following oral administration of curcumin to rats at a dose of 1 g/kg body weight, about 75% of dose was excreted in the faeces and only traces of the compound was detected in the urine. When a single 400 mg dose of curcumin was administered orally to rats, about 60% was absorbed and 40% was excreted unchanged in the faeces over a period of 5 days. Intraperitoneal administration resulted in fecal excretion of 73% and biliary excretion of 11% [7].

2.4.3 Volume of Distribution

- Following oral administration of radio-labelled curcumin to rats, radioactivity was detected in the liver and kidneys.

2.4.4 Excretion

- No pharmacokinetic data available.

- Oral & Ip doses of (3) H-curcumin led to fecal excretion of most of radioactivity. IV& ip doses were well excreted in bile of cannulated rats. When admin orally in dose of 1 g/kg, curcumin was excreted in feces to about 75%, while negligible amt appeared in urine. Measurement of blood plasma levels & biliary excretion showed that curcumin was poorly absorbed from the gut.

2.4.5 Biological Half-Life

- No pharmacokinetic data available [7].

2.5 Mechanism of Action

- Curcumin acts as a scavenger of oxygen species, such as hydroxyl radical, superoxide anion, and singlet oxygen and inhibit lipid peroxidation as well as peroxide-induced DNA damage. Curcumin mediates potent anti-inflammatory agent and anti-carcinogenic actions via modulating various signaling molecules. It suppresses a number of key elements in cellular signal transduction pathways pertinent to growth, differentiation, and malignant transformation; it was demonstrated in vitro that curcumin inhibits protein kinases, c-Jun/AP-1 activation, prostaglandin biosynthesis, and the activity and expression of the enzyme cyclooxygenase (COX)-2 [7].

2.6 Need Of Novel Drug Delivery System For Curcumin

- Curcumin is highly unstable in acidic pH of the stomach and degraded at alkaline pH before reaching to the blood and other constituents might be metabolized by the liver. Resulting, the optimum quantity of the Curcumin may not reach the blood resulting in no/less therapeutic effect. Nanocarriers applying to Curcumin will carry optimum amount of the drug to their site of action bypassing all the barriers such as acidic pH of

stomach, liver metabolism and increase the prolonged circulation of the drug into the blood due to their small size [11].

- Despite promising biomedical properties, free Curcumin molecules suffered from low water solubility, which in turn have resulted in poor bioavailability and clinical efficacy [12]. Hence, researchers have attempted to enhance water solubility and bioavailability of Curcumin by loading of Curcumin in biodegradable polymeric nanoparticles. For instance, Curcumin loaded poly (lactic-coglycolic acid) PLGA nanospheres were formulated for prostate cancer therapy [13].
- So Curcumin was selected as feasible drug candidate for delivery through a nano delivery system because of the following properties:
    - **i.** To improve the solubility.
    - **ii.** To enhance the bioavailability
    - **iii.** To reduce the dose.
    - **iv.** To target the site of action.
    - **v.** To control the release of the drug

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CHAPTER 3- REVIEW OF LITERATURE

Area of Review: Literature review is done on Starch (Definition, Chemical Structure, Applications, Fumctional Poperties, and Sources), Soft Rice or Kumol Chawl of Assam, Methods of Isolation of Starch from Rice, and Method of Preparation of Nano Particles.

3.1 Starch

- De borah Le et al. (2010), Okunlola A et al. (2013) state that Starch is a natural, renewable, and biodegradable polymer produced by many plants as a source of stored energy. The use of starches as natural polymers for pharmaceutical applications remains attractive because they are cheap, readily available, biodegradable and capable of modification .Native starch is a white powder with a bland taste and flavor, and is insoluble in cold water.
- Lin N et at. (2011) define starch as the major energy reserve of higher plants, is a mixture of two main components: amylose, a linear or slightly branched (1→4)-α-D-glucan, and amylopectin, a highly branched macromolecule consisting of (1→4)-α-D glucan short chains linked through α-(1→6) linkages.
- Suk Fun Chin et al (2014) Starch nanoparticles with particle sizes in the range of 10–1000 nm have been extensively studied as controlled release nanocarriers. Dialdehyde starch nanoparticles (DASNPs) conjugated with 5-fluorouracil (5-Fu) were found to have enhanced breast cancer cell (MF-7) inhibition *in vitro* as compared to free 5-Fu.
- Builders & Arhewoh et al. (2016) Starch is having the internal properties that are appropriate for pharmaceutical use. It is also used in a wide range of specialty drug delivery applications, such as delivering of complex molecules and targeting specific areas of the body. While there are several official native starch carriers with different identities, new sources are available that will continue to grow with the political,

economic, and scientific interest in starch and starch-based products. The size of the starch granules on the functional application is important for starches. Taking for example the rice starch grain is among the smallest in size which make it a desirable candidate to be used in both medicated powders and cosmetic powders for topical use. Due to the small size of starch, it can be used as an absorbent of oil from the skin; hence it is suitable as an excipients in dry shampoo and also as a lubricant in some diagnostic and surgical materials. Starch or starch-based as excipients have been revealed to offer several advantages in drug formulation and production in terms of the product safety lower in cost, and quality of product. The starch has been used as binder, diluent, disintegrant, lubricant and glidant in granules, capsules, and in tablet formulation

- Agboola et al., (2018). Starch a hydrophilic glidant can be used in the conventional capsules and tablets formulation with a concentration of 2-10 % w/w to reduce the inter-particulate abrasion and provides a better powder and granules flow. Different types of starch like Maize starch are mainly used as a glidant in capsules and tablets formulation, and starch from yam, cassava, and fonio have shown to be a potential glidants in the formulation of tablets. The flow rate, angle of repose, and flow factor are the properties of starch as a glidant that is evaluated in pharmaceutical powders and granules. Particle size of starch as a glidant is a significant parameter that controls the efficacy of glidant; that is the smaller the glidant particle size, the more efficient is the glidant properties
- Labelle et al., (2020). Lubricants are excipients that are added typically in little amounts to powders and granules during tablets and capsules formulation to decrease

the interfacial friction between the die walls and the tablet's surface needed in ejection, prevent sticking of the granules and tablets to the punch, also prevent sticking of powders in the dosators and tamping pins while filling the capsules. The concentration of starch is 2-10 % of the granules and powder weight. When starch is to be used as a lubricant in tablets and capsules formulation, enough trials need to be done to acquire an optimized concentration as starch can cause alteration in the flow and compaction of the mixed powder. Maize starch BP is commonly used as a lubricant, having dual mechanism like increasing the powder flow, prevent sticking of the tablets and granules to the die walls and punches

- Vo et al., (2021), (Odeku et al., (2013). As a novel application starch has been used as a drug carrier for controlled drug delivery. As starch is composed of amylose and amylopectin, it can be used as a coating or film forming polymer which makes it compatible for target delivery and control the release of drug from the system due to its mucoadhesive properties. Studies on maize starch has been evaluated as an effective film coat for tablets which retards or slow down the dissolution rate and confer controlled drug release and the starch coating can also be used in the matrix system and nanoparticles to carry the drug to the specific sites like the lungs, colon, and to target cancer cells
- Wojtasz et al., (2016). The use of starch as a coating polymer has made it to be used in delivering of drugs through microspheres system. The properties for starch as microspheres in the effect of cross-linking are the particle size increase with the increase in the time of cross-linking which will increase the concentration of drug loading, the particles swelling ratio was a function of the type of cross linker and not of

the cross linking time. The drug release from starch microspheres depend on the other cross-linking agent also, because when starch is cross-linked with epichlorhydrine release the drug within 30 min. but when link with gluteraldehyde or formaldehyde the drug release rate gets decreased

- Ablah et al., (2018). The cross-linking of starch can also be used in the targeting drug delivery system of protein drug matrix that is used in the targeting of drug to the colon. This is done by double modification of cross-linking the pregelatinized maize starch along with $POCl_3$ which will decrease the enzymatic degradation and can be used as a potential drug carrier to target the colon. The resistant starch shows similar release of drug and it is envisaged to be resistant to the amylase enzyme in the intestinal wall, without the formed nanoparticlesfast release of drug is shown when compared to the conventional or native starch. When the branch is reduced, it implies an idyllic precursor for directing ligand conjugation in design of oral colon-specific nano-particulate drug carrier.
- Santander-Ortega et al., (2010), Garcia et al., (2020). The starch nanoparticles can be prepared by different methods like cross-linking micro emulsion, ultra-sonication, nanoprecipitation, acid hydrolysis, recrystallization, and enzymolysis. Starch-based nanoparticles have been utilized for the transdermal drug delivery of the medications like testosterone, flufenamic acid and caffeine. The skin penetration information for the three medications recommends that starch nanoparticles have the potential for transdermal medication delivery applications. Exemplification and delivery properties of these nanoparticles were considered, showing high epitome effectiveness for these three tried medications (testosterone, flufenamic acid, and caffeine); likewise, a near

straight delivery profile was noticed for hydrophobic medications with an invalid starting burst impact

- Fausto et al. (1999). Industrially, starch is obtained from cereal grains such as wheat and maize (corn), or from tubers, such as potatoes, tapioca, and arrowroot, or from the pith of the sago plant. By far, the larger part of the starch is obtained from maize, wheat, potato, and tapioca. Starches from the different sources have different properties that affect their functionality and, therefore, it is in their end-of-use. In India only starches from maize and tapioca are produced in a significant amount .
- Bergthaller et al. (2007). Starch is present in almost all the tissue such as leaves, roots, tubers, seeds, stems, flowers, etc. of green plants however there are a small number of plants which are grown commercially for the starch which includes cereal such as wheat, corn, sorghum, and rice, tuber mainly potato, root like tapioca and arrowroot, stem of sago, and legume crops mainly pea. Worldwide production of starch is wholly depending on the use of cereals as the raw materials. Corn or maize is the main corp which supplies around 80% of the global starch market conquering the title of world largest industry situated in U.S.
- Wang p et al., (2015). Numerous studies have reported the isolation of starch from commercially important sources, such as cereals, grains, corn, etc. Starch derived from different sources exhibits different physicochemical properties. And, these physicochemical properties determine the application of starch in a different field. So, there is a growing interest for novel and unique starch sources. New botanical materials are gaining momentum as unconventional sources of starch, raising their commercial importance and, as a result, providing a higher demand for starch with novel and

unique characteristics to replace traditional starch sources. With the development of food and other industries, these non-conventional sources of starch have been investigated deeply in recent years.

- Guo K et al (2018) in their study, isolated starch from kernels of mango, jackfruit, litchi, longan, and loquat fruits and investigated the structural and functional properties (Table 2). Availability of 64, 56, 53, 59, and 71 % starch, respectively, in the five fruit kernels, indicates that they are good sources of starch. The starches were spherical, elliptical, and irregularly shaped with varying sizes containing approximately 25% amylose. Mango, jackfruit, and longan starches displayed A-type crystallinity whereas litchi and loquat starches showed C-type crystallinity. Significant differences were also observed in enthalpy, gelatinization temperature, viscosity, crystalline lamellar intensity, and susceptibility to enzyme hydrolysis.
- Huang J et al (2015) isolated starch from the rhizomes of *Curcuma longa*, *Canna edulis*, and *Canna indica*, and *Lilium lancifolium* bulb and compared the structural properties (Table 2). They observed variations in the structural and functional properties of the isolated starch. The isolated starches were different in morphology and size. *C. edulis* starch with the largest granule size had the highest breakdown viscosity, pasting peak, and swelling power. While *C. longa* starch with the smallest granule size possessed very high resistance to hydrolysis and digestion. A high content of amylopectin long branch chain and low content of amylopectin short branch chain was observed in C. longa. The L. lancifolium starch possesses low resistance to gelatinization, digestion, and hydrolysis. X-ray diffraction pattern revealed the B-type crystallinity of all the isolated starches

3.2 Soft rice or kumol chawl of Assam

- Shaptadvipa et al. (2009). The waxy rice of Assam has been classified in two groups viz., Bora (glutinous) and Chokuwa (Semi-glutinous) based on amylose content. Chokuwa (soft rice), is another class of rice used for instant preparations. This class of rice is not known in any other parts of the world. Its preparations are very popular in community feasts and festivals in Assam. "Soft rice (komal chawl)" are prepared from this class of rice by soaking the rice either in cold or hot water for a brief period of time and consume directly with milk, curd or sugar etc.
- Shefalika et al (2010). The rice variety 'Kumol Chawl' is an agricultural product of Assam region of North-East India. Rice is the staple diet for almost all sections of the Assamese community. This indigenous variety of rice does not require cooking or boiling to make it edible. Soaking the rice in cold water for half an hour makes it ready for serving, hence the name 'Kumol Chawl' or 'Soft Rice'. The typical recipe of 'Kumol Chawler Jalpan' ('Kumol Chawl with curd and jaggery) is simple. 'Kumol Chawl', parboiled rice from waxy paddy can be consumed after soaking in water for some time. Soaked and drained 'Kumol Chawl' mixed with curd and jaggery is ready to be consumed. This cereal based recipe has been analyzed for its nutrient composition per 100 gm as in basis and has been compared with another 12 cereal based recipes commonly consumed in Assam, India.4 The study reveals that 'Kumol Chawl' may fit into the category of health food.
- Borkakati et al. (2013). Chokuwa rice has special significance in its use in various delicious preparations in Assam. It is used in daily breakfast and also in social as well as religious ceremonies. Most of the *chokuwa* rice varieties do not become

sticky on cooking but become soft in consistency. Hence, they are also locally known as '*Komal Chaol'* or soft rice. The most important feature of this group of rice is that, the parboiled *chokuwa* rice become soft on just soaking in ordinary water and can be consumed directly with curd, milk, curry etc. without cooking. Thus, it assumes a very high commercial importance as this can be utilized for preparation of instant rice, which could be supplied to the soldiers camping at remote and inaccessible places, factory workers, tea garden labourers etc. This is also popular for its peach rice, puffed rice (*Akhoi*), beaten rice (*Chira*) and several palatable dishes. According to this study the % of starch present is 79.2%, amylose 16.86%, amylopectin 83.14%, crude protein 8.71, crude fat 2.60%, crude fibre 1.73%, ash 1.03%., phosphorous 0.29%, calcium 0.036%, iron 2.67%.

- Kashyap A et al. (2016)**.** The rice products of Assam are also convenience foods and are traditionally being consumed as ready-to-eat breakfast cereals. A unique characteristic of these rice products is that they soften and become consumable on simple soaking in water. The principal factor that governs the utilization of different rice products is the amylase to amylopectin ratio. The significant rice products of Assam are *kumal chawl, bhaja chawl, sandahguri* and *hurum*. *Kumal chawl* is parboiled rice made by normal parboiling method and is prepared from both *chowkua* and *bora* paddy. According to this study the % of carbohydrates present is 88.7 %, protein 8.9, fat 1.5 %, fibre 0.3 %, ash 0.6%, energy 403.9 %.

3.3 Isolation Methods

- Lim *et al*., (1999), Ashogbon et al., 2013) Rice starch was isolated from rice flour by using the alkaline deproteination method of with some modifications. Rice grain

was first dehulled and ground to powder using a laboratory grinder. Rice flour (200 g pass through 1 mm sieve screen) was mixed with 500 ml of 0.1% NaOH. The mixture was stirred on a magnetic stirrer for 3h, and stored at 4°C overnight. The supernatant was decanted, and fresh volume of sodium hydroxide was added to the solid phase and stirred for another 3h at ambient temperature. The procedure was repeated twice after which the solid phase was washed with 0.1% NaOH, blended and filtered. Distilled water was added to the filtrate and allowed to stand for 3 h. The supernatant was decanted and distilled water was added again. The procedure was repeated several times until the pH of the filtrate was between 6.0 and 6.5. The starch residue was collected and dried in a vacuum oven at 40°C for 48 h.

- Reddy D. Kodandaram et al. (2013)**.,** The residue obtained after protein extraction was sequentially extracted with 1 liter of each distilled water and 2% NaCl (each for 24 hrs at 4oC) followed by extraction with 300 ml of 0.1 N NaOH twice (48 hrs at 4oC). Subsequent to each of above the extraction, the slurry was centrifuged at 10000 rpm for 30 min. at 4oC and supernatant was discarded. The residue from the second NaOH extraction was further extracted with 80 % aqueous ethanol (100 ml) at 80 0C for 1hr, cool to room temperature and allowed to settle for 4 hr at 40C. The supernatant was discarded, and the residue was dehydrated and powdered.
- Iba´n˜ ez et al., (2007) Milled rice was soaked with three times its weight of deionized water for 12h.The rice and water were then blended in a Warring blender for 3min.The slurry was centrifuged at 6000g for 5 min. The supernatant was discarded and the rice flour dried. The regents used to remove protein from the rice starches were Pronase (67PUK/g, Calbiochem, SanDiego, USA), SDS (Bio-Rad,

Richmond, CA),and NaOH (Fischer Scientific Co., NJ). The four treatments for protein removal were by treatments of rice flour with five times its weight with solutions of (1) Pronase (0.2%), (2) NaOH (0.1%, 0.025M),(3) NaOH (0.4%,0.1M), or (4) SDS (1.0%). Each treatment consisted of two duplicate steps. Each step was a treatment of the rice flour with reagent at 37 degree delicious with continuous moderate shaking for 18h. Afterwards, the slurry was centrifuged at 6000g for 10 min. The supernatant and any brown surface layer of the starch were removed and the lower white starch layer was washed with deionized water. After repeating this step, the final starch residue was washed and centrifuged at least five times. The NaOH treatments were neutralized with HCl after the first washing. The final starch was freeze- dried, passedthrougha200-meshsieve, and stored until analyzed.

3.4 Method of preparation of nanoparticles

- Suk Fun Chin et al (2014) The solvent phases essentially consist of absolute ethanol as an organic solvent, oil (oleic acid, sunflower oil, and cyclohexane) as the continuous phase, and surfactant (Tween 80 or Span 60) as an emulsifying agent. About 2.5 × 10-4 M of curcumin was dissolved in 15 ml of ethanol solution containing of 0.8 × 10-3 M of surfactant, followed by the addition of 5 ml of oil and the mixture was stirred continuously for 1 h. After 1 h, 1 ml of starch solution (1% w/v) was added drop-wise to the mixture and stirred for an addition of 1 h. Curcumin was loaded *in situ* onto starch nanoparticles as the starch nanoparticles formed during the precipitation process. The curcumin loaded starch nanoparticles were collected by centrifugation and the samples were washed several times with

ethanol to remove any excess of curcumin that adhered at the surface of the starch nanoparticles**.**

- Chin SF et al., (2014). Preparation and Preparation of jackfruit seed starch nanoparticles (JSSN) loaded with curcumin was performed as follows by Nanoprecipitation method with some modifications: 10-50 mg Jackfruit seed starch at different concentration was mixed with a curcumin/acetone solution (10 mg curcumin/20 ml acetone) during 15 min by using a magnetic stirrer at 600 rpm. The 20 ml of water were added drop by drop with constant stirring. The resulting suspension was stirred at room temperature until acetone was completely vaporized. All experiments were carried out at 25 °C. Nanoparticles were separated by centrifugation at 4000 rpm during 40 min; samples were washed several times with ethanol to remove any excess of curcumin. Finally, the curcumin-loaded nanoparticles were dried in hot air oven at 30 °C during 48 h.

3.5 References

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CHAPTER 4- AIM & OBJECTIVE

Aim:

Isolation and Characterization of Starch from Assam Soft Rice (Kumol Chawl) and Preparation, Evaluation of Curcumin Loaded Starch Nanoparticles.

Objective:

- to isolate starch from soft rice
- to evaluate the physicochemical properties of the rice starch
- to prepare curcumin loaded nanoparticles using isolated rice starch
- to evaluate the prepared curcumin loaded starch nanoparticles

Plan of Work:

1. **Literature Review on National & International Context**

2. **Preformulation Study Of Pure Drug**

   - Physicochemical properties
   - Organoleptic property & Solubility
   - UV Spectrophotometry
   - Compatibility study by FT-IR

3. **Collection Of The Rice Sample**

4. **Isolation Of Starch**

5. **Characterization Of Isolated Starch**

6. **Formulation of Drug Loaded Nanoparticles**

7. **Evaluation of Drug Loaded Nanoparticles for Different Parameters**

   - Drug entrapment efficiency
   - Particles size distribution and polydispersity index
   - Fourier transform infrared spectroscopy
   - Scanning Electron Microscopy
   - In-vitro drug release study

Rationale Behind the Study

Nanocarriers have been extensively investigated over the past few decades as they have shown great optimism in the area of drug delivery. Nanocarriers, due to their high surface area to volume, can modify basic properties and drug performance or bioactivity of the drug. Improved pharmacokinetics and biodistribution, toxicity reduction, improved solubility and durability, controlled release, and site-specific or targeted delivery of therapeutic agents are some of the features that nanocarriers can incorporate into drug delivery systems. Generally, nanocarriers are prepared by using synthetic polymers. Though synthetic polymers are used in drug delivery but they are less biocompatible or biodegradable as compared to the natural polymer. Moreover, synthetic polymers are more toxic and high in cost as compared to natural polymers such as starch, cellulose, soya, and chitosan. Starch has been getting much more attention since the 1970s. Starch is the most attractive material among these renewable natural polymers; due to its low cost, availability, and capability of high production from renewable resources. Moreover, starch nanoparticles are produced by simple techniques, on a laboratory scale, they have proven to be a promising alternative for the formation of more stable emulsions, as a carrier agent for bioactive compounds and as agents to improve the barrier and mechanical properties of films based on plant polymers. The rice variety ‘Kumol Chawl’ is an agricultural product of Assam. This indigenous variety of rice does not require cooking or boiling to make it edible. Kumol Chawl is a parboiled rice variety from waxy paddy which can be consumed after soaking in water for some time. The study of characteristics by the Central Rice Research Institute, Cuttack, under the Indian Council of Agricultural Research (ICAR) helped to identity ‘Kumol Chawl’ as a possible GI candidate in the north-eastern region of

India, including West Bengal and Orissa other than Assam and Nagaland through their various substations in these provinces. Kumol Chawl' containing only 16% of amylose and 83% of amylopectin swells up after some time of soaking in water by absorbing it and make it edible within some period.

On the other hand, Curcumin is a natural polyphenol found in the rhizomes of turmeric (*Curcuma longa*) and exhibited antioxidant, antiinflammatory, antiproliferative, anti-invasive, and antiangiogenic activities. More recently, curcumin has been shown to have antitumor effects in many cancer cell lines and the clinical effects of curcumin are being studied in human clinical trials and animal models on various conditions and numerous myeloma. Despite these promising biomedical properties, free curcumin molecules suffered from low water solubility, which in turn have resulted in poor bioavailability and clinical efficacy. Hence, researchers have attempted to enhance water solubility and bioavailability of curcumin by loading curcumin in biodegradable polymeric nanoparticles.

Based on this report, to increase the bioavailability and basic properties of Curcumin novel formulations such as Curcumin loaded nanoparticles are have been developing to overcome these drawbacks. Keeping in this view, Curcumin loaded starch nano particles are prepared by using starch of kumol chawl or soft rice of Assam as a nanocarrier to improve the release property and bioavailability of Curcumin.

CHAPTER 5-MATERIALS AND METHODOLOGY

5.1 Chemical Used

All the drugs and chemicals used are of analytical grade and the manufacturers for respective chemicals are listed below

Table 5: **List of chemicals used and respective manufacturers**

Sl. NO.	**Chemicals**	**Manufacturer or supplier names**
1.	**Assam kumol chawl**	**Collected from local village (Azara, Guwahati, Assam-17)**
2.	**Methanol**	**Merck Specialities Pvt. Ltd. (Mumbai)**
3.	**Sodium hydroxide**	**Merck Specialities Pvt. Ltd. (Mumbai)**
4.	**Curcumin**	**Sisco Research Laboratories Pvt. Ltd.**
5.	**Oleic acid**	**Sigma Chemical India**
6.	**Ethanol**	**Merck Specialities Pvt. Ltd. (Mumbai)**
7.	**Tween 80**	**Merck Specialities Pvt. Ltd. (Mumbai)**
8.	**Liquid paraffin**	**Merck Specialities Pvt. Ltd. (Mumbai)**
9.	**Span 60**	**Merck Specialities Pvt. Ltd. (Mumbai)**

5.2 Instrument used

All the instruments used in the practical works conducted throughout the project and the respective manufacturers are enlisted below.

Table 6: List of instruments used and their manufacturers

Sl. No.	Instruments	Company name
1.	Digital weighing balance	Citizen
2.	UV spectrophotometer	Shimadju, Model no: UV 1800240V
3.	Magnetic stirrer	Rolex, India
4.	Homogenizer	IKA T25 Digital Ultra Turrax, Germany
5.	Hot air oven	International comrcial Traders, 18, Kolkata-001
6.	FT-IR	Bruker, Alpha E
7.	Melting point apparatus	Macro Scientific Works 10A/UA, Janwahar Nagar, Delhi-007
8.	Scanning Electron Microscopy	SEM@ CSIC, Dibrugarh University-JSM -IT 300
9.	Differential Scanning Calorimetry	NIPER GUWAHATI: Mettler
10.	Centrifuge	REMI-8C, Ireland
11.	Lyophilizer	IIC Industrial Corporation
12.	Zeta sizer	Malvern Instruments, Model No: Nano ZS 90, UK
13.	Viscometer	Brookfield LVDV-E
14.	pH meter	Indosati Scientific Lab Equipments
15.	Water bath	Jain Scientific Glass Works

5.3 Preformulation Study of Drug

The drug was subjected to the following preformulation tests.

5.3.1 Organoleptic Properties

The organoleptic properties such as color, odor were examined visually.

5.3.2 Solubility

On basis of polarity of the solvent system were selected and the solubility was observed. A minute quantity of the drug was taken in a test tube and solubility of the drug was determined by dissolving the drug in 10 ml various solvents like water, ethanol and methanol.

5.3.3 Melting Point

A little amount of the drug sample in a dry capillary tube of 1mm internal diameter forming a column about 3mm high. Heat the melting-point apparatus to a temperature 5- 10˚C below the expected temperature of melting and adjust the heating so that the temperature in the chamber rises about 1˚C per minute. Introduce the capillary tube into the melting point apparatus, and noted the temperature when the drug substance becomes completely melted to liquid state; this is considered to be the melting point.

5.3.4 UV Scanning

The various concentration of drug sample was prepared. The spectrum of this solution was run in 400-700 nm range in UV-visible spectrophotometer and the maximum absorption frequency was detected by using UV visible spectrophotometer.

5.3.5 Preparation of standard calibration curve in phosphate buffer 7.4

5 mg of drug was weighed accurately and dissolved in 50 ml of PBS. The further dilution was made to get a concentration 2, 4, 6, 8, 10µg/ml. The absorbance of each sample was measured by using UV-visible spectrophotometer at 420nnm.

5.3.6 Compatibility Study of Drug and Excipients

Fourier transform infra-red spectroscopy (FT-IR) of Starch curcumin and 1:1 starch, Curcumin ratio were studied for its functional groups in FT-IR spectroscopy in a Fourier–transform infrared spectrophotometer (Bruker, Alpha) in range of 4000–400 cm−1.

5.4 Isolation of rice starch

5.4.1 Alkaline deproteination method

Rice starch was isolated from rice flour by using the alkaline deproteination method with some modifications. Rice grain (fig) was first dehulled (fig) and ground to powder (fig) using a laboratory grinder. Rice flour 60g was mixed with 360ml of 0.1% NaOH (1:6 rice flour and NaOH solution). The mixture was stirred on a magnetic stirrer for 3h, (fig) and stored at 4°C overnight. The supernatant (fig) was decanted, and fresh volume of sodium hydroxide was added to the solid phase and stirred for another 3h at ambient temperature (fig). The procedure was repeated twice after which the solid phase was washed with 0.1% NaOH for several times and filtered. Distilled water was added to the filtrate and allowed to stand for 3h (fig).The supernatant was decanted and distilled water was added again. The procedure was repeated several times until the pH of the filtrate was between 6.0 and 6.5. The starch residue was collected and lyophilized at -35^{0C} for 5 hours (fig) [1-3].

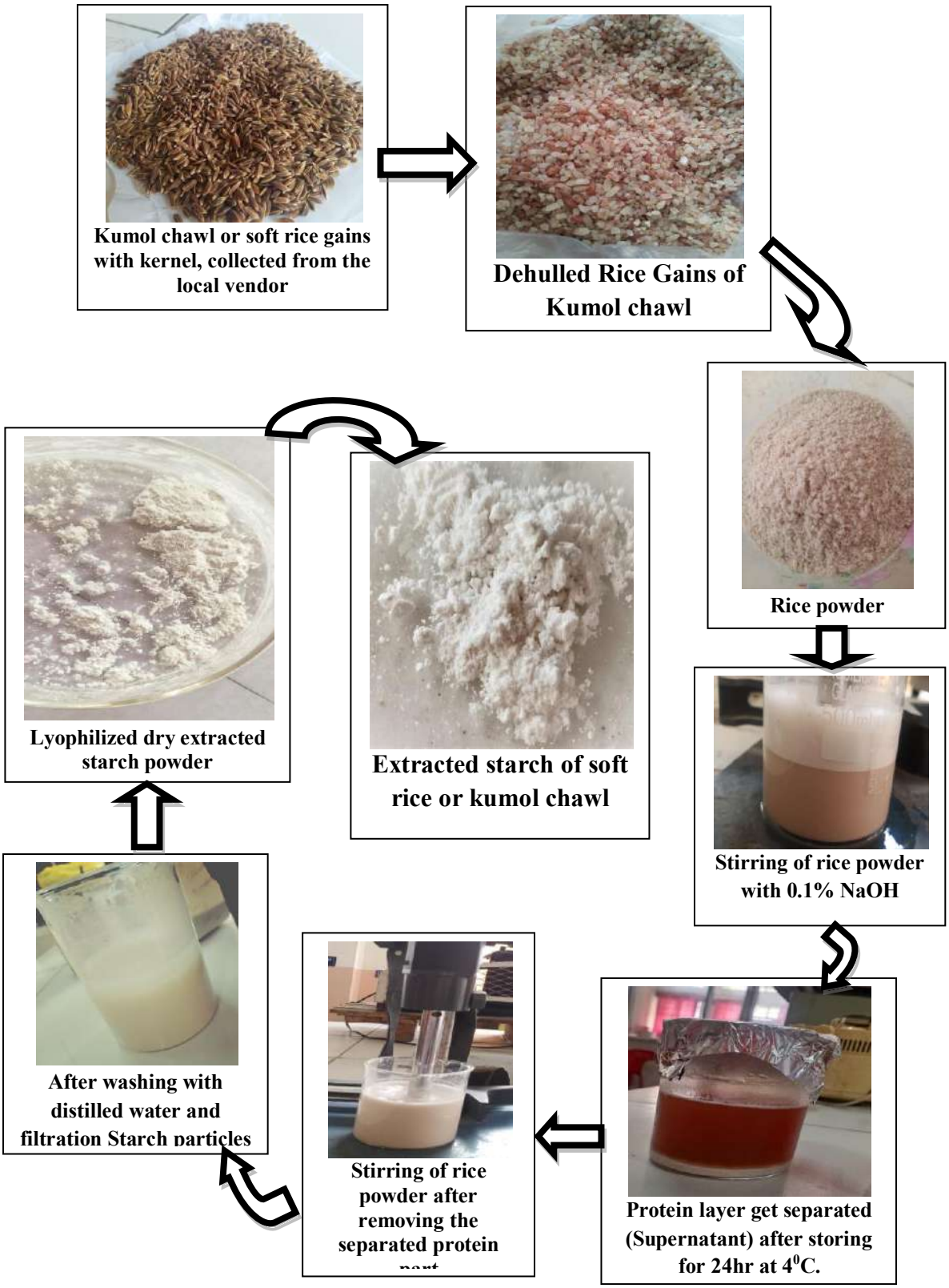

Fig. 5: Schematic presentation of extraction of Starch from rice.

5.5 Characterization of starch

5.5.1 Starch Yield

A known quantity of crude material was weighed first. After the polysaccharide was isolated the mass of the polysaccharide was determined. The yield was expressed as percentage of the mass of the dry precipitate against mass of the whole fresh crude material

5.5.2 Morphology of rice starch granules

The morphology of rice starch granules was evaluated by scanning electron microscope (SEM) (JSM-IT 300) and examined and photographed at an accelerating voltage of 20.0kv with a magnification of x3000, x500 and x1000.

5.5.3 Particle size

Particle size distribution of extracted Starch was dispersed in distilled water to get a stock solution of 1% (w/w).The solution was then homogenized at 8,000 rpm. This dispersion was then swiftly transferred in sample cell and particle size distribution was determined using Zeta Sizer (Nano-s90, Malvern Instruments, UK) with the help of software.

5.5.4 Differential Scanning Calorimetry

Extracted starch was studied using a differential scanning calorimeter (Perkin Elmer 4000) for glass transition, gelatinization and melting point temperature. About 3mg of the sample were crimped in a standard aluminum pan and heated in a temperature range of 10 ^{0}C to 280˚C at a heating rate of 10˚C per minute in nitrogen atmosphere

5.5.4 Physicochemical Characterization

i. Determination Of Organoleptic Properties

The physical appearance of a drug was observed.

ii. Determination Of Melting Point

Melting point of the starch was studied using a differential scanning calorimeter (Perkin Elmer 4000). About 3mg of the sample were crimped in a standard aluminum pan and heated in a temperature range of 10 ^{0}C to 280˚C at a heating rate of 10˚C per minute in nitrogen atmosphere

iii. Determination Of Solubility

Small increments of isolated starch were added to 10 ml of solvent Coldwater, Warm water, Ethanol, Methanol in a 25 ml Stoppard standard Flask with vigorous shaking [15].

iv. Determination of pH

1% w/w aqueous suspension of starch was prepared and the pHwas determined using a pH meter.

v. Viscosity

The viscosity of 1% starch suspensions was measured using a Brookfield viscometer.

vi. Percentage Moisture Content

Dried the empty dish and lid in the oven at 105ºc for 3 h and transferred to a desiccators to cool. Weigh the empty dish and lid. Weigh about 3 g of the sample to the dish. Spread the sample with uniformity. Dish with the sample Placed in the oven and dried for 3h at 105ºc. After drying the dish was transferred with a partially covered lid to the desiccators to cool. Weight of The

dish along with the dried sample was taken again. Percentage of Moisture Content was calculated by following formula.

Percentage of Moisture Content = $\frac{W1-W2}{W1} \times 100\%$

W1: Weight (g) of the sample before drying, W2: Weight (g) of the sample after drying

vii. Determination Of Swelling index

10 ml of distilled water and liquid paraffin was taken in two different test tubes; 3 g of Powder was added to both test tubes. The dispersions were allowed to stand for12 hours. The Volumes of the sediment in the tubes were recorded. The swelling index of the material was calculated using the following formula

$$S.I\ (\%) = \frac{\text{Volume of sediment in water} - \text{Volume of sediment in light liquid paraffin}}{\text{Volume of sediment in light liquid paraffin}} \times 100\%$$

viii. Water absorption index

For the determination of the water absorption index, the starch sample was suspended in 10 ml distilled water at 30◦Cin a centrifuge tube, stirred for 30 minutes, and then centrifuged at 3000 rpm for another 10 minutes. The supernatant was decanted, and the weight of the gel formed was recorded. The water absorption index was than calculated as gel weight per gram of dry sample.

$$\text{Water absorption index} = \frac{weig\,t\ of\ t\,e\ gel\ formed(g)}{weig\,t\ of\ t\,e\ sample} \times 100\%$$

ix. Gelatinization Temperature

Gelatinization Temperature of the starch was studied using a differential scanning calorimeter (Perkin Elmer 4000). About 3mg of the sample were

crimped in a standard aluminum pan and heated in a temperature range of 10 ^{0}C to 280˚C at a heating rate of 10˚C per minute in nitrogen atmosphere

5.5.5 FT-IR Analysis

Fourier transform infra-red spectroscopy (FT-IR) Starch was studied for its functional groups in FT-IR spectroscopy in a Fourier–transform infrared spectrophotometer (Bruker, Alpha) in range of 4000–400 cm−1.

5.5.6 Phyto-Chemical test

Table 7: Phyto- Chemical test of extracted starch

Serial No.	Name of the phyto constituent	Test
1.	Carbohydrates	Malice's test
2.	Reducing Sugar	Benedict test
3.	Monosaccharide	Barfoed's test
4.	Polysaccharide	Iodine test
5.	Protein	Biuret test Millon's test
6.	Alkaloids	Dragrondrof test
7.	Steroids	Salkowski test Liberman-Barchard Reaction test
8.	Glycosides	Killer – killani test Legal test

9.	**Saponin Glycosides**	**Foam test**
10.	**Flavanoids**	**Lead acetate test**

5.5.6 Stability Studies:

Stability testing plays a crucial role in the drug development process. The purpose of stability Testing is to provide evidence on how the quality of drug product varies with time under the Influence of a variety of environmental factors, such as temperature, humidity, and light, so as to recommending shelf life for the drug product and recommended storage conditions. Stability studies were conducted according to ICH guidelines by storing sample in 40˚C ± 2˚C/ 75% ± 5% RH to test the physical and chemical stability of the developed formulations. Throughout the study, extracted starch powder was stored in well-closed containers. The stored powder was evaluated for organoleptic properties, Physicochemical characteristic.

5.6 Preparation of Nanoparticles

5.6.1 Loading of Curcumin onto Starch Nanoparticles.

i. Method 1: Precipitation in water-in-oil micro emulsion

The solvent phases essentially consist of absolute ethanol as organic solvent, oil (oleic acid) as the continuous phase, and surfactant (Tween 80 and span 60) as an emulsifying agent. About 0.1g of Curcumin was dissolved in 15ml,20ml,25ml(in three different beaker) of ethanol solution containing of 1ml of surfactant, followed by the addition of 5ml Of oil and the mixture and was stirred continuously for 1 h. After 1 h, 1ml of starch solution (1% w/v) was added drop-wise to tall the three mixtures and stirred for another 1h. Curcumin

was loaded in situ on to starch nanoparticles as the starch nanoparticles formed during the precipitation process. The Curcumin loaded starch nanoparticles were collected by centrifugation and the samples were washed several times with ethanol to remove any excess of Curcumin that adhered at the surface of the starch nanoparticles [4].

ii. **Method 2: Without micro emulsion**

iii. To the extracted starch nanosuspension, 30 mg of Curcumin dissolved in 5ml of acetone was added with continuous stirring. The resulting solution was centrifuged and washed with methanol to remove any free Curcumin until the filtrate became colorless. The product obtained was air dried [6]

5.6.2 Characterization of nanoparticles

i. **FTIR Analysis of Curcumin loaded nanoparticles**

Fourier transform infra-red spectroscopy (FT-IR) of Curcumin loaded nanoparticles was studied for its functional groups in FT-IR spectroscopy in a Fourier–transform infrared spectrophotometer (Bruker, Alpha) in range of 4000–400 cm−1.

ii. Particle size

Particle size distribution and polydispersity index study of the nanoparticles. The Particle size analysis of the dried nanoparticles (average apparent diameter, D) and polydispersity index (PDI) were determined by dynamic light scattering using Zetasizer (Malvern Nano S90). Determinations were carried out at 25°C at a fixed angle of 90°. Nanoparticles were dispersed in distilled water solution before measurement.

iii. Loading Efficiency of Curcumin.

The Curcumin loaded starch nanoparticles were separated from the reaction medium by centrifugation and the UV absorbance of free Curcumin was measured at a wavelength of 422 nm. The concentration of Curcumin was calculated with reference to a regression equation (linear plot with slope of 0.005) obtained from constructed calibration curve of Curcumin in absolute ethanol solution. The percentage of loading efficiency of Curcumin onto starch nanoparticles was calculated based on the following equation [9, 10]:

$$\text{Loading efficiency (\%)} = \frac{[Curcumin]tot - [Curcumin]free}{[curcumin]tot} \times 100\%$$

iv. Loading capacity of Curcumin onto starch nanoparticles was calculated based on the following equation:

$$\text{Loading capacity (mg/mg)} = \frac{[Curcumin]tot - [Curcumin]free}{total\ weight\ of\ nanoparticle} \times 100\%$$

Where [Curcumin]tot is the concentration of Curcumin added and [Curcumin]free is the concentration of Curcumin present in the supernatant after centrifugation.

5.7 Curcumin Release Studies

About 50mg of Curcumin loaded starch nanoparticles was placed in 15ml of buffer solution at ph 7.4 at 37 ± 0.5°C [11]. At predetermined time intervals, the solution was centrifuged, and the supernatant was removed and replaced with the same volume of buffer solution. The amount of Curcumin released in the supernatant was determined using a UV/Vis spectrophotometer. The concentration of Curcumin released was calculated with reference to the regression equation generated from constructed calibration curve of Curcumin in PBS (linear plot with slope of

0.010).The percentage of Curcumin released at a specific time was determined based on the following equation [12, 13]:

Release of Curcumin (%) = $\frac{[\text{Curcumin}]\text{rel}}{[\text{Curcumin}]\text{load}} \times 100\%$

Where [Curcumin]rel is the concentration of Curcumin released and [Curcumin]load is the concentration of Curcumin loaded into the starch nanoparticles.

5.8 Swelling Studies

The swelling behavior of the Curcumin loaded starch nanoparticles was studied by immersed reweighted dried Curcumin loaded starch nanoparticles in 10ml of buffer solution (ph 7.4) at 37 ± 0.5°C. The weights of the swollen starch nanoparticles were determined at various time intervals. The swelling ratio was calculated based on the following equation [14]: Swelling ratio (SR) = $\frac{(\text{Ww}-\text{Wd})}{\text{Wd}}$

Where Ww and Wd are the wet and dry weight of the starch nanoparticles.

5.9 References

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CHAPTER 6- RESULT AND DISCUSSION

6.1 Preformulation Study of Drug

6.1.2 Organoleptic properties of drug

Organoleptic properties of the drug are described in Table 8.

Table 8: Organoleptic properties of drug

Drug	State	Color	Odor	Taste
Curcumin	Solid	Turmeric yellow	Characteristic	Characteristic

6.1.2 Solubility

Solubility of the drug are described in Table 9.

Table 9: Solubility of Drug in Various Solvents

Solvent System	Drug (Curcumin)
Water	Poorly soluble
Methanol	Redialy soluble
Ethanol	Redialy Soluble

6.1.3 Melting Point

The melting point of the drug was found to be 170^{o}C- 180 ^{o}C

6.1.4 UV Scanning

The maximum absorption frequency was found to be 420nm using UV Visible spectrophotometer.

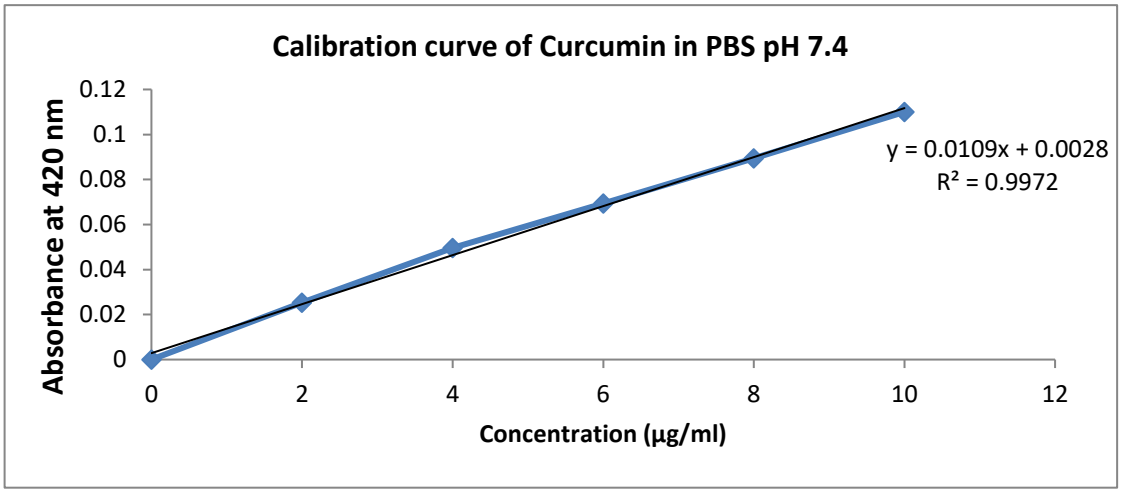


Fig. 6: Calibration curve of Curcumin in PBS pH 7.4

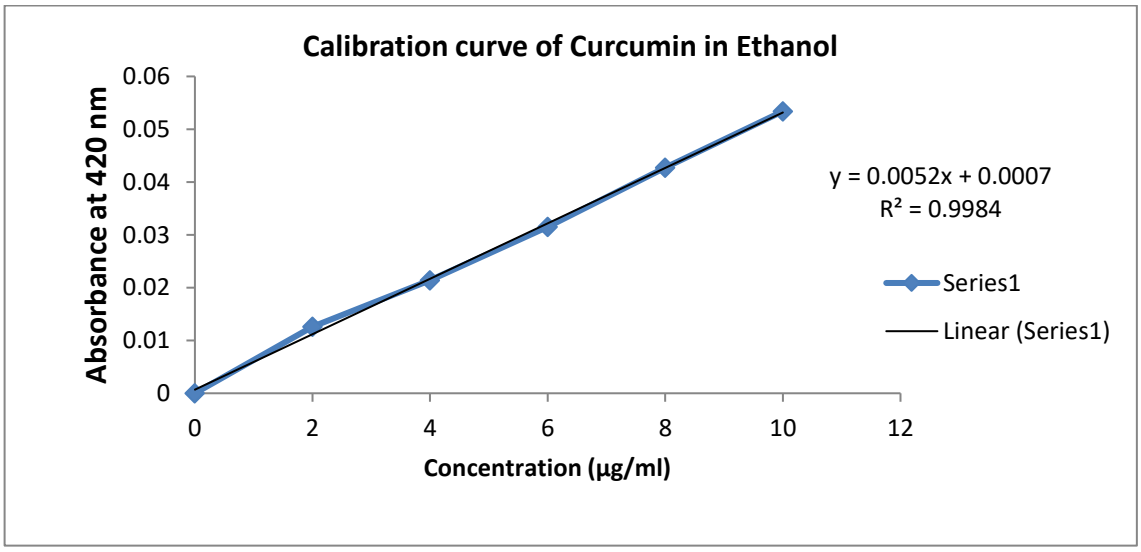


Fig. 7: Calibration curve of Curcumin in Ethanol

6.1.5 Compatibility study of drug and excipients (Curcumin and starch)

The physicochemical compatibility study of Curcumin and extracted starch was carried out by infrared spectroscopy study of Curcumin (Table 10), starch (Table 11) and 1:1 physical mixture of extracted starch and Curcumin. IR spectral analysis of Curcumin (Fig. 8) showed broad, strong peaks at 3506-3333 cm^{-1} representing

OH stretching (alcohol), similarly peaks at 2919 cm^{-1} , 1934-1818 cm^{-1} , 1934-1818 cm^{-1} , 1761 cm^{-1} and 1729 cm^{-1} [r]epresents CH stretching of alkanes or alkenes, aromatic compounds, C=O Stretching of aliphatic ketone respectively. Peaks at 1469 cm^{-1} and peaks at 1449 cm^{-1} represents C-H bending of methylene group and methyl group respectively. Peaks at 1206 cm^{-1} , 1150 cm^{-1} ,1107 cm^{-1} ,1074 cm^{-1} and 987 cm^{-1} represents C-O bending of alkyl aryl ester, aliphatic ether, secondary alcohol, primary alcohol and C=C bending of alkanes respectively. [1, 19]. The IR spectrum of starch (fig 9) showed different stretch and bend vibrations in respect of the peaks. The spectra of starch displayed a peaks at 3743-3588 cm^{-1} representing hydroxyl (-OH) groups (alcoholic), Peak at 2931-2899 cm^{-1} is associated to C-H stretching of alkanes. Peak at 1466 cm^{-1} CH bending of methyl group, 1417 cm^{-1} represents O=H bending (alcohol), 1340 cm^{-1} OH bending (alcohol), 1205 cm^{-1} C-O stretching (alkyl aryl ether), 1166 cm^{-1} C-O stretching (tertiary alcohol),1136 cm^{-1} represents C-O-C (stretching ether bond) and peaks at 1071 cm^{-1} C-O stretching (primary alcohol) [1, 3, 20, 21]. IR spectral analysis of 1:1 starch and Curcumin ratio (Fig.10) showed peaks at3743-3610 cm^{-1} , 3338 cm^{-1} representing OH stretching of Alcohol, peaks 3054 cm^{-1} , 17393338 cm^{-1} represents CH stretching of alkanes and C=O stretching of aliphatic ketone. Peaks at 1644 cm^{-1} , 1616 cm^{-1} , 1567 cm^{-1} ,1514 cm^{-1} represents C=C stretching of alkenes, conjugated alkenes, cyclic alkenes, benzene ring respectively. Peaks at 1460 cm^{-1}, 1411 cm^{-1} represents CH bending of methylene group and methyle group. Peaks at 1361 cm^{-1}, 1320 cm^{-1} O-H bending of alcohol and phenol, peaks at 1216 cm^{-1}, 1177 cm^{-1}, 1031 cm^{-1} represents C=O stretching of tertiary and primary alcohol

respectively. Lastly peaks at 987 cm^{-1}, 959 cm^{-1} represents C=C bending of alkenes. As we can see, in the IR analysis of physical mixture of Curcumin and starch all the functional group of Curcumin and starch are present and there is no arising of any new peak for new functional group or compound so the drug Curcumin and starch are compatible with each other.

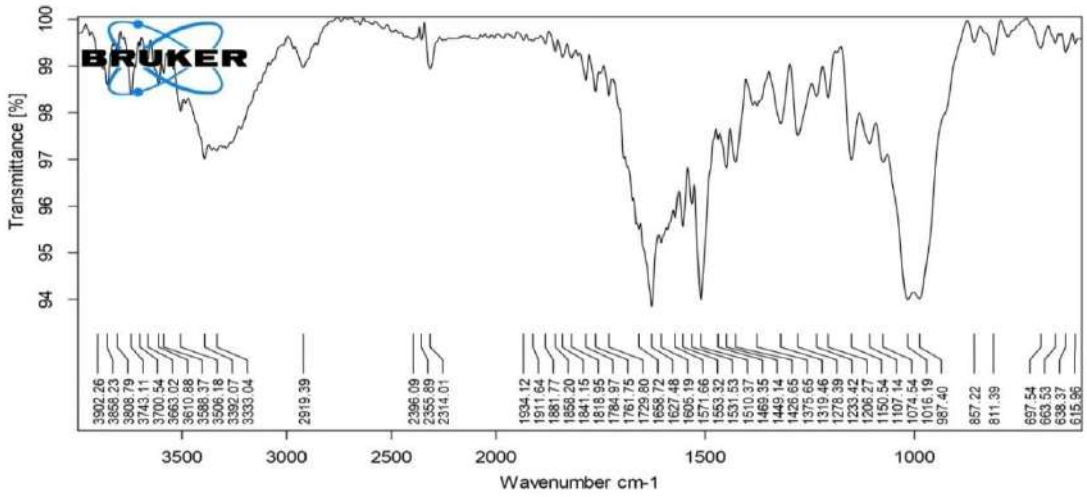


Fig. 8: FT-IR spectra of Curcumin

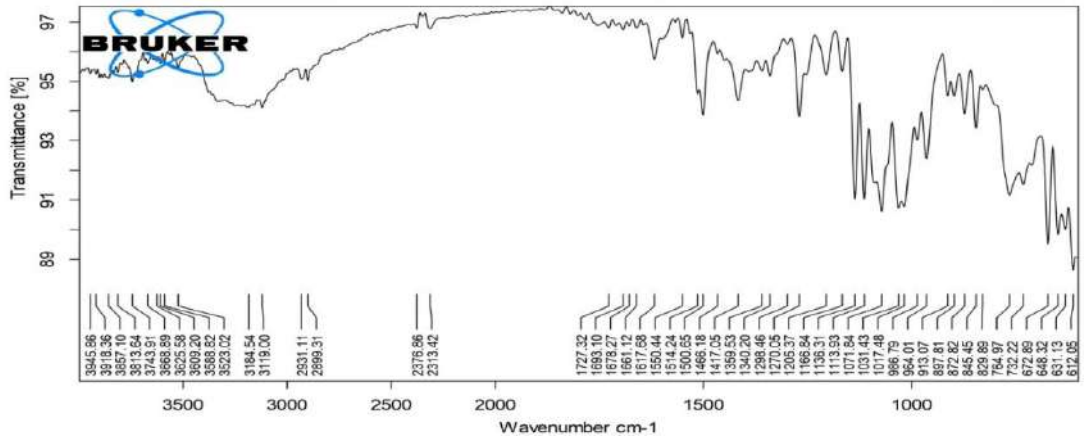


Fig. 9: FT-IR spectrum of extracted starch of soft rice

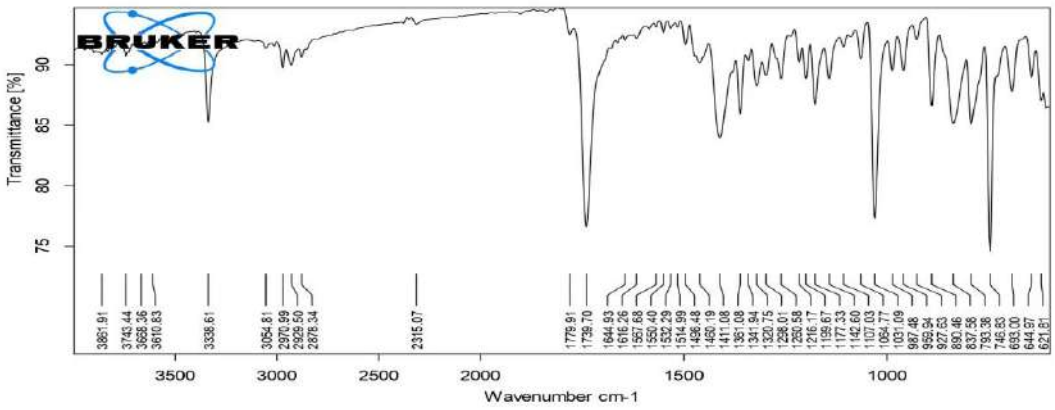


Fig. 10: FT-IR spectra of extracted starch & Curcumin Mixture

Table 10: IR absorbance spectrum of Curcumin

Sl no.	ABSORBANCE	APPEARANCE	GROUP	COMPOUND
1	3506-3333	Broad, Strong	O-H stretching	Alcohol
2	2919	Medium	C-H Stretching	Alkane, Alkene
3	1934-1818	Weak, Broad	C-H bending	Aromatic compound
4	1761, 1729	Medium	C=O Stretching	Aliphatic Ketone
5	1658,1627,1605	Strong, Sharp	C=C stretching	Alkene
6	1571	Strong, Sharp	C=C stretching	Cyclic Alkene
7	1469	Medium	C-H bending	Alkane (Methylene group)
8	1449	Medium	C-H bending	Alkane (Methyl group)
9	1206	Medium	C-O stretching	Alkyl aryl ester
10	1150	Medium	C-O stretching	Aliphatic ether
11	1107	Medium, Sharp	C-O stretching	Secondary alcohol
12	1074	Medium	C-O stretching	Primary alcohol

13	987	Strong, Broad	C=C bending	Alkane

Table 11: IR absorbance spectrum of extracted starch

Sl no.	ABSORBANCE	APPEARANCE	GROUP	COMPOUND
1	3743-3588	Broad, Strong	OH stretching	Alcohol
2	3523	Sharp, Medium	OH stretching	Alcohol intermolecular bonded
3	2931-2899	Medium	CH stretching	Alkane
4	1466	Medium	CH Bending	Methyl Group
5	1417	Strong	OH bending	Alcohol
6	1359,1340	Medium	OH bending	Alcohol
7	1205	Medium	C-O stretching	Alkyl aryl ether

Table 12: IR absorbance spectrum of extracted starch & Curcumin (1:1)

Sl. No	ABSORBANCE	APPEARANCE	GROUP	COMPOUND
1	3743-3610	Broad, Strong	OH stretching	Alcohol
2	3338	Sharp, Strong	OH stretching	Alcohol(intermolecular bonded)
3	3054	Medium	C-H stretching	Alkane
4	1739	Strong, Sharp	C=O stretching	Aliphatic Ketone
5	1644	Weak	C=C stretching	Alkene
6	1616	Weak	C=C stretching	Conjugated alkenes
7	1567	Medium	C=C stretching	Cyclic alkenes
8	1514	Medium	C=C stretching	Benzene ring
9	1460	Medium, Broad	C-H bending	Alkanes (methylene group)
10	1411	Strong	C-H bending	Alkane, Methyl Group
11	1361,1341	Medium	O-H bending	Alcohol
12	1320	Medium	O-H bending	Phenol
13	1216	Medium	C-O stretching	Alkyl aryl ether
14	1177	Medium	C-O stretching	Tertiary alcohol
15	1031	Strong	C-O stretching	Primary alcohol
16	987,959	Medium	C=C bending	Alkene

6.2 Characterization of Extracted starch

6.2.1 Yield percentage

Kumol chawl were procured from local vendors in the region of Azara, Guwahati-17, Assam. The rice starch extracted by using alkaline deproteination method were dried, sieved and stored in well closed container. The % yield value for the extraction process was found to be 25%. As during the process of rice starch isolation washing for a lot of times was necessary which may result in loss of dissolved polysaccharide.

6.2.2 Morphology of rice starch granules

Examined and photographed at an accelerating voltage of 20.0kv with a magnification of x3000, x500 and x1000 showed clear picture of particles which are homogenized in size and shape. They have crystalline structure with sharp surface, polygonal shape. The particles were dispersed throughout the area with no clumps.

6.2.3 Particle size

The average particle size and polydispersity index (PDI) of extracted starch were determined as it could be explored for nanoparticular applications. The PDI is a dimensionless number that indicates the width of the size distribution.The Z-average value for extracted starch was found to be 514±10.01 nm and the PDI was reported to be 1.000±.05. The particle size analysis provided a convincing approach for the extracted starch to be used as other novel (nanoparticulate) carriers.

6.2.4 Differential Scanning Calorimetry

From DSC report three graphs were generated; first graph shows the glass

transition temperature at 15-20 °C, second graph shows the gelatinization temperature with temperatures of 26.89 °C (onset), 69.36 °C (peak), 122.64 °C (endset), and the third graph shows the melting point with temperatures of 140.54 °C (onset), 142.35 °C (peak), and 144.47 °C (endset).

Fig 11: Microscopic view of Extracted Starch particle (100x magnificent)

6.2.5 Physicochemical Characterization

The starches obtained via extraction processes were dried under ambient conditions for 24 hours. Furthermore, these starches were evaluated for the physicochemical parameters and it revealed that the melting point obtained at >180^{0}C. The pH of the 1% solution was in the range of 7-7.50. Viscosity is 1.074cps. Moisture content is 0.23%. Swelling index is 150% and Water absorption index is more than 200%. Gelatinization Temperature was 75^{0}C. The results are given in Table 15. As for the organoleptic properties it was in solid state, off white in color (fig 12), did not have any characteristic odor and taste (table 13). In case of solubility isolated starch is soluble in warm water but insoluble in cold water and alcohol (table 14).

Table 13: Organoleptic properties of extracted starch

Sample	State	Colour	Odour	Taste
Extracted starch	Solid, powder	Off white	No odour	No taste

Fig 14: Extracted starch of soft rice or kumol chawl

Table14. Solubility of extracted starch

Solvent System	Extracted starch
Water	Insoluble
Hot water	Sparingly Soluble
Methanol	Insoluble
Ethanol	Insoluble

Table15. Physicochemical properties of extracted starch

Sl No.	Parameter	Results
1.	Melting point	142^0C

2.	**pH**	**7-7.5**
3.	**Viscosity**	**1.070cps**
4.	**Moisture content**	**0.23%**
5.	**Swelling index**	**150%**
6.	**Water absorption index**	**>200%**
7.	**Gelatinization Temperature**	**69.3^{0}C**

6.2.6 FT-IR analysis of starch

6.2.7 Spectrum of NaOH extracted starch found to be similar with native starch. The IR spectrum of starch showed different stretch and bend vibrations in respect of the peaks Table 11. The IR spectrum of starch (fig 9) showed different stretch and bend vibrations in respect of the peaks. The spectra of starch displayed a peaks at 3743-3588 cm^{-1} representing hydroxyl (-OH) groups (alcoholic), Peak at 2931-2899 cm^{-1} is associated to C-H stretching of alkanes. Peak at 1466 cm^{-1} CH bending of methyl group, 1417 cm^{-1} represents O=H bending (alcohol), 1340 cm^{-1} OH bending (alcohol), 1205 cm^{-1} C-O stretching (alkyl aryl ether), 1166 cm^{-1} C-O stretching (tertiary alcohol),1136 cm^{-1} represents C-O-C (stretching ether bond) and peaks at 1071 cm^{-1} C-O stretching (primary alcohol) [1, 3, 20, 21].

6.2.8 Phyto-Chemical Test

The starches obtained via extraction processes were dried under ambient conditions for 24 hours. Furthermore, these starches were evaluated for phyto-chemical studies as per the procedures described in the Method section. The raw rice powder and starches extracted from it were evaluated for the presence of carbohydrates,

polysaccharides, proteins, alkaloids, glycosides, steroids, flavanoids, and saponins. These phyto-chemical tests revealed that rice powder contained carbohydrates, polysaccharides, proteins, glycosides, and steroids. The extracted starches confirmed the presence of only carbohydrates and polysaccharides. The phyto-chemical tests used are given in Table 16.

Table 16: Phyto-Chemical test of extracted starch

Sl No.	Name of the phyto constituent	Test	Extracted starch	Rice powder
1.	Carbohydrates	Molisch test	Present	Present
2.	Reducing Sugar	Benedict's test	Absent	Absent
3.	Monosaccharide	Barfoed's test	Absent	Absent
4.	Polysaccharide	Iodine test	Present	Present
5.	Protein	Biuret test Millon's test	Absent	Present
6.	Alkaloids	Dragrondrof test	Absent	Absent
7.	Steroids	Salkowiesk test Libermann-Burchard Reaction test	Absent	Present
8.	Glycosides	Killer – killani test Legal test	Absent	Present
9.	Saponin Glycosides	Foam test	Absent	Absent
10.	Flavanoids	Lead acetate test	Absent	Absent

6.2.9 Stability Studies:

After storing for 3month at 40˚C ± 2˚C/ 75% ± 5% RH the extracted starch was stable in terms of particle size, physicochemical properties and FT-IR analysis.

There was no change in organoleptic properties also.

6.3 Characterization of nanoparticles

6.3.1 Loading of Curcumin into Starch Nanoparticles.

Figure shows that the Curcumin powder was insoluble in aqueous media and observed floating on the aqueous media. In contrast, Figure shows that Curcumin loaded starch nanoparticles were completely dispersed in aqueous media and rendered Curcumin in good dispersibility in aqueous media fig. 13. The loading of Curcumin onto the starch nanoparticles has increased the solubility of Curcumin in the aqueous media as the starch nanoparticles were very hydrophilic in nature with small in size and large surface area. It allows for a greater interaction with the aqueous solvent and thus resulted in the increase of Curcumin solubility in aqueous media.

15(a) **15(b)**

Fig 15: 15(a) solubility of free Curcumin in aqueous medium
15(b) solubility of Curcumin after loading on nanoparticles

6.3.2 FTIR Analysis of Curcumin loaded nanoparticles

The peaks at 3000-3700 cm^{-2} indicates –OH stretching frequency and the absorption band at 1166 cm-2 represents –C-O on polysaccharide skeleton. In case

of Curcumin the broad peak around 3333 indicates OH stretching. In the FTIR spectrum of Curcumin, the functional groups such as hydroxyl group, carbonyl group and the ethylene group showed peaks at 3506 cm^{-1}, 1729 cm^{-1} respectively [1]. For the Curcumin incorporated nanoparticles, the peaks corresponding to these functional groups were observed at 3311.14 cm^{-1}, 1709- cm^{-1} respectively, which indicates that the major peaks of Curcumin were retained in the case of Curcumin incorporated nanoparticles also. In the spectrum of Curcumin, the peaks at 987 cm^{-1} indicated the bending vibrations of C=C bond of alkanes group [2]. In the case of Curcumin incorporated nanoparticles also, these peaks were observed in the same region, 997cm-1. Both Curcumin and Curcumin incorporated starch nanoparticles showed a peak around 1150-1076 cm^{-1}, which corresponds to the C-O stretching frequency of ether group in Curcumin [4, 5]. The peak intensity was almost similar for nanoparticles loaded Curcumin and pure Curcumin. The absorption around 1500-1400 cm^{-1} indicated the –C-O elongation frequency of –OH groups in Curcumin and Curcumin incorporated nanoparticles [2]. In pure Curcumin absorbance at 2919 shows CH stretching of alkanes or alkenes which present in Curcumin loaded nanoparticles at absorbance 2923cm^{-1}. [6, 7]

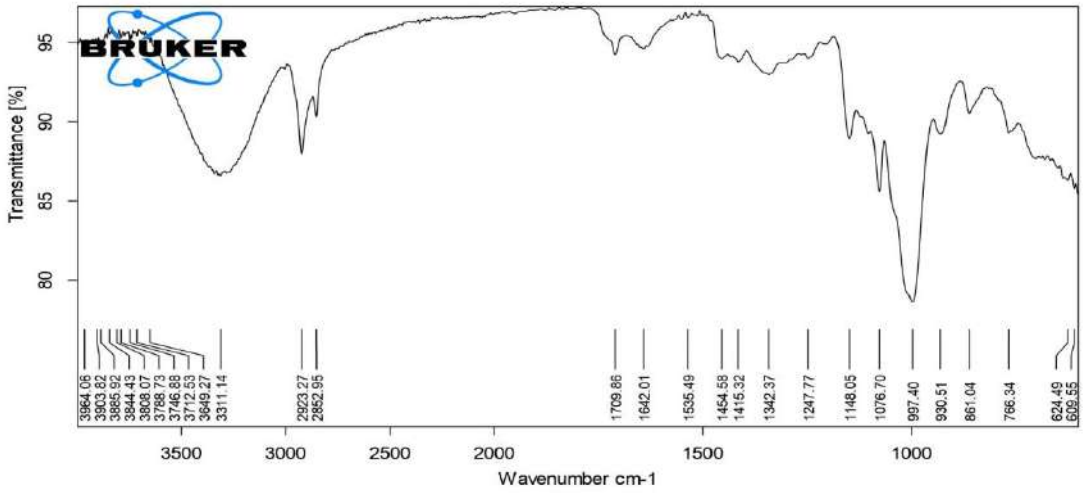


Fig. 16: FT-IR spectra of Curcumin loaded nanoparticles

6.3.3 Optimization of Particle size

The Z-average value for Curcumin loaded nanoparticles was found to be 242 ±10.01 nm and the PDI was reported to be 0.550±.05. Particle size is mostly depend on the amount of stirring speed during addition of starch suspension for drug loading, reaction media, concentration of surfactant.

i. Effect of Stirring speed particle size.

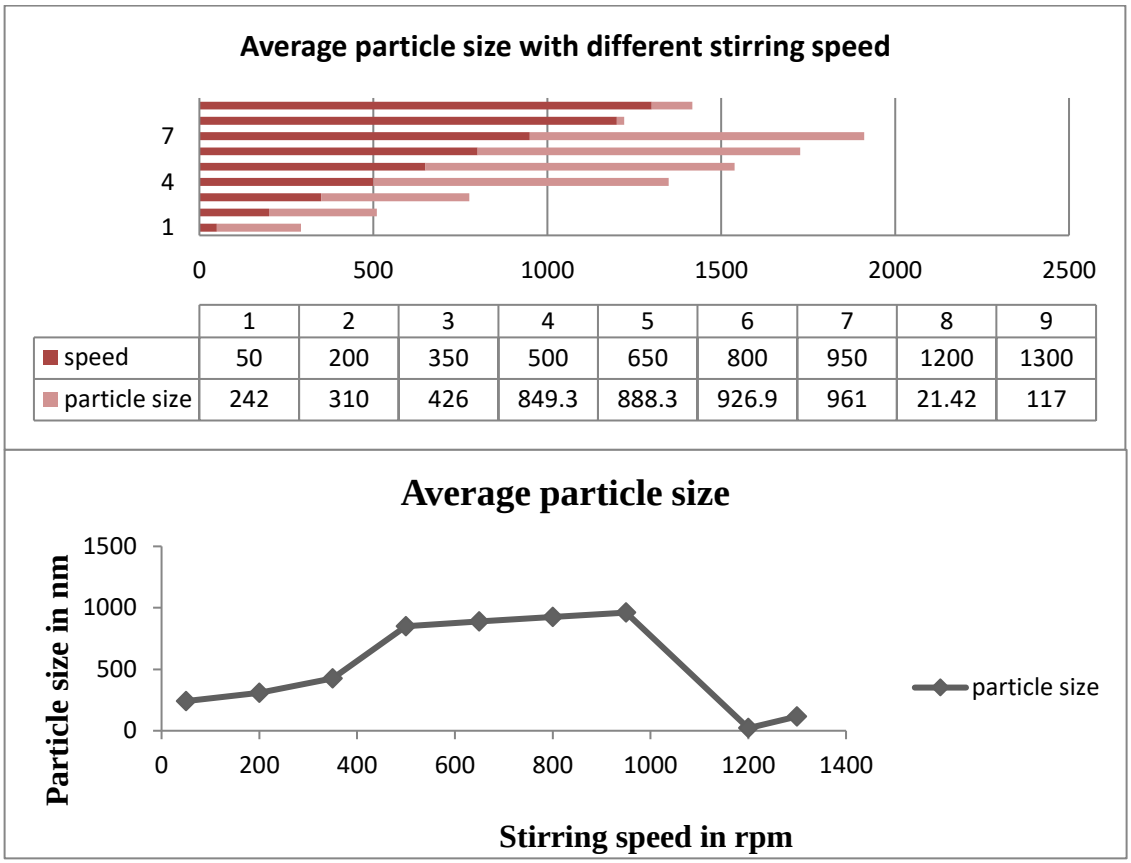
The effect of stirring speed of magnetic stirrer during drug loading of Curcumin into starch nanoparticles on particle size of the Curcumin loaded nanoparticles were studied by stirring the process at different speed (fig 15).When stirring speed of magnetic stirrer increases from 50 rpm to 1000rpm particle size of the Curcumin loaded nanoparticles also increases and at high speed more than 1000 rpm it gets breaks and scattered giving different peak of particle size in zeta size analyzer). At 50 rpm it shows average particle size of 117nm while at 1000rpm it shows particle size more than 1000nm. Below 50 rpm the drug loading efficacy is very low so we used 50 rpm as optimized speed.

ii. Effect of reaction media

The smallest mean particle sizes of Curcumin loaded starch nanoparticles were obtained when oleic acid/ethanol micro emulsion was used as the reaction medium. This was due to the deep penetration of oleic acid into the surfactant with its longest alkyl chain length and smallest lipophilic domains and thus resulted in the formation of the smallest mean particle sizes of starch nanoparticles by controlling the droplet size [8].

iii. Effect of concentration of surfactant

The mean particle diameter of Curcumin loaded starch nanoparticles prepared in oleic acid/ethanol micro emulsion reaction medium with 0.8 × 10−3Mof Tween 80 appeared to be smaller (242nm) as compared to sample without surfactant. The presence of optimum concentration of surfactant in oleic acid/ethanol micro emulsion reduced the surface tension between the starch nanoparticles, which had prevented the coalescence between starch nanoparticles in the micro emulsion system and led to the formation of smaller particle sizes. In contrast, Curcumin loaded starch nanoparticles of larger particle sizes were formed without the presence of surfactant in the micro emulsion system [8].



	1	2	3	4	5	6	7	8	9
speed	50	200	350	500	650	800	950	1200	1300
particle size	242	310	426	849.3	888.3	926.9	961	21.42	117

Fig 17: Average particle size with different rotating speed

6.3.4 Optimization of drug loading efficacy

The maximum loading efficacy of Curcumin onto starch nanoparticles was found to be 58.33% with drug loading capacity 97.22%, when we used oleic acid/ethanol as reaction media (method1i.e precipitation in water-in-oil micro emulsion). Loading efficacy of Curcumin is more in method1 as compared to the method 2 (26.43%).

i. Effect of Reaction media

The micro emulsion reaction media of oleic acid and ethanol have been shown to enhance the loading efficiency of curcumin onto starch nanoparticles due to the presence of lipophilic domains of the micro emulsion system which could dissolve the hydrophobic Curcumin molecules [6, 7]. Lipophilic domains

(hydrophobic) were formed due to the presence of oil phase (oleic acid) in the system. Preparation of Curcumin loaded starch nanoparticles in oleic acid/ethanol micro emulsion reaction medium gave rise to the highest loading efficiency as the highly hydrophobic nature of oleic acid (C18) would enable Curcumin molecules to be more soluble as compared to other reaction medium [8].

ii. Effect of Types of Surfactant

The effect of types of surfactant on the loading efficiency of Curcumin onto starch nanoparticles were studied by using two types of nonionic surfactant, namely, sorbitan monostearate (Span 60) and polysorbate 80 (Tween 80) (Fig 16). The concentrations of both Span 60 and Tween 80 were $0.8 \times 10{-}3$ M. Nonionic surfactants were used in this study because they are not ionic strength dependence and they are nontoxic in nature [9, 10]. Tween 80was shown to be the more effective surfactant as compared to Span 60 for loading of Curcumin onto starch nanoparticles as evidenced by the higher loading efficiency (58.33%) obtained when Tween 80 was used (Fig 16). The higher loading efficiency could be due to a more stable micro emulsion formed in the presence of Tween 80 as it was more soluble in water as compared to Span 60 [11, 12]. The hydrophilic polyesters groups of Tween 80 rendered them water soluble, whereas Span 60 was comparatively more hydrophobic and only soluble in hot water. Tween 80 surfactant formed strong hydrogen bonds between its oxyethylene groups and hydroxyl groups of water molecules resulting in a stable water-in-oil microemulsion system and enhanced Curcumin loading

efficiency. Besides, the longer hydrophobic chain length of Tween 80 (C_{64}) as compared to Span 60 surfactant (C_{24}) resulted in deeper penetration of the alkyl chain of the Tween 80 in the oleic acid oil (hydrophobic domains), which promoted the formation of a stable microemulsion system and enhanced the loading efficiency of Curcumin onto starch nanoparticles.

iii. Effect of Oil/Ethanol Ratio.

The effect of oil/ethanol volume ratios (v/v) on the loading efficiency of Curcumin onto starch nanoparticles was shown in Fig 17. It was found that as the ratio of oleic acid (oil phase) to ethanol increased, the loading efficiency of Curcumin onto starch nanoparticles decreased. This decrease was due to the destabilization of oleic acid/ethanol micro emulsion system by the excess oil volume which resulted in the formation of an inhomogeneous and cloudy solution [13].The optimum ratio of oil to ethanol (1: 5) had resulted in the highest loading efficiency of Curcumin onto starch nanoparticles. This could be attributed to the role of ethanol as a cosurfactant which have reduced the interfacial tension of oleic acid/ethanol micro emulsion and improved the interactions of Tween 80 surfactant monolayer with water and oil at the interface of oleic acid/ethanol micro emulsion system and consequently the formation of a stable micro emulsion [14]. Besides, ethanol also served as a co solvent to enhance the dissolution of Curcumin and Tween 80 surfactant in the oleic acid/ethanol micro emulsion reaction media and thus improved loading efficiency of Curcumin onto starch nanoparticles. Similar observations have been reported by other researchers [15, 16], where their studies showed that

most of the surfactants alone could not produce stable micro emulsion system and the addition of solvent such as alcohol was required to facilitate the formation of a stable micro emulsion system.

iv. Effect of Surfactant Concentration.

The concentration of surfactant in micro emulsion system was observed to affect the loading efficiency of Curcumin onto starch nanoparticles. The highest loading efficiency of Curcumin (58.34%) was achieved when Tween 80 was used as a surfactant in the micro emulsion system, whereas the lowest loading efficiency of Curcumin was obtained when no any surfactant was used in the micro emulsion system. This was due to the lower interfacial tension between oleic acid (oil phase) and water in the presence of surfactant in the micro emulsion system. This led to the formation of a stable micro emulsion system and facilitated a higher loading of Curcumin onto starch nanoparticles [17, 18].

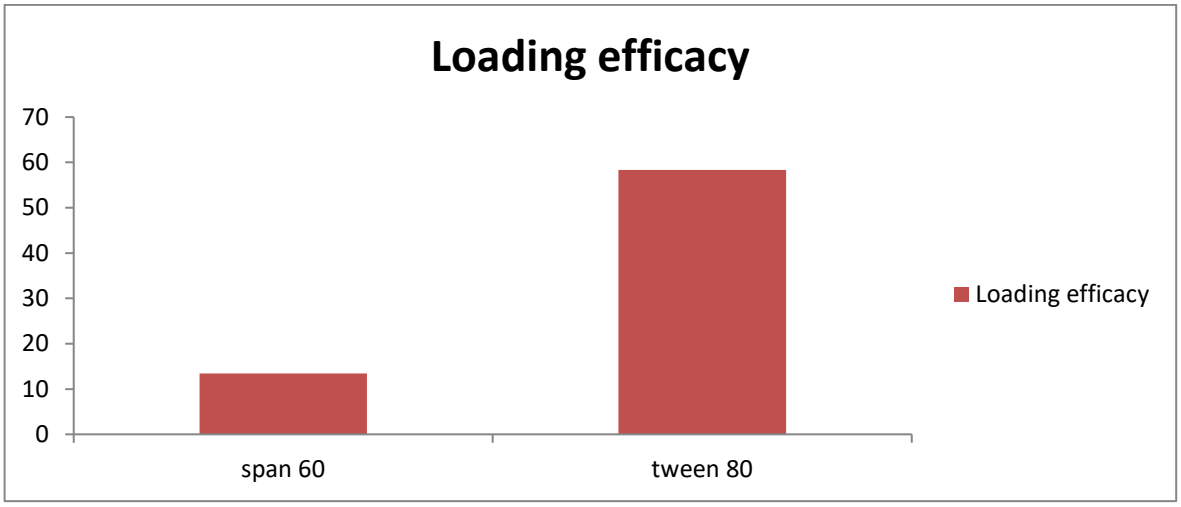


Fig 18: Effect of Types of Surfactant in loading efficacy

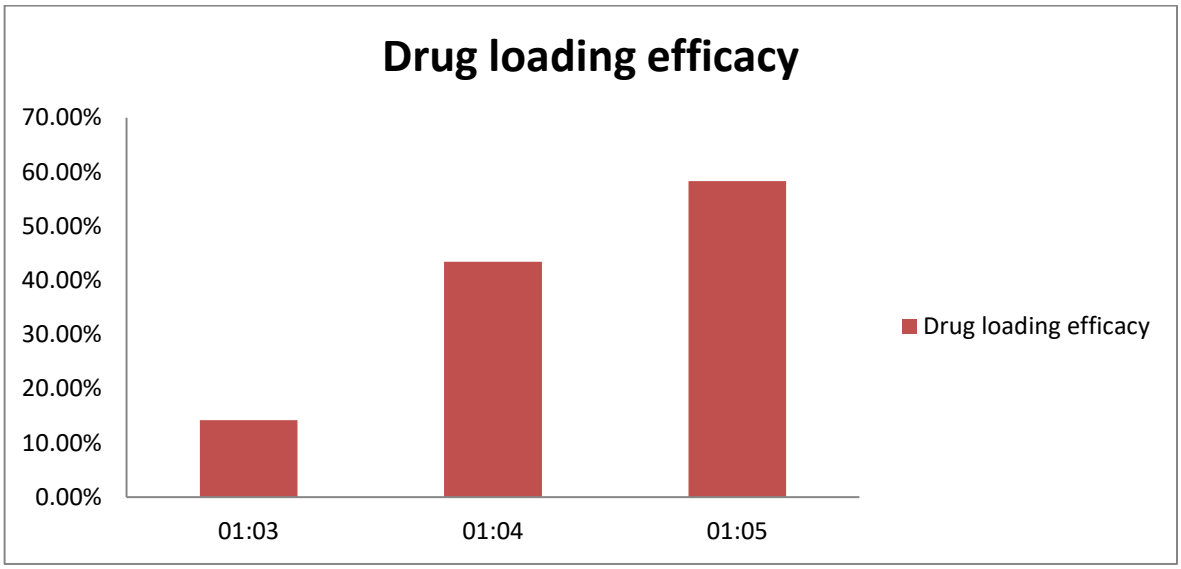


Fig 19: Effect of oleic acid/ ethanol ratio in drug loading efficacy

6.4 Curcumin Release Studies.

The Curcumin loaded starch nanoparticles which were prepared in oleic acid/ethanol micro emulsion reaction medium in the presence of 0.8×10^{-3} M Tween 80 surfactant were used for release studies. The release studies were performed in buffer solution at pH 7.4 at 37 ± 0.5^0C as a function of time with continuous stirring over 24hr. Figure 9 shows the swelling behavior of the starch nanoparticles and the release profile of Curcumin from starch nanoparticles. The release characteristics of Curcumin from starch nanoparticles were observed to be dependent on the swelling behavior of the starch nanoparticles. Starch nanoparticles are very hydrophilic with plenty of OH group starch nanoparticles swelled due to absorption of the buffer solution and Curcumin in the swollen part of starch nanoparticles were diffused out from the starch nanoparticles gradually. Starch nanoparticles were observed to afford a sustained release of Curcumin without initial burst release of Curcumin being observed. In the first 8h, starch nanoparticles showed a swelling ratio of 21.73, and a total of about

25.3% Curcumin was released gradually out from starch nanoparticles. As the swelling ratio of starch nanoparticles increased to 22.3, about 54% of Curcumin had been released within a period of 24 h. and in 32 hours swelling ration reached 23 and about 84.3% of Curcumin get released. After trial for 3 times average percentage of Curcumin release and swelling index is showed in table 17.

Table 17: Swelling ratio of starch nanoparticles and release profile of Curcumin from starch nanoparticles as a function of time.

Time (hour)	cumulative % of Curcumin Released	Swelling index
0	**0**	**0**
1	**0.6**	**6.3**
2	**3.6**	**10.4**
4	**6.4**	**19.13**
6	**12.3**	**21.2**
8	**25.3**	**21.73**
24	**54.3**	**22.3**
32	**84.3**	**23.83**

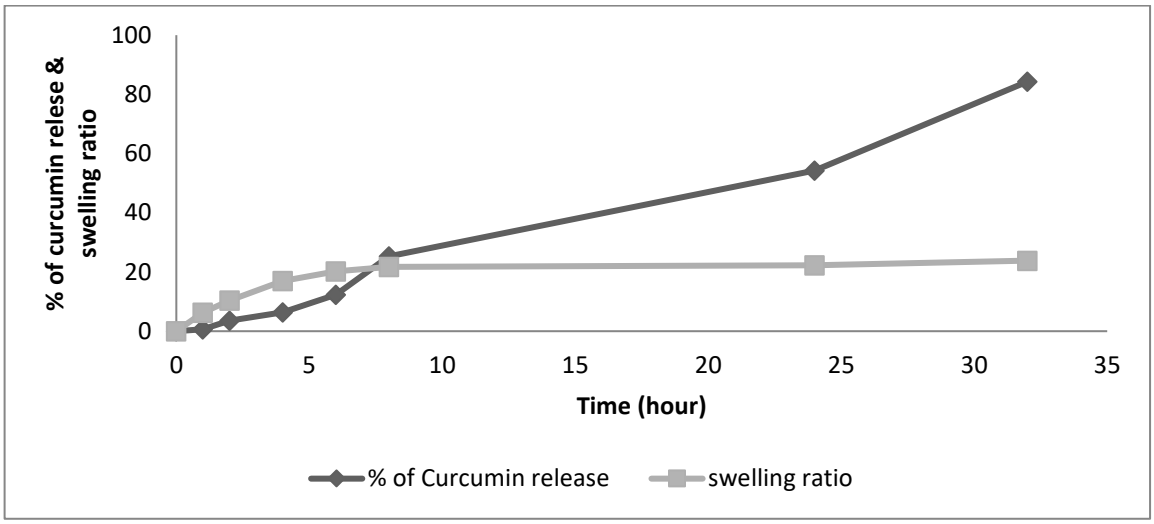


Fig 20: Swelling ratio of starch nanoparticles and release profile of Curcumin from starch nanoparticles as a function of time.

Table 18: Trial 1- for the study of release of Curcumin from Curcumin loaded starch nano particle

Time (hour)	cumulative % of Curcumin Released	Swelling index
0	0	0
1	0.5	6.3
2	3.6	10.4
4	6.0	16.9
6	13.0	20.2
8	25.1	21.80
24	52.6	22.3
32	84.3	23.7

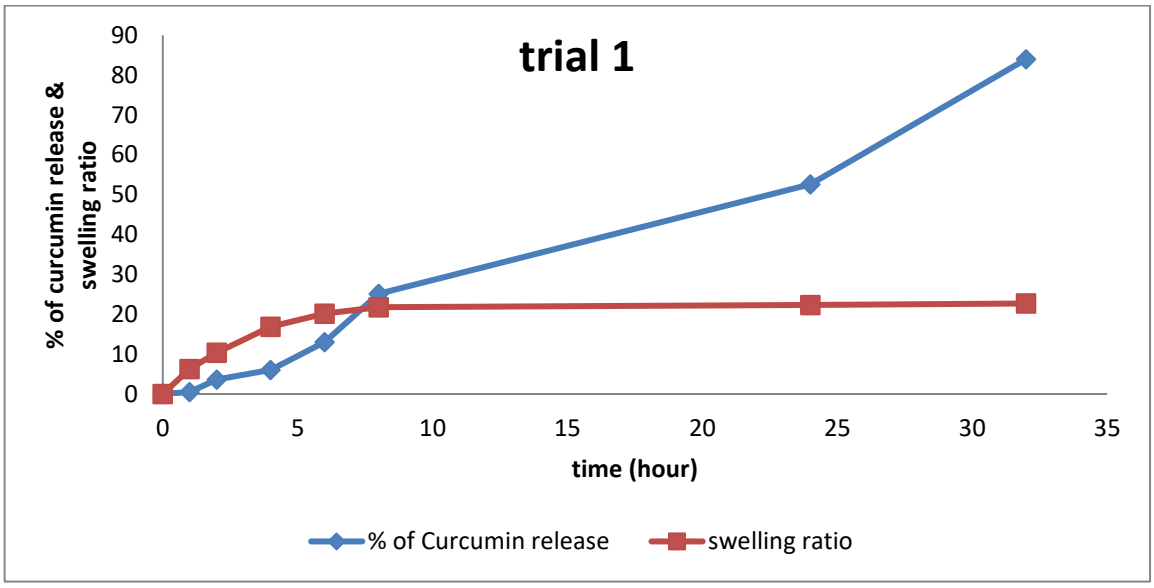


Fig 21: Swelling ratio of starch nanoparticles and release profile of Curcumin from starch nanoparticles as a function of time, Trial 1

Table 19: Trial 2- for the study of release of Curcumin from Curcumin loaded starch nano particle

Time (hour)	cumulative % of Curcumin Released	Swelling index
0	0	0
1	0.8	6.9
2	4.2	10.9
4	8.0	17.7
6	13.0	20.6
8	27.2	22.0
24	59.0	22.6
32	87.0	23.0

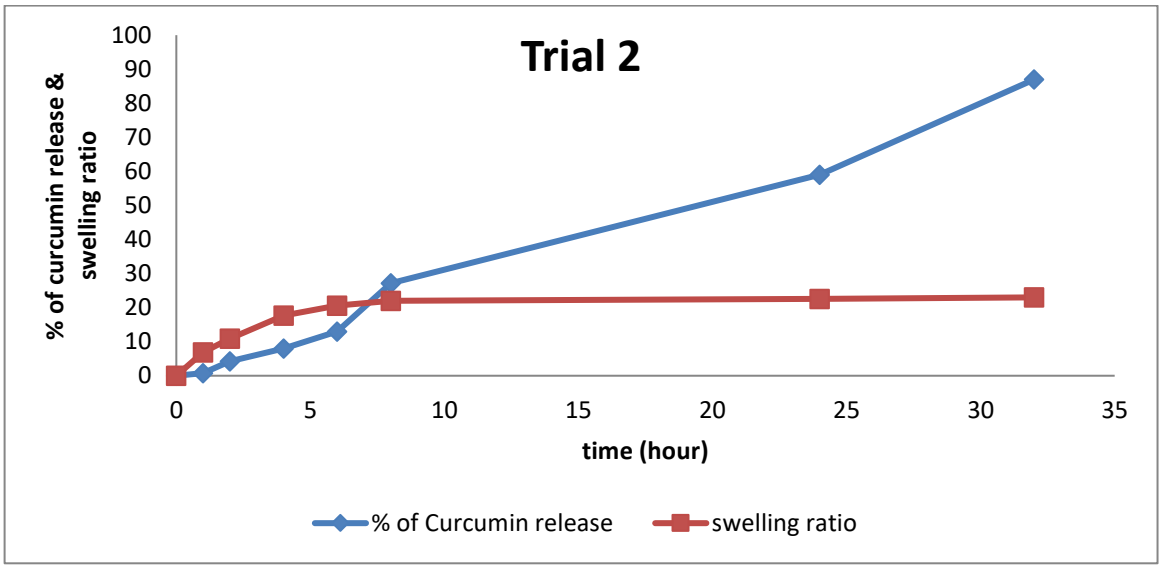


Fig 20: Swelling ratio of starch nanoparticles and release profile of Curcumin from starch nanoparticles as a function of time, Trial 2

Table 22: Trial 3- for the study of release of Curcumin from Curcumin loaded starch nano particle

Time (hour)	cumulative % of Curcumin Released	Swelling index
0	0	0
1	0.34	5.7
2	2.90	9.9
4	5.30	16.5
6	11.00	19.9
8	23.06	21.4
24	51.4	22.0
32	82.00	22.8

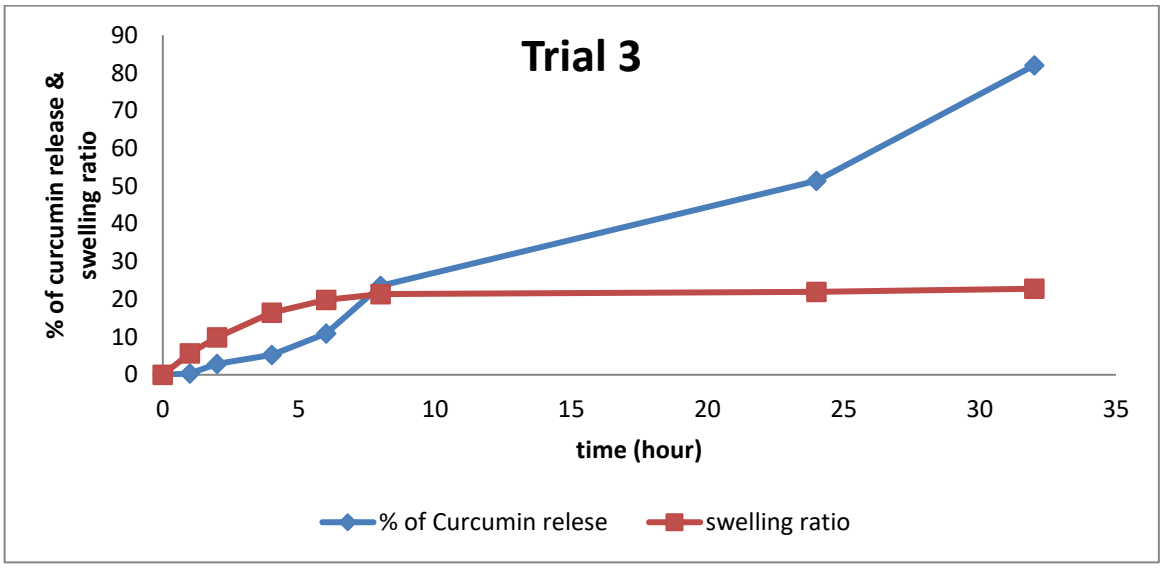


Fig 23: Swelling ratio of starch nanoparticles and release profile of Curcumin from starch nanoparticles as a function of time, Trial 3

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CHAPTER 7- SUMMARY & CONCLUSION

The study was conducted with the aim to extract and characterized starch; extracted from kumol chawl or soft rice of Assam and to prepare Curcumin loaded nanoparticles. The kumol chawl starch was successfully extracted with yield of 25% and particle size of 514 nm with PDI 1. Soft rice starch differed from traditional rice starches significantly in its chemical composition, rheological properties, particle size, and fine structure. We get starch with Low amylose content and higher swelling power.

Curcumin was successfully loaded in the starch by using a simple nanoprecipitation process in a micro emulsion system and Curcumin loaded nanoparticles has particle size 242 nm with PDI 1. Compatibility testing of drug and starch was done by using FT-IR. The loading performance can be optimized with a good choice between reaction medium, type and concentration of surfactant, oleic acid / ethanol ratio, and Curcumin concentration. Curcumin loading using 2.5 × 10−5M curcumin at ratio 1: 5 (v / v) oleic acid to ethanol micro emulsion system with 0.8 × 10−3M Tween 80 surfactant and time 2 h of drug loading gave 58.33% drug loading efficacy. In the in-vitro release study it was observed that curcumin loaded starch nanoparticles provides a sustained release of Curcumin by releasing 87% of Curcumin in 32 hours. The in-vitro release study shows that release of Curcumin from the nanoparticles depend on the swelling ration of the starch nanoparticles.

This loading of Curcumin on to the starch enhanced the solubility of curcumin in aqueous medium as free Curcumin are poorly soluble in aqueous medium. It can increase the bioavailability of the Curcumin as well as it can bypass the first pass metabolism by liver.

This study suggested that starch nanoparticles have the potential to be used as sustained release nanocarriers of Curcumin. Further study on in-vivo effect of Curcumin loaded nanoparticles in myeloma cell line could not be completed in this study due to shortage of

time but it can be conducted to study its use as anticancer targeting drug with higher modifications.

1. **Poster Presentation**

   a. Presented a Scientific Poster entitled "**Investigation of Adulterants and Curcumin Content in Different Marketed Brands of Turmeric Powder"** at two days national seminar on "current research in drug discovery and development"; Dibrugarh University, 13th and 14th November, 2019.

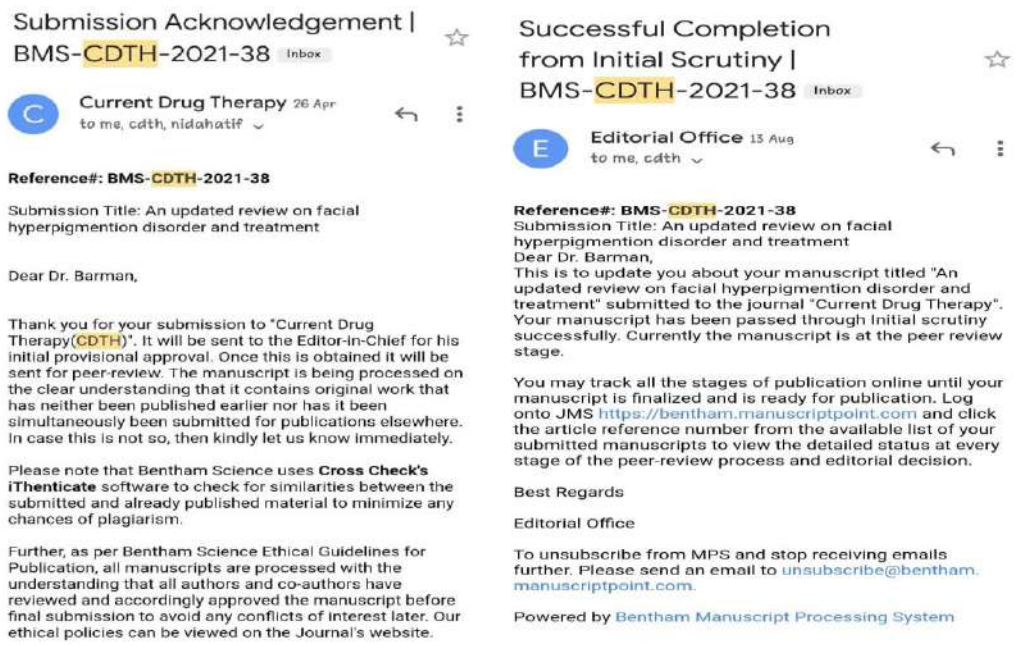
   b. Presented a poster entitled "**Functionalized Lipid Polymer Hybrid Nanoparticle mediated targeted Drug Delivery**" at two days national seminar on "PharmaNanotech-2018"; Dibrugarh University, 23rd and 24th November, 2018.

2. **Review Article Published**

   a. Bharali, A., Deka, B., Sarma, H., Sarma, S., Ahmed, A., Bhattacharjee, B., Das, G., Das, B., Upadhyaya, **M., Phukan**, M. and Gogoi, B., 2021. Integrating Recommendations to Improve Treatment Outcomes in the Clinical Management of Allergic Conjunctivitis. *Pharmaceutical and Biosciences Journal*, pp.22-40.

3. **Review Article Under Process:**

   a. The article **An updated review on facial hyperpigmentation disorder and treatment** is under review process in **Current Drug Therapy** with corresponding author Kamallochan Barman and co-author: Bhupen Kalita, **Mayuri Phukan**, Robina Jidung and Payal dasgupta.



Submission Acknowledgement | BMS-CDTH-2021-38 Inbox

Current Drug Therapy 26 Apr
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Submission Title: An updated review on facial hyperpigmention disorder and treatment

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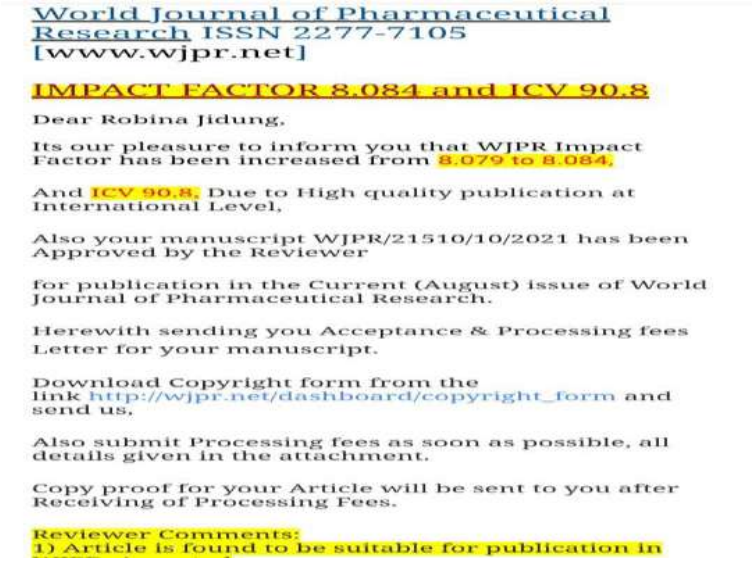
b. The article **Nanosponges an Advance Technique of Drug Delivery System: A Review** is accepted in **World Journal of Current Pharmaceutical Research** with corresponding author Robina Jidung and co-author: Bhupen Kalita, Trishna Das, Kamallochan Barman and **Mayuri Phukan.**



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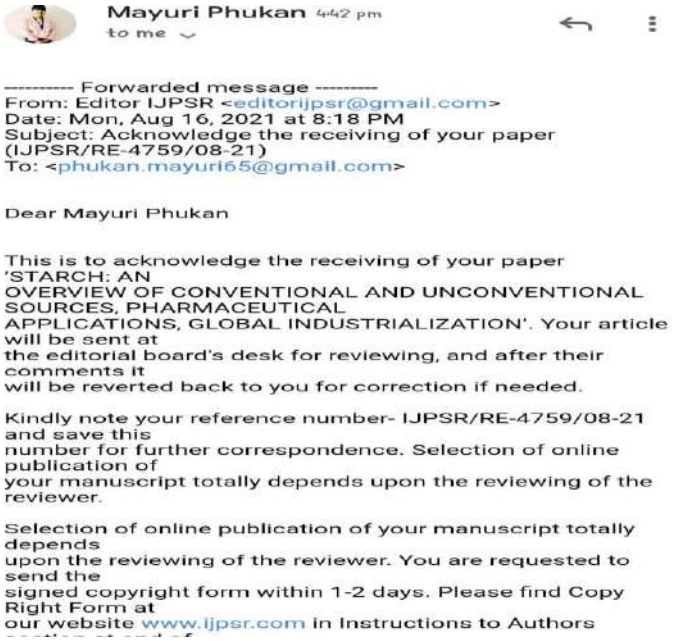

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