EXTRACTION OF ALLICIN FROM GARLIC FOR FORMULATION AND DEVELOPMENT OF BILAYER TABLET FOR ANTIHYPERTENSIVE ACTIVITY

DISSERTATION SUBMITTED TO
Assam Science and Technology University (ASTU), Guwahati, Assam

FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTER OF PHARMACY.

Under the supervision of
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CERTIFICATE FROM THE PRINCIPAL

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I wish him all success in life.

Prof (Dr.) Gouranga Das

Principal

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CERTIFICATE FROM THE GUIDE

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled **"EXTRACTION OF ALLICIN FROM GARLIC FOR FORMULATION AND DEVELOPMENT OF BILAYER TABLET FOR ANTIHYPERTENSIVE ACTIVITY"** a bonafide and genuine research work carried out by me under the supervision of **Dr. Bhanu Pratap Sahu**, Associate Professor, Department of Pharmaceutics, ***Girijananda Chowdhury Institute of Pharmaceutical Science, Hatkhowapara, Azara, Guwahati-17.*** The work embodied in this thesis is original and has not been submitted for the award of degree, diploma, associateship or fellowship of any other university or institution.

Date: **Toufikananda Rabha**

Place: **GIPS Guwahati**

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This dissertation is the end of journey in obtaining my post graduation degree. So,before the beginning of the project ;I would like to express my deep sense of gratitude to all the teachers to whom i am obliged.At the very onset, I would like to offer my sincere gratitude to my esteemed guide, Dr Bhanu P Sahu associate professor of Department of Pharmaceutical Sciences ,GIPS and Dr. Sheikh Sofiur Rahaman assistant Professor,GIPS who guided me till the accomplishment of work.I will remain indept for being humble and patience through out my work.

I am extremely grateful to Dr.Gouranga Das , principal of GIPS.I am very much grateful to all teachers of GIPS, for their guidance helps throughout my course and also extent my heartiest thanks to my friends for their constant inspiration.

Words cannot express my gratitude for my Parents for making me capable of reaching this height of education. They always stood beside me through good times and bad times, through suffering and happiness. Without them, I would never have been succeeded in achieving my goals.

CONTENTS

Lists of Tables

Abbreviations

AgI-Angiotensin I

AgII- Angiotensin II

UAE-Ultrasonic assisted extraction

NF-KB- Nuclear factor kappa light chain enhancer of activated B cells

PVP-Polyvinyl pyrolidone

MCC- Microcrystalline cellulose

HPMC-Hydroxypropyl methyl cellulose

Abstract

Its has been known that garlic have many health benefits and people have been using it traditionally for curing various disease.It has been found that garlic contains allicin which is the most active components of all other constituents of garlic.Garlic have antimicrobial , antioxidant ,antifungal and antihypertensive properties. Since, allicin is not directly present in garlic it has to undergo chemical reaction to produce allicin from alliin .In this study ultrasonicated assisted extraction is used which is cost effective and environmental friendly.As we known hypertension is common now a days which hamper the quality of life.So to treat hypertension various conventional dosage form are available in the market which requires frequent administration for maintaining the therapeutic plasma level of drug and have some side effects. As allicin have antihypertensive properties it can be formulated into bilayer tablet.Bilayer of allicin is formulated for immediate release as well as for sustained release for maintaining the plasma level for long period of time.Thus, improves the quality of life for the patients.

CHAPTER -1

INTRODUCTION

1.INTRODUCTION.

Conventional dosage forms are accused of repetitive dosing and unpredictable absorption window that cause wide range of fluctuation in drug concentration in the blood stream and tissues with subsequent undesirable toxicity and poor therapeutic efficiency.This dynamic such as repetitive dosing and erratic absorption led to the concept of controlled drug delivery systems. Formulation of layers from different polymers allows manipulation over more than one rate-controlling polymer, thus enabling different types of drug delivery of one or more drugs.

1.1 BILAYER TABLET.

Developed and developing countries are moving towards combination therapy for treatment of various diseases and disorders requiring long term therapy such as hypertension and diabetes. The problem of dose dependent side effects is minimized by combination therapies and is advantageous over monotherapy. From last few years, interest in developing a combination of two or more active pharmaceutical ingredients in a single dosage form has increased in pharmaceutical industry. Bi-layer tablets can be a primary option to avoid chemical incompatibilities between APIS by physical separation. Bi-layer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances and also for sustained release tablet in which one layer is immediate release as initial dose and second layer is maintenance dose.

Controlled release dosage form is a term used to describe the dosage forms having drug release features based on the time, course and/or location and which are designed to accomplish therapeutic or convenience objectives which are not offered by conventional release dosage forms. However, controlled release dosage form does not provide a rapid onset of action of drug entity.Whereas immediate release drug delivery

system is intended to disintegrate rapidly and exhibits instant drug release. However,it is also associated with fluctuations in drug plasma levels,which leads to reduction or loss in drug effectiveness or increased incidence of side-effects. Therefore, to compensate the dip in drug plasma concentration due to metabolism and excretion, it is necessary to administrate the dosage form several times per day.A relatively constant plasma level of a drug is often preferred to maintain the drug concentration within the therapeutic window.However, it is difficult to achieve, especially for once-daily dosage forms.

Bilayered tablet is suitable for combination therapy,i.e., for sequential release of two different drugs, separate two incompatible substances and also for sustained release dosage form in which one layer is immediately released as a loading dose and second layer act as a maintenance dose.On the basis of these considerations, the bilayered tablet have been specially developed to provide two different release rates or biphasic release of a drug from a single dosage form in which one layer is formulated to obtain immediate release effect of the drug, with the aim of reaching a high plasma concentration in a short period of time while the second layer is designed as sustained released layer, which provides effective plasma concentration by a maintenance dose of drug for an extended period of time. The design of bilayered tablet dosage form holds many advantages over conventional dosage forms such as a reduction in frequency of drug administration, improved patient compliance, reduction in drug level fluctuation in blood and quantitative reduction in total drug usage when compared with conventional therapy.

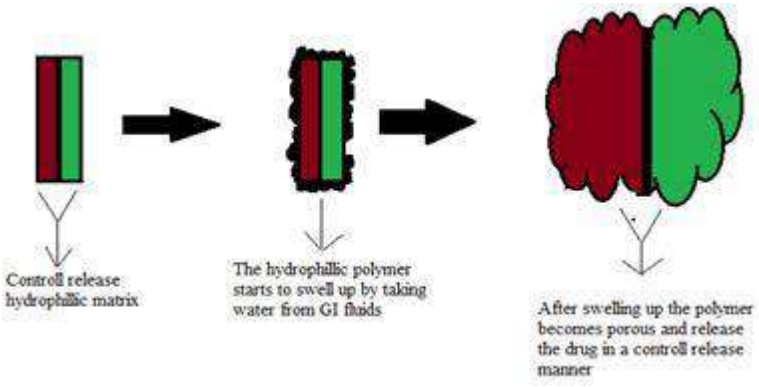
1.11 Various approaches used in bi-layer tablet

A. Floating drug delivery system

They are designed to have a low density and thus float on the gastric contents after administration until the system either disintegrates or the device absorbs fluid to the point where its density is such that it loses buoyancy and can pass more easily from the stomach with a wave of motility responsible for gastric emptying.The bi-layer tablet is designed in such way that, one layer gives immediate dosing of the drug which gives faster onset of action while other layer is designed as a floating layer which forms a gastro retentive system.

B. Polymeric bioadhesive system

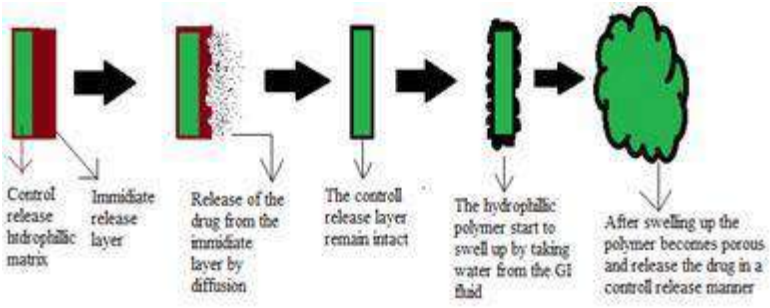
These are designed to imbibe fluid flowing administration such that the outer layer becomes a viscous, tacky material that adheres to the gastric mucosa/mucus layer. This should encourage gastric retention until the adhesive forces are weakened. These are prepared as a one layer with immediate dosing and other layer with bioadhesive property.



C. Swelling system

These are designed to be sufficiently small on administration so as not to make ingestion of the dosage form difficult (e.g., less than approximately 23 mm long and less than 11 mm wide for an oval or capsule-shaped tablet whereas 10-12 mm in

diameter for round tablets). On ingestion they rapidly swell or disintegrate or unfold to a size that precludes passage through the pylorus until after drug release has progressed to a required degree. Gradual erosion of the system or its breakdown into smaller particles enables it to leave stomach. The simple bi-layer tablet may contain an immediate release layer with the other layer as extended release or conventional release or both as controlled release layer.



1.12 Advantages of bilayer technology

- Bilayer tablets can be designed in such manner as to modify the release as either of the layers can be kept as extended and the other as immediate release.
- The Bi - layer tablet is suitable for preventing direct contact of two drugs and thus to maximize the efficacy of a combination of two drugs.
- Separation of incompatible components. Prospective use of single entity feed granules. Greatest chemical and microbial stability over all oral dosage forms. Objectionable odour and bitter taste can be masked by coating technique. Bilayer execution with optional single – layer conversion kit.
- Low cost compared to all other dosage forms.
- Offer greatest precision and least content uniformity.
- Easy to swallow with least hang up problems.

- Flexible concept.
- Suitable for large scale production.
- Lighter and compact. Patient compliance is improved leading to improve drug regimen efficiency.
- They are a unit dosage form and offer the greatest capabilities of all oral dosage forms for the greatest dose precision and least content variability. Patient compliance is improved fewer daily dose are required compared to the traditional delivery system.

1.2 ALLICIN

Garlic (*Allium sativum L.*) belongs to the *Alliaceae* family has been cultivated around the world for centuries and used for both culinary and therapeutic properties . Garlic exhibits high biological activity when the fresh garlic is cut or crushed that attributed to sulphur compounds and particularly to thiosulphates and it is considered to be one of the best disease-preventive foods . These compounds are responsible for garlic's therapeutic properties, such as cardiovascular disease hypertension, cholesterol, and diabetic . Organosulfur compounds like alliin, allicin, diallyl disulfide, and *S*-allycysteine are found in garlic while allicin is the most dominant among others active compound . The primary precursor for the flavor of garlic is allicin (C6H10S20) with the molecular weight of 162.273 g/mol, density of 1.1 g/cm3 and boiling point of 248.6 °C at 1 atm. The appearance of allicin is colorless liquid. Allicin or diallyl thiosulfinate in the scientific name is the most abundant of thiosulfinate that found in garlic bulb, typically accounting 70% w/w of total thiosulfinate . Although allicin is the main bioactive compound produced by garlic, it is not physically present in a whole garlic bulb. Allicin is only formed when the cell wall membrane of garlic bulb was ruptured,

or the simple word is crushed, chopped, or cut. The enzymatic reaction through alliinase produces allicin from alliin Figure 1 shows the enzymatic reaction of alliin and biosynthesis of allicin.

Upon the cell wall broken, alliin hydrolyzed by enzyme alliinase, and in this case, alliin reaction leads to the production of allyl sulfenic acid, pyruvic acid, and ammonia. Two molecules of allyl sulfenic acid condense spontaneously, by removing water become one molecule of allicin. A study by Touloupakis and Ghanotakis shows the allicin yielded approximately 2.5mg/g of fresh garlic or about 5-20 mg per clove. Aside of enzymatically process allicin can be prepared chemically , and biologically . Nonetheless, allicin at the molecular level is challenging to carry out due to its chemical instability and decomposes to others organosulfur compound such a diallyl sulfide, diallyl disulfide, and ajones.

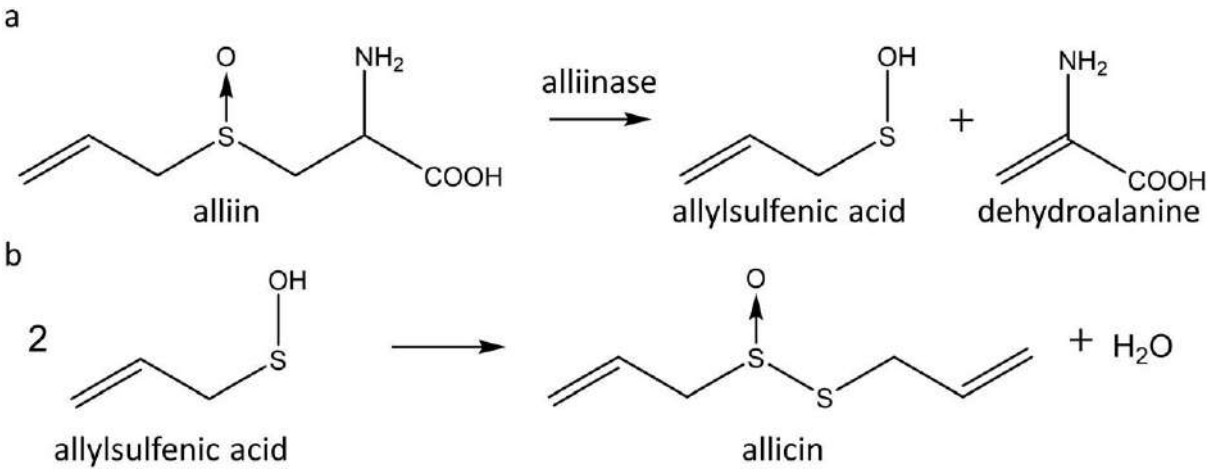


Figure 1. Biosynthesis of Allicin from Alliin.

1.21 Allicin as antihypertensive agent:

Allicin acts by reduction in vascular resistance and thereby fall in total peripheral resistance ,Contributing substantially to antihypertensive action by activating nitric oxide synthase which convert agrginin to nitic oxide.Nitric oxide is a vasodialator which relaxes the inner muscle of blood vessel.Allicin inhibit angiotensin converting enzyme which convert AgI to AgII .Ag II constrict the muscle of blood vessel leading to narrowing therefore decrease in AgII will lead to somewhat fall in blood pressure.Decrease in angiotensin II will lead to decrease in production of aldosterone which inturn decrease reabsorption of sodium and water from distal convulated tubule therefore decrease plasma volume.

Red blood cells will produce hydrogen sulphide when provided with allicin which contain sulphur group.The sulphur contribute with RBC to increase production of hydrogen sulphide.

Hydrogen sulphide will bind and activate vascular potassium channel therby resulting in hyperpolarization of vascular smooth muscle cells.

1.22 Other properties of allicin.

Antibacterial properties

Dially sulphide found to be more effective in fighting the campylobacter bacterium.Campylobacter bacterium is one of the most common causes of intestinal infections. It has been understood that allicin have pontential antibiotic activity and Subramanyan et al ,studied the *in vitro* activity of different spices on intestinal bacteria

in healthy and disease and understood that garlic have certain activity towards decreasing the growth of bacteria. Sharma et al, reported *in vivo* property of *Allium sativum* extracts on gram negative and gram positive bacteri of the gastro-intestinal tract and demonstrated its effectiveness in inhibiting the bacteria.It has been seen that Garlic also inhibited bacterial flora, which were resistant to some of the common antibiotics . It has been found that the effect of garlic on enterotoxigenic *Escherichia coli* was prominent. Generally it is known that fresh garlic produced the greatest antimicrobial activity followed by freeze-dried powder. It is showed that temperature and time had major effects on gaining the most active components for inhibition of bacterial growth.Garlic content allicin has been reported to have antimicrobial activity towards Helicobacter pylori which the main cause of gastric ulcer and have been found that helicobacter was resistant to certain antibiotics but was sensitive to allicin content of garlic extract.

Immuno modulator

Garlic helps to enhance the immune system the body and prevent diseases caused due to immune dysfunction. Garlic constituents shows a modulation of cytokine production as mediator of inflammation.The nuclear factor-KB (NF-KB) is a central transcription factor and it has a centralrole in the expression of genes that control immune response. NF-KB is involved in the activation and regulation of key molecules responsible for inflammatory diseases. and it also increases the expression of the genes of some cytokines. The inhibition of NF-KB by garlic was indirectly controlled by a modulation of pro- inflammatory cytokines and anti-inflammatory cytokines.Allicin is known to inhibit TNF-α induced secretion of pro-inflammatory cytokines and chemokines from intestinal epithelial cells and thus reduced inflammation.

Antioxiants

Allicin reacts with free thiol containing enzymes and act as a antioxidant by trapping the free radicals. Allicin scavenge hydroxyl radicals and inhibit superoxide production by phorbol ester-activated human granulocytes. Allicin shows modification of SH-dependent activities, an additional therapeutic property, and inhibitory effect on NO formation.The inflammatory environment in human atherosclerotic lesions resulted in an expression of the inducible form of nitric oxide synthase and lead to the formation of peroxynitrite. A potent oxidant peroxynitrate is formed when the synthesis of large amounts of NO coincides with superoxide production. Peroxynitrite initiate LDL oxidation and promote platelet aggregation.Peroxynitrite is formed when NO is produced in high enough concentrations to compete endogenous superoxide dismutase for superoxide.

Heart Disease

Dially trisulfide content of garlic helps to protect the heart during cardiac surgery and after a heart attack.Hydrogen sulphide gas is known to protect the heart from damage.But it is highly volatile therefore dially trisulfide is the safer way to deliver the benefits of hydrogen sulphide. Cardiomyopathy is the leading cause of death among diabetes patients.It is a chronic disease of myocardium,which is thickened,enlarged and stiffed.Allicin content of garlic have significant potential in protecting hearts from diabetes induced cardiomyopathy. Cardiomyopathy is the leading cause of death among diabetes patients.It is a chronic disease of myocardium,which is thickened,enlarged and

stiffed.Allicin content of garlic have significant potential in protecting hearts from diabetes induced cardiomyopathy.

Although allicin is short-lived and poorly stable, it can easily cross cell membranes due to its hydrophobic nature. Allicin reacts with free thiol groups rapidly in cellular compartments. Alinase converts alliin to allicin at pH 7.0. It can be inactivated by heating or at a pH below 3.5 . Therefore, an enteric-coated formulation has been applied to hamper stomach disintegration of many commercial garlic supplements and protect against allinase enzymes.

CHAPTER-2

LITERATURE REVIEW

Literature review

Ranitha Mathialagan *et al, 2017* extracted allicin from garlic by using ultrasound assisted extraction . In this paper, UAE method was studied for optimisation based on the evaluation of several experimental parameters, namely particle size, extraction time and extraction temperature. The best combination of response function was sliced garlic, at a temperature of 25 °C and 90 min of extraction time with ultrasonic irradiation. Under the optimal conditions, the yield of allicin reached 112 μg/mL.

Yardfon Tanongkankit *et al ,2019* extracted allicin by Ultrasound assisted extraction .According to him UAE at frequency of 45 kHz and 30˚C for 40 min was recommended since it provided the highest yield of allicin. UAE gave 4.8 times greater in the yield of allicin than conventional method .

Harikesh Dubey *et al,* concluded invivo studies on rats to evaluate the antihypertenive activity of allicin .Dexamethaone induced hypertenion rats were given the prepared allicin extract for 2 months and from the results it has been found that allicin treated rats have significant decrease in blood pressure.

Udayakumar *et al*, 2013 have developed bilayer tablet dosage form containing combination of immediate and sustained release layer prepared using "Glibenclamide and Metformin Hydrochloride" respectively for the treatment of Type-11 diabetes mellitus. From this research work it is evident that the formulated bilayer tablet has ability to release the Glibenclamide immediately and Metformin hydrochloride for

longer period of time, which can be used for treatment of type II diabetes mellitus compared to Marketed formulation.

Mohammad Shafiur Rahman *et al*,2007 reported thatAllicin plays a major role in the antiproliferative effect of water-soluble garlic preparations and this effect may be attributed to the ability of allicin to transiently deplete the intracellular gluthathione (GSH) level. The extent of the decrease in GSH levels correlated well with the growth inhibitory activity of allicin.The antiproliferative effect of garlic is also clear. Allyl sulphur compounds are important antitumorigenic agents and diallyl disulfidereduced the size and the number of preneoplastic foci in rats liver induced by AFB1. The protection of garlic against cancer aroused from several mechanisms including the blockage of nitrosamines formation and bioactivation

Darekar *et al* 2015 have formulated Nateglinide sustained release (SR) and immediate release (IR) bilayer tablet by different concentration of Hydroxypropyl methylcellulose (HPMC) and HPMC K 100 M to control the release pattern. The sustained release layer of Nateglinide was prepared by using different grades of HPMC like, HPMC K-100, HPMC along with other excipients by direct compression technique. The immediate release layer of Nateglinide was prepared by Cross carmellose sodium and Sodium starch glycolate. Nateglinide is a poorly water soluble (BCS class 2) ant diabetic drug. Due to the poor water solubility of this drug, its bioavailability is dissolution rate-limited.

Sharmin *et al* 2012 have prepared by direct compression technique incorporating an immediate release layer and a sustained release layer. An immediate release layer was

successfully designed to release the bolus dose instantaneously. Water soluble Xanthan gum, water insoluble Kollidon SR and Eudragit L 100 were used as carriers in the sustained release layer of the matrix tablet. All the tablets were evaluated for thickness, diameter, weight variation, hardness and friability. The *in vitro* drug release was studied for eight hour, first two hours dissolution in acidic medium followed by six hour dissolution in buffer medium. Matrix tablet showed a sustained release rate with a controlled fashion as a function of the quantity of polymer used. The *in vitro* drug release data were fitted with several mathematical models and mean dissolution time along with fractional dissolution time values (T25%, T50% and T 80%) were calculated. Xanthan gum was found to be the most effective rate retarding agent compared to Kollidon SR and Eudragit L 100, when used at same ratio in the formulations.

Chauhan *et al* 2014 have formulateed and evaluate the bilayered tablets containing Ketorolac Tromethamine floating sustained release layer and Omeprazole as immediate release layer in order to produce a single tablet containing two different classes of drugs widely prescribed by doctors to have better patient compliance and reduced side effects. The sustained release layer of Ketorolac Tromethamine was prepared by wet granulation technique using polymers like HPMC K100M and Carbopol 940 in different concentrations. Sodium bicarbonate was used as a gas generating agent. Immediate release layer of Omeprazole was prepared by wet granulation technique using sodium starch glycolate, Crosspovidone and Crosscarmelose sodium as super disintegrants in different concentrations. The results of the evaluation tests indicated that the optimized formulation SF1 showed desired release along with optimum floating lag time and desired floating lag time for the ketorolac layer and good disintegration

time and desired release rate for the omeprazole layer. Also the stability data of the optimized formulation indicated that the formulation showed sufficient stability upon storage.

Yatin N Dholariya *et al***,** Designed and optimized bilayered tablet of Hydrochlorothiazide. *In vitro* dissolution studies were carried out using USP dissolution test apparatus I at 100 rpm, 37 ± 0.5°C. The dissolution medium was simulated gastric fluid adjusted to pH 1.2 with HCl for initial 2 h and later it replaced with pH 6.8 phosphate buffer and dissolution

continued for another 10 h. At different time intervals, samples were withdrawn and filtered using 0.45 μm membrane filter and was further analysed by UV spectrophotometer at 272 nm. *In vitro* study revealed initial burst release up to 30% from the immediate release layer of bilayered tablet; further study was carried out for 12 h to determine release profile of sustained release layer. Drug release from sustained release layer was mainly dependent on polymer concentration. T100% was obtained in 9 h for formulation containing low level of polymer blend. It was reported that drug release was retarded up to 12 h with an increase in concentration of polymer blend of HPMC K4M and HPMC K 100M.

HIROYUKI FUJISAWA *et al***,** quantified allicin with the application of chromatographic . The allicin produced its peak area in good proportion (*r*2) 0.9894) to the quantity loaded onto the column over the range of 0.1-2.0 mg/mL. He reported that there was a good correlation between the amount of authentic allicin and its peak area produced, indicating a high credibility of this method.

MARWA NCIR *et al*,2017 studied in vitro and in vivo studies of Allium sativum extract against deltamethrin-induced oxidative stress in rats brain and kidney. His study showed that the administration of the garlic extract appeared to protect the kidneys and brain due to presence of high antioxidant phytochemicals and properties of female rats from deltamethrin- induced oxidative stress by reducing the intensity of LPO, conjugated dienes and AchE and by enhancing the activities of enzymatic antioxidants.

CHAPTER - 3

OBJECTIVES

AND

PLAN OF WORK

3.1 Objectives

- To extract allicin from raw garlic cloves.
- To make the extracted garlic into powderized form.
- To formulate into immediate release and sustained release bilayer tablet.
- To study the antihypertensive activities of allicin.

3.2 Plan of work

- Literature Review
- Preformulation Studies
- Extraction of allicin from garlic.
- Formulation of Bilayer Tablet of Allicin
- Evaluation of Tablets
    - Pre and Post Compression characterization.
    - Weight Variation
    - Friability
    - Hardness
    - Disintegration
    - Drug Release
- Animal study

CHAPTER- 4

MATERIAL

&

METHODOLOGY

4.1 MATERIAL USED IN THE EXPERIMENT

1	API	Allicin	Extracted from garlic by ultrasonic assisted method
2	Buffering agent	Sodium citrate	GIPS store
3	Diluent	Microcrystalline cellulose	GIPS store
4	Polymer	HPMC E15 PVP	GIPS store
5	Superdisintegrant	Sodium starch glycolate	GIPS store
6	Binder	Xanthum gum	GIPS store
7	Lubricant	Magnesium stearate	GIPS store

8	Glidant	Talc	GIPS store
9	Colour	Iron red	GIPS store

Table-1 List of materials

4.2 EQUIPMENTS USED.

SL. No.	Name	Company
1	Ultrasonic bath	PCiANALYTICS
2	Digital weighing balance	Citizen, Denver Instument
3	Melting point determination	Macroscientific works 10A/UA, Janwahar Nagar, Delhi-11007
4	Tablet compression machine Bilayer	Parle Elizabeth Tools Pvt Ltd
5	Hardness tester	Monsento,Pfizer
6	Friability tester	Roche friabilator

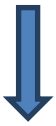
7	Bulk density apparatus	Konark Intrument

4.3 METHODOLOGY:

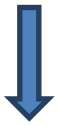
4.31 Extraction of Allicin

Allicin was extracted by ultrasonic assisted extraction method from raw garlic cloves.

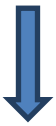
Garlic cloves were crushed using mortar pestle and kept for 1hr in a beaker covered with alluminium foil.



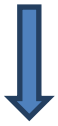
10g of crushed garlic were mixed in 100 ml distilled water



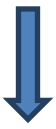
Placed the mixture in ultrasonic bath for 1hr at 25 degree celcius



The mixture was then filtered through watman filter paper.



The filtrate was then allowed to freeze over night.



The freezing filtrate was then allowed to freeze dry in lyophilizer.

4.32 PHYSICO CHEMICAL PARAMETERS

Solubility determination:

A small amount of the extracted allicin powder was taken in a test tube and solubility is observed in 1ml of various solvents like water, methanol, ethanol, phosphate buffer.

4.33 ESTIMATION OF ALLICINE

Preparation of standard calibration curve of Allicin in phosphate buffer pH 7.4 :

100 mg of allicin was weighed accurately and dissolved in 100 ml of phosphate buffer in 100 ml volumetric flask . 10 ml was taken from the stock solution and transferred into 100 ml volumetric flask and diluted up to 100 ml with phosphate buffer. From above standard working solution 1ml, 2 ml, 4 ml, 6 ml, 8 ml, 10 ml was withdrawn and diluted up to 10 ml Phosphate buffer in 10 ml volumetric flask to get concentration of 10 ug/ml, 20 µg/ml ,40 µg/ml, 60 µg/ml,80 µg/ml,100 µg/ml respectively. The absorbance of each solution was measured by UV-visible spectrophotometer at 247nm using phosphate buffer as blank.

4.34 Formulation of Tablet:

The immediate release layer was prepared by using superdisintegrants- sodium starch glycolate and xantham gum as binder and the sustained release layer was prepared by using hydrophilic polymer like HPMC E15 and PVP. The various ratios of the ingredients used are shown in the formulation table, Table-3 . The drug and excipients were mixed using mortar and pastle to get a perfect blend for direct compression into tablets. And the mixture was then evaluated for various pre-compression parameters such as Bulk Density, Tapped Density, Angle of repose, Hausner's Ratio, Carr's Index.

4.35 PRE COMPRESSION PARAMETERS:

Bulk density (Db):

Accurately weighed powder was carefully transferred into graduated measuring cylinder. The power bed was then made uniform and the volume occupied by the powder was noted as per the graduation marks on the cylinder as ml. It is expressed in gm/ml and is calculated using the following formula.

$$Db = M/Vb$$

Where, M - Mass of the powder

Vb - Bulk volume of the powder

Tapped density (Dt):

It is the ratio of total mass of powder to the tapped volume of powder. The graduated measuring cylinder containing accurately weighed powder was manually tapped for 50 times. Volume occupied by the powder was noted. It is expressed in gm/ml and is calculated by following formula.

$$Dt = M/Vt$$

Where, M - Mass of the powder Vt - Tapped volume of the powder

Angle of repose (θ):

The frictional forces in a loose powder can be measured by the angle of repose. This is the maximum angle possible between the surface of a pile of powder and the horizontal plane. Sufficient quantities of Allicin powder were passed through a funnel from a particular height (2 cm) onto a flat surface until it formed a heap, which touched the tip

of the funnel. The height and radius of the heap were measured. The angle of repose was calculated using the formula.

Angle of repose (θ) = tan-1 (h/r)

Where, h – Height of the pile in cm

r – Radius of the pile.

Compressibility index (I) and Hausner's ratio

Carr's index and Hausner"s ratio measure the propensity of powder to be compressed and the flow ability of granule. Carr"s index and Hausner'sratio were calculated using following formula.

$$I = Dt - Db/Dt \times 100$$

Where, Dt –Tapped density of the powder

Db –Bulk density of the powder

$$\text{Hausner's ratio} = Dt/Db = Vb/Vt$$

4.36 Compression of Bilayer tablets:

The bilayer tablets of Allicin were prepared by direct compression method and were evaluated for post compression physical characteristics like hardness, weight variation, drug content and friability.

4.37 POST COMPRESSION PARAMETERS:

Tablet Dimensions:

Thickness and diameter were measured using calibrated digital Vernier callipers. Five tablets of each formulation were picked randomly and thickness and diameter was measured individually .

Hardness test:

The prepared tablets were subjected to hardness test. It was carried out by using Monsanto hardness tester and expressed in kg/cm2.

Friability test:

Tablet friability was tested by Roche friabilator. Pre weighed tablets were given 100 revolutions in 4 min and were dedusted. The percentage weight loss was calculated by reweighing the tablets.

Friability=(initial weight- final weight/initial weight)100

Weight variation test:

20 tablets were selected at random from the lot, weighed individually and the average weight was determined. The percent deviation of each tablets weight against the average weight was calculated. The test requirements are met, if not more than two of the individual weights deviate from the average weight by more than 5% and none deviates more than 10%.

CHAPTER - 5

RESULTS

&

DISCUSSION

5.1 PHYSICO CHEMICAL PARAMETERS

Solubility-

Allicin is soluble in Alcohol, partially soluble in water, freely soluble in methanol and phosphate buffer. Allicin is polar in nature.

Organoleptic properties-

The bulk characteristic of the drug is crystalline, color is light yellow and have a characteristic odor which gives garlic its odor.

5.2 CHARACTERISATION OF FORMULATED TABLETS:

5.21 Pre Compression Parameters:

The extracted allicin powders were mixed with other ingredients with the help of mortar and pestle. The prepared powder were evaluated for angle of repose, bulk density, tapped density and compressibility index, hausner's ratio.For immediate release layer the bulk densities and tapped densities of the powder were found to be in the range of 0.328 to 0.474 gm/ml and 0.410 to 0.488 gm/ml and for sustained release layer it was found in the range between 0.140 to 0.175 gm/ml and 0.188 to 0.237 gm/ml

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The angle of repose of immediate release layer varied from 19.53 to 21.92 and for sustained release layer varied from 18.23 - 21.48 which indicates good flow properties of the powder.

Hausner ratio was found in the ranged between 1.1 to 1.21 for immediate release layer and for sustained release layer it was found in the range between 1.22 to 1.26.

The compressibility index for immediate release layer was in the range of 14.6 to 16.53 and for sustained release layer 13.13 to 16.75 these values indicates that the powder mixture of all batches of formulation exhibited good flow properties. {Table- 5}

5.22 Formulation of Tablets:

Allicin and other ingredients are accurately weight and compressed by direct compression method using 6mm dye. Six formulation are made and it is given in Table-3

5.23Post Compression Parameters:

The tablets were evaluated for Weight variation, Hardness, Friability, Thickness, drug content .Thickness was found to be in the range of 3.5 to 3.7 mm and the hardness of the tablets was in the range of 5.3 to 5.7 kg/cm2 which was sufficient for the handling of tablets throughout the shelf life. Friability was found in between 0.2 – 0.4% which is within the acceptable limit. From the weight variation test the % weight variation for all 20 tablets were found 243±4.5-247±2.1 .{Table-6}

Parameters	Observed
Nature	Crystalline
color	Light yellow
odor	Characteristic
Solubility	Partially insoluble in water Freely soluble in methanol Soluble in alcohol
Melting point	25 degree celcius
Absorption Spectra	247nm

Table-4: Solubility and organoleptic properties

For immediate release formulation

Formulation	Bulk density(gm/ml)	Tapped density(gm/ml)	Carr's index	Hausner ratio	Angle of repose
F1	0.338	0.421	15.67	1.21	19.81
F2	0.346	0.410	15.85	1.10	20.32
F3	0.394	0.465	16.37	1.21	23.63
F4	0.436	0.479	14.38	1.10	18.73
F5	0.328	0.437	14.74	1.10	18.89
F6	0.471	0.488	15.84	1.21	19.74

For sustained release formulation

Formulation	Bulk density(gm/ml)	Tapped density(gm/ml)	Carr's index	Hausner ratio	Angle of repose
F1	0.149	0.190	14.16	1.22	18.86
F2	0.144	0.188	15.65	1.24	20.83
F3	0.168	0.196	14.87	1.22	18.59
F4	0.157	0.227	17.54	1.26	21.48
F5	0.154	0.198	14.11	1.22	18.23
F6	0.173	0.235	16.79	1.25	21.14

Table- 5: Pre Compression Parameters for immediate release formulation and sustained release formulation.

Immediate release layer

Name	F1	F2	F3	F4	F5	F6
Allicin	10	10	10	10	10	10
Sodium citrate	5	5	5	5	5	5
Sodium starch glycolate	10	10	10	10	10	10
Microcrystalline cellulose	45	45	45	45	45	45
Xanthum gum	5	5	5	5	5	5
Magnesium stearate	2	2	2	2	2	2

Talc	2	2	2	2	2	2
Iron red	1	1	1	1	1	1

Sustained release Layer

Name	F1	F2	F3	F4	F5	F6
Allicin	20	20	20	20	20	20
HPMC E15	15	15	15	20	20	20
|PVP	20	25	30	20	25	30
MCC	86	81	76	81	76	71
Xanthum gum	20	20	20	20	20	20
Magnesium stearate	5	5	5	5	5	5
Talc	4	4	4	4	4	4

Table -3

Formulation	Wt variation %	Hardness(kg/cm2**)**	Thickness (mm)
F1	243 ± 4.1	5.6	3.5
F2	246 ± 2.03	5.5	3.4
F3	243 ± 4.5	5.3	3.6
F4	246 ± 2.4	5.7	3.5
F5	247 ± 2.1	5.5	3.6
F6	243 ± 3.8	5.6	3.7

Table – 6 Post Compression Parameters of the prepared bilayer tablet.

CHAPTER – 6

CONCLUSION

Conclusion

This research study incorporate the extraction of allicin from garlic,compressed into bilayer tablet for immediate release aswell as sustained release .And parameters for the dosage form were evaluated to known whether the dosage form prepared can be acceptable and safe to use.

This study demonstrated that allicin can be extracted by ultrasonic assisted extraction method which is environmental friendly aswell as cost effective and is less sophisticated then other methods.

In this it was found that allicin content in garlic after extraction can made into powderised form with the application of freeze dryer lyophilizer.It was observed that because of the powder form of allicin there was no problem in compression of the tablet.

It was observed that the pre-compression parameter and the post compression parameters were in the acceptable range which indicated the formulated dosage form is acceptable and safe .

As we came to know that allicin present in garlic have antihypertensive activity,It is not possible to get sustained action if we take large amount of raw garlic at once .So, this study is a noble approach in delivering the drug allicin to the body continuously for long duration of time .So,bilayer tablet having immediate release and sustained release layer is better option for treatment of hypertension for better quality of life for hypertensive patients.

CHAPTER – 7

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REFERENCE-

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