IDENTIFICATION AND MOLECULAR DOCKING STUDY OF NATURAL COMPOUNDS FOR TREATMENT OF COVID 19

Thesis Submitted to

K.R. Mangalam University in partial fulfilment of the

requirement for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

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This is to certify that the dissertation entitled "**IDENTIFICATION AND MOLECULAR DOCKING STUDY OF NATURAL COMPOUND FOR TREATMENT OF COVID 19**" submitted to K.R. Mangalam University in partial fulfilment of the requirement for the award of Degree of MASTER OF PHARMACY in Pharmaceutics, embodied the original research work carried out by MOHD KAFEEL under our supervision and guidance.

It is further stated that no part of this dissertation has been submitted, either in part or full for any other degree of K.R. Mangalam or any other university/institution.

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LIST OF ABBREVIATIONS

1. SARS-CoV-2- Severe Acute Respiratory Syndrome Coronavirus 2
2. ACE-2- Angiotensin-converting enzyme-2
3. TNF-α-Tumor Necrosis Factor
4. IL-6 - Interleukin-6
5. CHM- Chinese herbals medicine
6. M protein- Membrane Protein
7. S protein- Spike Protein
8. E protein- Envelope Protein
9. N protein- Nucleocapsid Protein
10. RdRp- RNA-dependent RNA polymerase
11. M pro- Main Protease
12. HCQ- Hydroxychloroquine
13. COVID-19- Coronavirus Disease 2019
14. MERS-CoV- Middle East Respiratory Syndrome Coronavirus

CHAPTER 1: INTRODUCTION

The first case of the newly emerged Human Coronavirus (HCoV) was reported in the month of Dec 2019 in Wuhan city of China (COVID-19) [1]. On 12th January 2020 World Health Organization (WHO) declares COVID-19 is a pandemic and caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is a non-segmented RNA virus and is mainly identified in the broncho-alveolar secretions, sputum, and saliva [2]. The virus belongs to the family Coronaviridae and genus Betacoronavirus which is interrelated to severe acute respiratory syndrome coronavirus (SARS-CoV) and (MERS-CoV) [3,4]. The various symptoms of COVID-19 are chest congestion, fever, respiratory distress, myalgia, fatigue loss of appetite, shortness of breath, cough and headache, the 1st case of COVID-19 in India was reported on 30 Jan 2020 in Kerala and as on December 2020 more the 95 Lakhs peoples were suffered from the diseases with more than 1 lakh of deaths [5,6].

Critics of the specific Coronavirus CSS idea have guaranteed that"cytokine storm has basically no definition" [7] and that there is absolute "no proof that will Coronavirus will provoke cytokine storm" in the patient [8]. Cytokine storm is a clinical aggregate of immune dysregulation recognized by perpetuated service of lymphocytes and macrophages that leads to secretion of large quantities of cytokines which causes systemic swelling and multi-organ failure with high mortality.

1.1 Sign and symptoms

Fever, Dry cough, Tiredness, Sore throat, Diarrhoea, Conjunctivitis, Headache, Loss of taste or smell, A rash on the skin, or discoloration of fingers or toes, [9,10]

Serious symptoms: Difficulty breathing or shortness of breath, Chest pain or pressure, Loss of speech or movement[11]

1.2 Genomic structure of SARS-CoV-2

The novel coronavirus is composed positive single-stranded ribonucleic acid (RNA) structure which acts as a molecular message that allows the assembly of proteins required for other elements of the virion [12,13]. The virus is large in size is about 9 to 12 nm with a diameter of 60 to 140 nm and the particles of this virus are spherical and polymorphic.HCov is divided into 4 different protein which plays a critical role to bind with the human receptor that are namely spike (S), membrane (M), nucleocapsid (N), and envelope (E) proteins and all these protein is used to protect the genome of this virus in which spike protein (S) is located on the surface of the virus having three-segment which are ectodomain (ED) region while (E) proteins are the smallest (8.4–12 kDa size) protein of this virus which is situated on the endoplasmic reticulum and Golgi complex in the host cells [14-16]. Viral morphogenesis, CoV assembly, and budding formation is the main role of the (E) proteins and act as as a virulence factor [17,18]. (M) protein mainly used for viral assembly which hundred times bigger than (E) proteins [19,20]. The function of (M) protein is to maintain the shape of the viral envelop and also responsible for viral intracellular homeostasis. The N protein plays an important role when the virus is ready for packaging its viral genome into a helical ribonucleocapsid (RNP) [21]. It regulates the replication and transcription of viral RNA and maintains the formation of RNA complexes.[22,23]

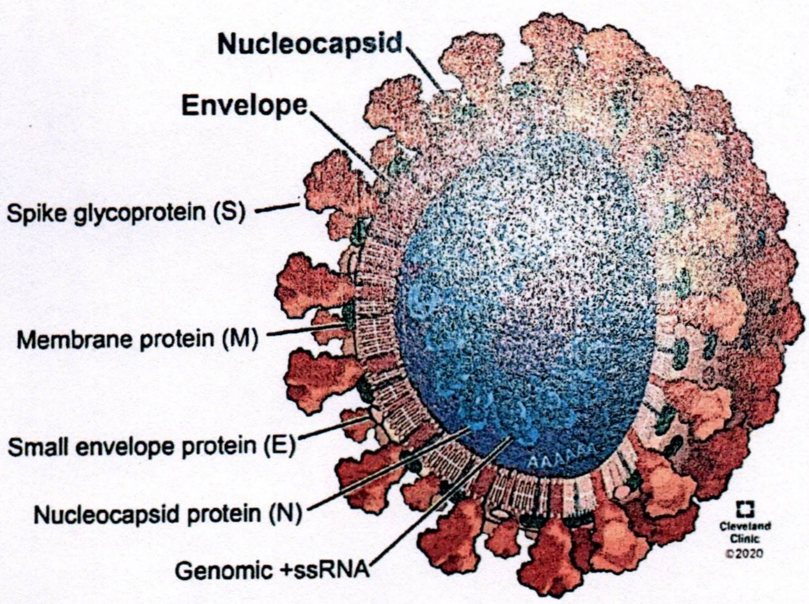

Fig. 1 structure of COVID 19

1.3 Life cycle of COVID-19

The life cycle of COVID-19 begins in host cells once it reaches to lungs and binds to the type-II pneumocytes of the alveoli using its spike proteins (S protein) to the cellular receptor Angiotensin-converting enzyme-2 (ACE-2) [24-26]. After binding to the receptor conformational changes in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway [27,28]. Following fusion with host cells, the virus injects its positive single-stranded RNA (+ssRNA) into the host cell which then translates into viral replicase polyproteins pp1a and 1ab and results in cleavage to small products by viral proteinases [29]. The polymerase produces a series of sub-genomic mRNAs by discontinuous transcription and is finally translated into relevant viral proteins. Host RNA-dependent RNA polymerase converts the viral RNA into a new virions genome [30,31]. Viral proteins and genome RNA are subsequently assembled into virions in the endoplasmic reticulum and Golgi apparatus and then transported via vesicles and released out of the cell (Fig. 2).

A healthy person gets infected when they come in contact with an infected person [32]. Once at the surface, the virus reaches in alveolar type-2-pneumocytes where it attaches with spike protein with ACE2 receptor of type-2 cells [33]. Following fusion with host cells, the virus injects its positive single-stranded RNA (+ssRNA) into the host cell. Host cells, ribosome's translates the viral into large viral proteins which are further chopped into smaller proteins and used to make the structural proteins of progeny virions [34,35]. Host RNA-dependent RNA polymerase converts the viral RNA into a new virions genome. Progeny virus after completing the life cycle bursts the host cells starts infection into nearby cells. In severely infected patients, the virus also affects vital organs like the kidney and heart [36,37]. To date there is no effective medicine available for the treatment of COVID-19 hence in this review we focused on repurposing drugs used in traditional Chinese and Indian as well as an allopathic system of medicine (Fig. 3) which may be used alone or in combination to get maximum advantage of therapy [38,39].

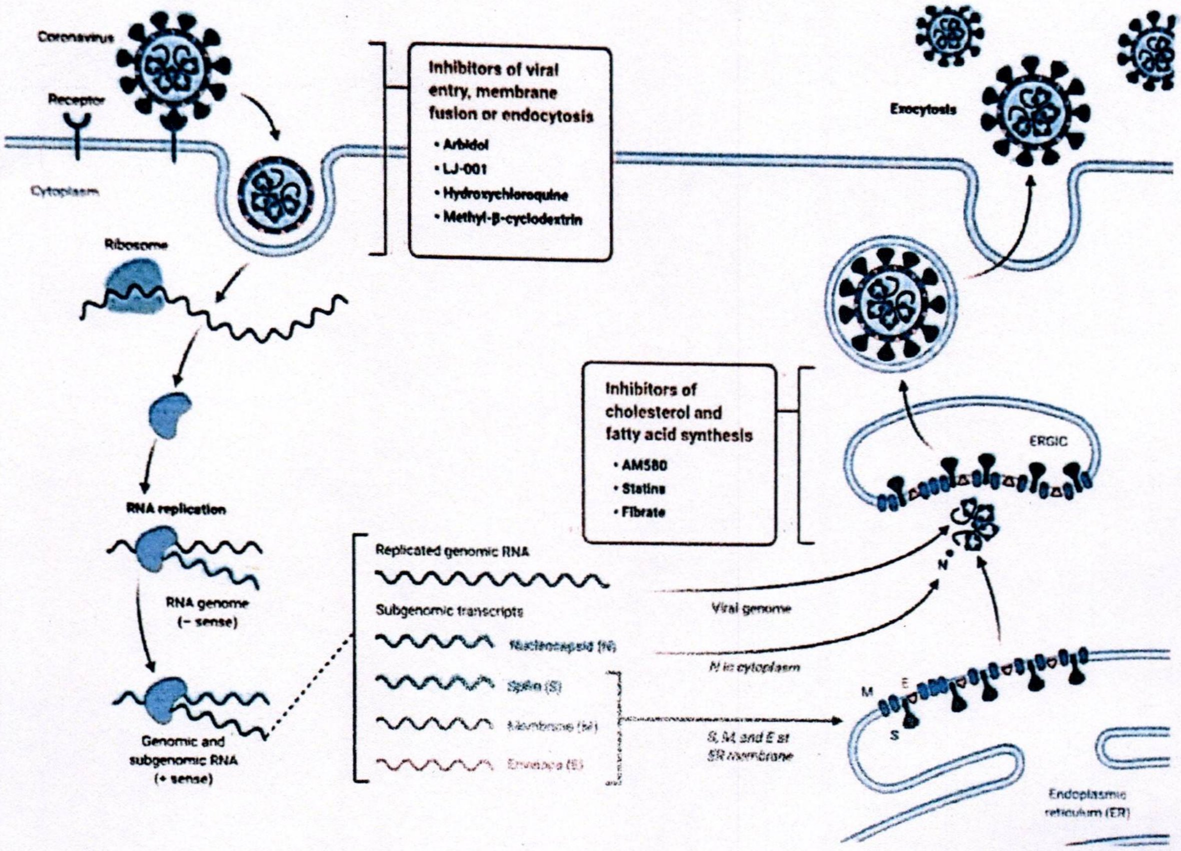

Fig. 2 Life cycle of COVID -19

.4 Pathophysiology

nterleukin -6 and COVID -Cytokines storm

n early scientific studies, another mediator in COVID-19 is an elevated level of interleukins-6 IL-6) [40-42]. In COVID-cytokines storm numerous inflammatory cytokines like IL-1, IL-10, ınd tumor necrosis factor(TNF)-α are raised approximately 2-100 times above normal levels, vhereas IL-6 shows much higher concentration as compared to other interleukins. Some studies eported a marked elevation in serum IL-6 levels in the 100-10, 000 pg/mL range in patients with he serious disease [43-45]. These markedly elevated IL-6 levels in COVID-CSS are similar in nagnitude to serious CAR T-cell CRS [19] and higher than other hyper-IL-6 syndromes such as netacentric cattleman disease, where IL-6 is elevated but typically <100 pg/mL [46]. HLH is said o encompass a varied spectrum of "hyper ferritinemia hyper-inflammatory syndromes with a :ommon terminal pathway but with different pathogenetic roots"[47].

IL-6 is difficult disks immune dysregulation plus respiratory failing within COVID-CSS will be quickly accumulating. Raised serum IL-6 will be linked with lymphopenia, reduced lymphocyte cytotoxicity, and endothelial service. These types of defense defects may become partially renewed simply by therapy with IL-6 blockade with tocilizumab [48-51]. Study-related to IL-6 pointed out that the concentration of IL-6 > 80 pg/mL and C-reactive proteins concentration > 97 mg/L is very predictive and is associated with respiratory system failure [52-55].

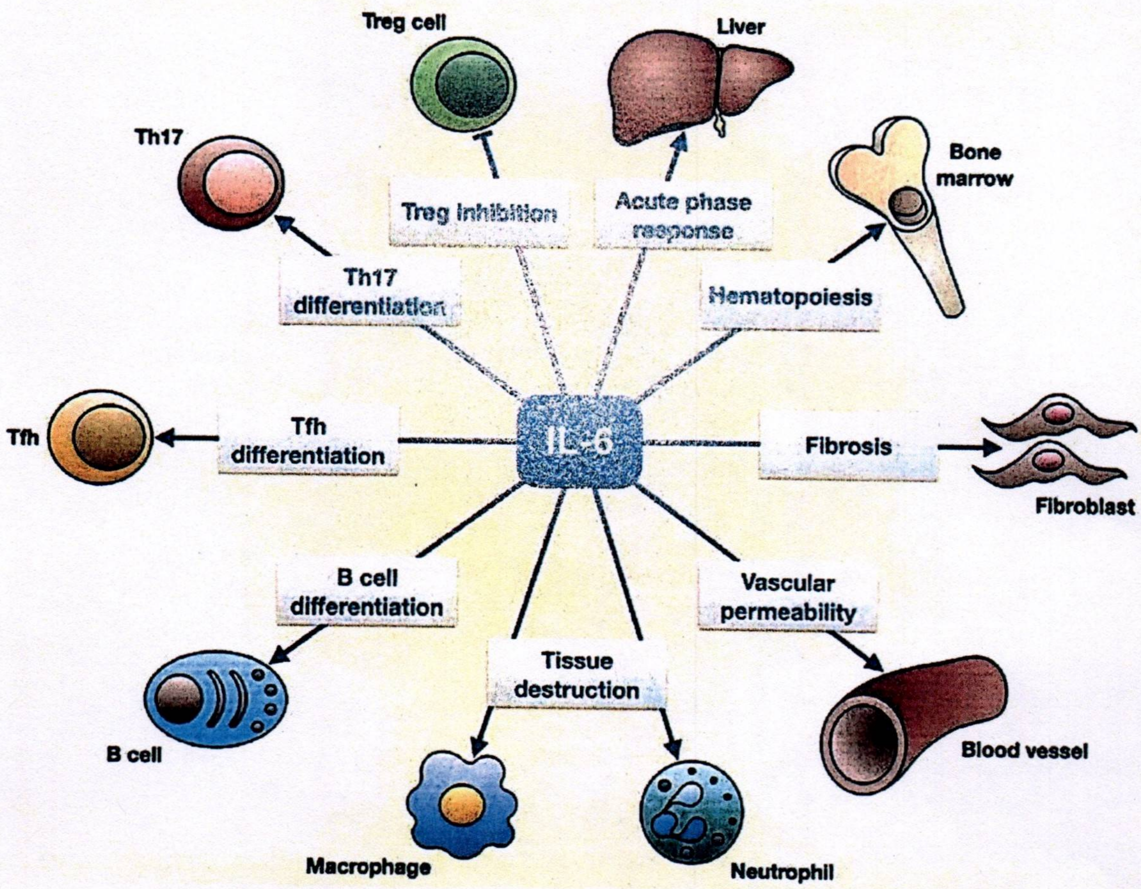

Fig. 3. Interleukin -6

1.5 Treatment strategiesfor COVID-19

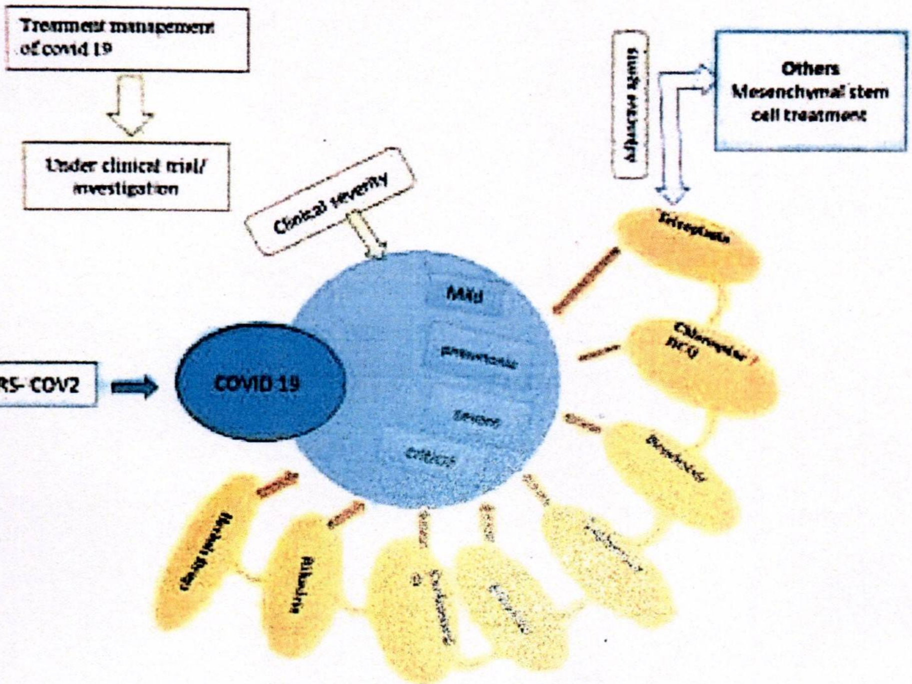

Fig. 4. Treatment strategiesfor COVID-19

Antibacterial drug

Teicoplanin

It is a semisynthetic glycopeptide antibiotic used to treat serious infections caused by gram-positive bacteria. This drug showed prominent effects when taken at a dose of 400mg once a day to treat infection associated with coronavirus [56]. It acts by inhibiting spike viral protein by cathepsin L which is responsible for the release of genomic viral RNA [57].

Antiprotozoal drug

Chloroquine/hydroxyl chloroquine

Chloroquine acts by inhibiting the virus at the entry-level to the host cell while if it already inside the cell then it prevents the replication of the virus by changing the acidic pH of DNA replication and its organelles. Some researchers found that hydroxyl chloroquine is more safer as

FORMULATION AND EVALUATION OF CIPROFLOXACIN LOADED ALGINATE-PECTIN INTERPENETRATING POLYMER NETWORK BASED SUSTAINED RELEASE ANTIMICROBIAL MICROSPHERES

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INTRODUCTION

1.1. Challenges with conventional drug delivery systems

Drug delivery systems are created using design techniques for controlled or targeted release. of medical treatments. The process of administering a pharmacological substance is known as drug delivery to have a therapeutic impact on either people or animals. It demonstrates a significant effect on the treatment for a range of diseases [1]. There are issues with bioavailability, unusual distribution mechanisms for medications, and rational formulation design for treatments with limited solubility. The delivery of pharmaceuticals containing proteins as well as those intended for children and the elderly are important additional problems with drug distribution. The best hope of increasing the oral bioavailability of drugs that aren't very water-soluble lies in SEDDS, or self-emulsifying drug delivery systems [2].

1.2. Definition of NDDS

This drug delivery system combines advanced methods with novel dosages techniques to increase medication safety, manage drug release, and introduce better drug potency are among them a medication to a desired tissue selectively [3].

To obtain the desired therapeutic result, a pharmacological or medical medication is administered by a process known as drug delivery. The distribution technique plays a significant role in a drug's efficacy. Examples of novel drug delivery systems include products that mix drugs and devices or are medical equipment. Pharmaceutics, molecular biology, and polymer science are all used in the development of novel drug delivery systems (NDDS). The method of administration of a drug can have a substantial impact on its effectiveness. Some medications have an ideal concentration range within which they function optimally; dosages outside of this range may be harmful or have no therapeutic benefit [4].

1.2.1 Advantage of NDDS [5]

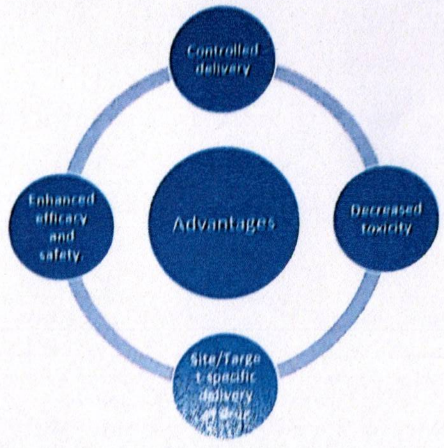

Fig. Advantages of NDDS

1.2.2 Disadvantages of NDDS [6]

- Dose dumping.
- Limited potential as carrier to non-phagocyte target tissue.
- Low solubility and permeability
- Poor bioavailability.

1.3 Microspheres in drug delivery system

Microspheres are solid, free-flowing particles with spherical shapes that range in size from 1 to 1000 m. Microparticles is also occasionally used as a synonym for microspheres [7]. These are composed of biodegradable synthetic polymers or proteins that are utilised for the targeted delivery of antibiotics, hormones, vaccines, and medicines. They also have a wide surface area and make mass transfer behaviour and diffusion easier to assess [8]. To improve drug absorption, microspheres for oral usage have also entered the market. Basically, microspheres are of two types: -

1. **Microcapsules-** The enclosed material is clearly encompassed by a distinct capsule wall.

2. Micromatrices- The entrapped material is spreading throughout the matrix of the microspheres [9-10].

1.3.1 Ideal characteristics of microspheres [11-12]

- The ability to incorporate reasonably high concentrations of the drug.
- Susceptibility to chemical modification.
- Long shelf-life.
- Controlled particle size and dispersibility in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale.
- Biocompatibility with good biodegradability.

1.3.2 Advantages of microspheres [13-14]

- Provide prolonged therapeutic effect.
- Protects GIT from irritant effects of the drug.
- Reduces dose and toxicity.
- Improves bioavailability of drug.
- Provides better patient compliance.
- Delivers the drug at specific site of action.

1.3.3 Disadvantages of microspheres [15]

- Unknown toxicity of beads.
- Difficulty of large-scale manufacturing.
- Less reproducibility.
- These type of dosage forms should not be chewed or cracked.

1.4 Types of microspheres

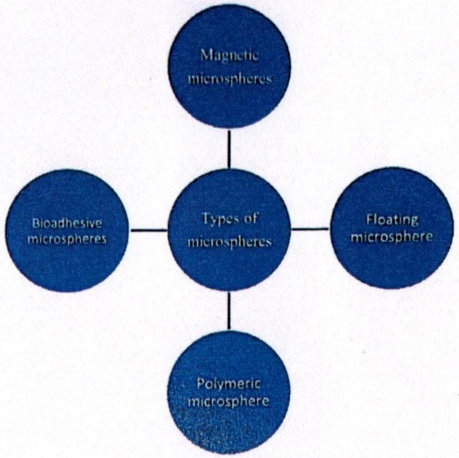

Fig 2: Types of microspheres [16]

.4.1 **Floating microspheres**– These microspheres are tiny and hollow having no center and are free flowing cells which differ in range from 1-1000 µm. The bulk density in this type of microsphere is less than gastric fluid so these stay buoyant in stomach without disturbing the gastric emptying rate [16]. The drug is released deliberately at the desired rate, if the system is floating on gastric content and therefore increase variation in plasma concentration and gastric residence time [17]. It decreases the chances of dumping of dose and diminishes the dosing frequencies and offers extended therapeutic effect [18-19].

Patel A et al. (2006) prepared and evaluated the floating microspheres of metformin hydrochloride and optimized drug release pattern to match target release profile. By the use of non-aqueous emulsification solvent evaporation method using ethyl cellulose floating microspheres were formulated and which extended the release of drug for at least 8 hours in stomach hence enhancing the patient compliance and bioavailability.

1.4.2 Bioadhesive microspheres- Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water-soluble polymers [20]. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc can be termed as bio adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action [21-23].

Ye Zhang et.al formulated chitosan coated alginate/ gelatin microspheres loaded with Berberine hydrochloride and evaluated them for their pharmaceutical characteristics and pharmacokinetics. These bio adhesive microspheres were prepared by emulsification method. Three batches were prepared and then evaluated for stability and are used for sustained delivery to treat duodenal and benign gastric ulcers [24].

1.4.3 Polymeric microspheres– These microspheres are of two types and are classified as follows:[25]

1. Biodegradable polymeric microspheres
2. Synthetic polymeric microspheres

Fajun Zhao et al., in a low-permeability reservoir by using polymeric microspheres to improve in-depth profile control. Distillation precipitation was utilised to create polymeric microspheres with a nanometer-sized particle size. Infrared spectroscopy, scanning electron microscopy, thermogravimetry, high-pressure and high-temperature rheometry, and dynamic light scattering were employed to test and analyse the structure, apparent pattern, thermal endurance, particle size, hydration, and swelling capability of the microspheres. With a centred size distribution, the synthesized polymeric microspheres were all uniformly round.

Synthetic Polymeric Microspheres – These microspheres generally have clinical applications and also used as fillers, embolic particles, and bulking agents. These can also be used as drug delivery vehicles. These are safe and biocompatible. But their major drawback is that they have a habit of migrating away from the site of injection and leads to potential risk which results in organ damage [26].

Biodegradable Polymeric Microspheres - Starch which is a natural polymer is used with the perception as they are biocompatible, biodegradable, and bioadhesive. These biodegradable polymers extend the residence time when they interact with the mucous membrane as it has great swelling properties with the aqueous media which further results in the development of gel. By the concentration of polymer, the extent and rate of drug is controlled in sustained way. The drug loading efficiency of biodegradable microspheres in clinical use is very complicated and is problematic to control the drug release and this is the major disadvantage of these microspheres [27-28].

1.4 **Magnetic microspheres–** These are molecular particles which localizes the drug to the target site and are very short enough to cross the capillaries without creating an esophageal obstruction [29]. In this system freely circulating drug present in huge amount can be substituted by a small volume of magnetically targeted drug. Magnetic responses to a magnetic field are received from magnetic carriers from the incorporated substances which are used in magnetic microspheres are dextran, chitosan and many more [30-31]. There are two types of magnetic microspheres which are: - 1. Diagnostic microspheres 2. Therapeutic magnetic microspheres

Fengxia Li et al., formulated magnetic polylactic acid microspheres loaded with curcumin by O/W emulsion solvent evaporation method to obtain a targeted drug delivery system. FTIR was used to characterize functional groups. Scanning electron microscopy was used to check morphology of microspheres while dynamic light spectroscopy for size distribution of microspheres. The microspheres were found spherical with smooth surface with a diameter of 0.55–0.75 μm and showed sustained release effect on in vitro drug release.

1.5 Methods of formulation of microspheres: -

Incorporation of solid, liquid or gases into one or more polymeric coatings can be done by microencapsulation technique. The different methods used for various microspheres preparation depends on particle size, route of administration, duration of drug release and these above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, co precipitation etc [32].

Methods of preparation-

1. Ionic gelatin technique
2. Spray drying method
3. Emulsion cross linking method
4. Emulsion solvent diffusion technique
5. Multiple emulsion method
6. Emulsion solvent evaporation technique

1.5.1 Ionic-gelatin method- Alginate/chitosan particulate system for diclofenac sodium release was prepared using this technique. 25%(w/v) of diclofenac sodium was added to 1.2%(w/v) aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it was added drop wise to a solution containing Ca^{2+} /Al^{3+} and chitosan solution in acetic acid. Microspheres which were created were held in reserve in original solution for 24 hr for internal gellification followed by filtration for partition. The complete discharge was obtained at pH 6.4-7.2 but the drug did not liberate in acidic pH [33].

1.5.2 Spray- drying method- This technique which involves separating the core material in liquefied covering material, was utilised to develop polymeric mixed microspheres loaded with the medication ketoprofen. The combination is then sprayed into the surroundings to solidify the coating, and the solvent quickly evaporates after that. Ketoprofen was added to an organic solution of poly (epsilon-caprolactone) and cellulose acetate butyrate (CAB) in different weight ratios, which was then produced and sprayed under various experimental conditions to produce drug-loaded microspheres. Due to the principle of rapid drying, this method is speedy but may lack crystallinity.

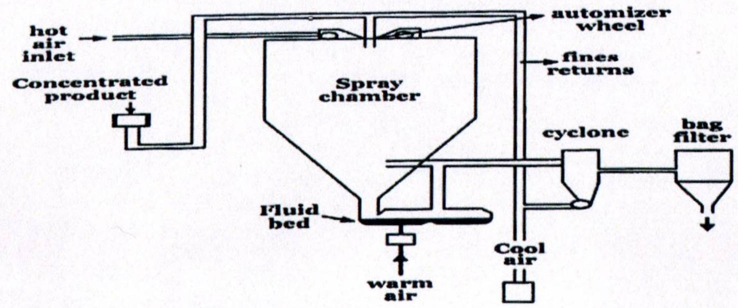

Fig. 3: Spray drying method

1.5.3 Emulsion cross-linking method- In this method drug was dissolved in aqueous gelatine solution which was previously heated for 1hr at 40°C. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500rpm for 10 min at 35°C results in w/o emulsion then further stirring is done for 10 min at 15°C.Thus the produced microspheres were washed respectively three times with acetone and Isopropyl alcohol which then air dried and dispersed in 5mL of aqueous glutaraldehyde saturated toluene solution at room temperature for 3 hrs for cross linking and then was treated with 100mL of 10 mm glycine solution containing 0.1%w/v of tween 80 at 37°c for 10min to block un reacted glutaraldehyde. Examples for this technique is Gelatin a microsphere [34].

1.5.4 Emulsion solvent diffusion technique- By using this technique the floating microparticles of ketoprofen were formulated to enhance the colon residence time. The mixture of drug and polymer was dissolved in a mixture of dichloromethane and ethanol in 1:1 and this mixture was further added to solution of sodium lauryl sulphate drop wisely. At room temperature, with the help of propeller type agitator the solution was stirred for 1 hour at 150 rpm. The formulated microspheres were washed and at room temperature were dried in desiccator and later were sieved and collected [33].

1.5 **Multiple emulsion method-** This technique is used for preparation of oral controlled release medication delivery for a variety of pharmaceuticals. The powder medication was first disseminated in methyl cellulose solution, then emulsified in ethyl cellulose solution in ethyl acetate. In an aqueous medium, the first emulsion was then reemulsified. under optimal conditions [35].

1.6 **Emulsion solvent evaporation method-** In this technique the drug is dissolved in polymer which was previously dissolved in chloroform and the resulting solution is added to aqueous phase containing 0 .2 % sodium PVP as emulsifying agent. The above mixture was agitated at 500 rpm then the drug and polymer (eudragit) were transformed into fine droplet which solidified into rigid microspheres by solvent evaporation and then collected by filtration and washed with demineralised water and desiccated at room temperature for 24hrs [36].

1.6 **Particle shape and size-** Traditional light microscopy (LM) and scanning electron microscopy (SEM) are the two most used methods for visualising microparticles (SEM). Microparticles' shape and exterior structure can be determined using both methods. In the case of double-walled microspheres, LM allows you to modify the

CERTIFICATE FROM THE DEAN

This is to certify that research work embodied in this thesis entitled "*Formulation and Evaluation of Ciprofloxacin Loaded Alginate- Pectin Interpenetrating Polymer Network Based Sustained Release Antimicrobial Microspheres*" submitted to K. R. Mangalam University, Gurugram, Haryana, for the award of the degree of **M. Pharmacy (Pharmaceutics)** has been carried out by Itisha Budhiraja under at Department of Pharmaceutics, School of Medical & Allied Sciences,

K. R. Mangalam University from September 2021 to August 2022.

To the best of my knowledge and belief, this work is original and has not been submitted so far in part or in full for the award of any degree or diploma of any University/ Institute.

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