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Targeting Cancer by Natural Polymeric Nanoparticles

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ABSTRACT

Delivering a therapeutic agent to the desired site is the major problem in the treatment of many diseases such as cancer. Conventional utilization of drug is characterized by its limited bio-distribution, its side effect, and lack of its selectivity. The poor solubility and large volume of distribution of anticancer drugs are the major problems in cancer therapy. Reducing size of drug and designing it into a suitable formulation and selecting a pathway for drug uptake is fundamental basis of nanotechnology. Great interest of nanoparticles is due their nanometer scale range. Their reduced particle size entails high surface area and hence a strategy for faster release. These particles are capable of deep penetration into the tissue without disrupting its functions. Thus, it has been suggested that nanoparticles should improve the therapeutic efficacy while decreasing the toxic side effects of anticancer drugs. The need for polymers with specific physical and biological properties has generated continued interest in novel polymer screening from natural resources.

Keywords: Target delivery, cancer, nanoparticles, natural polymer, biodegradable polymers.

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INTRODUCTION

Cancer is one of the most devastating diseases across the world; its affecting more than 10-million people every year, one out of three people will develop some form of cancer in their life. However death rate have come down in the past two years due to its better understanding of tumor biology and improved diagnostic devices and treatments. Current practice of cancer treatments include surgical intervention, radiology and chemotherapeutic agents which often kill healthy cells and causes toxicity to the patients. It would there for desirable to develop chemotherapeutics that can either passively or actively target cancerous cells. Passive targeting applies the characteristics attribute of tumor biology that allow nanoparticles to accumulate in the tumor by the enhanced permeability and retention (EPR) effect. Passive targeting nano carriers first reached clinical; trials in the mid-1980s. [1]

Finding appropriate targets is based on a detailed understanding of the molecular changes that underlying cancer. These approaches includes the conventional, more empirical approach used to develop cytotoxic chemotherapeutics- the main stay of cancer drug development in past decades.[2]

The recent research areas include development of drug carriers to allow alternative dosing routes, new therapeutic targets such as blood vessels fueling tumor growth and targeted therapeutics that are more specific in their activity. Clinical trials have shown that patients are open to new therapeutic options and the goal of these new chemotherapeutics is to increase survival time and the quality of life for cancer patients. [3]

Commonly, nanoparticles will trigger certain tissues strictly because of their nanosize and/or their physico-chemical properties; but new types of nanoparticles that respond to an externally applied field, be magnetic, focused heat, or light, in such ways that may make them ideal therapeutics or therapeutic delivery system, under examination. For example, iron oxide nanoparticles, which can act as the foundation for targeted magnetic resonance imaging (MRI) contrast agents, can be heated to temperatures lethal to a cancer cell merely by increasing the magnetic field at the very location where they are attached to tumor cells. [4]

Over the last few decade pharmaceutical laboratory have been actively engaged in synthesizing biodegradable nanoparticles using various natural products. Although the synthetic polymers display chemical stability, their bio-incompatibility still limits their potential use in clinical applications. The natural polymers always show low immunogenicity low/non toxicity, and most important good biocompatibility, they have been the most preferred polymers in drug delivery

systems. Among the natural polymers, alginate, chitosan have become one of the most popular materials used to form nanoparticles. Recently, scientists have turned their attention on tuning starch and chitosan for use in nano-drug delivery. [5]

The polymeric nanoparticles (PNPs) are prepared from biocompatible and biodegradable polymers in size between 10- 1000 nm where the drug is either dissolved, entrapped, encapsulated or attached to a nanoparticles matrix. PNPs act as promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to specific target; such an advantage improves the drug safety by reducing dose related side effects.

The aim of this review article is to summarize about various natural polymeric material, methods to prepare nanoparticles, mechanism of drug release and localization of drug in target tissue, characterization of nanoparticles and its biomedical applications.

POLYMERS USED TO DESIGN NANOPARTICLES

Today, polymer nanotechnologies have emerged an important part of the more promising future to achieve drug delivery challenges based on drug targeting and on the delivery of undeliverable molecules such as oligo-nucleotides or RNA interfering effectors. [6]

Table 1: Various novel polymers to design nanoparticles

Polysaccharides	Starch
	Dextran/ dextrin, cyclodextrim
	Chitosan
	Pectin
	Alginate
Protein	Albumin
	Gelatin
	Collagen
Lipids	Phospholipids

In present situation there has been an increased interest towards formulation nanoparticles (NPs) loaded with selected drugs for use in drug delivery. Particularly, polymeric NPs have obtained increasing attention in pharmaceutical and in the fields of drug delivery. The polymeric NPs have shown high efficacious drug delivery agents due to their site specific properties, as extending the drug release, decreasing drug degradation, increasing bioavailability and reducing drug toxicity. [7]

Polymers which obtained from natural sources have been extensively employed not only in the food industry but also in pharmaceutical technology. Polysaccharide polymers have emerged as being one of these because these are biocompatible, biodegradable and are less toxic. Incorporation of the therapeutic agent into a polymeric matrix, particularly of a natural origin, might potentiate

the protection of the pharmaceutically active compound from degradation, control drug release, improve absorption, enhance the therapeutic effect, and lead to the consequential decrease in the frequency of administration. [8]

Considering the increased potential offered by polymer chemistry today, there are only a few limited numbers of polymers which can be used as constituent of nanoparticles designed to deliver drugs. In order To explain this fact clearly, one should consider that a selected polymer needs to fulfill several requirements to be used in such an application. Firstly, it must be biodegradable or at least totally eliminated in a short period of time allowing repeating administration without any risk of uncontrolled accumulation inside the body Secondly, it must be non immunogenic and non toxic. Its degradation products, if any, must also be non toxic and non immunogenic. Thirdly, it can be formulated under the form of polymer nanoparticles with required properties regarding the drug delivery goal for which the nanoparticles are designed. [9]

METHODS TO PREPARE POLYMERIC NANOPARTICLES

Solvent Evaporation

Solvent evaporation method was the first developed method for preparation of nanoparticles. In this method, first nanoemulsion formulation prepared. Polymer dissolved in organic solvent as dichloromethane, chloroform or ethyl acetate, after that drug is also dispersed in this solution. Then this mixture emulsified in an aqueous phase containing surfactant such as polysorbates, poloxamers sodium dodecyl sulfates polyvinyl alcohol, gelatin to make an oil in water emulsion by using either mechanical stirring, sonication, or micro fluidization (high-pressure homogenization through narrow channels). After formation of nano-emulsion the organic solvent can be evaporates by increased the temperature and reduced pressure with continuous stirring. [10, 11]

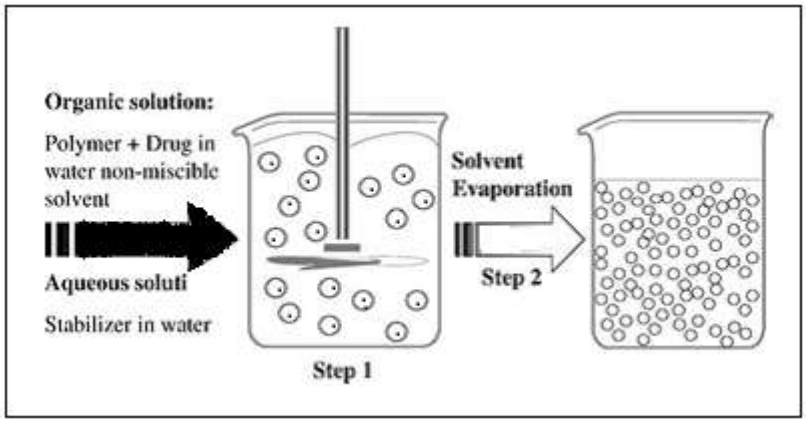

Figure 1: Solvent Evaporation method

Ref: Anil Mahapatra et.al, Journal of Nanobiotechnology, 2011

Dialysis

Dialysis is one of the effective methods for preparation of nanoparticles. In this method first polymer and drug dissolved in an organic solvent. This resulting solution was then added to a dialysis tube and dialysis carried against a non-solvent miscible with the former miscible. The displacement of the solvent inside the membrane leads to the progressive aggregation of polymer due to a loss of solubility which results in the formation of homogeneous suspensions of nanoparticles. [12]

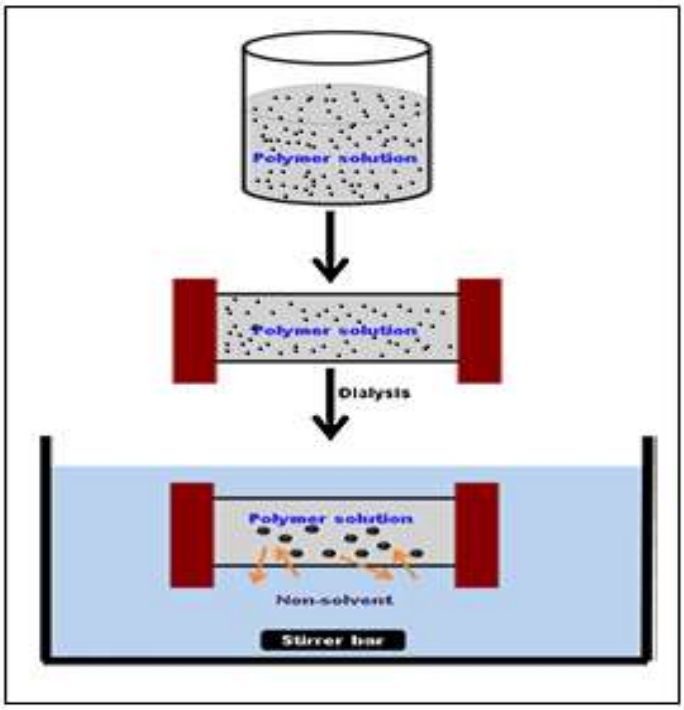

Figure 2: Dialysis

Ref: swati tyagi et.al, department of biotechnology

Supercritical fluid technology

Supercritical fluid technology is an alternative method, because in this method organic solvents are not used which are hazardous to the environment as well as to physiological systems in the body. Supercritical fluids are defined as a solvent at a temperature above its critical temperature remains a single phase fluid regardless of pressure. Supercritical CO_2 is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1 °C, Pc = 73.8 bars), it is non-toxicity, non-flammability, and low price. [13]

Mainly supercritical fluid used in two main techniques:

1. Supercritical anti-solvent (SAS)
2. Rapid expansion of critical solution (RESS)

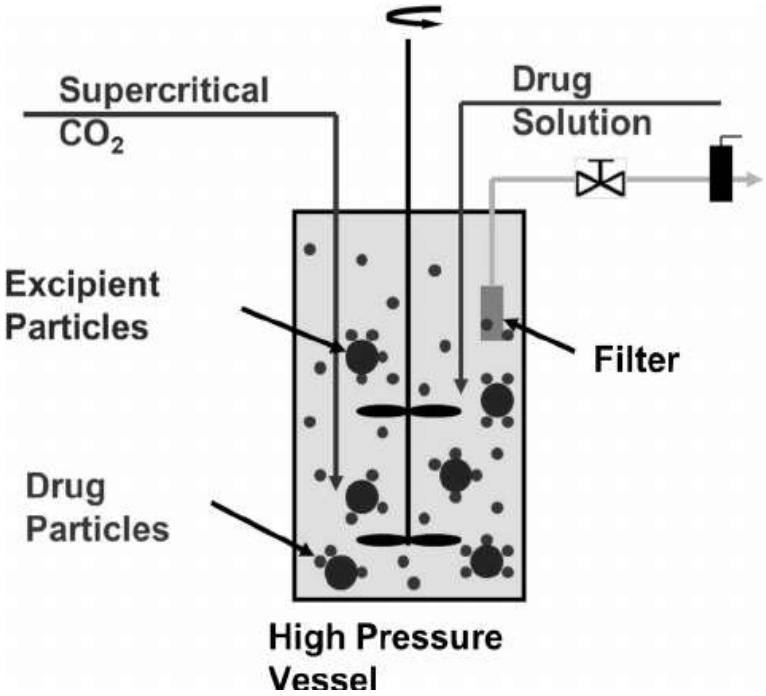

Figure 3: Supercritical fluid technology

Ref: Rishi paliwal et.al, 15 Jan, 2014

Nanoprecipitation method

This is another method for preparation of nanoparticles. This method is also called solvent displacement method. This technique was first described by Fessi at al. In this method precipitation of polymer and drug obtained from organic solvent which is diffused into the aqueous medium with or without the presence of a surfactant. Firstly drug was dissolved in water, and then a cosolvent (acetone used for make inner phase more homogeneous) was added to it. Then another solution of polymer (ethyl cellulose, eudragit) and propylene glycol with chloroform is prepared, and this solution was dispersed to the drug solution. This dispersion was slowly added to 10 ml of 70% ethanol solution (aqueous). After 5 minutes of mixing, the organic solvents evaporated at 35° under normal pressure, nanoparticles were separated by using cooling centrifuge (10000 rpm for 20 min), supernatant were removed and nanoparticles washed with water and dried at room temperature in a desicator. [14]

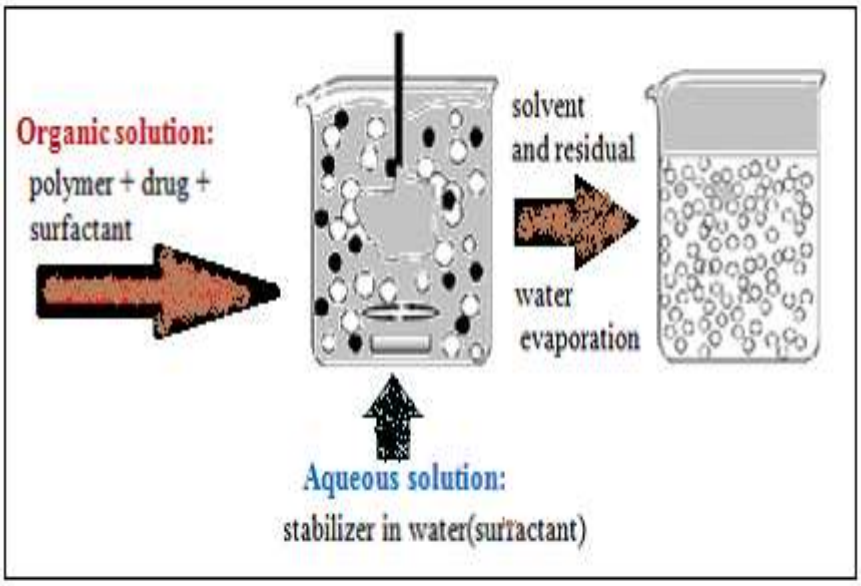

Figure 4: Nanoprecipitation method

Ref: Anjali et.al, Shri Guru Ram Rai Institute of Technology and Sciences

Ionic Gelation Method

In this method the polymer was (Rawat S et al., 2008) was first dispersed in organic solvent (5% glacial acetic acid) the solution was stirred continuously for 4 hours then it was stabilized by keeping overnight to obtain clear gel.. polymeric nanoparticles formed spontaneously upon addition of cross linking agent under high speed stirring (3000 rpm) using high speed stirrer. The resulting nano particle suspensions were centrifuged at 10,000 rpm for15 minutes. The particles were washed with distilled water and freeze dried. [15]

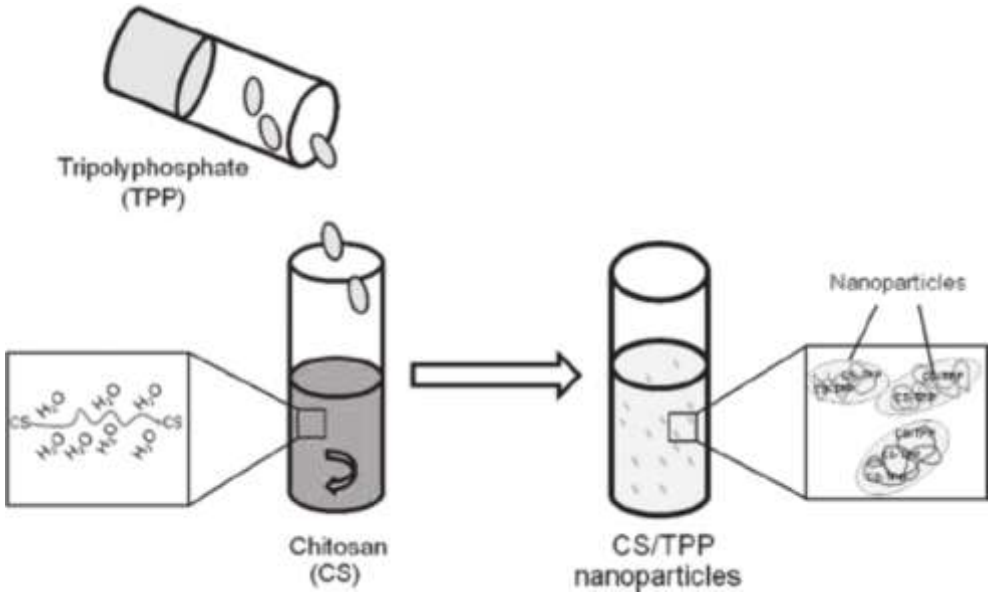

Figure 5: Ionic Gelation method

Ref: AnaGrehna et.al, Feb 2012

Salting Out Method

This technique was introduced and patented by Bindschaedler et al. and Ibrahim et al. It is based on the principle of separation of water-miscible solvent from aqueous solution by salting out effect (Catarina PR et al., 2006). Generally, acetone is used because it is totally miscible with water and is easily removed. In this method, polymer and drug are dissolved in a solvent which is emulsified into an aqueous solution containing salting out agent (electrolytes, such as magnesium chloride and calcium chloride, or non- electrolytes such as sucrose) but salting out can also be produced by saturation of the aqueous phase using colloidal stabilizer/ emulsion stabilizer/ viscosity increasing agent such as polyvinyl pyrrolidone or hydroxyl ethyl cellulose, PVA, Poly(ethylene oxide), PLGA and poly(trimethylene carbonate). After preparation of o/w emulsion is diluted with the addition of sufficient water to allow the complete diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. [16]

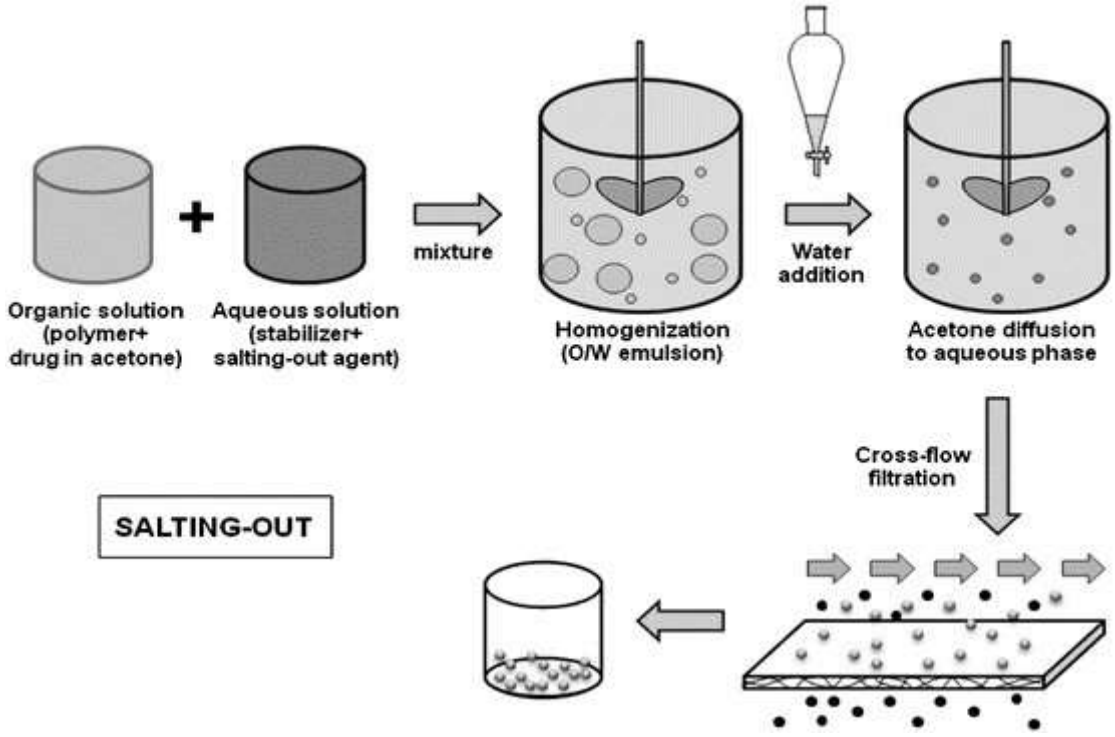

Figure 6: Salting Out method

Ref: Ana.c. fonseca et.al, jan 2013

Coacervation method

This method is used for the preparation of nanoparticle using biodegradable hydrophilic polymers (such as chitosan, gelatin and sodium alginate etc). Calvo at al prepared these nanoparticles by ionic gelation method which involves preparation of two aqueous phases. First phase contain

polymer like chitosan, a di-block co-polymer like ethylene oxide or propylene oxide (PEO-PPO). Second phase contain polyanion sodium tripolyphosphate. Between these two phases electrostatic interaction occurs which forms coacervates. [17]

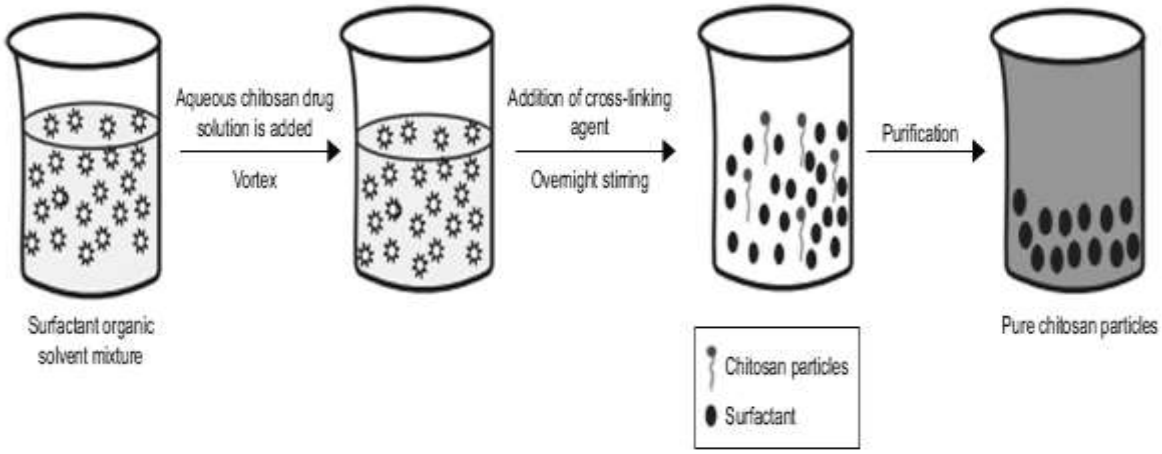

Figure 7: Coacervation method

Ref: Ahmed.Tet.al,January 2016, volume- 2016:10

Double Emulsification method

As emulsification and evaporation method have their limitation of poor entrapment of hydrophilic drugs, hence double emulsification technique is used. First, w/o emulsion prepared by addition of aqueous drug solution to the organic polymer solution with continuous stirring. To this prepared emulsion another aqeous phase is added with vigorous stirring, resultanting w/o/w emulsion. Then organic solvent removed by high centrifugation. [18]

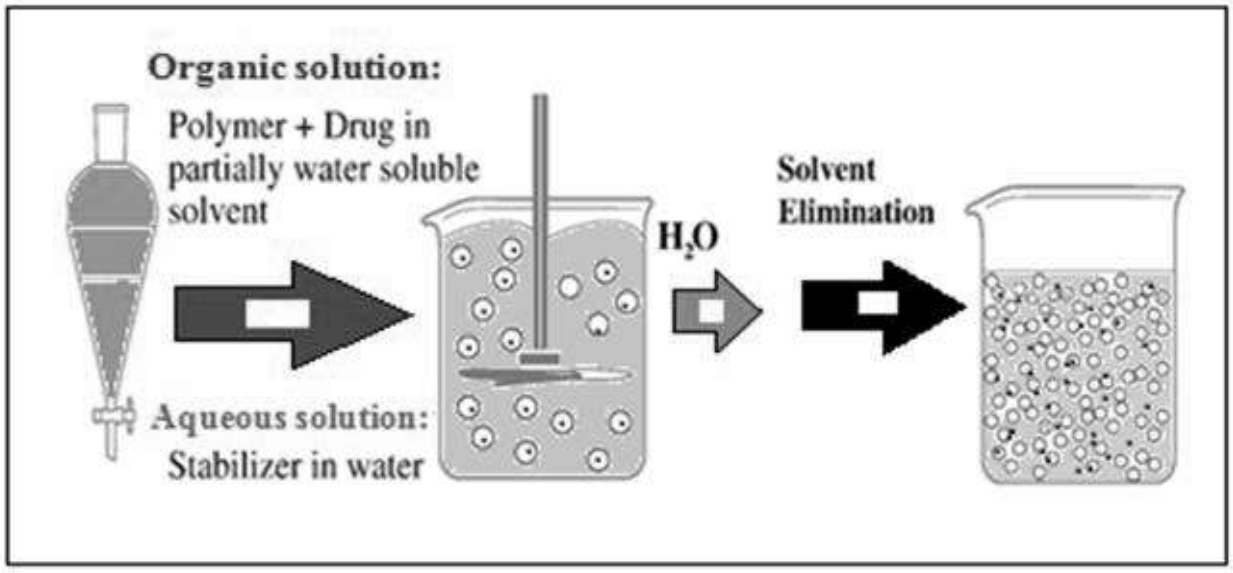

Figure 8: Double Emulsification method

Ref: Renu Tiruwa, Indian J. Pharma bio/Res 2016

MECHANISM OF DRUG RELEASE AND OF DRUG UPTAKE IN TARGET TISSUE:

There are several mechanisms have been proposed to govern release of drug from polymeric nanoparticles, such as swelling of the polymer and diffusion of the adsorbed drug, drug diffusion through the polymeric matrix, polymer erosion or degradation and a combination of both erosion and degradation as represented in Figure 9. The initial burst release from the nanoparticles is either because of swelling of the polymer, creating pores, or diffusion unbound drug from the surface of the polymer. Sometime nanoparticles also exhibit a pH-dependent drug release because of the solubility of polymer. Chitosan derivatives alter the release of drug from the NP, affording tunable drug release and impacting the pharmacokinetic profile of the loaded drug.

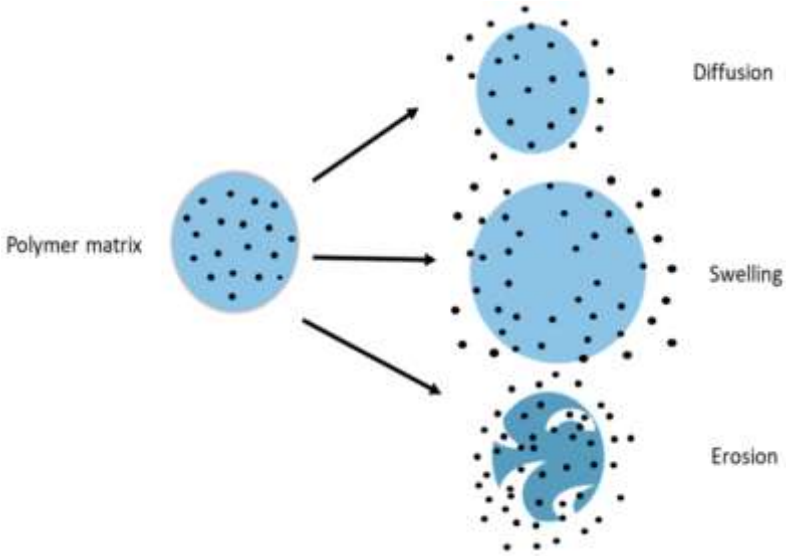

Figure. 9: Diagram representing the possible mechanisms of drug release by diffusion, swelling and erosion of polymer (chitosan) matrix.

Ref: Munawar A. Mohammed et.al. pharmaceutics, 2017

One of the example about the mechanism of alginate-enclosed chitosan-calcium phosphate-iron-saturated bovine lactoferrin nanocarrier (AEC-CP-Fe-bLf NCs) internalization and its action inside human body shows that the alginate coating of orally directed AEC-CP-Fe-bLf NCs is degraded in the alkaline environment offered in the small intestine.Then, the free alginate coating C-CP-Fe-bLf NCs enter the blood circulation via endocytosis and/or transcytosis. After that, C-CP-Fe-bLf NCs is released in the tumor site by using enhanced permeability retention effect. Finally, the entry of C-CP-Fe-bLf NCs inside the cancer cells is based on oligosaccharide and/or lactoferrin receptor-mediated endocytosis. [19, 20, 21]

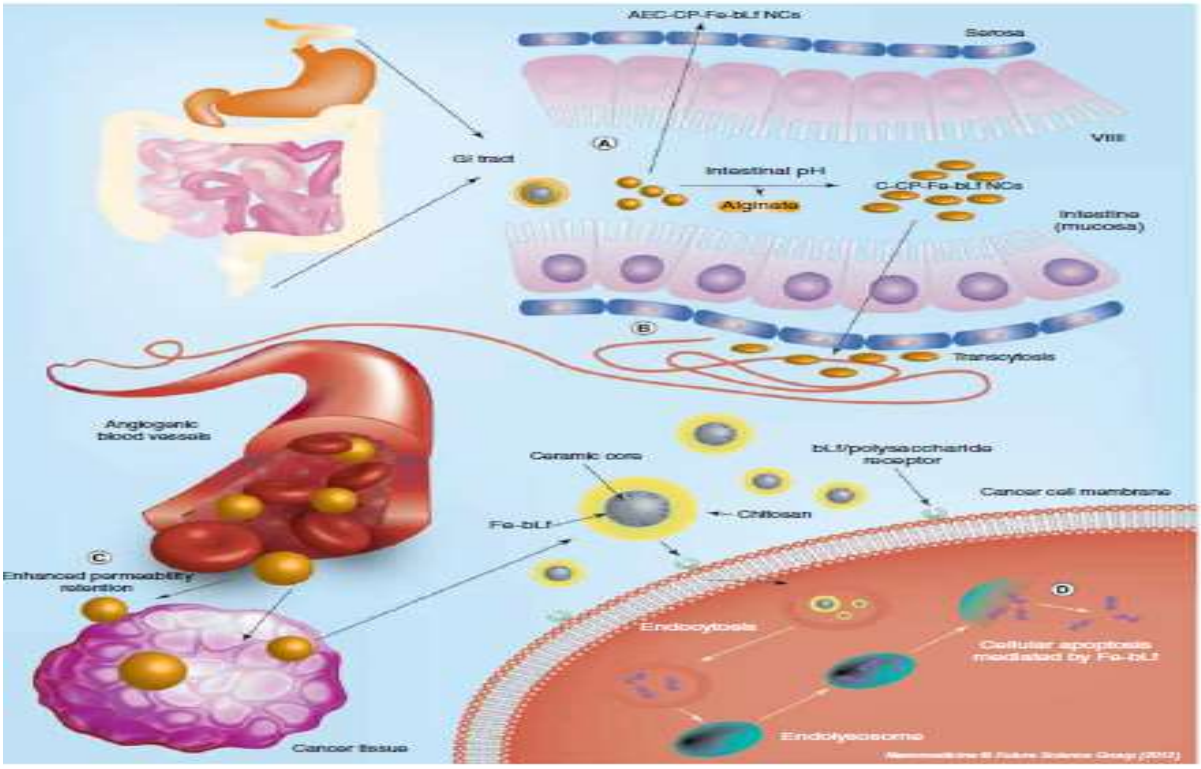

Figure 10: Mechanism of drug release

Ref: Ida Idayu Muhamad et.al. Nano medicine. Pg.no. 303

Both passive and active targeting can be used for nano-particle delivery. Passive targeting is based on the its unique pharmacokinetics of nanoparticles including increased volume of distribution, minimal renal clearance and enhanced permeability and retention (EPR) through the porous angiogenic vessels in tumor. Surface adherence of polymers allows nanoparticles to prevent uptake by mononuclear phagocytes in the liver, spleen, and lymph nodes, thereby improving drug accumulation in the tumor. Active targeting depends on ligand mediated binding of nanoparticles to receptors of tumor cells. Binding of ligands to the vasculature occur immediately, as it is directly accessible to nanoparticles circulating in the blood, particles penetrate into the tissues where receptors expressed on cancer cells and in the interstitium can be used for localization. [22, 23, 24, 25]

CHARACTERIZATION OF NANOPARTICLES:

In-vitro drug release study

In-vitro drug release study of nanoparticles dispersion was carried out in diffusion cell apparatus in phosphate buffer pH 6.8. At predetermined time intervals the samples was withdrawn and replenish with fresh medium and the absorbance was measured by HPLC at its lambda max. Data obtained from the in-vitro drug release for formulation in different release medium were fitted to

various kinetic models. Each experiment was performed in triplicate. The drug release mechanism and linearization were determined by finding the best of fit (R2) and sum squared of residuals (SSR) for each kinetic model. [26]

Drug Efficiency and Drug Loading

Drug efficiency and drug loading can be determined by subjecting freshly prepared solution nanoparticles to centrifuge at 6000 rpm/min for 20 min to get sediments of solid NPs. Next, the drug in the supernatant was analyzed; assuming that drug not present in the supernatant was capsulated into polymer. The encapsulation efficiency (EE) and the drug loading (DL) were calculated from the Equations (1) and (2). [27]

$$EE(\%) = \text{Amount of drug encapsulation} / \text{Amount of drug} \times 100 \quad (1)$$

$$DL(\%) = \text{Amount of drug (mg) in NPs} / 100 \text{ mg of NPs.} \times 100 \quad (2)$$

Morphology of nanoparticles

Morphology of the NPs was investigated by scanning electron microscopy (SEM). The nanoparticles which show spherical shape and smooth surface and absence of different NPs size and narrow size distribution will be considered for evaluation. In this study the mean size diameter measured by Dynamic Light Scattering (DLS) technique, which based on measuring the Z-Average of nanoparticles. [28]

Entrapment Efficiency (%EE)

Percent entrapment efficiency of the drug was determined by suitable analytical technique such as High Performance Liquid Chromatography (HPLC). In this method first formulation was centrifuged at 10,000 rpm for 30 min. Supernant was so collected is analyzed for drug content by HPLC. The percent entrapment efficiency was calculated as follows:

$$\%EE = (Sa-Sb)/Sa*100$$

Where, Sa is the total amount of drug in system, Sb is the amount of drug in supernatant after centrifugation. [29]

Conductivity study

The conductivity study was performed to confirm the crosslinking reaction by conductivity meter. In this method the change in conductivity was measured after each ml of addition of TPP solution to the nanoparticle solution. [30]

Stability of Prepared Single and Dual Loaded NPs.

All nanoparticles must be evaluated for their thermal stability under storage conditions. In general, long-term and accelerated storage conditions recommended by ICH guidelines for drug products intended for storage in a refrigerator are 5 ± 3 °C and 25 ± 2 °C/60 ± 5 % RH, respectively. If drug

products are intended for storage in a freezer, only long-term storage conditions of –20 ± 5 °C are recommended. Long-term stability is generally to be at least 12 months. No formulation should show little to no change in, or zeta potential after one year. This stability was also observed for, NPs stored at −70∘C for one year. [31]

Particle size and Zeta potential

The size of the particles is generally determined by dynamic laser scattering technique, using particle size analyzer at 25°C with an angle of 900. The zeta potential is crucial parameter for stability in aqueous nanoparticulate dispersion. The zeta potential measures the surface charge of the particles. The pellet obtained after centrifugation of the nanoparticulate dispersion was redispersed with water. The diluted sample was taken in a capillary type cell and the zeta potential was determined. [32]

APPLICATIONS:

1. Currently nanoparticles being developed in medicine, involves employing nanoparticles to deliver drugs, heat, tight or other substances to specific types of cells (such as cancer cells) particles. These are engineered in such a way that they are attracted to diseased cells, which allows direct treatment of those cells. This technique reduces damage to healthy cells in the body and allows early detection of the disease. Another goal of Nanotechnology involves delivery of the drug in the right place at right time. [33]
2. Several anti-cancer drugs including paclitaxel ,doxorubicin 5-fluorouracil and dexamethasone have been successfully formulated using nanomaterials. These nanoparticles can be used in targeted drug delivery at the site of disease such as cancer to improve the uptake of poorly soluble drug and targeting of drugs to a specific site, thereby increasing drug bioavailability. A schematic comparison of untargeted and targeted drug delivery systems is shown below. [34]
3. A novel approach for this technology is to use oligonucleotides for sensitizing tumor cells to chemotherapy. The oligonucleotides are being combined with Nano liposomes to target and deliver the nucleic acids to the cancer cells (102) and block production of the alpha folate receptor. This block was shown to decrease cell survival of breast cancer cell lines, and sensitized a cell line by 5-fold to doxorubicin. This is a good example of how nanotechnologies can be used to increase the effectiveness of existing drugs, facilitating the use of lower dosages to decrease toxicity.
4. Recent research has developed number of NPs such as metal, semiconductor and polymeric particles to be use as imaging probes, diagnosis and as delivery vehicles in

cancer therapy. NPs play an important role in cancer diagnosis. The particles such as organic dye, doped polymer, liposomes, and quantum dots are used in cancer diagnosis. Multi functional NPs have the capability to simultaneously carry therapeutic agents, squares imaging contrast agent, diamonds and targeting moieties (circle) that can be used as anti cancer agents. Drug loaded NPs can also be used in treating cancer in animal model. [35]

5. In recent years, chitosan–anticancer drug conjugates have been popular delivery devices to be investigated. In this type low molecular weight chitosan conjugated with paclitaxel (LMWC-PTX) which can also be synthesized by chemical conjugation of LMWC and PTX through a succinate linker, which get cleaved at physiological conditions. These chitosan linked conjugates were evaluated as a carrier for the oral delivery of paclitaxel, exhibited more favorable characteristics than high molecular weight chitosan, such as lower toxicity and higher water solubility. Moreover, LMWC could quickly and reversibly open the tight junctions between human epithelial colorectal adenocarcinoma cells. [36]
6. Treatment of Bladder Cancer Paclitaxel-loaded gelatin nanoparticles was prepared using the desolvation method, and their physicochemical and biological properties were characterized. The size of the particles ranged from 600 to 1,000 nm and increased with the molecular weight of the gelatin polymer. Under optimal conditions, the yield was >80%, and the drug loading was 0.7%.Wide-angle X-ray diffraction analysis showed that the entrapped paclitaxel was present in an amorphous state, which has higher water solubility compared with the crystalline state. Identical, rapid drug release from nanoparticles was observed in PBS and urine, with 90% released at 37°C after 2 hours. [37, 38]

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