Layer-By-Layer Microcapsules for the Delivery of Lipophilic Drugs

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Abstract

About 40% of new compounds have low water solubility, however they are therapeutically active. For such drugs, different methods of formulation development should be proposed. Layer by layer assembly has recently been studied to solve this problem in which lipophilic drugs have been encapsulated. In this layer by layer process, adsorption of different components is done which is facilitated by the electrostatic attraction resulting in the formation of multilayer shells of nanometer size. These drug delivery systems include nano- and micro-particles and emulsions. In this review article, the formulation methodology, advantages, and uses of layer-by-layer assembly approach have been discussed.

Keywords: Lipophillic drugs, Layer-by-layer microcapsules, Drug delivery

1. Introduction

There is an increase in the drug formulations containing poor aqueous solubility drugs. Nowadays there are about 40% compounds which have poor aqueous solubility, however they are pharmacologically active. These molecules face the consequences of low bioavailability in the systemic circulation after oral administration, due to which they are not often used.

After administration of drugs in body, it is absorbed into the systemic circulation from where it is transported to various organs. At this point equilibrium is maintained. For the therapeutic effects, the drug is then distributed to the target sites. The drug are then metabolized and excreted from the body. Drugs sometimes cause adverse effects because they are transported to the site which is not the therapeutic site for the drugs. Drug absorption in gastrointestinal tract can be predicted from the drug dissolution.

A class II and IV drug compound in the Biopharmaceutical Classification System (BCS) is considered to be poorly aqueous soluble drug. The absorption of class II drugs is predicted by the dissolution because their permeability is good but solubility is less. The BCS class IV drugs have both poor solubility and poor permeability and are not good drug candidates. The following systems represent the examples of these classes. In 1970's, for the enhancement of biological activity of compound, phospholipid nucleoside conjugates and nucleosides having hydroxyl and amino groups were used. When lipid groups were attached to the ara-cytidine or when linked to phospholipids, these formed a pro-drug which was having more cytotoxic action compared to ara-cytidine only employed in animal tumor models.

The new molecules of ara-cytidine showed different pharmacologic profiles like reduced catabolism by cytidine deaminase, higher plasma half life, and release of nucleoside monophosphate, this process skip the nucleoside phosphorylation which is rate limiting. These new ara-cytidine molecules easily penetrate the blood brain barrier and are active to tumor cells [5].

Nicotinic acid is a wonder drug for the cure of dyslipdemia which is risk in atherosclerosis. It is also known as a wide spectrum lipid drug [6]. It decreases the level of lipoproteins i.e. VLDL and LDL and subcategory Lp(a) and also increases the level of protective HDL lipoproteins. It has been noted that nicotinic acid treatment decreases the progression of atherosclerosis, symptoms and death rates from coronary heart

disease. Nicotinic acid is now considered to be important in the drug delivery system because it has shown to stimulate the ABCA 1 membrane cholesterol transporter [7].

Phospholipids analogs are active against HIV infection both alone or conjugated with other pharmacologic agents [8]. AZT nucleoside analog, when conjugated to the phospholipids, this complex stops viral replication by inhibiting viral reverse transcriptase and increasing the production of defective virus particles that do not have group120 on the surface of virus which reduced the amount to attach to CD4+ cells and to stop the infected cell-cell fusion. The interest is in that data which shows that phospholipids show activity against drug resistant variants which is major problem in HIV treatment in world. Due to the development of new delivery mechanisms for HIV drugs it has been shown that this approach will reduce side effects and fatal effects and hence the transport of drugs to lymphatic systems and CVS and thus preventing resistant variants of HIV.

DDS with new approach has gained much popularity. These are applied to set pharmacokinetic parameters (ADME) to enhance the safety and efficacy of drugs. Since we are considering the properties of the encapsulated drugs, following properties should be present in the good delivery system [9,10].

a.   Increase dissolution and solubilization: Poor solubility drugs should be subjected to particle size reduction to enhance solubilization during its transport through Gastrointestinal tract

b.   Increasing gastric residence time: By doing so we can increase absorption and dissolution

c.   Increase drug payloads, decrease toxicity, immunogenicity and the stability of drugs in extracellular fluids

d.   By regulating transport to lymphatic system can increase the Bioavailability of lipophillic drugs

e.   Targeted delivery of encapsulated drugs reduces side effects and damage to the body.

f.   The span of treatment can be reduced by enhancing Pharmacokinetics or Pharmacodynamic profile

g.   Drug delivery system should be versatile to be able to deliver combination of anti-parasitic agents

h.   Affordable in cost when compared to benefit ratio

The approaches followed so far does't elaborates the above requirements.

Most viable feature of drug delivery systems is controlled release delivery of encapsulated drug at the specific site. Different ways are employed to increase the release of encapsulated drug i.e. changes in pH (e. g. pH 6.8 in tumor interstitium or 5.0 in endosomes) [11] or the enzyme action in capsule shell [12]. Abrupt and immediate release of drug is most wanted but still the release may be slow. So for this purpose change in temperature (hyperthermia) [13], light (photodynamic therapy), or mechanical disruption (ultrasound) [14,15] are used to open the shell of delivery system.

The various delivery systems e.g. emulsions, micro emulsions, surfactants, liposome and nanoparticles are now mostly considered because these are versatile and have many benefits. Liposomes particles are now mostly used delivery system for lipophillic drugs. Liposomes delivery has a pivotal role in drug delivery. These formulations are mostly used to decrease toxicity and to enhance targeting into the specific site for therapeutic benefits. From this we can assume that liposomes can be used for targeting tissues whether we incorporate recognition molecules or not. Gene therapeutic, vaccines, anti-fungals, anti-tumor cells and antimicrobials are the classes for which safety and efficacy has been established [16,17].

Nanoparticles are solid colloidal particles containing various biocompatible polymeric materials, inside which a drug is entrapped or attached covalently. For the delivery of nanoparticles biocapsules and biodegradable polymers of synthetic nature like poly (D, L-lactic co-glycolate) polyalkylcyanoacrylates are used for fabrication of nanoparticles, polysaccarides like curdlan and macromolecules chitosan, albumen and gelatin are widely described in literature [18]. Traditional colloidal carrier system e.g. liposomes, polymeric nanoparticles and emulsions are advantageous of solid lipid nanoparticles, but here they also reduced the draw backs related to it [19]. These nanoparticles have been used with success for the transport of various drugs e.g. lipophilic and hydrophilic by different routes. The delivery for lipid based drugs has usually single function and do not have diversity (i.e. the incorporation of many drugs in one capsule and

for magnetic delivery). Layer-by-layer assembly technology is used for this purpose i.e. fabrication and surface modification.

This method is applied for the stepwise absorption of various compounds (i.e. polyelectrolytes, nanoparticles, proteins, enzymes etc) electrostatic attraction helps in the growth of multilayer shells with nanometer (thickness) precision [20,21]. The release can be controlled from the enclosed substances if layer-by-layer method is applied. In this review mainly the emphasis is on the utility of Layer-by-layer for nanoparticles, emulsions and capsule delivery.

2. Preparation of Layer-by-layer systems

2.1 Standard methods of emulsification

In order to gain the desired results from the preparation technique special attention should be paid to the properties of the container to be used. After the incorporation of drug to the microcontainer the release and permeability is vital. While encapsulating lipophillic compounds, repetition of various steps is required in which the polarity of the dispersion medium is decreased. These repeated steps are very tiring and time consuming and also gives less output that's why they are not advantageous actually.

Hence to overcome the disadvantage of the repeated open-close procedure, one step technique on the basis of accurate deposition of polyelectrolyte Layer-by-layer has an increase practical value. In this article a new procedure for preparing the filled micro and nanocapsules which are an O/W emulsion, in which the droplets are coated with Layer-by-layer in the disperse phase. The oil loaded small containers for emulsion encapsulation have several benefits; very less work has been done in this regard [22-24], for which results were given.

Many steps are conducted to form Layer-by-layer carriers. Emulsion core by the process of emulsification were prepared first in which lipophillic material was dissolved. The process for emulsification is Ultra Turrax homogenizer. The change on the core of emulsion is noticed and according to that deposition of Layer-by-layer was done with layer of polyelectrolyte. To increase the separation of the disperse droplets which have strong electrostatic repulsion, low rpm centrifugation cannot be used as substitute. This is because the droplets of disperse phase have high coalescence and deformation. For the purpose of change the creamed emulsion layer, is drop-wise added to the polyelectrolyte solution with proper stirring. To precede the process of encapsulation further addition of the polyelectrolyte aqueous solution is done. To get the desired number of layers charged polyelectrolyte, alternate deposition was carried out.

As mentioned in the start, the fluid core in this encapsulated emulsion has a role of capsule load. This liquid oil is incompressible, deformable and mobile as compared to solid one. Now, on a glass substrate if the encapsulated particles of emulsion are transferred there will be interaction of the outer layer of polyelectrolyte in the capsule shell and the substrate and these interactions are strong enough to de-shape and disrupt the capsule. The capsule destroys after the drying process [25] which in turn releases the oil which was loaded in the capsule. Further additions of coats are done until the desired thickness of the shell is achieved. Different oils like artificial fats, fish oil, triglycerides, soybean, vitamin B12 or dissolved drugs in oil can be used in oil core. Also it depends on the application of the container demand in the various fields like medicine, biology, pharmacy or industry of food.

Previously it was studied that instead of bio-emulsifiers, the biopolymers like proteins can be essentially used for the encapsulation of emulsion [22,26,27]. There is an important class of proteins which are meant for the encapsulation of Layer-by-layer emulsion. These protein compounds are polyelectrolyte in nature as well as the interfacially active because of the lipophilic parts in their structure. At the interface of adsorption site these proteins provide a charge and the deposition of the Layer-by-layer occurs by conventional polyelectrolytes or biopolyelectrolytes. There is a question about the preparation of primary emulsion which is prepared from the water-soluble surfactants whose stability is because of the molecules of surfactants on the interface of oil/water and this is related to concentration in bulk.

About a micrometer scale, multilayer biogenic capsules of proteins were formed while also the lipids on the emulsion drops interface [28]. The capsules which are spherical in shape have uniform wall around the

fluid interface and the mechanical power is high for that of dry capsule, this was shown by the images taken from optical microscopy. If the protein layer is adsorbed the properties will alter at the interface. Rhodamine B dye was used to color the capsule shells so as to view the shape, by using florescence microscopy. The droplets of chloroform which are present in the aqueous solution having serum albumin of human were covered with the co-adsorption of lipids and proteins. As the time passes the chloroform will evaporate and the size of the droplets will shrink and a surface with folds will be formed. When the whole solvent gets evaporated we will get a capsule which is elastic and hollow from inside.

Using a single process interfacial polymerization of dopamine for the preparation of monodisperse polymer (polydopamine, PDA) on the emulsion droplets of dimethyl diethoxysilane (DMDES) and after this these DMDES were removed with ethanol, this study was done previously [29] if the condensation time for emulsion is changed the diameter of PDA can be changed from 400 nm to 2.4 um. If we adjust the concentration of the emulsion then we can have a capsule ranging from 10-30 nm. By repeating the interfacial polymerization steps we can get a high thickness of dopamine, with three cycles we will have a thickness of capsule up to 140 nm. In these emulsion droplets we can load thicoraline like lipophillic and stabilized nanoparticles of quantum dots i.e. CdSe/CdS. By means of magnified resolution images of TEM, one capsule have quantum dots of 2000 was observed. The PDA capsules which were loaded without aggregation were monodispersed.

Capsosomes having liposomes which are compiled like in cargo were reported to be a new delivery system of drugs by Caruso et al. [30]. The characterization of the film of multilayer assembly was carried out for poly (allylamine HCI) (PAH), DOPC liposomes (50 nm 1, 2 dioleoyl-sn-glycero-3-phosphocholine) and poly electrolytes like PSS (polystyrene sulfonate), this was done by using microbalance of quartz crystal along dissipation monitoring, the results were then compared to the growth of the film obtained on silica particles of intact liposomes and polyelectrolytes. The film assembly of liposomes on the substrates which are planar was then made onto the colloidal spheres of silica and after that the capsosomes were obtained when the core of silica was removed. These polyelectrolyte shells of liposome are best as barrier for the safeguard of the low molecular weight molecules, fragile compounds and therapeutic agents. Especially it is best for the protection of enzymes which easily get denatured in tough conditions and have different interactions with the surfaces. The integrity to the structure of liposomes is also provides by the polyelctrolyte shells but this depends upon the liposomes (50-200nm) size

The stable capsosomes were obtained when the silica core was removed, the adsorption on the surface of the silica resulted in the rupture of the liposomes, and this was not observed with the multilayers of polyelectrolytes. The number of adsorbed liposomes can be increased by incorporating another layer of liposomes and also with the high number of polyelectrolyte layers in liposomes layers. This is the diverse function of capsosomes that it gathers the benefits of both polyelectrolyte shells and liposomes, and it has applications in the formation of organelles and artificial cells in a closed atmosphere.

2.2 Ultrasonic emulsification

The way the sonochemical effects occur can be best studied by acoustic cavitation that is the formation of bubbles and its increase in size and its rupturing [31]. High energy regions are generated by means of these cavitations which have capability of forming pressure and heating in very short time. About 5000 K heat has been recorded inside the cavitating microbubble; this was confirmed by the metal carbonyl substitution and spectra of sonoluminescence [32]. The rate at which these were cooled was 1010 K/s and the pressure was 1000 atm. To keep the temperature low at the surface of the solution in bulk the cavitation process can help in this by forming intense conditions of physical an chemical processes at the interface of gas/liquid.

These intense conditions by cavitation process of microbubble can be used for various transformations of physical and chemical nature, this leads to the making of spherical shells which have in its inside cavity either liquid or gas. This shows a different microbubble having physical and chemical properties at the surface. The protein shelled non-aqueous microcapsules with liquid filled and microbubbles with air can be formed by subjecting serum albumin from human, bovine and hemoglobin solution of proteins to an ultrasound of increased intensity i.e. 20KHz for 3minutes [34]. These microspheres are smaller in size than the size of erythrocytes i.e. about 2.5 in diameter and can be kept for months and also easily pass through

the systemic system. These are formed by two processes like cavitation and emulsification. The microbubbles of albumin can be used experimentally and it shows that they are cross-linked with disulphide of cysteine which is present in the center of molecules of proteins. Oxygen and water generates superoxide in the acoustic cavitation process which acts as an oxidizing agent in cross-linking. Due to this the proteins in the microsphere shell do not denatured prominently and the hemoglobin will retain the heme part in it. The thickness of the cross-linked microsphere shell is approximately 6 protein molecules. While performing irradiation there is a dependency of microsphere yield on the solutions temperature. While we are configuring any experiment we should first optimize the temperature for that. In order to measure the concentration of oxygen in biological system the microspheres made from serum albumin of bovine filled with nitrogen are used [35]. The size for microsphere is usually 3um and oxygen easily permeates through the protein shell. The response of resonance signal of electron paramagnetic to oxygen increases about 40 fold after its encapsulation of nitroxides into microsphere, this also safeguard the nitroxide from bio-reduction. When the microspheres were injected into mouse, and it was observed after 70 min there was no significant reduction in the signal of paramagnetic electron resonance.

To target the tissues or any specific organ and to enhance the stability, we can change the properties of the ultrasonic microspheres. By using layer-by-layer process for the surface modification of microspheres these change characteristics can be obtained. The polyglutamate/polyethyleneimine (PEI)/polyacrylic acid (PAA) and hydrophobic loaded nano-containers silver dye/polyglutamate/PEI/PAA and 5,10,15,20-tetraphenylporphin in toluene were obtained both by the process of layer-by-layer and by ultrasonic method, which gives stable, about 600nm uniform microspheres [36]. A large forms of drugs which are insoluble in water are inside the lipophillic core which is in the container while the permeability is taken over by the shells of polyelectrolyte, these have multi-functions and are utilized for the addressing of drugs that are water insoluble, example is the targeting of anti-tumor drugs.

With the pH values of 4, 6 and 7 and with the addition of hydrochloric acid dilute, the 5% (w/v) aqueous solution of glutamate was layered by the toluene solution 5% (w/v). At the interface of organic and water an increased intensity horn of ultrasonic was placed. By the 150W power at the frequency of 20 kHz the mixture was sonicated at standard contidions for 3 minutes. In the aqueous phase the remaining polyglutamate containers loaded with toluene were suspended. When the polyglutamate shells loaded with toluene are formed the polyethyleneimine/polyacrylic acids which are biocompatible shell was performed immediately on Layer-by-layer assembly. Alternating layers of PEI/PAA/silver were used to form shells of nanostructured Layer-by-layer. The nanocontainers of original polyglutamate which are not coated with polyelectrolyte are less stable than the composite nanocontainers. These can be maintained for a month at about 2-5 ℃, as compared to this the nanocontainers of polyglutamate which are fresh are stable at 2-5 ℃ for many hours. When polyelectrolytes with opposite charged were used while Layer-by-layer deposition, no agglomeration was observed.

By changing the ionic strength and pH value we can bring variation in the shells of polyelectrolyte/polyglutamate which will ultimately release the lipophillic material loaded in the shells. The container of Polyglutamate gives a high yield if the values of pH are changed. When the pH is stabled at 6 or 7 the containers are formed while the treatment with sonochemical will not yield containers at 4.0 pH. The polydispersity and the capacity of the container can be increased if the values of the pH are decreased from 7 to 6. A drug carrier with a bio-friendly nature was prepared by using a hydrophobic drug i.e. rifampicin and vegetable oil, this was studied by Teng et al [37]. The antibiotic, rifampicin is a type of rifamycin and is semisynthetic in nature, it is indicated in the treatment of viral and bacterial infections because it the RNA synthesis is disrupted by it. Besides the active drug and oil in the core of the carrier, an emulsifier is also used that is lecithin. The multilayers of polyethyleneimine/polyglutamate/polyacrylic acid were used to make the containers shell, and the lipophillic drug can be released from this shell by changing its permeability by means of changes in the pH values of the medium. About 0.15 is the index of polydispersity of the containers loaded with polyglutamate oil and for about 4 months at 4 ℃ they used to be stable.

In order to improve the treatments for therapeutic purposes, platforms of nanomedicine of multifunctional nature can be developed by combining the various functional materials. An approach of nanomedicine for

the formulation of many active agents in one form and for the targeting a specific location, for the ease of administration and for the protection of drugs Han et al. [38] gave a combination which comprises of protein containers and magnetic nanoparticles. For this purpose the layer by layer process was used and on the protein surface the magnetic nanoparticles were coated. The magnetic containers used to flocculate so, to stop this we should add polyelectrolyte to the outer layer. On the side of the vial a magnet was placed which after 30 minutes turned the brown solution transparent, close to the magnet and near from the brown precipitates the containers were collected. When the magnet was removed the magnetic containers after little agitation were feasibly redispersed in solution.

The sonication time, process and volume are the acoustic variables of acoustic power which are important for subjecting materials in the sonication process and this also provides an input of energy for the size determination of microspheres [39]. The size of microspher is inversely proportional to the time of sonication. The equilibrium in the suspension is observed after few minutes when the plateau for size is reached. When the amplitude power is reduced there we observe that the equilibrium time also increases. The acoustic amplitude and the volume reaction are the factors on which the timings of equilibrium mainly depend. The bigger and small microspheres are formed due to low and high amplitude respectively. In the ultrasonic vessel, the power distribution affects the size of microspheres. The microspheres formed can be arranged by their sizes because of the unequal distribution of acoustic power in the vessel, so by this way a broader microspheres size distribution can be achieved.

The ultrasonic field produces fluctuations in pressure to which the microbubbles gas-filled protein shells gave response and these are produced ultrasonically. These gas filled microbubbles coated with polymers have a responsive mechanism to the ultrasound. During the acoustic cycle an expansion phase occurs and due to this expansion the wall of gas/shell breaks down, this expansion is due to acoustic pressure. Gas microspheres with protein shell can be used for target delivery which is accelerated by ultrasound.

By the use of ultrasound of high intensity in aqueous solution we can form microbubbles which are functioning and stable. These microbubbles are coated with lysozymes. Cavalieri et al. [40]. If the sonication time and the bonds of intramolecular disulphide are controlled then we can control the extent of cross-linking present in the molecules of lysozymes. Optimal distribution of size can be achieved if in the sonication process the treatment for denaturation is coupled with the range of 2-5 minutes and 30 seconds. The antimicrobial activity of microbubbles coated with lysozymes can remain for many months and also stay stable. The properties of microbubbles at the surface can be changed by the deposition of polyelectrolyte in layer by layer form on the air-core template protein shell as this is the versatile technique and is useful for both diagnostic and therapeutic purposes, also these microbubbles can be used for the adsorption of various biolabels and potent drugs. The biological activity of encapsulated epigallocatechin gallate (EGCG) nanoparticles and also it inhibit the hepatocyte growth factor as effectively as free form of EGCG. About 20-70% weight is of loaded polyphenol. The release time for EGCG is more rather than the nanoparticles which are non-encapsulated and also they maintain its activity against breast cancer which is created by the HGF intracellular signaling.

2.3 Polyelectrolyte microcapsules

Water based systems were usually used in layer-by-layer capsules of polyelectrolytes which are dispersed in water based solution in which the materials are encapsulated containing aqueous interior. Many examples of non-water based material into polyelectrolyte encapsulation can be mentioned based on the technique of changing either of the inner solvent or by using immiscible substances like hydrophobic with hydrophililic ones. D. May et al. [42] described a unique delivery agent which comprise of three agents having outer layer of   lipid and an inner layer of oil and the core consisting of gaseous agent. The oil layer which then investigated is composed of soybean oil, corn oil or the triacetin. The total diameter of these three agents is 0.5-8 μm and so can bear a big payload in comparison to nanomin diameter shells. These oil shells can be filled with lipophillic substances like paclitaxil ones. The thickness of oil layer in the active liposheres range from 0.3-1.5 um. Some of the components of microcapsules like chitosan and sodium alginate, magnetite nanoparticles, and lipid dipalmitoyl-sn-glycero-3-phosphate (monosodium salt) (DPPA), (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) (DPPC) or their composite 10% DPPA/90% DPPC are

natural polyelectrolytes [43]. The concentration and temperature of the drug affect their power of incorporation of substances. At certain temperature like at 60 ℃ the power of incorporating substances reaches to 22.4%. The phase transition of lipid DPPA film was lower and higher than the properties of microcapsules which releases drug at 37 ℃ and 40 ℃, respectively. As a result it was shown that lipid film base microcapsules are thermo sensitive and so can efficiently prevent the drug from releasing into the non-targeted sites.

The layer- by -layer adsorption of polyelectrolytes on sacrificial particles [44] can be used for producing polyelectrolyte multilayer capsules templates and this can be utilized for production of different range emulsions of predetermined size and surface chemistry. In case of sacrificial particles silica material of mesoporous and nonporous nature were used. The silica core were pierced by the HF and the oil was infiltrated through these semi permeable walls and if oil is in excess then it would be removed by PEM, uni-disperse layer-by-layer coated oil droplets can be obtained by this technique. The capsules obtained by this procedure would have size range from 3, 8 and 10 μm. The emulsions of the drugs filled with oleic acid inside the capsules of PMA were studied in the process of encapsulation [45]. Capsules having disulfide linkages are oxidation-reduction sensitive and that were studied in the release of drugs from encapsulated doxorubicin. Viability analysis demonstrated that encapsulated emulsions of these polymers are effectively engulfed by targeted tumor cells and this engulfment was pronounced with the 0.5 μm drug loaded capsules as compared to 1um or non-encapsulated drugs.

Human erythrocytes were used as base for polyelectrolyte capsule according to which different polymers of poly (sodium styrenesulfonate)-poly (allylamine hydrochloride) were made of the same shape and were used for a solvent exchange technique by using different non-aqueous solvents like methanol, ethyl alcohol, pentanol, hexanol, octanol, octane and decane and then the outer solvent was alternated with the inside one [46]. The scanning laser microscopy has shown the encapsulation of organic solvent in the capsules. If the solvent is exchanged the multilayer of the polyelectrolyte will be damaged and the interior part of the capsule will be collapsed. The sulphate and amino parts of multi-layers will disappear and the result will be cross-linking. For the encapsulation of organic solvents, poly (allylamine hydrochloride), melamine formaldehyde (MF) and poly (sodium styrene sulfonate) are also used. Several capsules are lost in the process of solvent exchange and the one which have less output is noted. Majority of the capsules will float on the surface of the capsule and n-octanol, if n-octanol is loaded inside the MF capsules. The deformation of capsules shell happens when the centrifugation speed is raised up to 5000 for 10 min. Mechanically stable and heavier capsules should be produced to overcome the problems of yield and efficiency [47]. If the bilayers of polyelectrolytes are increased, thicker capsules will be formed and there will be prevention of deformation. This process is considered to be time consuming. Also the release from the core of the thick capsule is difficult.

For the carrying of organic solvent i.e. toluene in water, a new matrix capsules were shown which gives stable dispersion without the use of surfactants [47]. By the use of opposite charged polyelectrolytes the matrix capsules can be formed by the method of Layer-by-layer adsorption. This adsorption is done on the calcium carbonate and the removal of core is also done. As the surface inside the calcium carbonate is rough and forms matrix capsule, so they have increased efficiency of microencapsulation which remains stable for several time. The first layer accumulated on particles was the polycation poly (allylamine hydrochloride) after that the anionic layer of poly (styrene sulfonate) is used to coat the particles of calcium carbonate modified to poly (allylamine hydrochloride). For achieving desired thickness the process is repeated again. EDTA can be used to remove the coating of CaCO3, followed by dispersion of these capsules in 96% ethanol solution, followed by keeping for 30min incubation period, followed by dispersion in the 1:2 mixture of ethanol and toluene and so on gradually reducing the concentration of ethanol at last the resulting matrix form are dispersed in pure toluene.

By the use of cross-linking reagents, different molecular weights and functions of polymers and template particles of mesoporous silica a capsule with the thickness of 15-60nm and diameter of 270-400 nm can be formed [49]. The macromolecule's molecular weight and nature will set the thickness of the capsule shell. To adsorb the poly (L-glutamic acid) PGA combined with doxorubicin (Dox), the positively charged amine-functionalized particles of mesoporous silica was used. Mesoporous shells were used for the

encapsulation of polymer drug conjugate (PGA-Dox). These were then cross-linked in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride using 2, 2'- diaminodiethyl disulfide dihydrochloride (cystamine). Homogenous sized nanocapsules of PGA-Dox were gained after removing mesoporous shells. The diameter of the capsule is 370nm and has thick wall i.e. 55-60 nm. The delivery system of PGA-Dox capsule is different i.e. they resist deconstruction and stay intact in physiologic pH because of the chains present in the PGA-Dox have lysosomal reducing conditions. It also prevents degradation by lysosomal hydrolases which occurs due to stimulus from any chemical inside the cells. They cause the death of tumor cells by the transport of Dox to LIM 1215, which are tumor cells of human colorectum. They also have a quality of targeted delivery.

According to Layer-by-layer technique the natural polyelectrolytes of sodium alginate and chitosan having water dispersed media were used to constitute the hydrophobic photodynamic drug like Hypocrellin B (HB) [48], by this method the HB encapsulation is effective, efficiently delivered to the targeted tumor cells. In vitro the intracellular HB loaded microcapsules gives possibility of controlled delivery to the MCF-7 tumor cells of breast by a very affective biocompatibility under light prohibited conditions while become toxic to the cells under light. The transportation of the HB inside the cells is also affected by the presence of ions on the outer layer of the cells and positive charges are responsible for transporting the drug inside the cells.

2.4 Coated drug crystals

To prolong the release of microparticles of drug formed by the process of microencapsulation is a good method. Also to target the drug to specific region, the microparticles can be surfaced with antigens. These properties of microparticles result in the low dose of drug, the adverse events can be reduced and the concentration of drug will be achieved quickly. The crystal of drugs which have less aqueous solubility can be changed to enhance the control release and to target the delivery of drug in dispersed phase by the technique of layer by layer which is perfect for this procedure.

In aqueous solution the release of the drug was controlled by encapsulating gelatin and polyions along with the microcrystals of furosemide [50]. Using layer-by-layer technique the gelatin and polyions were accumulated step wise on the microcrystals of drug of 5um. About 2-6 bilayers of PSS/gelation were adsorbed on the capsule whose thickness vary from 45-115nm and then stepwise the coatings of poly (styrenesulfonate) PSS and poly (dimethyldiallyl NH4Cl) PDDA was done. The capsules coated with 2-6 bilayer reduced the release of furosemide from microcapsules about 50-300 folds which was not seen in uncoated furosemide. The release of furosemide was maintained for about 3 hours in the simulated physiologic conditions and this was because of the enhancement in the composition and thickness of the walls of microcapsules. While coating furosemide microcrystals step 1 includes the addition of PSS which is negatively charged, and then a PDDA polycation was coated on it, this coating was repeated 2 times. After that 2-6 layers of gelatin/PSS were coated on the microcrystals, the release at different pH values was observed in step 3.

The partial release of drug at pH 1.4 for 2, 4 and 6 layers of gelatin was 0.11, 0.29 and 0.51%, respectively, while the release time of 0.71 h was observed for the unchanged microcrystals of drug. Compared to the uncoated micro-crystals, time for encapsulated furosemide was 2, 6 and 10 times greater. For 2, 4 and 6 bilayers of gelatin, the cumulative release were 1, 5, 3 and 5 h, respectively. The release time was not prominently increased by the coating of furosemide with the bilayers of 6-10 PDDA/PSS. A three staged release was observed. In the initial few seconds there was a quick release which gradually declined linearly with time. The total dissolution of drug is achieved when there is an asymptotic condition; this is observed in the last stage. A linear region is not obtained for the release of drug in a solution having 1.4 pH.

Polysaccharides having 10, 20 and 30 multi-layers were used to encapsulate microcrystals of ibuprofen having 5-40 μm size [51] to make the ibuprofen crystals coatings with thickness of shells ranging from 20-60 nm, the negative charged carboxymethylcellulose, sodium alginate and dextran sulphate and positive charged chitosan polysaccharides were used. Before the alteration of crystal surface the coating to the crystal was applied directly. By the use of micro-electrophoresis and dispersability measurement the extent of first layer was studied. The dispersability of microcapsules of ibuprofen in aqueous solution was greater. The thickness of the microcapsule shell reduces the release of ibuprofen but in the dispersion solution,

when solubility is increased the release also increases. At pH 7.4, fast release less than one minute is observed for drugs which have increased solubility. At pH 1.4, release of drug is very less taking several hours showing less solubility of the encapsulated drug. The microcapsules crystals of small, medium and large size shows complete release at 20, 30 and 60 sec, respectively at pH 7.4 and the release is about 1, 2 and 4 h at pH 1.4. The larger the crystals size increased will be the time for release of drug from microcapsules. As the ratio of surface to volume is less due to which the release is slower in large crystals. If smaller crystals for same extent of ibuprofen are formed it will have increased dissolution in bulk solvent as the surface area will be high. A novel approach for the formulation very less aqueous solubility components was shown for curcumin [52]. The organic solvent which is miscible with ethanol was used for the dissolution of drug. Further, if the aqueous polyelectrolyte was added to the solution then by the process of ultrasonication drug nucleation can be induced. Depending upon the power of sonication, initial solvent used and the concentration of components the size of the curcumin crystals i.e. 60-100 nm was gained. If the adsorption of polycations is done on the particles and then the formation of layer-by-layer nano-shell polyelectrolyte, the aggregation between the nanoparticles can be avoided. About 10-20 hours sustained release of drugs can be achieved from Layer-by-layer polyelectrolyte encapsulation. In 60% of water/ethanol solution the powder of curcumin was dissolved. The addition of protomine which is biodegradable or the aqueous poly (allylamine hydrochloride) polycation was then done. Water was slowly added during sonication in the solution. This results in the crystal nucleation of curcumin. In the initial stage of ultrasonication the growth of the drug particle was stopped. When the sonication was ceased the aggregation did not occurred and the particles of crystal were stable. The size of the crystal was affected by two factors, one is the addition of water and the other is the concentration of curcumin. At the addition rate of 0.4 mL of water/min, particle size of 320 nm was gained and at 0.05 mL/min rate the particles obtained were of the size120 nm. The large nanoparticles can also be obtained if the initial concentration of curcumin is increased. About 80-90% of the drug can be encapsulated in curcumin nanoparticles because it has a thin coating while liposome and micelle have a capacity of 3-5%.

3. Conclusion

Prominent progress has been made in the past hundred years for the use of layer by layer technology for the release and action of lipid based drugs, limited research has been performed in the development of long duration delivery medium with shell components which have high compatibility with biological system. The latest main disadvantage for the applications are large size of particles which causes release of drug (they should have 100-300nm range, and should not be in range of micrometers), there is little compatibility of the multilayer component with the biological system (only in some cases biocompatible layer-by-layer shell was presented) and the procedure for constructing the delivery particles is very difficult and time consuming. To reduce these problems many additional efforts will be required. All the techniques discussed in this article have good perspective for further research and study. For further work in this field in vivo and in vitro studies should be done on delivery systems. Also development can be done on the vehicles which will be Layer-by-layer based, as it is a versatile method and have accuracy of nano-scale for various things.

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