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Microspheres for colon targeted drug delivery: A comprehensive review

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Abstract
Colon targeting is a comparatively new approach for the localized delivery of drugs to obtain maximum possible output for the treatment of ailments. In order to target a drug inside the body it is required to be in the form of a dosage form. Various conventional dosage forms are there to target drugs to the colon but they don't prove to be efficient in the controlled and sustained release. Different Novel drug delivery systems are developed in order to overcome the disadvantages of conventional dosage forms such as liposomes, microspheres, nanoparticles etc. Microsphere is the novel drug delivery system having the particle size of less than 200µm. The materials used, polymers used and method of preparation have a characteristic influence on the therapeutic efficacy of microspheres. This review targets the various advantages of microspheres over the conventional dosage forms for colon targeting of drugs, their different method of preparation, properties of microspheres which are deciding factors for the efficient delivery of drugs and their evaluation techniques used. Microspheres are not only beneficial in sustained drug delivery but also serve as an efficient tool for localized therapy.

Keywords: Microspheres, colon targeted, sustained drug delivery, review

Introduction

Colon targeted drug delivery refers to the delivery of drugs directly to the colon in order to improve various complications encountered during drug administration by oral route. A number of colonic diseases like ulcerative colitis, amoebiasis, colonic cancer, IBD and various other colonic pathologies require the drug to be delivered at the target site for their improved treatment and reduced side-effects. Colonic route of drug administration is not only used for the local treatment of colonic diseases but it can also be used for the systemic delivery of protein and peptide drugs [1]. Colon targeted delivery is also recommended for the drugs susceptible to the enzymatic and chemical degradation in the upper G.I.T. Now a days vaccines are also delivered to colon by oral route as colon is rich in lymphoid tissue [2]. The diseases which are sensitive to the circadian rhythms such as angina, asthma, arthritis etc. are also treated effectively with reduced side effects by delivering the drug to the colon as it offers a longer transit time [3]. The colon targeted drug delivery system should ensure the drug to be protected in the upper G.I.T and releasing it on reaching the colon by the help of some triggering mechanism. Various approaches are used to target the drug to the colon, some most frequently used are pH dependent approach, microbially triggered approach, prodrug approach, time dependent release, pressure controlled systems etc. Novel systems developed for colon targeted drug delivery are liposomes, hydrogels, multiparticulate systems which include nanoparticles, microspheres, microcapsules, microtablets and some more [4]. The conventional dosage forms have a disadvantage of inefficacy and toxicity, so focus has been laid on the development of Novel Drug Delivery Systems which shows comparative steady state plasma concentration [5]. By using the NDDS, the drug molecule becomes more efficient and also its market value and the product patent life increases [6]. Controlled and targeted drug delivery is most focused systems with respect to the patient's compliance and the latest market demands all over the world. Earlier researches on controlled drug delivery focused on the development of zero order devices [7]. The zero order device is the system which maintains the drug concentration for a prolonged period of time in blood. Microspheres are found to be effective in the development of controlled and sustained release drug delivery systems [8, 9]. Biodegradable polymers are used for the controlled drug delivery for the last two decades.

Anatomy and Physiology of Colon

The length of human large intestine is about 1.5m. It starts from the distal end of ileum to anus [10]. Colon is present in the upper five feet of large intestine and is located in the abdomen. The outermost layer which lines the cylindrical colon is serosa, which is composed of aerolar tissue covered by single layer of squamous mesothelial cells. The next layer of large intestine is muscularis externa which consist of inner circular layer of fibres surrounding the bowel. The other two layers are the mucosa and the submucosa respectively [11]. The transverse colon is the longest and the most mobile part. The colon starts from ceacum which is spherical in shape which continues into the ascending colon situated under the liver. The ascending colon then leads to the transverse colon present beneath the left rib cage which turns downwards at splenic flexure forming descending colon. The last part of colon is the sigmoid colon which is S- shaped present in the lower part of the abdomen. The colon is approx 2-3 inches in diameter [12]. The physiology of the proximal and distal human colon is different thereby affecting the drug absorption. The luminal contents of the colon also differ from being liquid in the caecum to semisolid in the distal colonic region. The anatomy of colon is shown in figure 1.

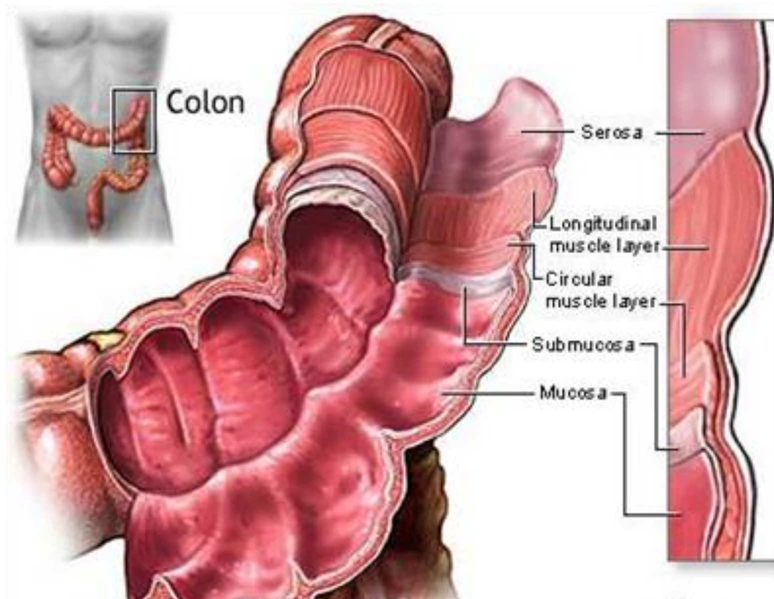

Fig 1: Anatomy of colon

Applications of Microspheres in Colon diseases:

Microspheres have been widely used for the treatment of various colonic ailments like ulcerative colitis, cirrhosis, IBD, colon cancer etc. Colon targeting is done for the drugs which are susceptible to damage by stomach acid and pancreatic enzymes [13]. Colon is also considered to be a potential site for the systemic absorption of peptides and proteins because it has less hostile environment in comparison to stomach and small intestine [14]. Drugs like nifedipine, theophylline, and isosorbide show better systemic absorption in the colon [15, 16] .Colon targeted drug delivery is also beneficial when the delay in systemic absorption is required from a therapeutic point of view and also for diseases which shows circadian rhythm [17]. Colon offers long residence time of drugs thereby increasing the absorption of drugs. Colon targeting of some drugs is also done to prevent their first pass metabolism after oral administration [18].

Factors to be considered for colonic delivery of drugs [19-21]

The physicochemical and biopharmaceutical properties of the drugs like solubility, permeability, stability should be taken into consideration. The drug release and its absorption should be prevented in the upper part of GIT, so that it reaches colon and get absorbed there. The release of drug should be under controlled rate. The colon is more viscous than the upper GIT because of low fluid content, so the drug should be in solution form before it reaches the colon.

Limitations of colon targeted delivery systems [22]

Anatomical position of colon is at the distal portion of the alimentary canal making it difficult to access. The pre requisite for the absorption of drugs is that they should be in solution form, but colon having less fluid content and more viscosity than that in the upper GIT offers difficulty for the absorption of poorly soluble drugs. Drug transport across mucosa is restricted in colon as it has lower surface area and relative tightened tight junctions. The reliability and efficiency of the drug delivery to colon is doubtful as the drug has to pass different range of pH and enzymes before reaching the target site.

Different dosage forms can be utilized for targeting drugs to the colonic region like tablets, capsules, multiparticulate systems etc. Multiparticulate systems is advantageous than conventional dosage forms because of showing more predictable gastric emptying and less localized adverse effects [23]. The detailed description of microsphere based colon targeted systems is given below.

Microspheres

Microspheres are generally the micro particulate systems which are intended for oral and topical use [24]. The classification of micro particulate systems is shown in the figure 2. Microspheres may be defined as free flowing powders made up of biodegradable synthetic polymers or proteins and having a particle size of less than 200μm [25]. Microspheres have a potential of delivering the drug to the target site and also prolong the release of drugs, hence improving the treatment. The technique of microencapsulation depends on the properties of drug, polymer and the intended use [5]. For the efficient entrapment of the drug substance, the selection of appropriate method of microencapsulation is most important [26]. Microspheres can be of different types which include bioadhesive microspheres, magnetic microspheres, floating microspheres, radioactive microspheres and polymeric microspheres [27]. Microspheres are normally polymers which are classified into mainly two types which include synthetic polymers and natural polymers. Synthetic polymers can further be of two types, biodegradable polymers e.g. Lactides, Glycolides, Polyanhydrides etc. and non-biodegradable polymers e.g. Poly methyl methacrylate, Acrolein, Epoxy polymers etc. Some of the examples of natural polymers are Albumin, Gelatin, Agarose, Chitosan etc. The loading of drug in the microsphere is done either during the preparation of microspheres or after their formation by incubating them with the drug to be entrapped. Although it is observed that maximum loading of drug occurs during the preparation of microspheres. The process variables like method of

preparation, heat of polymerization, intensity of agitation affect the loading capacity. The type and properties of the polymer and the drug entrapped used influences the release of drug from the microspheres.

Different types of microspheres are categorized as mentioned below [28-30]:

a) Magnetic Microspheres: These are further of two types
   i. Therapeutic magnetic microspheres
   ii. Diagnostic microspheres
b) Bioadhesive microspheres: polymers used for bioadhesion are
   i. Carboxymethyl cellulose
   ii. Carbopol
   iii. Tragacanth
   iv. HPMC
   v. Sodium alginate
   vi. Gelatin
   vii. Pectin

Out of these, gelatin has fair and pectin shows poor bioadhesion property while the rest mentioned above shows excellent bioadhesion [31].

c) Radioactive microspheres: These are sized from 10-30nm, which is a larger size than capillaries. So when injected to the artery of interest leads to the tumor. Different types of radioactive microspheres are α, β and γ emitters[32,33]
d) Floating Microspheres: These microspheres have less bulk density than the gastric fluid thus remains buoyant in stomach for longer time thereby releasing the drug slowly [34].

Different method of preparation of microspheres using various polymers has been mentioned in table1. [35-46].

Advantages of colon targeted microspheres over conventional method of drug delivery are mentioned as below:

1. Microspheres show much better drug dispersion in the G.I.T and also more uniform drug absorption.
2. Microencapsulation makes the drugs available for a prolonged period of time when given by the oral route accompanying with the decreased G.I.T irritation
3. With the microencapsulated dosage form the problem of polymer retention in the intestinal region which is encountered with the matrix tablets on frequent dosing is avoided [47].
4. As the size of microspheres is small, they get widely distributed throughout the G.I.T which prevents the side effects caused by the localization of the drug at the intestinal mucosa [48].
5. Delivery of drugs through microspheres decreases the inter- and intra- subject variability.
6. Microspheres show much consistent transit through the G.I.T [49].
7. Microspheres show enhanced efficacy and pharmacokinetic effects.
8. The microspheres are more stable due to encapsulation [50].

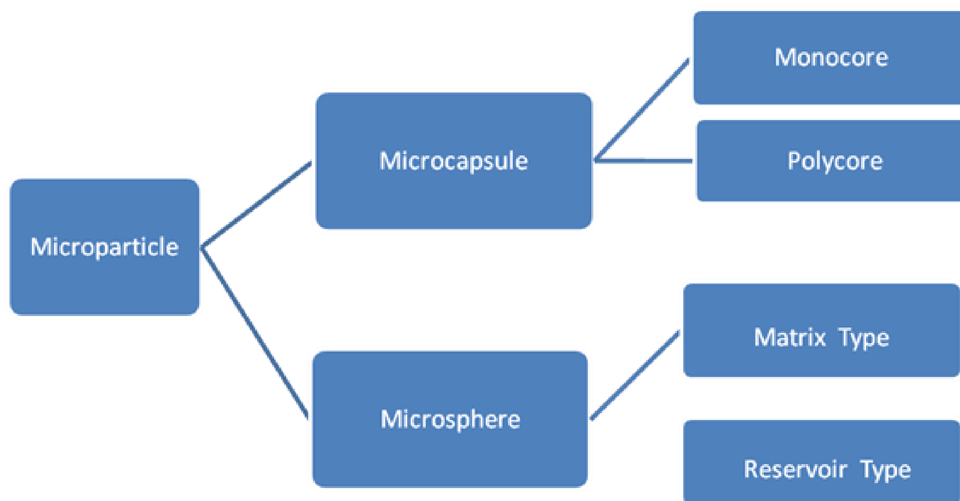

Fig 2: Classification of multi particulate system

Table 1: List of different method of preparation of microspheres along with the polymers used

S. No	Method of microsphere Preparation	Drug Used	Polymer Used
1.	Solvent Evaporation Technique (K. Kannan *et al*., 2009)	Acetazolamide	Eudragit RS, Eudragit RL
2.	Emulsion Solvent evaporation Technique (A.Swapna *et al*.,2011)	Mesalamine	HPMC, Ethyl Cellulose
3.	Ionotropic Gelation Technique (Rana Mazumder *et al*., 2010)	Metronidazole	Guar Gum, Sodium alginate.
4.	Emulsion Cross- Linking Technique (Mohanraj Palanisamy *et al*., 2009)	Metoprolol Succinate	Chitosan
5.	Emulsion solvent diffusion technique (S.D Barhatel, *et al*. 2009)	Ketorolac Trometamol	Ethyl cellulose, Eudragit S100, Eudragit R100.
6.	Emulsification Heat Stabilizing Method (Y. Phalguna *et al*. 2010)	Zidovudine	Hydroxyl Propyl Methyl Cellulose
7.	Co-Acervation Phase Technique (Arunachalam. A *et al*., 2010)	Ofloxacin	Gelatin
8.	Solvent evaporation method (M.Najmuddin *et al*.,2010)	Flurbiprofen	Eudragit L 100 and S-100
9.	Emulsion cross-linking method (Poonam Kushwaha *et al*., 2011)	Tramadol Hcl	Pectin, Shellac
10.	Emulsification Technique (Desiree Kietzmann *et al*., 2010)	Carboxyfluorescein	Eudragit S 100
11.	W/O emulsion solvent evaporation method(Prasant Rout *et al*., 2009)	Losartan	------
12.	Emulsion solvent evaporation method(Vimal Kumar Varma M. *et al*.,2009)	Diclofenac	PEG-10000, Ethyl Cellulose

Method of preparation of microspheres

1. Emulsion solvent evaporation technique

In this technique the polymer used is dissolved in chloroform and to this solution the drug is dissolved. This solution is then added to the aqueous phase containing the emulsifying agent. When agitated at 500 rpm, the drug and the polymer are converted into the rigid microspheres which are collected, washed and dried at room temperature for 24hrs [51].

2. Phase separation emulsion polymerization method

The drug was added to the egg albumin solution and stirred continuously until the uniform polymeric drug solution was made. The organic phase was formed by adding sodium lauryl sulphate in the coconut oil in a separate beaker. Now the polymeric drug solution was added to the organic phase drop by drop by using 22 gauge needle and the whole mixture is stirred continuously to form uniform dispersion. The stirring was continued for the next 2 hours while raising the temperature of the solution to 80 °C. Then the solution was cooled to room temperature keeping stirring continued with the help of mechanical stirrer. When the temperature of the solution reached the room temperature, formaldehyde and n-Hexane was added to separate the formed microspheres from the organic phase. Separated microspheres were then washed thrice with n-Hexane followed by distilled water, dried and stored in air tight container.

3. Ionotropic gelatination method

In this method, the guar gum was allowed to swell for 2 hrs in the drug solution. Sodium alginate solution in water was made in another beaker. The drug guar gum solution was added to the sodium alginate solution while stirring resulting into a viscous state. Then glutaraldehyde was added to the above solution. The drug polymer solution was then added drop wise into the beaker containing $cacl_2$ solution by using the 22G size needle. Solution was dropped from a height of 5 cms into the beaker. This all adding procedure was done under the continuous stirring of magnetic stirrer. Later the formed microspheres were separated using whattman filter paper no-1, dried at 30-40 °C and then stored in well closed container.

4. Emulsion cross-linking method:

In this method the drug was dispersed completely in the solution of 2% chitosan (in 1% aqueous solution of glacial acetic acid). A small volume of this solution was then injected through a syringe No. 23 into the oil phase having Span 80 (1% v/v) while stirring with a mechanical stirrer and this resulted into a w/o emulsion. The composition of external oil phase was heavy and light liquid parrafins in 1:1 ratio. Toluene saturated gluteraldehyde (8:1) was added to the emulsion after 30min of stirring and left for 7hrs for cross-linking and stabilization. The centrifugation of the formed microspheres was done at 4000 rpm and the sediment formed was washed with petroleum ether and acetone. It was then left for drying at 50 °C in hot air oven [52, 53].

5. Sieving method

The chitosan solution was made in acetic acid (4%) and drug was dispersed in into the solution. Then a crosslinking agent glutaraldehyde was added. This cross-linked mass, non-sticky in nature, was sieved through a suitable mesh size in order to obtain microparticles. The washing of formed microparticles was done with 0.1 N NaOH solution to remove the excess gltaraldehyde and dried at 40 °C for overnight period. In this method the entrapment efficiency was found to be high. Microparticles resulted were of irregular shape but the in vitro studies revealed the extended release of drug upto 12 hrs [54].

6. Spray- drying method

In this technique first of all the chitosan solution was made in aqueous acetic acid solution and then the drug was dissolved completely into it. A suitable cross-linking agent was also dispersed into the above solution of drug and chitosan. This solution was then atomized in a hot air stream. Small droplets were formed due to the process of atomization, and evaporation of the solvent from the droplets occurred due to the passage of hot air. This is how the free flowing particles are formed. Various physical factors like nozzle size, atomization pressure, spray flow rate and degree of cross-linking influences the particle size of formed microspheres. Microspheres formed by this method were spherical in shape having smooth surface and narrow size distribution [55].

7. Orifice ionic gelation method

The polymer solution was formed by dispersing sodium alginate and a mucoadhesive polymer in purified water. The drug was later added to the formed polymer solution and mixed properly using a stirrer. This resulted solution was then added into the calcium chloride (10% w/v) solution through a spray device having air compressor keeping the stirring continued. The droplets added into the calcium chloride solution were kept there for 15 mins to form rigid spherical microspheres. The formed microspheres were collected, washed repeatedly with purified to remove excess calcium present over their surface and then left for air drying [56].

8. Emulsion dehydration technique

In this method the drug and pectin were dissolved in water and stirred continuously to form homogenous solution. This drug polymer solution was then dispersed in isooctane containing span 85 present in it and the whole dispersion was stirred until a stable emulsion was formed. Then the solution was cooled up to 15 °C and then acetone was added to dehydrate the pectin droplets. Stirring was continued for the period of 20 to 40 minutes at a speed of 1000 rpm at room temperature to completely evaporate the solvent. The formed microspheres were collected, washed with acetone, freeze dried overnight and then stored in air tight container for carrying out further studies [57].

9. Emulsion/Aggregation process

The first step of this method was the stabilization of polymer in water. The size of polymer particles should be in range of nanometers. Different resins could be used like acrylates, polyesters, styrene based materials. Then in the next step the nanometer sized particles were grown in size by mixing them in deionized water having an aggregating agent. Now the other ingredients were added into the dispersion and mixing of the above solution was done to ensure a homogenous solution and it was continued throughout the growth process. When we observed that the desired size range was achieved the growth process was stopped. The shape of the microspheres formed depended upon the type of resin used. The formed

microspheres were collected, washed to remove the excess surfactants used in the process [58].

10. Co-acervation phase separation technique

In this method, initially the gelatin was dissolved in 10ml of water at 50 °C temperature. After the gelatin was mixed completely the drug was added and the whole solution was stirred at 300 rpm for 15 mins using magnetic stirrer to form a stable dispersion. Meanwhile 10ml of sunflower oil was heated upto 50 °C on a water bath. When the oil was heated sufficiently, the above formed dispersion was poured drop wise into it. Now the complete mixture was stirred at 300 rpm for 2 hrs using a magnetic stirrer at room temperature. After 2 hrs of stirring, the cross-linking agent formaldehyde (0.5ml) was added to the above dispersion and left for stirring for another half an hour. The formed microspheres were refrigerated for 24 hrs to make the microspheres rigid [59].

Evaluation and characterization parameters of microspheres

1. **Fourier Transformed Infrared spectroscopic analysis**: FT-IR spectral analysis is done using KBr pellet method by applying 6000kg/cm^2 pressure. The FT-IR study is done to study the polymer- drug interactions and the physical state of drug in the microsphere. The FT-IR of pure drug, empty microspheres, and the drug loaded microspheres is done separately to judge accurately.
2. **Micromeritic study of microspheres:** The particle size of microspheres is determined to obtain the average particle size to check their suitability for i.v administration and it is done by using particle size analyzer [60]. The particle size distribution of microspheres can also be determined by using the optical microscope having a calibrated stage micrometer. The equation for calculating size distribution is $Xg = 10 \times [(n_i \times \log Xi) / N]$, where Xg is the geometric mean diameter, n_i is the number of particles in range, X_i is the midpoint of range and N is the total number of particles.
3. **SEM:** Scanning Electron Microscopy of microspheres is done to study their morphological study. In this the microspheres are coated with 20nm gold palladium under an argon atmosphere in a vacuum evaporator using a gold sputter module. Then those coated microspheres are observed with scanning electron microscope [61]. The SEM photographs obtained are examined for the shape and surface morphology of microspheres. The Microspheres under Scanning Electron Microscope (SEM) are shown in figure 3.

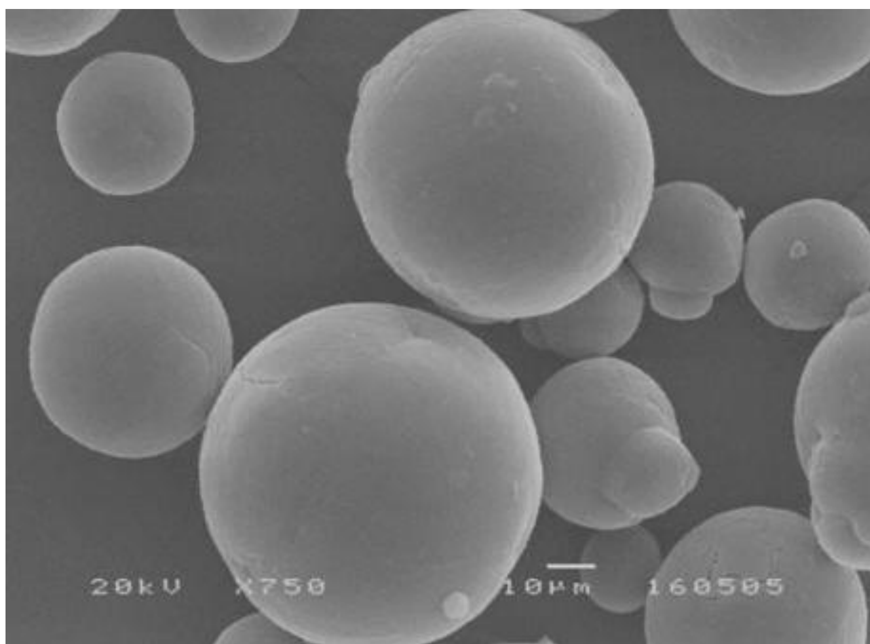

Fig 3: Microspheres under SEM

4. **Microencapsulation efficiency**
   The weighed amount of microspheres are crushed and then suspended in the suitable solvent in which the drug is soluble in order to extract the drug from the microspheres. The solution is then filtered through whatman filter paper and kept for 24 hrs. After 24 hrs the filtrate is then analyzed for absorbance using UV- spectrophotometer and concentration of drug is calculated. Microencapsulation efficiency is then calculated using the formula:
   Microencapsulation efficiency = (Practical drug content/Theoretical drug content)×100
5. **Swelling capacity**
   The swelling behavior of microspheres is determined by keeping them in the phosphate-buffer (7.4pH) for about 24 hrs. The swollen microspheres are blotted with the filter paper to remove surface water and then the wet weight of microspheres is determined. The formula for calculating the percentage swelling of microspheres is:
   $S = [(We-Wo)/Wo] \times 100$, where We is the weight of the microspheres at equilibrium swelling and Wo is the initial weight of the microsphere [62].

6. **Percentage yield**
   The percentage of the microspheres can be calculated by the following formula:
   Percentage yield=(Obtained mass of microspheres/initial mass of drug + initial mass of polymer)×100

7. **Loading capacity**
   The weighed amount of microspheres are kept overnight in a solvent in which the drug is soluble to ensure maximum extraction of drug into the solvent. The solvent is then filtered and the absorbance of the filtrate is then analyzed spectroscopically. The equation for calculating the loading capacity of microspheres is:
   Loading Capacity = (Mass of drug in microspheres/ Mass of microspheres)×100

8. **Density Determination**
   To determine the density of microspheres, an accurately weighed sample is kept in the multivolume pycnometer. A chamber is maintained at low pressure by introducing helium gas. The readings of reduced pressure are noted at different initial pressure is noted. From these two pressure readings, volume is determined which gives the density of microspheres.

9. **Isoelectric point**
   Isoelectric point of microspheres is determined by their electrophoretic mobility which is calculated by the apparatus micro electrophoresis. Electrical mobility is interperated by the mean velocity at pH values ranging from 3-10 which is calculated by measuring the time of particle movement over a distance of 1 mm. The electrophoretic mobility of microspheres is because of their surface charge and ionisable behavior.

10. **Electron Spectroscopy for chemical analysis(ESCA)**
    The electron spectroscopy is done to determine the surface chemistry of microspheres. The surface chemistry means the atomic composition of the surface and the surfacial degradation of the biodegradable microspheres which is predicted by the spectra obtained by ESCA [63].

Release kinetics of drug from microspheres

For the better efficiency of microspheres, the drug entrapped should be release on time and with adequate rate. Theoretically different mechanisms which can be considered for the release of the drug from microspheres are:

a) Polymer erosion: The polymer erosion method takes place mainly in case of biodegradable polymers. When water penetrates the microsphere structure, the microstructure of the carrier changes leading to the plasticization of the matrix. The plasticization leads to the cleavage of hydrolytic bonds causing the erosion of polymer. The rate and extent of water uptake determines the release profile of the drug as polymer erosion can be superficial or bulk. The superficial erosion leads to slow release of drug and bulk erosion leads to rapid release of drug.
b) Diffusion through pore: In this method the water continues to diffuse towards the core making the drug dissolve and creating water filled pore network for the diffusion of drug in a controlled manner [64].
c) Release through polymer surface
d) Pulsed release due to an applied oscillating or sonic field.

The drug release from the microspheres depends upon the structure of matrix entrapping the drug and the chemical properties of polymer and the drug. The drug could be released from the microsphere by any of the above methods.

Conclusion

Colon targeting has been proved beneficial in many ways by various research work done for the last two decades. Different approaches are designed in order to target drugs to the colon making use of different colonic parameters like pH, microbial content, pressure, transit time etc. Microspheres have proved to be efficient drug delivery system than many other types of delivery systems because of target specificity and better patient compliance. Microspheres offer a number of advantages over other conventional dosage forms like protection, reduced dissolution rate, masking of drug etc. Microspheres made from biodegradable polymers shows good results in controlled drug delivery. One of the important characteristics of microspheres is to engulf macrophages which lead us to target drug to the pathogens residing intracellularly. Peyer's patches also shows the uptake of microspheres, so vaccines are loaded into microspheres in order to target them there. Lastly I would like to conclude that microspheres present a number of advantages over conventional dosage forms for the drug targeting to the colonic region.

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