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Research Article

Preparation and Characterization of Eudragit Coated Chitosan Microspheres of Anticancer Drug for Colon Cancer

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Abstract:

Carrier technology provides an interesting as well as an intelligent approach for the delivery of drug. It offers delivery of drug by coupling the drug to a carrier particle such as microspheres, mucoadhesive microspheres, nanoparticles, liposomes, etc. Mucoadhesive microspheres constitute an important part of this particulate drug delivery system because of their small size and other efficient properties. Mucoadhesive microspheres play a vital role in the novel drug delivery system. Some drug delivery problems are overcome by producing controlled drug delivery system which enhances the therapeutic efficacy of a drug. From various approaches one approach is to using mucoadhesive microsphere as a carrier system for drug delivery. Mucoadhesive microspheres exhibit a prolonged residence time at the site of application or absorption and facilitate an intimate contact with the underlying absorption surface and thus contribute to improved and better therapeutic performance of drugs and also mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, controlled and sustained release of drug from dosage form and specific targeting of drugs to the absorption site. Mucoadhesive microspheres have been developed for oral, buccal, nasal, ocular, rectal and vaginal for either systemic or local effects.

Keywords: Mucoadhesion, mucoadhesive microspheres,mucoadhesive polymers,application.

Introduction

Cancer[1, 2, 3]:

Cancer is a category of diseases characterized by uncontrolled cell growth. Cancers are classified according to the major cell type they attack, and there are more than a hundred of them. Cancerous cells may expand out of control and form tumors, which are a leading cause of sickness (except in the case of leukemia where cancer prohibits normal blood function by abnormal

cell division in the blood stream). Some tumors emit hormones with widespread consequences, while others may impair digestive, nervous, or circulatory systems as they develop. Slow-growing tumors that don't metastasize are usually safe to ignore.

Malignant tumors, the most serious kind, develop when:

1. As cancer cells invade, they travel through the blood and lymph systems and wreak havoc on healthy organs and tissues all across the body.
2. Angiogenesis is the process through which a cell is able to multiply and grow by forming new blood vessels.
3. Cancer metastasizes when malignant cells from an original tumor spread to other parts of the body and kill healthy tissue there.
4. This process is often referred to as "metastasis." Those three features are what set cancers apart from benign tumors, which stay in one place and don't invade or spread. Leukemia, like other malignancies, develops into a tumor.

Oncology is the branch of medicine concerned with cancer research, diagnosis, and treatment. Cancer may affect anyone of any age, however the risk is higher for the majority of cancers in the elderly. Cancer was responsible for almost one-fifth of all deaths in 2007.

Bioadhesion :

Basic Concepts[23]: There are potentially four forms of bioadhesion in biological systems. As a first step, Adhesion of healthy cells to other healthy cells. Adhesion of cells to a surface. A healthy cell's attachment to a malignant one forms a triangular adhesion. Number four, the link established by the adhesive with the live organism.The capacity of a drug carrier system (composed of synthetic or biological macromolecules) to attach to a target in a living organism is referred to as its "bioadhesion." The biological surface might be made of epithelial tissue. Epithelial cells themselves, or the outermost layer of cells they surround, may provide an adhesive bond. Adherence to a mucus layer is known as mucoadhesion, whereas adhesion between cells is known as cytoadhesion. It is possible to think of bioadhesion and mucoadhesion as an analogue of bacterial adhesion to a tissue surface and mucus attachment to epithelial cells, respectively.

Bioadhesion: [24, 25]

To visualize the two steps required for a polymer to adhere to a biological membrane or its covering, keep in mind the following:
The first contact between two objects. Non-covalent interactions may also lead to the formation of secondary bonds. The interfacial layer between the adhesive and the biological membrane is just as important to the bonding process as the adhesive's surface.

Mucoadhesive Microspheres As Novel Drug Delivery System [28, 29, 30, 31]

A smart approach to drug delivery, carrier technology involves attaching the drug to a carrier particle such a microsphere, nanoparticle, liposome, etc., in order to regulate the drug's release and absorption qualities. Microspheres, with their small size and useful carrier qualities, are a crucial component of these particulate DDS. One important limitation of these modern DDS is their short half-lives at the absorption site. Having a system in place to guarantee that DDS makes physical contact with absorbent membranes is a major bonus. Coupling mucoadhesion properties to microspheres might lead to a new kind of delivery strategy. Mucoadhesive microspheres include microparticles and microcapsules (with medication in the core) that are either made entirely of a mucoadhesive polymer or have an outer coating of it and have a

diameter of 0.1 to 1 mm. However, microspheres with mucoadhesive properties expand the scope of their use as a targeted and controlled release pharmaceutical delivery method. As mucosal layers in the buccal, oral, vaginal, nasal, rectal, and ocular routes of administration all offer attachment sites for bioadhesive systems, the mucoadhesive drug delivery system may be adapted to any of these routes. A smart approach to drug delivery, carrier technology involves attaching the drug to a carrier particle such a microsphere, nanoparticle, liposome, etc., in order to regulate the drug's release and absorption qualities. Because to their advantageous size and carrier properties, microspheres are often used in these particulate DDS. One major drawback of the new DDS is that they tend to disappear soon after being released from the absorption site. A mechanism to ensure that DDS really contacts the absorbent membranes would be a huge improvement. Microspheres with added mucoadhesion capabilities might provide a novel method of drug administration. Microparticles and microcapsules (containing medicine in the core) with a diameter of 0.1 to 1 mm and formed wholly of a mucoadhesive polymer or coated on the outside are considered mucoadhesive microspheres. Yet, microspheres' mucoadhesive qualities increase their potential as a targeted and controlled release drug delivery option. Because of the mucosal layers present in the buccal, oral, vaginal, nasal, rectal, and ocular routes of administration, the mucoadhesive drug delivery system may be customised for usage in various locations.

Preformulation studies

Selection of drug and excipients

Disease status, pathophysiology, symptoms, rationale for adjunctive therapy, currently marketed formulations, their limitations, the mechanism of drug transport to colorectal tumors, lipid nano-formulations that aid in selecting the drug and excipients, and so on were all thoroughly reviewed in the literature. Oxaliplatin, oxaliplatin (oxaliplatin), and irinotecan are the basis of current pharmacological treatment for colorectal cancer.

Due of its potential anticancer activity and its still-existing limitations, oxaliplatin was chosen among them. The formulation type and the necessity for the designated carriers informed the subsequent selection of excipients. The lipid based formulation consists of stearic acid, DSPE-PEG-2000, soyalecithin, DSPE-PEG-3400mal, and cholesterol. For this purpose, the polymeric nanoparticles were prepared using chitosan.

Analytical method development

Selection of detection wavelength

Separately, the stock solution (10 g/ml) was made in ACN. In UFLC (Shimadzu LC 2010A HT), a photodiode array (PDA) was used to capture the spectrum of the solution as it was scanned in the UV-Visible range of 200-800nm.

Preparation of standard stock solutions

A 1mg/ml (1,000 g/ml) stock solution of oxaliplatin was prepared by carefully weighing 10mg and transferring it into a 10ml volumetric flask, where it was dissolved in ACN and brought up to volume using the same solvent. After adding 10 ml of the corresponding drug solution (1 mg/ml) to 100 ml of volumetric flask with ACN, the stock solution was diluted to 100 g/ml. After being prepared, these solutions were refrigerated at 40 degrees Celsius until analysis.

Linearity and range of oxaliplatin

Calibration curves were generated by varying the concentration of the standard solution to examine linearity and dynamic range. The standard drug solution's

concentration (y) was plotted against the calibration curve's mean peak area (x). Oxaliplatin's linearity was determined using weighted least squares regression analysis spanning the concentration range of 0.1 ng/ml to 1 ng/ml. Aliquots of 0.1, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 ml were pipetted from the stock solution (1000 g/ml) into 10 ml volumetric flasks, and the volumes were brought up to 10 ml with ACN to produce concentrations of 1-1000 g/ml for UFLC (Shimadzu LC 2010A HT) analysis at 240 nm. Linear regression analysis was applied to calibration curve data, yielding the intercept, slope, and regression equation. The mean and standard deviation (S.D.) were calculated from measurements taken three times.

Preparation of Calibration Curve of Oxaliplatin

After carefully measuring out 10mg of the medication, it was mixed with a phosphate buffer (pH 4.7) solution in a volume of 100 mL of phosphate buffer saline to get the desired final concentration (pH 4.7). Thus, a stock solution of 100 g/mL was prepared. Aliquots of 0.5 mL, 1 mL, 1.5 mL, 2 mL, and 2.5 mL were removed and placed in a 10 mL volumetric flask, where they were further diluted to a final concentration of 10 mL in phosphate buffer. The solution was analysed in the visible spectrum with a maximum wavelength of 266 nm.

Table 1: Calibration curve of oxaliplatin

Sn.No.	**Concentration**	**Absorbance**
1.	5µg/mL	0.275
2.	10µg/mL	0.594
3.	15µg/mL	0.877
4.	20µg/mL	1.161
5.	25µg/mL	1.484

Infrared Spectra of Oxaliplatin

The IR spectroscopy was performed using the FT/IR Spectrometer at the MPCST Quality Assurance Laboratory, and when compared to the reported value, the produced curve between the % transmittance and frequency (cm-1) proved the validity of the study. [68, 70, 71]

Chromatographic Technique

High Performance Liquid Chromatography

High performance liquid chromatography was used to measure the content of oxaliplatin in phosphate buffer (pH 3). Phosphate buffer was used to dilute the medicine to a concentration of 10mg/mL. (pH 3). Hence, a stock solution with a concentration of 1mg/mL (1000 g/mL) was prepared. Next, two millilitres of the stock solution were pipetted into a volumetric flask of 100 millilitres, and the remaining volume was filled with phosphate buffer. A study was performed on the resulting pharmaceutical solution.

Methods of Preparation of Chitosan Microspheres44, 45, 46Easily mixed together Chemical denaturation is the preferred method for manufacturing chitosan microspheres because of the technique's flexibility and versatility**.**

Result And Discussion

Drug Identification Test

Physical Appearance

The supplied sample of oxaliplatin IP/BP was white, porous mass, odorless, non-hygroscopic powder[50].

Melting Point

Melting point of oxaliplatin was found to be 260^0C and matched with literaturevalue[50].

Solubility

Solubility of oxaliplatin in different solvent is given in table 7.1 on the basis of soluble and insoluble. oxaliplatin is hydrophilic drug and soluble in some organic solvents[50].

Table 2: Solubility of oxaliplatin in various solvents

Sr. No.	Solvents	Solubility
1.	Water	Soluble
2.	Methanol	Soluble
3.	Ethanol	Soluble
4.	Acetone	Insoluble
5.	Chloroform	Insoluble
6.	Phosphate buffer(pH7.4)	Soluble
7.	Phosphate buffer(pH4.7)	Soluble

Determination of Λ Max

UV spectrum of oxaliplatin was interpreted absorption maxima (λmax) as given procedure in previous chapter and is shown in table 7.2and fig 7.1.

Table 3: Determination of absorption maxima of oxaliplatin

Wavelength(nm)	Interpretation	Inference
200-400	Scanning range	Drug absorption maxima (λmax) 266.5nm of oxaliplatin
266.5	Highest peak	

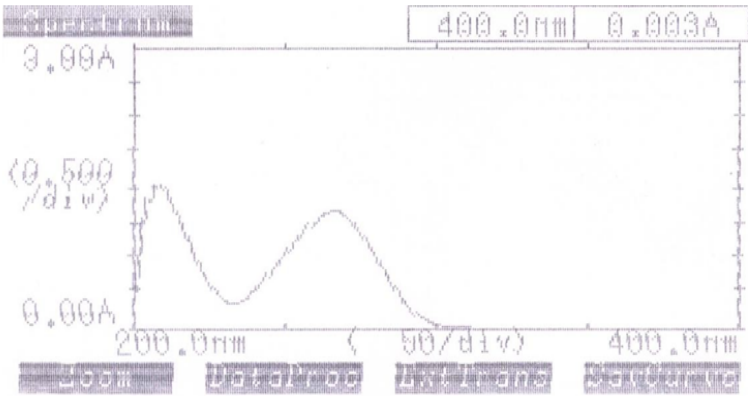

Figure 1: Determination of absorption maxima (λmax)

The drug's reference spectra from IP 2007 is similar to the spectrum seen after scanning the drug across the authorised scanning range (200-300 nm), with an absorption maximum at 266.5 nm.

Partition Coefficient

Drug distribution estimates may be made with the use of partition coefficients. Hydrophobic medications with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells whereas hydrophilic drugs (low partition coefficients) preferentially are located in hydrophilic compartments such as blood serum. Lipophilic drugs have partition coefficients (K) significantly higher than 1, whereas hydrophilic drugs have partition coefficients (K) significantly lower than 1.

By solving for the partition coefficient in the n-octanol: PBS (4.7) system, the value of 1.044 was determined for the medication.

The partition coefficient, K = Amount Of drug in organic layer

Amount of drug in aqueous layer

Partition coefficient, K = 24.484 / 25.566 = 0.96

The drug's 0.96 partition coefficient is consistent with the existing literature. [46]

Preparation of Calibration Curve of Oxaliplatin

This result was obtained using the strategy discussed in the previous chapter. oxaliplatin's calibration curve is extremely linear between 2 and 12 micrograms per millilitre. The drug's standard curves were created at a wavelength of 266.5 nm. After doing regression, a linear calibration curve was created. With an R2 of 0.9995, we know that the drug follows the Beer-rule Lambert's between 2 and 12 g/mL. The results45 are shown in table 7.3 below.

Table 4: Calibration curve of oxaliplatin

Sn.No.	Concentration	Absorbance
1.	2µg/mL	0.274
2.	4µg/mL	0.595
3.	6µg/mL	0.879
4.	8µg/mL	1.162
5.	10µg/mL	1.482
6.	12µg/mL	1.781

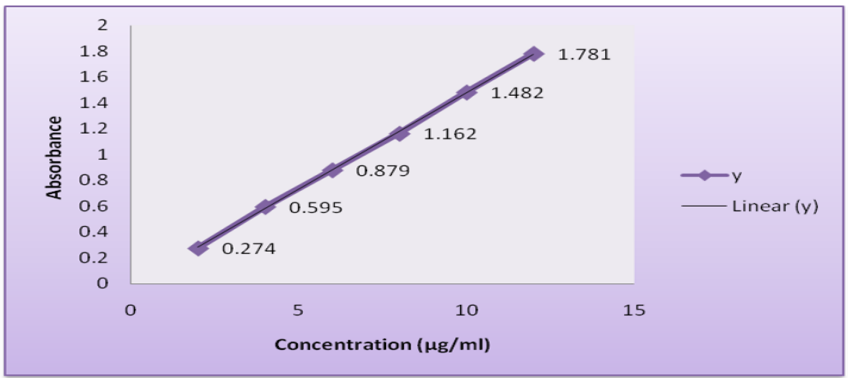

Figure 2: Calibration curveofoxaliplation

Correlation coefficient (R^2)=0.9995 Intercept = 0.019

Equation of regressed line; Y= 0.149X+ 0.019

Where X= value of concentration

Y=value of absorbance Slope of regressedline = 0.149

Infrared Spectra of Oxaliplatin

Using an FT/IR Spectrometer, we were able to get an IR spectra that was very close to the reference spectrum of oxaliplatin by the use of IR spectroscopy. Results were shown in table7.4 and fig7.3[50].

Table 5: IR spectral characteristics oxaliplatin

Sr.No.	Wave Number(cm^{-1})	Interpretation
1.	2967.90	NH Stretching
2.	1602.25	C=O Stretching
3.	1054.63	CH in plane deformation
4.	851.97	CH out of plane deformation

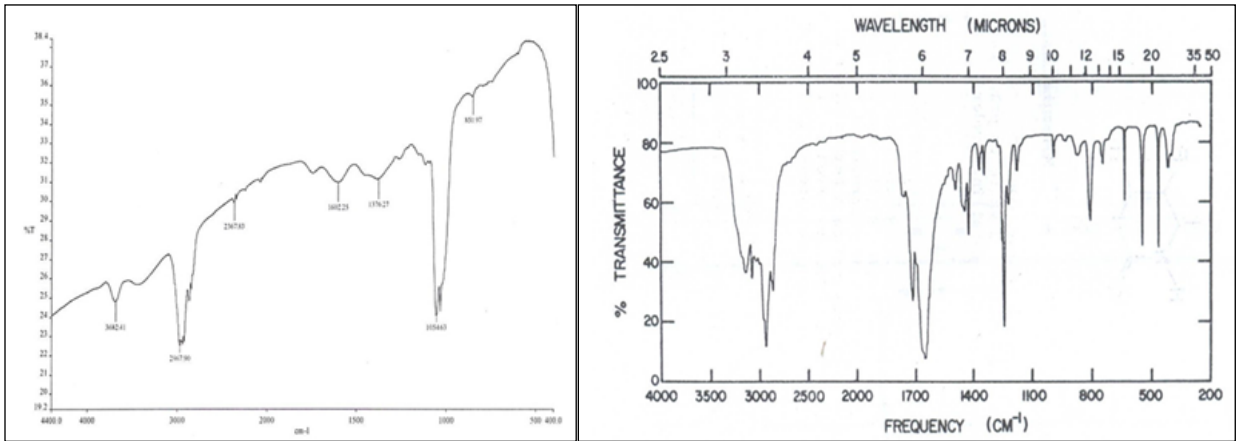

Figure 3:Reference IR spectrumof oxaliplatin

When compared to the standard IR spectra published in IP2007, the IR spectrum of the drug sample was confirmed to be consistent with it.These results showed that oxaliplatin was genuine.

High Performance Liquid Chromatography:

Phosphate buffer (pH 3) was used to dilute the medicine to a concentration of 10mg/mL. So, a stock solution with a concentration of 1mg/mL (1000 g/mL) was prepared. After that, 2 mL of the stock solution was pipetted into a 100 mL volumetric flask, and the rest of the flask was filled with phosphate buffer. The resulting pharmaceutical solution was subsequently evaluated. In this investigation, the retention length of oxaliplatin was measured to be 2.921 min, which is less than the 3.5 min reference value. The retention duration is also determined by the surrounding temperature and humidity, as well as the quality and amount of the chemicals utilised for the same goal. HPLC examination revealed a retention time of 2.921 for the medication, which is quite near to the norm. The estimation approach was shown to be trustworthy and sensitive enough over the relevant concentration range.[44]

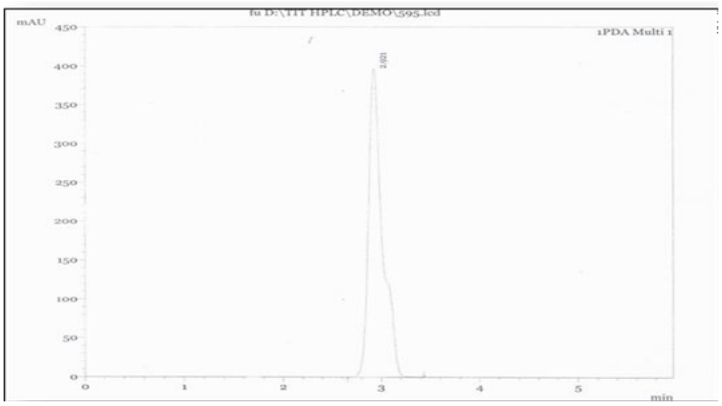

Figure 4: HPL C curve of oxaliplatin

Evaluation of Chitosan Microspheres Prepared By Both The Methods

Table 6: Evaluation parameters of CMS-1 & CMS-2

Sr.No.	Evaluation parameters	CMS-1	CMS-2
1.	Percentage drug entrapment	25.89	14.57
2.	Particle size and size distribution	4352.53nm	2761.80nm
3.	Zeta potential	+41.0mV	+51.2mV
4.	Swelling properties	Less	More
5.	*Invitro* wash-off test	>24hrs	<24hrs

The drug entrapment rate was greater in Method 1 (25.89%) than in Method 2 (14.57). Although the efficiency of entrapment is enhanced by increasing the polymer concentration, the concentration of chitosan is about the same in both methods. However, entrapment effectiveness decreases as drug concentration rises (33, 37), since there isn't enough chitosan to completely encapsulate the molecule.

It is possible that incomplete emulsification of the two stages contributed to the lower entrapment efficiency seen in procedure 2. Therefore, microspheres were selected for optimisation based on their size homogeneity, high entrapment efficiency, and predictable release profile44.

It was found that, even though the polymer concentration was kept constant, increasing the amount of polymer resulted in larger microspheres. The microspheres' particle size changed as the amount of cross-linking increased; the particles became smaller as the cross-linker concentration increased. The method 2 microspheres were more compact because of the elevated glutaraldehyde57 content.

Due to the positive zeta potential of the chitosan employed in both formulations, electrostatic interaction with the negatively charged sialic acid and fucose residue of mucin prolongs the residence time in the intestines. The positive charge on the surface of chitosan microspheres may be related to the number of free amino groups present. After 15 minutes of cross-linking with glutaraldehyde, the microspheres' zeta potential decreased from +55 mV to about +47 mV. However, the surface charge remains constant after this first stage.

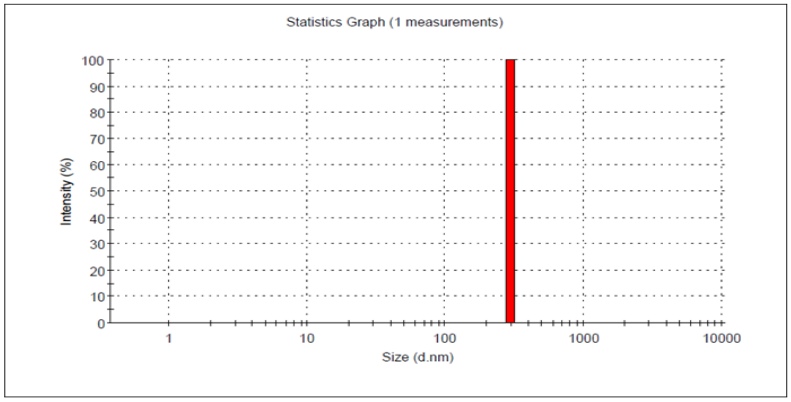

Figure 5: Particle size distribution of chitosan microspheres prepared by method-1

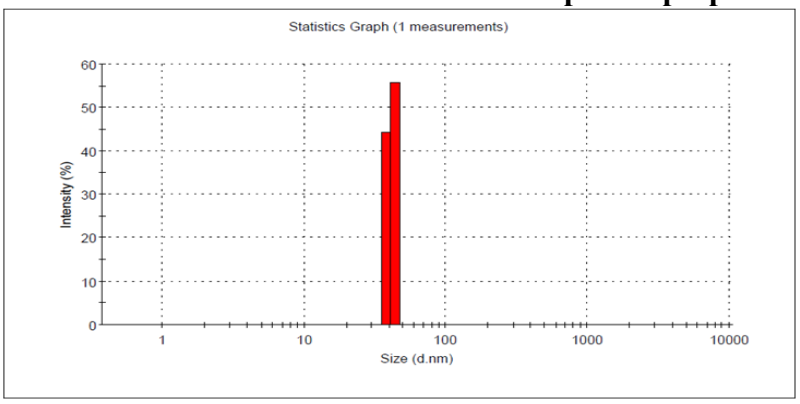

Figure 6: Particle size distribution of chitosan microspheres prepared by method-2

Morphological Study of Microspheres

The degree of sphericity and clumping of unloaded chitosan microspheres was evaluated using digital optical microscopy images. Some clumping was seen between 2000 and 2500 rpm, but overall, the 1% chitosan solution produced perfectly spherical microspheres.The degree of sphericity and clumping of unloaded chitosan microspheres was evaluated using digital optical microscopy images. Some clumping was seen between 2000 and 2500 rpm, but overall, the 1% chitosan solution produced perfectly spherical microspheres.[33]

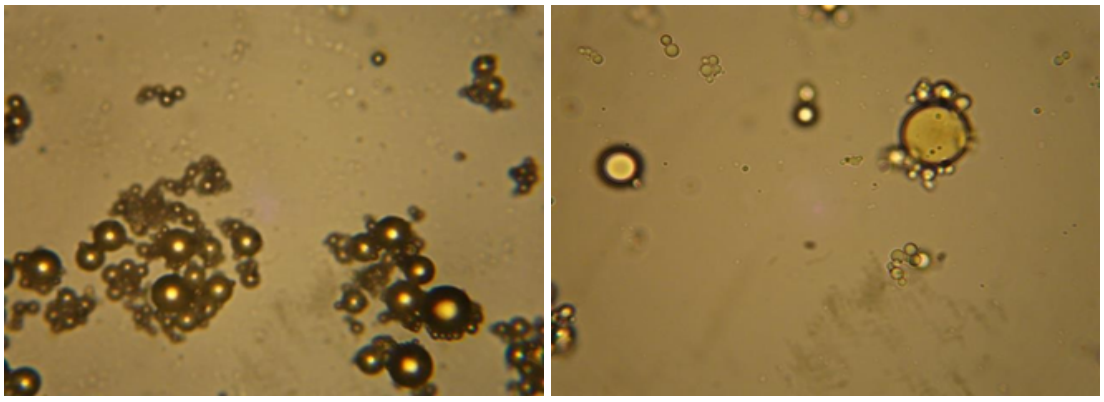

Figure 7: Photographs of unloaded chitosan microspheres

***Invitro* Release Profile of Cms-1 and Cms-2:**

Table 7: *In vitro* release profile of oxaliplatin from CMS-1 & CMS-2

Sr. No.	Time(hrs)	% Drug Release of CMS-1	% Drug Release of CMS-2
1.	1	8.96	7.56
2.	2	17.25	17.78
3.	3	22.64	21.34
4.	4	28.46	26.49
5.	5	33.49	31.56
6.	6	38.43	33.56
7.	7	44.37	40.78
8.	8	50.46	45.18
9.	9	57.64	48.64
10.	10	63.49	50.87
11.	11	67.18	55.67
12.	12	72.34	57.64
13.	24	80.64	60.49

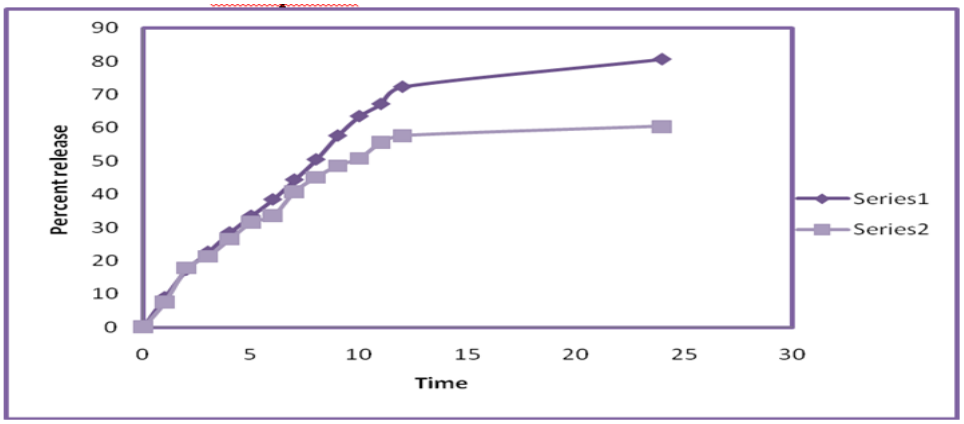

Figure 8: Percent release of oxaliplatin from CMS-1& CMS-2

Due to the use of adequate cross-linking agent and reduced cross-linking time, the release pattern of microspheres made using method-1 was determined to be satisfactory and possessing good linearity.[57]

Due to the use of adequate cross-linking agent and reduced cross-linking time, the release pattern of microspheres made using method-1 was determined to be satisfactory and possessing good linearity.[57]

Optimization and Evaluation of Chitosan Microsphere Sprepared By Method-1

Effect of Drug/Chitosan Ratio on Entrapment Efficiency of Microspheres

Chitosan microspheres with different drug/polymer ratios were made using Method-1 and then examined for their ability to entrap and release oxaliplatin. It seems that not enough chitosan was used to completely encapsulate the medication in formulations A1 and A2, since the entrapment efficacy declined precipitously as the drug concentration increased. Entrapment efficiency was also shown to be proportional to chitosan content (formulations B1, B2, and B3). Researchers observed that entrapment efficiency declined as cross-linking and concentration times rose. It is possible that the decrease in entrapment effectiveness with increasing cross-linking time and concentration is due

to partial emulsification as a result of the oil phase becoming more viscous. As the drug loading grew, the viscosity of the polymer solution rose, resulting in larger polymer/solvent droplets. The larger particles' slower hardening rate meant that less medication could be entrapped inside of them. Aggregation occurred at higher polymer concentrations, leading to less drug entrapment. As a result, B2 is superior to other formulations in terms of entrapment efficiency.[33, 37, 44]

Table 8: Effect of drug/chitosan ratio on entrapment efficiency of microspheres

Formula	Drug/chitosan ratio	Cross-linking agent amount (mL)	%Entrapment Efficiency
A2	2:1	5	30.72
A1	1:1	5	32.13
B1	1:2	5	34.23
B2	1:3	5	38.72
B3	1:4	5	35.57

***In Vitro* Release Profile of Oxaliplatin Chitosan Microspheres Prepared by Method-1**

The in-vitro release profile of several core chitosan microspheres formulations is shown in table 7.7 and figure 7.10. In vitro drug release testing was performed for 24 hours in a 7.4-pH phosphate buffer. The proportion of drug release was drastically reduced at higher polymer concentrations. At greater concentrations, the more dense polymer matrix necessitated longer diffusional routes. This means the drug may be released at a lower rate from the polymer matrix. Since more of the microsphere's surface area comes in contact with the solvent, the medicine may be released more rapidly from microspheres with lower polymer content. No matter what medication concentration is employed in the A2 formulation, the oxaliplatin release profile remains same. Since the diffusional routes between A2 and A1 are comparable in length, their respective releases are also quite similar. As chitosan concentration increased, the fraction of liberated microspheres gradually decreased.

Table 9: Percent Release of oxaliplatin showing the effect of drug/polymer ratio

Sr.No.	Time	Percent Release of oxaliplatin showing the effect of drug/polymer ratio				
		A1	A2	B1	B2	B3
1.	1	9.05	8.73	7.15	6.91	6.18
2.	2	18.46	16.92	14.49	12.84	11.94
3.	3	24.97	21.56	18.16	17.49	15.37
4.	4	30.42	28.64	24.94	22.91	19.49
5.	5	34.81	32.67	29.73	28.37	25.94
6.	6	39.72	37.48	34.16	32.94	29.76
7.	7	45.91	43.64	40.93	36.94	33.49
8.	8	50.34	49.37	45.94	41.97	38.47
9.	9	58.49	56.49	52.91	49.37	41.97
10.	10	65.43	61.72	57.94	52.86	48.18
11.	11	68.43	64.28	60.49	56.48	52.49
12.	12	74.46	69.67	64.18	59.67	55.94
13.	24	81.34	79.13	74.94	63.49	1.94

Based on these findings, either formulation B2 or B3 was chosen for the subsequent coating of the Eudragit S-100 on the chitosan microspheres used to create the pH-dependent, extended-release system. This coating shields the drug from the harsh gastric environment of the intestine. Because of this, enteric coated formulation was made using a B2 formulation (1:3)

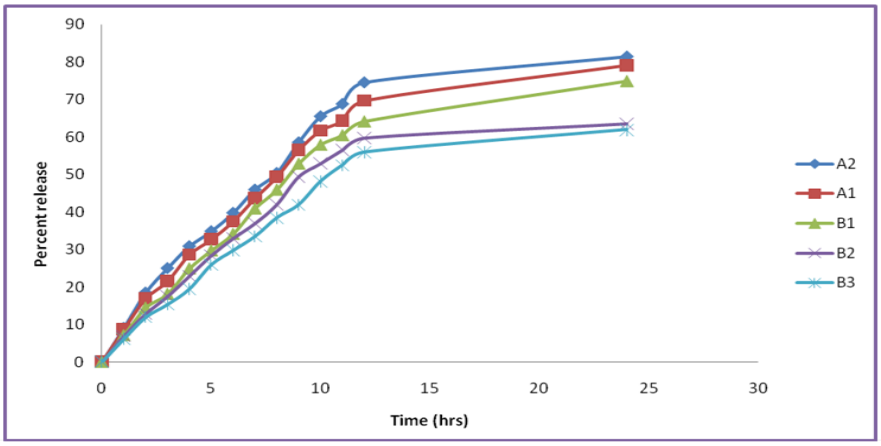

Figure: 9: Comparative study of release profile of different formulation

Eudragit Coated Chitosan MicrospheresAfter emulsifying the core microspheres dispersions in liquid paraffin containing span-80, the chitosan microspheres (B2) were coated with Eudragit S-100 using a solvent evaporation technique and then dispersed in the Eudragit S-100 solution. Encapsulated microspheres were created using a filter and n-hexane washing technique, then dried at 600C for 1 hour and stored.73, 75

Microspheres coated with Eudragit were tested for their efficacy as an enteric coated delivery method.

Summary and Conclusion

To help pave the way for the safe and specific prevention of colon cancer using Eudragit-coated chitosan microspheres, this study was designed to converge on all possible influencing parameters through sequential evaluation, taking(or using) necessary experimental findings towards final selection of eligible dosage form, their preparation, and subsequent characterization. Oxaliplatin passed all formal identification tests, showing a high degree of agreement with available data, raising hopes that it may be utilised safely in research.

To find the most practical, high yielding, and reproducible method for preparing oxaliplatin containing Eudragit coated chitosan microspheres, we compared the solubility of oxaliplatin and chitosan in acidic medium, the regularity in shape and uniformity in size of prepared microspheres, the drug entrapment efficiency of microspheres, and the invitro release profile after 24 hrs studies between the two methods (I & II).

Acknowledgement

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