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Research Article

MICROSCALE BASED DRUG DELIVERY SYSTEM FOR TARGETING VINBLASTINE IN COLORECTAL CANCER

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ABSTRACT

The poor specificity of drugs in while targeting the cancerous cells and occurrence of systemic toxicity is a undesired aspect of conventional cancer therapy. Damage of normal tissues, due to release of drug at sites other than tumor leads to severe side effects. Development of drug-resistant tumors is also observed in patients. These situations lead for development of novel carriers which release chemotherapeutic agents at the target site. Advanced drug Microcarriers have been explored as a way of lessening or overcoming these problems. In this study, Vinblastine loaded targeted Microparticles, were prepared and evaluated for their effects on the viability of colon tumor cells. Vinblastine loaded microparticles has a rough surface and particle size ranging between 316±0.25µm to 389±3.22 µm. The zeta potential of microparticles was -18.77mV to -21 mV and FTIR study confirmed absence of drug polymer interaction in microparticles. Optimized formulation had shown satisfactory invitro release in comparison to other formulations. Cytotoxic activity was evaluated by cell line study on HT29. These results demonstrate that Vinblastine loaded Microparticles easily penetrate to tumor cells through and lead to tumor cell death.

Keywords: Vinblastine, Chitosan, Eudragit, Colorectal cancer, Microparticles

INTRODUCTION

Colorectal cancer is a cancer of the colon and rectum of GIT system. A large number of population is fighting with colorectal cancer in north America, Europe and australia. A higher incidence of colorectal cancer has also been observed in peoples having irregular lifestyle as well as due to genetic abnormalities in human. Exposure to ionizing radiation and organic chemicals, irregular eating habits, GIT disorders, piles, immune-suppressants and the antibiotics are also considered risk factors for developing colorectal cancer[1].

Chemotherapy is the standard treatment for many types of colorectal cancer. Even when a cure is not possible, chemotherapy may help you live longer and feel better[2]. Treatment plan will include the kind of medicine that works best for the specific type or subtype of colorectal cancer include prednisone, methotrexate, l-asparaginase, vincristine, doxorubicin, daunorubicin, cytarabine, Idarubicin, mitoxantrone fludarabine, chlorambucil, rituximab, alemtuzumab, imatinib, dasatinib, nilotinib, Cytarabine, hydroxyurea, busulfan, etc.

For the treatment of colorectal cancer we use the anticancer[3]. To prevent from the toxicity of the drug we elucidate a new Microscale based drug delivery system which containing Vinblastine drug entrapped in enteric polymer. For drug with poor solubility, bioavailability, targeting limitations novel drug delivery systems have emerged as a site specific tool in comparison to conventional drug delivery system[4]. Concavalin A acts as a selective targeting agent for the tumor cells. When the formulation is administered in the body then Concavalin A leads the microparticles specific toward the tumor cells and these cells are killed due to the action of anticancer drug (Vinblastine sulphate). So that the formulation is targeted towards the tumor cells of colon leaving normal cells intact.

MATERIALS AND METHODS

Drugs, and chemicals: chitosan, ethanol, acetone, HPLC water, were purchased from Loba Chem pvt ltd, Mumbai (India), Eudragit S100 and L 100 were procured as a gift sample from Evonik Degussa India Pvt. Ltd., Mumbai, India Rhodamine B dye was purchased from HiMedia Laboratory Pvt. Ltd., Mumbai and Vinblastine drug was purchased from Sigma Aldrich, Mumbai. All reagents and chemicals used in this study were of analytical grade.

Method of Preparation

Microparticles were formed according to Solvent emulsification-evaporation method[5,6]. To prepare microspheres polymer and Vinblastine Sulphate were dissolved in 5 ml aqueous solution of glacial acetic acid and ultrasonicated for 5 minutes. The resultant solution was dispersed drop-wise in aqueous medium containing 0.1% w/v Tween 80 (stabilizer), in liquid paraffin, while stirring at 700 rpm using mechanical stirrer with vaccum evaporation. Then heating was provided to stabilize the polymer This system was maintained under mechanical agitation at room temperature for 2 hrs to allow the complete solvent evaporation. The microspheres were filtered and washed with acetone for 3 times. The microspheres were air dried and kept in an airtight desiccator for further studies. These microspheres are then again suspended in a eudragit S100 and L100 in organic solvent. This solvent is again emulsified in another mixture of light liquid paraffin and heavy liquid paraffin at ratio 1:1. All the formulation were prepared varying microsphere to polymer ratio using both the polymers Eudragit L100 and S100. Throughout the process, a constant stirring was maintained. The remaining solvent is evaporated under reduced pressure resulting in dry Microparticles.

Particle Size of Microparticles

The size of Vinblastine targeted Microparticles was determined by Malvern Particle Analyzer. The samples were diluted with distilled water and measured at 25º C at 90 degree a scattering angle[7]. Analysers measure the particle size and size distribution of different batches of formulation by dynamic light scattering and analyze particles in between range of 0.1µm - 2000µm. Size distribution[8] was characterized by a polydispersity index (PI) and it varies from 0.0 to 1.0. Polydispersity index (PI) value closer to zero means the particles are the more homogenous. Morphological characteristics were examined using Scanning electron microscope[8]. In order to determine the stability of the Micro carriers when dispersed in aqueous solution, they were stored at room temperature for 21 days. Each formulation was carried in triplicate.

Product Yield

The product yield of VEMLs was determined by calculating percent ratio of practical mass to theortical mass of the VEMLs obtained[9]. The following formula was used to calculate product yield.

$$\text{Product Yeild} = \frac{\text{Practical mass (Microparticles)} \times 100}{\text{Theoretical mass (Drug +Polymer)}}$$

Optimization of Formulation Parameters

Effect of drug (VBL) to polymer (CH) ratio The CH and VBL in the ratios as mentioned in Table 1, were taken to prepare different microparticle formulations. In each formulation, the amount of CH (10 mg), SPAN (1% v/v) were kept constant. The microparticles were prepared using REMI mechanical stirrer at a stirring rate of 500 rpm for 6 hr.

Characterization of Microparticles

FTIR analysis Fourier transform infrared (FTIR) spectra were obtained using FTIR-8400S (Shimadzu Co., Kyoto, Japan) combined with Quick Snap sampling modules. Infrared spectra of the samples were recorded in the solid state by the KBr disc method over the wavenumber range of 4,000– 400 cm−1 . CH, VBL and VEML were run as controls.

Determination of Particle Size and Surface Morphology

Shape and surface morphology of Microparticles was done by Scanning Electron Microscopy (Leo 435 VP) at SAIF AIIMS New Delhi. Small volume of Microparticulate was placed on an electron microscope. The samples were placed briefly in a vaccum drier and then within microscope. Pictures of Microparticles were taken by random scanning of the sample. The particle size and morphology of the particle was examined by SEM.

Drug Content and Encapsulation Efficiency

The weighed amount of VEMLs (10 mg) was suspended in 100 mL of methanol for 12 hr and it was subjected to continuous stirring (100 rpm). Then the sample was centrifuged at 2000 rpm and filtered using filter paper[10]. The absorbance was noted at 268nm using UV spectrophotometer (Shimadzu 1800, Japan). The values of drug content (%) and drug entrapment (%) are shown in Table 2.

Dynamic Swelling Study

The dynamic swelling behavior of the microparticles was studied by mass measurement[11]. The microparticles were incubated with 25 ml of simulated gastric buffer solution (pH 7.4) in a Petri dish at 37°C. The microparticles were taken out at different time intervals without pressing hard to remove the excess surface liquid. The swollen microparticles were weighed on an electronic balance (PBG 200, WENSAR) having an accuracy of 0.001 mg. The studies were performed in triplicate and average values were taken for data analysis.

$$\text{Dynamic swelling} = \frac{\text{Initial weight of microparticles}}{\text{Weight of microparticles at time 't'}}$$

Percent Mucoadhesion

Albino rats (400–500 g) fasted overnight were sacrificed to and their colon removed[12,13], which was divided into pieces of 2 ± 1 cm and rinsed with 2 ml of physiological saline. Previously counted microparticles (100 mg) were scattered uniformly over colonic mucosa and placed in a chamber for 20 min, and then maintained at room temperature with relative humidity of 93%. They were then transferred to a polyethylene support and fixed at an angle of 45°, followed by rinsing with physiological saline solution (pH 7.4) for 5 min at a rate of 20 ml/min. The microparticles remaining at the surface of colonic mucosa were counted and the percentage of the drug remaining microparticles was calculated[14]. The experiment was performed in triplicate, and the percentage of binding was calculated by the following formula:

$$\text{Binding (\%)} = Wr / W0 \times 100$$

Where Wo is initial number of microparticles; and Wr is number of residual microparticles.

Rheological Characterization of VEMLs

Angle of repose was determined using funnel method. Microparticles (5gm) were allowed to pass through a funnel that was raised vertically until a maximum cone height, h, was obtained. Diameter of heap, D, was measured. The angle of repose 'θ' was calculated using the following formula[15]

$$\tan \theta = 2h\ D$$

In Vitro Drug Release Study

A modified dialysis method[16] was used to evaluate the in vitro release of Vinblastine -loaded microparticles. One end of the dialysis sac was tied with the thread and examined for any leaks. Later it was filled with 2.5 mg of microparticles in packed sac acted as a donor compartment. The sac was then immersed in glass beaker containing 25 ml of 0.067 compartments at 37°C ± 2°C. The content of beaker was stirred using magnetic bead stirrer and the beaker was stirred using magnetic bead stirrer and the beaker was covered with an aluminum foil to prevent loss of solvent during the experiment. At selected time interval, 3 ml samples were withdrawn from the release medium and replaced with the same amount of freshly prepared phosphate buffer maintained at the same condition. The sample was assayed spectrophotometrically at 268 nm and the percent drug release was calculated. All of the formulations were studied for in vitro drug release in triplicate.

Kinetic Treatment of Dissolution Data

In order to describe the kinetics of the release process of drug from the different formulations, models were fitted to the dissolution data of formulations using non-linear regression analysis. In vitro release data was fitted to various release models. The determination coefficient (R2) and Sum of Squares of Residuals (SSR) were used as the indicators of the best fit to release data for each model.

In vitro Cellular Cytotoxicity Assay

The cytotoxicity of Microparticles was evaluated by MTT assay on HL-29 cell lines. Microparticles or Microparticles were added at equivalent vinblastine per well in medium. Microparticles without vinblastine were added as control. Absorbance was measured at 590 nm using a microplate reader (ELX 800; BIO-TEX Instrument, Inc.). The cell viability (%) was calculated and compared with the untreated control

Cellular Uptake Experiment and Subcellular Localization

Rhodamine B was encapsulated in Microparticles as a probe for the uptake study and confocal laser scanning. [17] Free Rhodamine B was removed from the Rhodamine B loaded Microparticles via ultrafiltration. HL – 29 cells were seeded in 24-well plates and incubated for 24 h before use. Rhodamine B labeled Microparticles was added at equivalent Rhodamine B per well and incubated with cells for 2 h. To investigate the endocytotic mechanism that was responsible for internalization of Microparticles, prior to incubation with Rhodamine B labeled Microparticles. Cells were collected, washed and resuspended in PBS. Subcellular localization was determined using confocal microscopy.

Stability Studies

Stability studies of the developed formulations were carried as per ICH guidelines[18]. The formulations were potted in Aluminum foils and set aside in the stability chamber (REMI Environmental Test Chamber, India) maintained at 25 ± 2°C and 40 ± 2˚ Cwith relative humidity of 60 ± 5% and 75 ± 5% RH. The samples were analyzed at an interval of 15 days for the period of 3 months for the drug content and particle size. The results of the stability study are shown in Figure 7.

Statistical Analysis

All results were expressed as mean ± standard deviation (SD) and statistical analysis was performed with ANOVA. In vitro drug release kinetics model fitting was carried out using Sigma Plot. A difference with $p \leq 0.05$ (i.e. 5% level of significance) was considered to be statistically significant.

RESULT

Particle Size

The size distribution along the volume mean diameter of the targeted Microparticles was measured by Microtrac particle size analyzer. The estimated size of average particle was 34.26±1.12 µm to 48.12±0.06 µm. Particle of formulation were in Microsize having rough surface. Addition of a small volume of ethanol to the mixture of acetone leads to smaller Vinblastine Microparticles and this alteration can prevent the aggregation of particles effectively.[19] Lowering the amount of solvent resulted in formation of larger particles. The particle size was found to be 42.2±0.02µm respectively. As the drug concentration increased there was an increase in particle size, which may be as a result of drug acting as a core and covering of drug by polymer.[13, 20] Results are shown in Table 2.

Surface Morphology

Shape and surface morphology of Microparticles was studied by Scanning Electron Microscopy[15]. Photographs of formulations were shown in Figure No.3**.** Vinblastine Microparticles have shown smooth and spherical shape with different sizes depending on the ratios of the drug and polymer used. The stability and Zeta potential is tested by Zeta PALS, Brookham Instruments. The measurement of the product is done by adding it in mannitol as Lyoprotectant.

Optimization of Formulation and Process Variables of Microparticles Preparation

The effect of VBL: CH ratio of as mentioned in Table 1, on the particle size, product yield and encapsulation efficiency were studied. It was observed that as on increasing VBL: CH ratio from 1:10 to 1:5, the microparticle size was changed from 389.56 ± 0.60 µm to 316.6 ± 1.22 µm. The change in drug: polymer ratio also showed effect on drug content (%) and encapsulation efficiency (%). The drug content (%) and encapsulation efficiency found to increase from 59% to 76% and 82 to 86 % respectively. On these findings, formulation VEML3 was selected for further optimization study. The product yield was observed to be 75.52±1.21%. Upon decreasing the amount of polymer a decreased product yield from 75.52 ± 1.24% to 64.54 ± 0.51%, respectively was observed.

The compressibility index values were less than 20 for all batches, suggesting good flow characteristics of beads. The tapped density was found to be 0.30 ± 0.01 g cm^3 to 0.52± 0.02 g cm^3, while the apparent density was found to be between 0.27 ± 0.03 g cm^3 and 0.45 ± 0.03 g cm^3.The angle of repose of microparticles was also below 20 (Table 3).

FTIR spectra of the vinblastine, chitosan and vinblastine loaded microparticles are shown in Figure 3. The assignments of bands are as follows: 3437 cm−1 (O–H stretching vibrations), 2935 cm−1 (overlapping C–H stretching vibrations peaks of methyl, methylene and –CH), 2879 cm−1 (methylene C–H stretching vibrations), 1715 cm−1 (stretching vibration of carbonyl group). In FT-IR analysis showed that pure drug present in the microparticulate systems, indicating no existence of different forms of drug with polymers. The XRD studies of the formulated microparticles confirmed that drug was present in the microparticles in crystalline form. the polymer Chitosan and Eudragit RS/RL100 and chitosan was found to be in amorphous, anhydrous, and crystalline state.

The photomicrograph as shown in figure 3 indicates that the VEMLs formed were rough and spherical in shape. The VEMLs were observed with no precipitation of drug on the surface as shown in Figure 4. It also showed numerous small pores present on the surface of the microparticles which seems like a matrix structure.

Drug Content and Entrapment Efficiency

The drug %EE of formulations Microparticles was found to be satisfactory high. The data showed that drug entrapment efficiency and loading capacity of Microparticles were increased from 82.56±0.78 to 86.32±0.88% and from 0.83±0.08 to 0.86±0.09%, respectively by increasing the Drug concentration[10].

Dynamic Swelling Study and Percent Mucoadhesion

Polymer cross linking significantly affects the swelling behavior of Microparticles. Low swelling was observed with increase in chitosan concentration, due to the formation of a dense structure. At low density, crosslinking a loose polymer structure found which has high hydrodynamic area that can entrap more solvent into it. The swelling property of the formulation followed the order: VEML > VEML-9 > VEML-8 > VEML-7 > VEML-6 > VEML-5 > VEML-4 > VEML-3 > VEML-2 > VEML-1. These results are in agreement with the results obtained by Felipe and coworkers[14]. Mucoadhesion studies were carried out to ensure adhesion of the formulation for prolonged period of time. Higher concentration of the mucoadhesive property, results

in better adhesive characteristics, as shown by the formulation VEML-5, to an extent of 82.25% in comparison to other formulations (Table 4). Data are represented as mean ± standard deviation (SD) (n = 3). VEML, Microparticles.

In-Vitro Drug Release Study

The controlled release potential and diffusion of the microparticles containing vinblastine was evaluate each sample over 24 hr in triplicate[12]. The release rate of vinblastine depends on the total concentration of drug present in the formulation. It is observed that drug release more quickly when the concentration of polymer is lower and drug concentration is higher. The vinblastine loaded Microparticles confirmed drug-enriched entrapped matrix model suggested for sustained release[17]. It is observed that the degree of diffusion of Microparticles was decreased due to increase in polymer and drug ratio. The drug enriched core is surrounded by a thick chitosan coated with Eudragit which regulates the diffusion and promotes sustained drug release.[21]

The kinetic treatment of in vitro by release data revealed the biphasic drug release pattern[22] (Jiang et al., 2014). The initial burst effect could be due to two reasons: the first was the drug near or on the surface of the microparticles and the second was the well-known porous nature of microparticles, the pores provided a channel for release of the drug[23]

In- Vitro Cytotoxicity Assay

In order to know the activity of Vinblastine -loaded Microparticles, in vitro cellular cytotoxicity was evaluated by MTT assay. The viability of HT29 cells treated with Microparticles did not demonstrate a significant difference compared with microparticles at low vinblastine concentrations (<10 nM, $p > 0.05$), but the inhibiting activity of Microparticles increased with vinblastine concentration from 10 to 50 nM, and demonstrated a significant difference at 50 nM. In addition, blank Microparticles without Vinblastine showed trifling cytotoxicity. It could be speculated that the higher cytotoxicity of Ligand banded Microparticles compared with Microparticles was mainly result from better loading. Confocal microscopy was used to observe the intracellular distribution of the internalized Microparticles. After incubation with either microparticles, the fluorescence was mainly localized in cytoplasm. Fluorescence intensity of cells treated with microparticles was much higher than that of cells treated with microparticles, which was consistent with above result of cellular uptake.

Stability Studies

Stability study was carried out at 25 ± 2°C and 40 ± 2° C with relative humidity of 60 ± 5% and 75 ± 5% RH for 3 months. The changes in % drug content and particle size of different formulations were noted (Figure 7). There was no significant decrease in drug content as well as particle size i.e. nearly constant which indicates high stability of the formulation. The obtained data was subjected to t-test at 95% level of significance. No significant difference in relation to drug content and particle size was observed in the formulation.

DISCUSSION

Emulsification solvent evaporation-emulsion solvent diffusion method was used for the preparation of microparticles. The method was simple and reproducible and has provided uniform microparticles avoiding solvent toxicity. The effect of VBL: CH ratio on the particle size, product yield and encapsulation efficiency were studied on formulations. It was observed that drug: polymer ratio has considerable effect on the size of microparticles. On increasing the ratio of drug to polymer, the particle sizes of the microparticles increased and later get constant. This may be due to increase in drug to polymer ratios, the amount of polymer available per microparticle get less to entrap drug properly. Thus smaller quantity of polymers surrounded the drug. The alteration in drug: polymer ratio had significant effect on drug content (%) and encapsulation efficiency (%). The particle size increased upon increasing the concentration of the polymer solution. The change in size of microparticles could be attributed to increased viscosity. The FTIR studies showed that the drug and the polymer were compatible with each other. The SEM showed that the particles were uniform and spherical in size. Due to this uniformity the flow property of the VEMLs were found to be excellent. The in vitro drug release study of Eudragit coated VEML microparticles was carried and the kinetic parameters of drug release were evaluated. The results showed that the Eudragit coat protected the core microparticle for approximately 320 minutes which is equivalent to the desired time for formulation to reach the colon and then under the influence of the colonic environment the Eudragit coating on micropaticle eroded resulting to delivery of drug to the colon. It was observed that for each formulation the drug release decreased with decrease in the drug content. This may be due to the fact that as the drug content decreases the surface of polymer increases which leads to decrease in release of drug from the microparticles.

In all studies, value of n was approximately 0.5 which indicated Fickian diffusion and biphasic release pattern. The biphasic release involves of two phases i.e. immediate release phase followed by sustained release phase. In immediate release phase initial burst effect is observed with drug release at 180 min. The drug has sustained release for sustained manner till 24th hr indicating of biphasic release pattern.

The stability of VEML during storage is an important requirement for its successful clinical purpose. Degradation is likely to occur under conditions of elevated ambient temperature and humidity. The microparticles were subjected to accelerated stability testing and it was found that there was no significant decrease in drug content or particle size of VEML and physical stability of the polymers used in the preparation of VEML. This present study helped in formulating suitable colon targeting drug delivery system. It is a novel approach which should be explored in future for its better application.

CONCLUSION

We can conclude that the use of VEML microparticles to the colorectal site has positive consequence in treatment of tumour cells. The varying ratio of drug and polymer help in developing a optimised formulation. The polymers help in regulating the release of drug at target site excluding non target sites, thereby preventing unintended toxicity to normal tissues. The drug delivery system in future can be used as carrier for targeting more drugs with better therapeutic value. Targeted drug delivery systems have a great importance in targeting drugs with a stealth characteristic to target sites in field of therapeutics.

AKNOLEDGEMENT

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Table 1: Composition of formulations.

Sr No.	Formulation Code	Vinblastine (mg)	Chitosan (mg)
1	VEM-L1	1	10
2	VEM-L2	1	20
3	VEM-L3	1	30
4	VEM-L4	2	10
5	VEM-L5	2	20
6	VEM-L6	2	30
7	VEM-L7	3	10
8	VEM-L8	3	20
9	VEM-L9	3	30
10	Void	-	10

Table 2: Product yield, Drug Content, Drug entrapment, particle Size and in vitro drug release study of microparticles

Formulation	Product yield (%)	Drug content (%)	Particle Size (µ)	% Drug entrapment	Drug release (%)
VEM-L1	64.51±0.051	59.44±0.35	316.16±1.22	86.32±0.88	53.81.98
VEM-L2	74.37±0.62	69.14±0.921	343.4±0.24	85.45±0.26	55.4±2.63
VEM-L3	71.27±0.57	76.33±0.69	342.28±0.21	84.38±1.2	64.6±2.86
VEM-L4	75.52±1.24	62.33±2.31	358.12±0.6	82.56±0.78	68.4±3.15
VEM-L5	72.14±1.03	64.22±1.33	353.34±0.86	83.44±0.61	56.4±3.21
VEM-L6	70.08±1.06	62.44±2.81	342.58±0.34	85.44±2.18	59.5±2.45
VEM-L7	63.4±1.23	65.4±2.61	346.54±0.45	86.72±3.14	58.4±3.10
VEM-L8	65.16±2.33	63.42±2.12	381.48±0.43	84.84±1.24	60.6±2.44
VEM-L9	63.41±0.61	64.14±1.96	370.28±0.98	82.62±2.48	61.08±3.22

Table 3: Rheological characterization

Formulation code	Evaluation parameters				
	Angle of repose (□)	Poured density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner's ratio
VEM-L1	16.64±1.04	0.42±0.03	0.48±0.02	12.5±0.05	1.15±0.03
VEM-L2	18.42±1.12	0.30±0.02	0.339±0.01	13.09±0.04	1.01±0.02
VEM-L3	19.35±0.60	0.32±0.02	0.368±0.01	13.04±0.05	1.15±0.02
VEM-L4	17.35±0.80	0.27±0.01	0.30±0.01	10.01±0.03	1.11±0.03
VEM-L5	17.84±0.91	0.45±0.02	0.52±0.02	13.46±0.04	1.16±0.01
VEM-L6	23.61±0.42	0.39±0.03	0.47±0.01	15.76±0.03	1.19±0.03
VEM-L7	26.88±1.24	0.37±0.02	0.46±0.01	19.56±0.14	1.25±0.02
VEM-L8	28.34±1.63	0.41±0.03	0.533±0.13	22.64±0.16	1.30±0.04
VEM-L9	23.45±1.74	0.35±0.04	0.427±0.04	18.03±0.27	1.22±0.04

Table 4: Polydispersity index, Zeta potential and mucoadhesion of formulations.

Formulation Code	Polydispersity index	Zeta potential (mV)	Mucoadhesion (%)
VEM-L-1	0.224	-18 ±0.6	74.02 ± 1.04%
VEM-L-2	0.219	-17 ±1.2	76.54 ± 0.76%
VEM-L-3	0.221	-19±1.3	77.98 ± 0.32%
VEM-L-4	0.226	-22 ±1.4	79.62 ± 0.38%
VEM-L-5	0.221	-16 ±1.1	82.25 ± 0.14%
VEM-L-6	0.211	-19 ±2.3	82.95 ± 0.53%
VEM-L-7	0.213	16 ±1.9	84.53 ± 0.42%
VEM-L-8	0.214	-18 ±2.1	87.64 ± 0.61%
VEM-L-9	0.208	-17 ±1.2	89.43 ± 0.21%

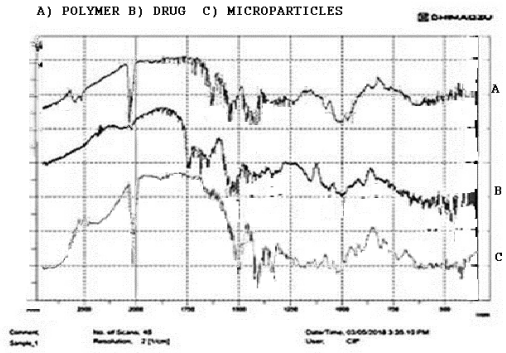

Figure 1: FTIR of Polymer, Vinblastine and microparticles

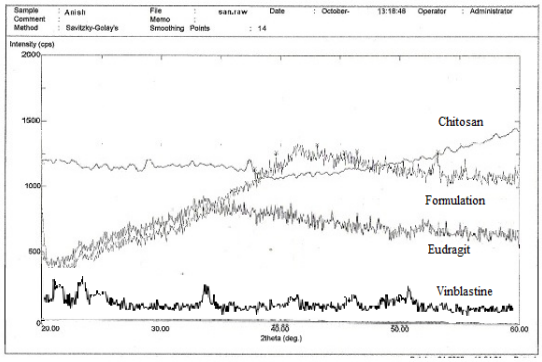

Figure 2: XRD of formulation, drug and polymers

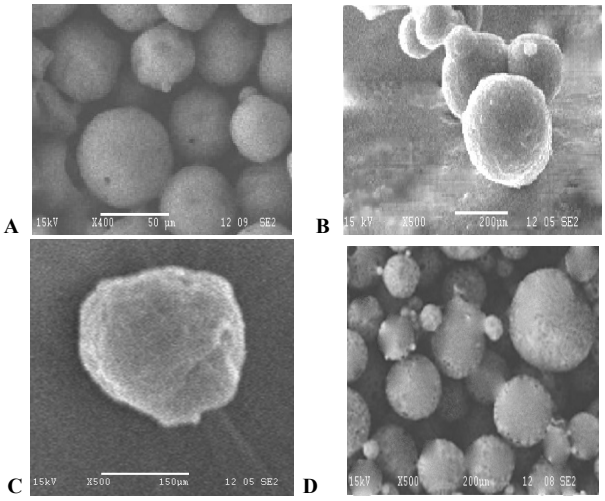

Figure 3: Scanning electron microscopy (SEM) of VEML Microparticles.

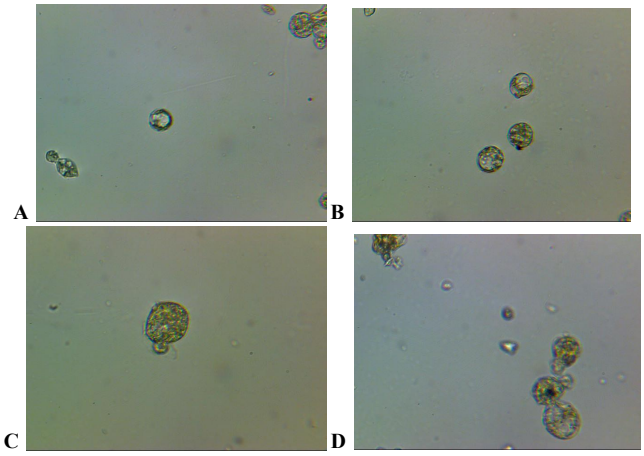

Figure 4: Photo micrograph of VEML Microparticles.

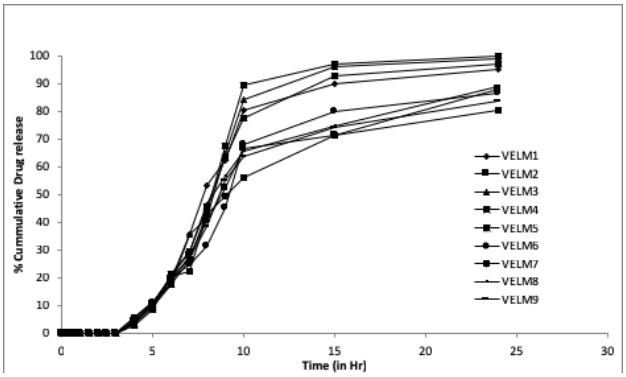

Figure 5: In- vitro percent drug release of Vinblastine Microparticles.

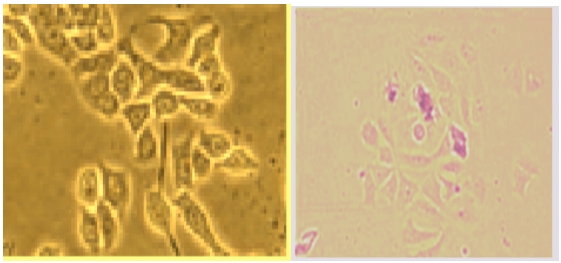

Figure 6: Confocal images of HT29 cells after treatment of Microparticles (A) and Microparticles (B) or after the triple fluorescence-labeling experiments: red fluorescence from Rhodamine B

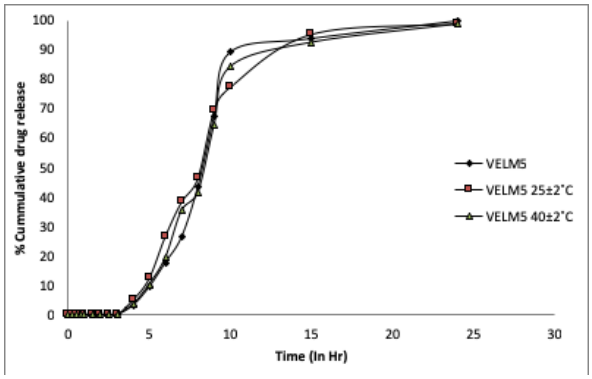

Figure 7: Stability study of Microparticle VEML5

REFERENCES

1. Remesh A. Toxicities of anticancer drugs and its management. International Journal of Basic & Clinical Pharmacology 2017; 1:2-12.
2. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microparticles. International Journal of Pharmaceutics 2006; 318: 103-17.
3. Verma A, Laakso I, Seppänen-Laakso T, Huhtikangas A, Riekkola ML. A Simplified Procedure for Indole Alkaloid Extraction from *Catharanthus roseus* Combined with a Semi-synthetic Production Process for Vinblastine. Molecules 2007; 12: 1307-1315.
4. Chandy A, Ahirwar D, Ahirwar B. Microspheres for Targeting an alkaloidal anticancer drug in colon cancer, Research Journal of Pharmacy and Technology 2013,6(6),618-621.
5. Chandy A, Ahirwar D. Ex-Vivo Evaluation of Enteric Coated Microparticles Containing an Anti-Cancer Drug. Research Journal of Pharmacy and Technology 2018; 11: 3509-3513.
6. Onishi H, Kikuchi H, Machida Y. Comparison of simple Eudragit microparticles loaded with prednisolone and Eudragit-coated chitosan-succinyl-prednisolone conjugate microparticles: Part I. Particle characteristics and in vitro evaluation as a colonic delivery system. Drug development and Industrial Pharmacy 2012; 38: 800-807.
7. Debuigne F, Jeunieau L, Wiame M, Nagy JB. Synthesis of Organic Nanoparticles in Different W/O Microemulsions. Langmuir 2000; 16: 7605–7611.
8. Masarudin MJ, Cutts SM, Evison BJ, Phillips DR, Pigram PJ. Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [(14)C]-doxorubicin. Nanotechnology, Science and Applications 2015; 8: 67-80.
9. Smith AW, Segar CE, Nguyen PK, MacEwan MR, Efimov IR, Elbert DL. Long-term culture of HL-1 cardiomyocytes in modular poly(ethylene glycol) microsphere-based scaffolds crosslinked in the phase-separated state. Acta Biomaterialia 2011; 8: 31-40.
10. Yeo Y, Park K. Control of encapsulation efficiency and initial burst in polymeric microparticle systems. Archives of Pharmacal Research 2004; 27: 1-12.
11. Notario-Pérez F, Martín-Illana A, Cazorla-Luna R, et al. Influence of Chitosan Swelling Behaviour on Controlled Release of Tenofovir from Mucoadhesive Vaginal Systems

for Prevention of Sexual Transmission of HIV. Marine Drugs 2017; 15: 50.
12. Jain V, Jain D, Singh R. Factors effecting the morphology of eudragit S-100 based microparticles bearing dicyclomine for colonic delivery. Journal of Pharmaceutical Sciences 2011; 100: 1545-1552.
13. Jain SK, Jangdey MS, Lectin Conjugated Gastroretentive Multiparticulate Delivery System of Clarithromycin for the Effective Treatment of Helicobacter pylori. Molecular Pharmaceutics 2009; 6: 295-304.
14. Felipe JOV, Francisco JBV, Joao S, Abdul WB. Mucoadhesive platforms for targeted delivery to the colon. International journal of pharmaceutics 2011; 420: 11-9.
15. Jain V, Singh R. Design and characterization of colon-specific drug delivery system containing paracetamol microparticles. Archives of Pharmacal Research 2011; 34: 733-40.
16. Costa P, Sousa LJM. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences 2001; 13: 123-33.
17. Qin L, Xue M, Wang W, Zhu R, Wang S, Sun J, Zhang R, Sun X . The in vitro and in vivo anti-tumor effect of layered double hydroxides nanoparticles as delivery for vinblastine. International Journal Pharmaceutics 2010; 388: 223–230.
18. Guideline IHT. Stability Testing of New Drug Substances and Products Q1A (R2). Current step 2003; 4:1-26.
19. Giri TK, Choudhary C, Ajazuddin, Alexander A, Badwaik H, Tripathi DK. Prospects of pharmaceuticals and biopharmaceuticals loaded microparticles prepared by double emulsion technique for controlled delivery. Saudi Pharmaceutical Journal 2012; 21: 125-141.
20. Devrim B, Canefe K. Preparation and evaluation of modified release ibuprofen microspheres with acrylic polymers (eudragit) by emulsification solvent evaporation method: Effect of variables. Acta Poloniae Pharmaceutica.Drug Research 2006; 63: 521-34.
21. Destrée C, Nagy JB. Mechanism of formation of inorganic and organic nanoparticles from microemulsions. Advances in Colloid and Interface Science 2006, 123–126, 353-367.
22. Jiang T, Wu C, Gao Y, Zhu W, Wan L, Wang Z, Wang S. Preparation of novel porous starch microsphere foam for loading and release of poorly water soluble drug. Drug development and Industrial Pharmacy 2014; 40: 252-9.
23. Mahboubian A, Hashemein SK, Moghadam S, Atyabi F, Dinarvand R. Preparation and In-vitro Evaluation of Controlled Release PLGA Microparticles Containing Triptoreline. Iranian Journal of Pharmaceutical Research 2010; 9: 369-78.

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