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Formulation and Evaluation of Colon Targeted pH Dependent Microcapsules of Thymoquinone for Colorectal Cancer

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Original Research Article

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Abstract: Aim of the present work was to prepare the colon-targeting pH dependent microcapsules of Thymoquinone for the treatment of colorectal cancer. pH dependent polymer Eudragit L100,and S100 were used to formulate the microcapsules by solvent evaporation technique using two methods. Microcapsules were evaluated for particle size, shape, flow properties, surface morphology by scanning electron microscopy, percentage yield, drug content, and *in vitro* drug release behaviour, *in vivo* targeting efficiency. The formulated microcapsules were discrete, almost spherical with somewhat folded and invaginated surface, and with good flow properties. Thymoquinone loaded microcapsules demonstrated good entrapment efficiency (of 86.438% in method 1 and 92.49% in method 2). Result of *in* vivo targeting efficiency showed that the formulation can able to target colon effectively. Formulation done by method 2 gave most promising result compared to first method. It is concluded from the present study that pH dependent Eudragit microcapsules are promising carriers for oral colon-targeted delivery of Thymoquinone for colorectal cancer.

Keywords: Thymoquinone, Eudragit L-100, Eudragit S-100, microcapsules, pH dependent, colon targeting, colorectal cancer

INTRODUCTION

The word new or novel in the relation to drug delivery system is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be major – advance towards solving the problem associated with the existing drug delivery systems [1].

The most convenient route for the administration of drugs to the patients is oral route. When the conventional dosage forms are administered orally they dissolve in the stomach fluid or intestinal fluid and depending upon the physicochemical properties of the drug they get absorbed from the regions of the GIT. It has a serious drawback in conditions where localized delivery of the drug in the colon is required or in conditions where a drug needs to be protected from the hostile environment of upper GIT. So, the dosage forms that deliver drugs into the colon rather than upper GIT have a number of advantages. Colon-specific drug delivery system has gained increased importance not just for the delivery of the drugs for treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides [2, 3].

Multi-particulate approaches tried for colonic delivery includes formulations in the form of pellets, granules, micro particles and nanoparticles. Because of their smaller particle size compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to low inter and intra subject variability. Moreover, multi-particulate systems are to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption. Multi-particulate systems also avoid the vagaries of gastric emptying and different transit rates; thereby release the drugs more uniformly.

The most preferred route for CDDS is oral route. But some other routes may be used. Rectal administration offers the shortest route for targeting drugs to the colon. However, it is difficult to reach the proximal part of colon via rectal administration. Rectal administration can also be uncomfortable for patients and compliance may be less than optimal. Drugs can be supplied as solutions, foam, and suppositories for intra rectal administration. The intra rectal route is used for both systemic dosing and for the delivery of topically active drug to the large intestine.

Today, colon specific drug delivery is challenging task to pharmaceutical technologists. In general, seven primary approaches have been proposed

for targeted colon delivery, namely Transit time dependent colonic DDS, pH Dependent colonic DDS, pH- and time-dependent colonic DDS, Bacterial enzyme dependent colonic DDS, pH and bacterial enzyme dependent colonic DDS, Colonic pressure controlled DDS and Osmotic pressure controlled colonic DDS [4].

Cancer of the colon and rectum is one of the most common internal malignancies. Colorectal cancer is the second-leading cause of cancer deaths in the United States. In 2005, estimated 1, 45290 new cases of colon cancer were diagnosed in the United States. Almost all cases of colorectal cancer begin with the development of benign or noncancerous polyps. When colon cancer cells spread outside the colon or rectum to lymph nodes, they may also spread to other lymph nodes, the liver, or other organs. Surgery is still a mainstay of the treatment of colorectal cancer.

Chemotherapy is also used to treat advanced colorectal cancer. But conventional chemotherapy is not as effective in colorectal cancer as it is in other cancers, as the drug does not reach the target site in effective concentrations. Thus, effective treatment demands Chemotherapy is also used to treat advanced colorectal cancer. But conventional chemotherapy is not as effective in colorectal cancer as it is in other cancers, as the drug does not reach the target site in effective concentrations. Thus, effective treatment demands increased dose size, which may lead to undue consequences. To improve this situation, pharmaceutical technologists have been working on ways to deliver the drug more efficiently to the colon, where it can target the tumour tissues. It is possible that delivery of small quantities of antineoplastic agent to the inner surface of the colon could destroy small tumours that arise spontaneously in this region, reducing the need for surgery [5].

Various approaches, namely microcapsules, compressed microcapsules, and modified tablets can be used for the colon specific drug delivery [6].

Here microcapsules are formulated based on the pH dependent colonic drug delivery. The pH-dependent CTDDS exploit the generally accepted view that pH of the human GIT increases progressively from the stomach (pH 1-2 which increases to 4 during digestion), small intestine (pH 6-7) at the site of digestion and it increases to 7-8 in the distal ileum. The coating of pH-sensitive polymers to the tablets, capsules or pellets provide delayed release and protect the active drug from gastric fluid [7]. pH dependent drug delivery is achieved by Coating the extract with a pH sensitive polymer, Basically it is poly (meth) acrylate base polymers and these are co-polymers derived from esters of acrylic and meth acrylic acid, whose physicochemical properties are determined by functional groups (R). Eudragit polymers are available in a wide range of different physical forms (aqueous dispersion, organic solution, granules and powders). For achieving local treatment of bowel disorders such as Crohn's disease, ulcerative colitis or intestinal cancer drug needed to deliver at inflammatory site in colon without loss in upper GI this is accomplish by coating the tablet core with pH dependent polymer Eudragit S100 but this type of formulation lead to premature drug release in distal part of small intestine this problem is overcome by coating with solution containing mixture of both Eudragit L100 and Eudragit S100.

Thymoquinone (THQ) is one of the important compound found in *Nigella sativa (*Family: *Ranunculaceae*;). The oral delivery of THQ is valuable, although it is limited because of its solubility-related poor oral bioavailability. The induction of apoptosis in tumor cells is a key mechanism for the effectiveness of chemopreventive drugs. TQ has been shown to induce apoptosis by p53-dependent [8] and p53-independent [9] pathways. In HCT-116 human colon cancer cells, the pro-apoptotic effects of TQ are dependent on p53 [10] while in myeloblastic leukemia HL-60 cells, TQ-induced apoptosis is p53-independent and occurs through the activation of caspase 8, 9 and 3 [11]. TQ treatment of HCT-116 cells resulted in a marked increase in p53 and p21 protein levels and in a significant inhibition of the anti-apoptotic Bcl-2 protein [10]). Co-incubation with the specific p53-inhibitor, pifithrin-alpha, restored the mRNA and protein levels of Bcl-2, p53 and p21 to control levels and suppressed TQ-induced apoptosis. P53-null HCT-116 cells are less sensitive to TQ-induced apoptosis [8].

Various technologies used for preparation of the microcapsules include coacervation- phase separations, multi orifice centrifugal process, spray drying and spray congealing, pan coating, solvent evaporation, interfacial polymerization etc. [9].

Advantages of microcapsules are better bioavailability, improve patient compliance, requirement of lower dose of drug, longer retention time, mucosal protection from irritating drug, enhanced absorption of poorly absorbed drug, reduce incidence of side effects etc [12-15].

MATERIALS AND METHODS

Thymoquinone was obtained as a gift from Leonid chemicals (Bengaluru, India). Eudragit L100 and S100, liquid paraffin, methanol, petroleum ether, and other solvents were purchased from Chemika biochemicals, Kearala. All other materials used in studies were of analytical reagent grade and were used as received.

Preparation of Thymoquinone Loaded Microcapsules

Solvent evaporation method

Two types of methods were used here, Solvent evaporation by method 1 and solvent evaporation by method 2.

Method 1 [15]

Accurately weighted Eudragit L-100, S-100 and Magnesium stearate in 1:1:2 ratios were dissolved in acetone to form a homogenous polymer solution. Core material, i.e. thymoquinone was added to the polymer solution and mixed thoroughly. This organic phase was slowly poured at 15^{o}C into liquid paraffin with stirring at 1000 rpm to form a smooth emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone evaporated and smooth-walled, rigid and discrete microcapsules were formed. The microcapsules were collected by decantation and the product was washed with petroleum ether (40-60^{0}C), four times and dried at room temperature for 3 hrs. The microcapsules were then stored in a desiccators over fused calcium chloride.

Table 1: Formulation Details of pH dependent Microcapsules- Method 1

Ingredients	Quantity
Thymoquinone	25 mg
Eudragit L 100	500 mg
Eudragit S 100	500 mg
Magnesium Stearate	1000 mg
Acetone	20 ml
Liquid Paraffin	50 ml

Method 2 [16]

Accurately weighted Eudragit L-100, S-100 and Magnesium stearate in 1:1:2 ratios were dissolved in dichloro methane to form a homogenous polymer solution. Core material, i.e. thymoquinone was added to the polymer solution and mixed thoroughly. This organic phase was slowly poured into liquid paraffin containing n-Hexane (9:1) ratio with stirring at 1000 rpm to form a smooth emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual dichloro methane evaporated and smooth-walled, rigid and discrete microcapsules were formed. The microcapsules were collected by decantation and the product was washed with n-Hexane, four times and dried at room temperature for 3 hrs. The microcapsules were then stored in a desiccators over fused calcium chloride.

Table: 2. Formulation Details of pH dependent Microcapsules- Method 2

Ingredients	Quantity
Thymoquinone	25 mg
Eudragit L 100	500 mg
Eudragit S 100	500 mg
Magnesium Stearate	1000 mg
Dichloro methane	20 ml
n-Hexane	5 ml
Liquid Paraffin	45 ml

EVALUATION OF MICROCAPSULES

Particle size and surface morphology

Thermal emission scanning electron microscopy (instrument model TESCAN VEGA 3 SBH) has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. Dry microcapsules were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microcapsules were taken by random scanning of the stub.

Percentage yield [17]

The total amount of microcapsules obtained was weighed and the percentage yield calculated taking into consideration the weight of the drug and polymer [17].

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Flow properties

Flowability of microcapsules investigated by determining, bulk density, tapped density, Carr's index and Hausner ratio.

Loose bulk density

It is measured by putting the accurately weighed microcapsules into a graduated cylinder and the volume was calculated using following formulae: [18]

$$\text{Loose Bulk density} = \frac{\text{Weight of powder} \times 100}{\text{Volume occupied by powder}}$$

Tapped density

The tapped density was determined by three tap method. Weighed quantity of Microcapsules is carefully introduced into a 10 mL graduated cylinder. The cylinder is dropped onto a hard wood surface three times from height of 2.5 cm. It can be calculated by using formula: [19]

$$\text{Tapped density} = \frac{\text{Weight of powder} \times 100}{\text{Final volume after tapping}}$$

Compressibility index

It is indirectly related to the flow rate, cohesiveness and particle size. It can be calculated by Following formula: [20]

Compressibility index=

$$\frac{\text{Tapped density} - \text{Loose bulk density} \times 100}{\text{Tapped density}}$$

Hausner's ratio

Hausner's ratio is an index of ease of powder flow; it was determined by using the following formula: [20]

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Loose bulk density}}$$

Drug content

Microcapsules were soaked in ethanol for 24 hours. Centrifuged at 5000rpm for 60 minutes. The supernatant was collected and from this 1 ml was pipetted out and made up to 10 ml with ethanol. Absorbance was measured at 254 nm. [21**].**

***In-vitro* dissolution studies**

In-vitro dissolution profile of each formulation was determined by employing USP XXIII rotating basket method (900 ml pH 1.2, pH 6.8 and pH 7.4 phosphate buffer, 100 rpm, 37o±0.5^{o}C).Microcapsules o thymoquinone equivalent to 50 mg was loaded into the basket of the dissolution apparatus. The first two hour study was performed in pH 1.2 buffer(0.1 N Hydrochloric acid).Samples were withdrawn every 1 hour and the next two hour study was performed in phosphate buffer pH 6.8. In every one hour 1ml of sample was withdrawn from pH 6.8 medium, diluted with phosphate buffer and made up to 10ml.At the same time 1ml of phosphate buffer was added to the dissolution medium to maintain the sink condition. The same procedure was repeated for entire 8 hour study with pH 7.4 buffer. The absorbance of the sample was determined at wavelength of 254nm against pH 7.4 blank. The amount of drug present in the sample was then determined from the calibration curve and cumulative percent of drug release was calculated data obtained was also subjected to kinetic treatment to obtain the order of release and release mechanism. [22, 23].

Determination of *in vivo* targeting efficiency

Before commencement of the experimentation on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee, and it was approved by the committee. Institutional Animal Ethical Committee, University College of Pharmacy, Cheruvandoor, Kottayam has given consent for conducting the animal experiment–No IAEC/02/2017 dated 03/04/2017.

This study is carried out to check the *in vivo* targeting efficiency of the formulation. Rabbits were selected as an animal model for evaluating the colon-specific delivery.

Roentgenography study, a comparatively safer technique was carried out in healthy male albino rabbits. The behaviour of microcapsules in rabbit was observed using a radiographic imaging technique. It involves the use of radio-opaque marker barium sulphate, incorporated in the formulation to determine the position of microcapsules. Healthy rabbits fasted overnight and on the next day morning microcapsules enclosed in a capsule shell was administered with the help of a veterinary doctor. At different time intervals, X-ray images were taken under the supervision of a radiologist. [24].

RESULTS AND DISCUSSION

Preparation of Thymoquinone loaded pH dependent Microcapsules

pH dependent microcapsules of Thymoquinone was successfully prepared by solvent evaporation methods. In these methods, drug polymer mixtures were dispersed into an immiscible vehicle to form a homogenous solution. As the solvent is evaporated, the droplets become gradually concentrated and the nucleation takes place to produce microcapsules. Eudragit L100 and Eudragit S100 and Magnesium stearate (1:1:2) were used to prepare pH dependent microcapsules.

Micrometric properties of pH Dependent Microcapsules

The micromeritic properties of the prepared microcapsules were given in table 3.

Table 3: Micromeritic properties of the prepared microcapsules

Physical properties	Method 1	Method 2
Loose bulk density	0.304mg/ml	0.318mg/ml
Tapped density	0.318mg/ml	0.333mg/ml
Carr's index	4.40	450
Hausner's ratio	1.046	1.047
Percentage drug loading	86.438%	92.49%
Percentage yield	85.28%	89.26%

The results demonstrated that method 2 give better properties compare to method 1.

Optical microscopic images -Thymoquinone loaded microcapsules

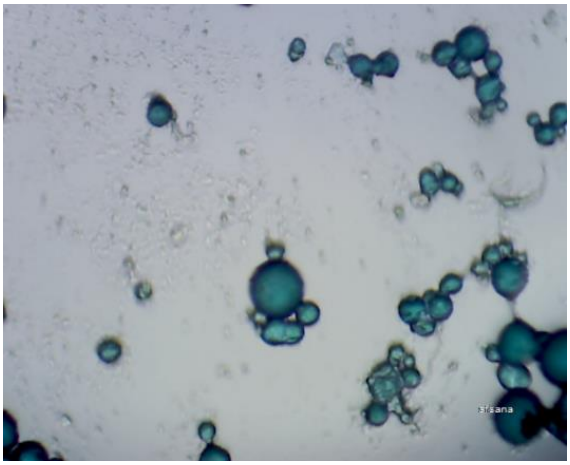

Fig-1: Method 1

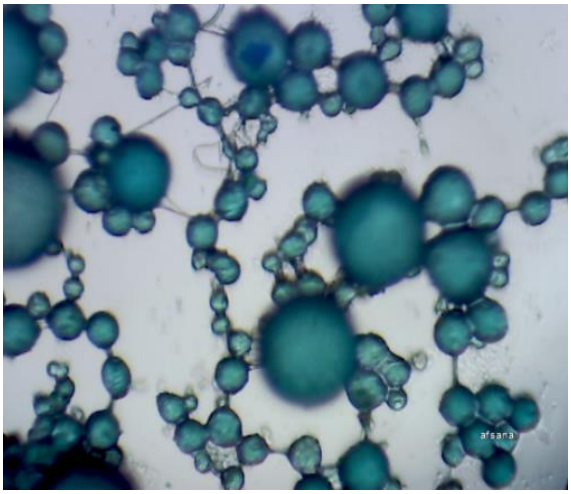

Fig-2: Method 2

Scanning electron microscopic images

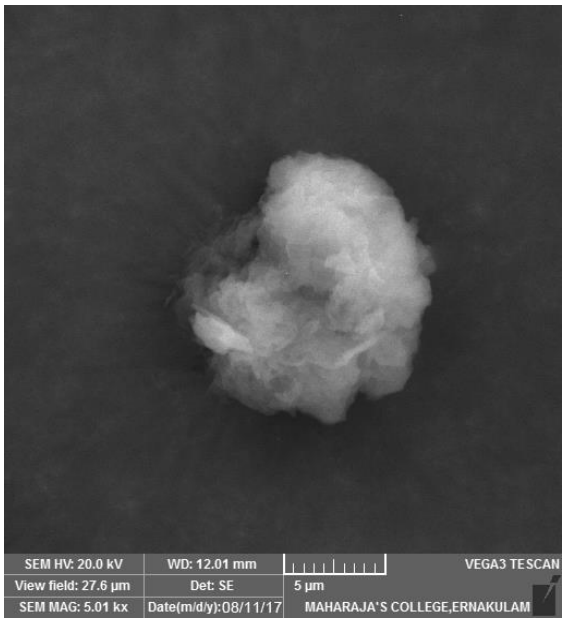

Fig-3: Method 1

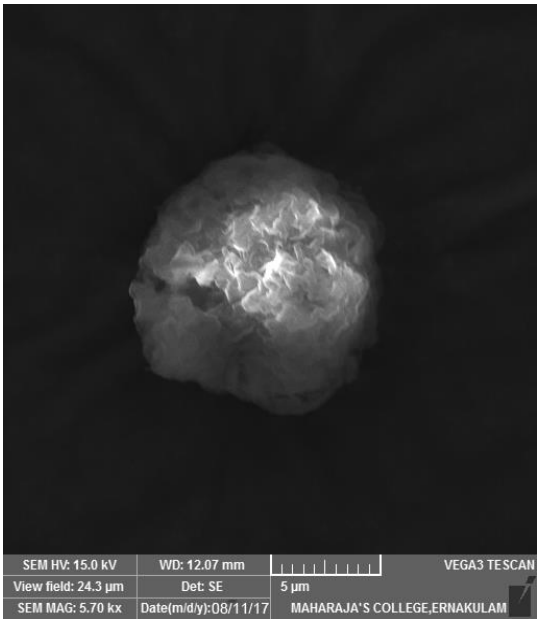

Fig-4: Method 2

It can be clearly observed from the photographs of the microcapsules prepared by solvent evaporation technique, that the microcapsules are small, spherical and discrete. The shape and surface morphology was further confirmed with the SEM photographs. The microcapsules were almost spherical and have somewhat folded and invaginated surface. By comparing the SEM images and physical properties it was also found that method 2 is better when compared with method 1.

***In vitro* drug release studies of Thymoquinone loaded microcapsules.**

Table 4: *In vitro* drug release of Thymoquinone loaded microcapsules

Time(hour)	Dissolution media	Cumulative% release of TQloaded microcapsules (Method 1)	Cumulative % release of TQloaded microcapsules (Method 2)
0	pH 1.2 buffer	0	0
1		0	0
2		0	0
3	pH 6.8 buffer	9.76	16.39
4		13.41	24.16
5	pH 7.4 buffer	17.65	32.3
6		19.98	35.4
7		27.63	39.22
8		30.11	43.22
9		37.48	52.1
10		46.4	65.86
11		59.16	70.79
12		68.96	81.69

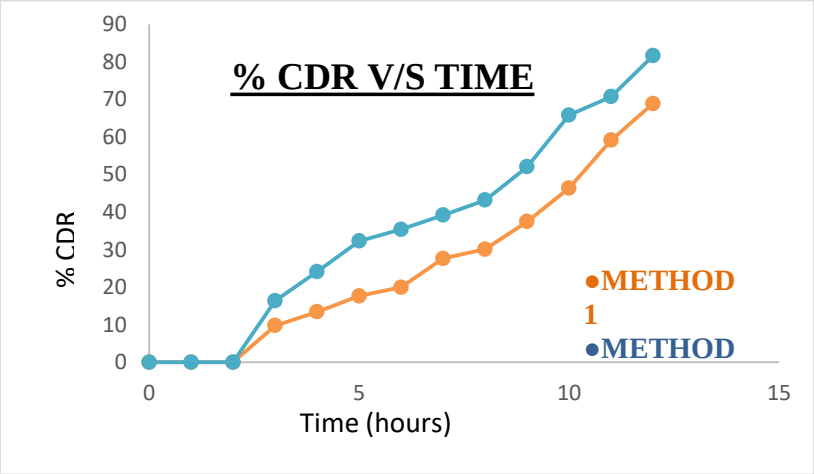

Fig-5: Cumulative drug release v/s time of Thymoquinone loaded microcapsules

From the drug release profile it was found that formulation done by method 2 is better than method 1.So kinetic assessment of method 2 was done.

DATA FOR KINETIC ASSESSMENT

Table 5: *In vitro* drug release of Thymoquinone loaded microcapsules

Time(T)	√T	Log T	% CDR	Log %CDR
0	0	0	0	0
1	1	0	0	____
2	1.414	0.3010	0	____
3	1.732	0.4771	16.39	1.2145
4	2	0.6020	24.16	1.3830
5	2.236	0.6989	32.3	1.5092
6	2.449	0.7781	35.4	1.5490
7	2.645	0.8450	39.22	1.5935
8	2.828	0.9030	43.22	1.6356
9	3	0.9542	52.1	1.7168
10	3.162	1	65.86	1.8186
11	3.316	1.0413	70.79	1.8499
12	3.464	1.0791	81.69	1.9121

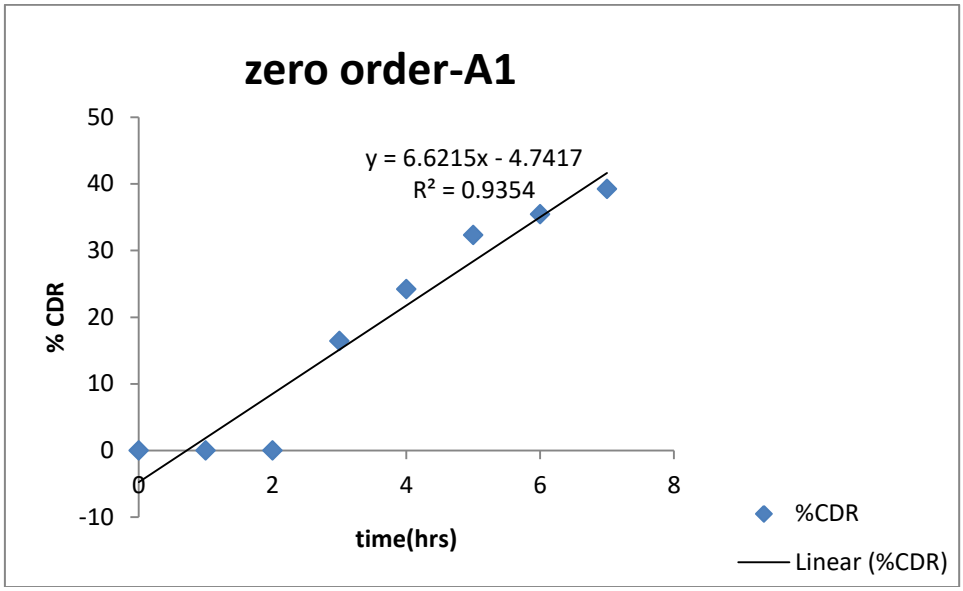

Fig-6: Zero order plot of drug release

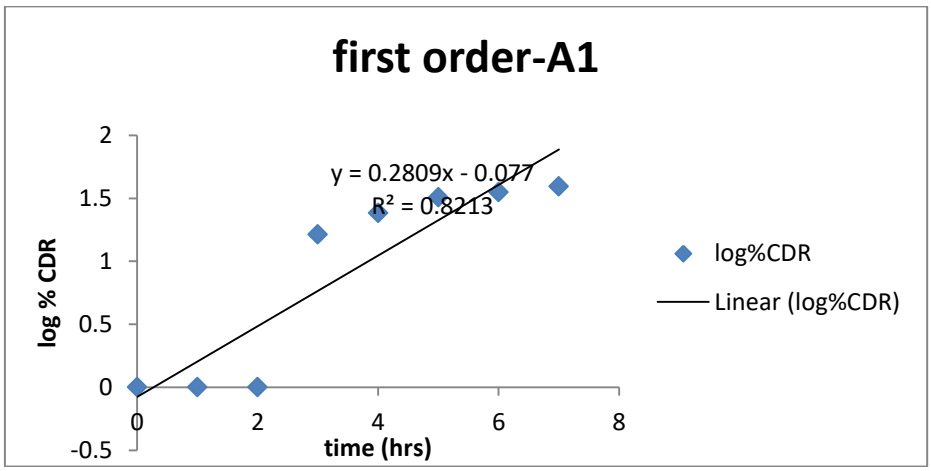

Fig-7: First order plot of drug release

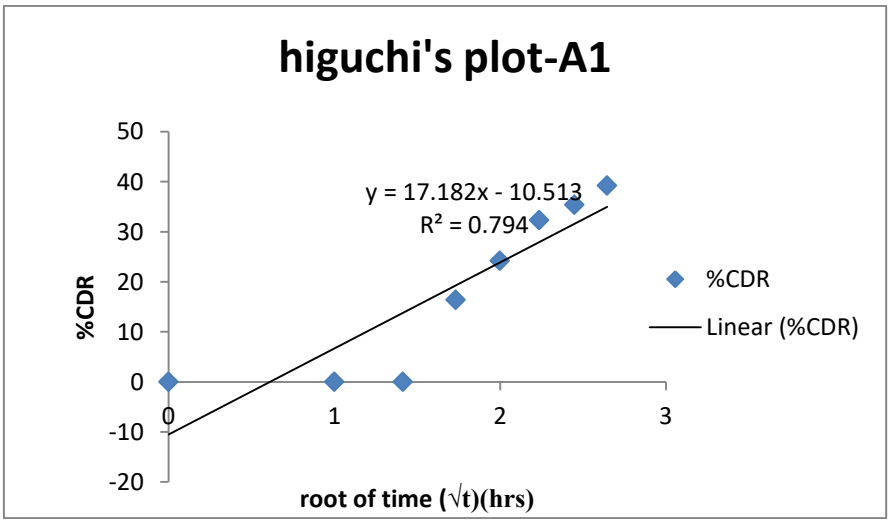

Fig-8: Higuchi plot of drug release

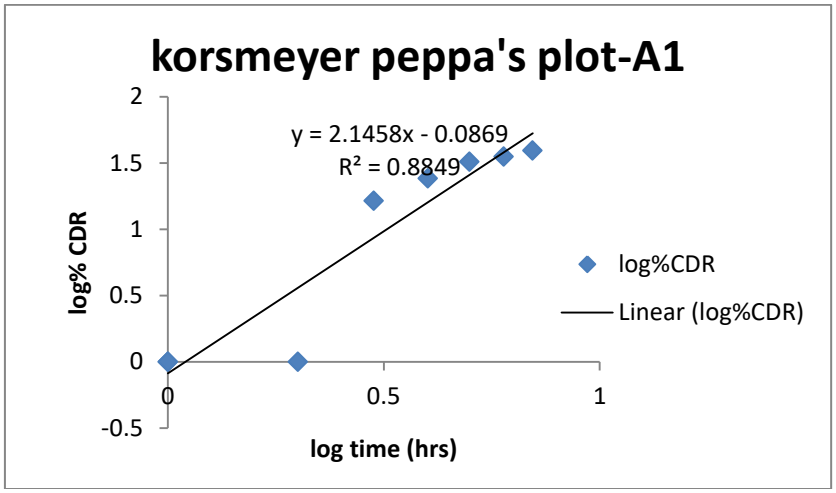

Fig-9: Korsmeyer peppas plot of drug release

From the kinetic assessment it was found that the formulation followed zero order kinetics and korsmeyer peppas model. From the 'n' values it was found that the formulated microcapsules follows super case ll transport that is erosion controlled drug delivery system.

Determination of *in vivo* targeting efficiency

Since the particles are small, and the amount of radio-opaque material is also less, the amount of microspheres visible by X-rays is also minimal. The observations were confirmed by a veterinary surgeon. It can be concluded that the formulation is able to target colonic region effectively.

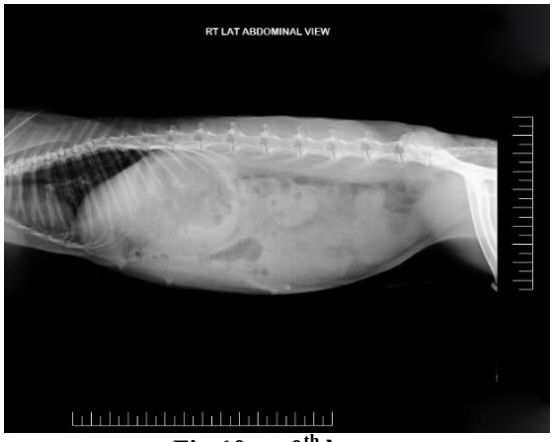

Fig-10: at 0th hour

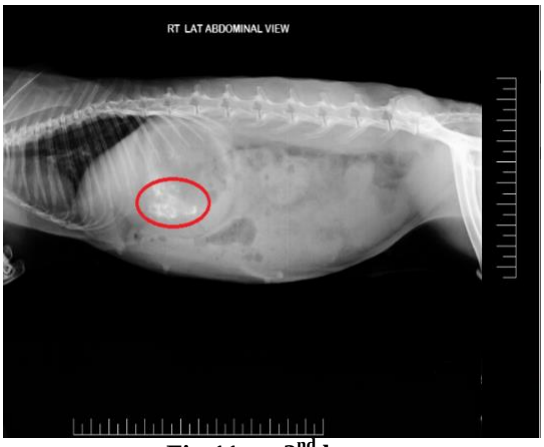

Fig-11: at 2nd hour

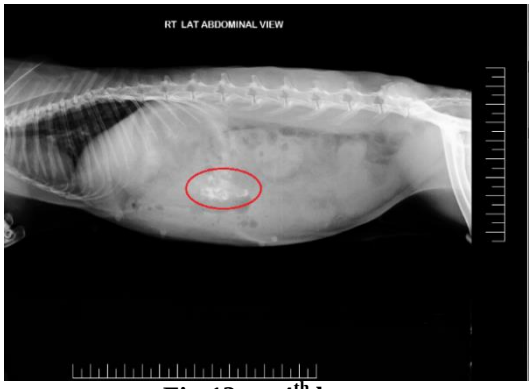

Fig-12: at 4th hour

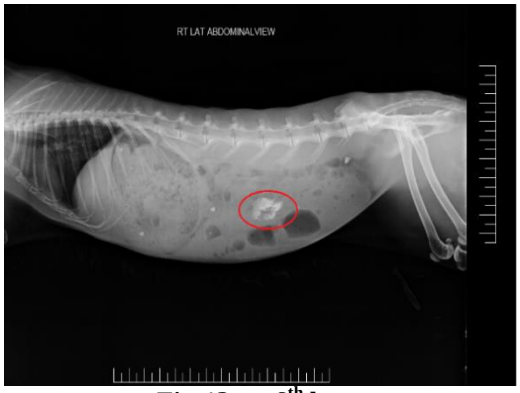

Fig-13: at 6th hour

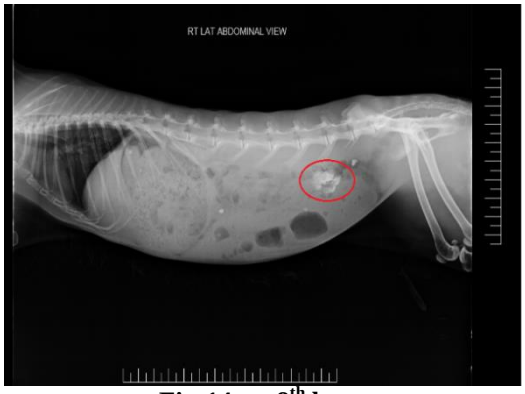

Fig-14: at 8th hour

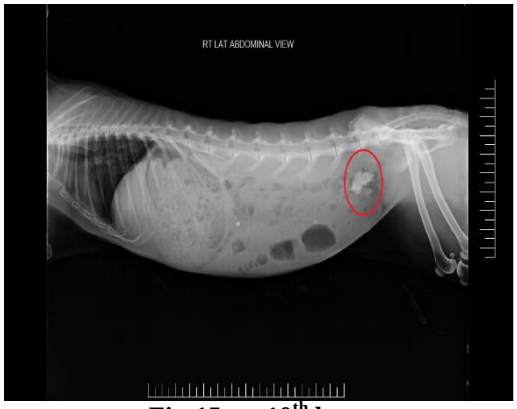

Fig-15: at 10th hour

Figure 10-15 shows the x-ray images of rabbit administered with radio opaque microcapsules

X-ray studies revealed that the microcapsules remained in the stomach for the first 2hours. Microcapsules get released from the capsule shell by dissolving the gelatine shell and distributed in the small intestine as observed in the 4th hour. Microcapsules are visible at 4th h in the small intestine as observed image and some are also seen in the X-ray image taken at the 8th h in the large intestinal region. X-ray image at 10th h indicates the presence of microcapsules in the colonic region. The observations were confirmed by a veterinary surgeon. It can be concluded that the formulation is able to target colonic region effectively**.**

CONCLUSION

The colonic region of the gastro-intestinal tract has become an increasingly important site for drug delivery and absorption of drugs used in the treatment of diseases associated with large intestine. Colon Drug Delivery System offers considerable therapeutic benefits to patients in terms of both local and systemic treatment. Although the surface area of colon is small, it has long retention time. In this work Thymoquinone microcapsules were prepared successfully by using the solvent evaporation methods. The preparation method influences the micrometric properties as well as drug release pattern of microcapsules. The yield was high and encapsulation efficiency was good for both preparation, but was highest for the formulation prepared by method 2. The assessment of release kinetic showed that drug release from Thymoquinone microcapsules followed the Korsmeyer peppas model and super case Ⅱ tansport (erosion controlled drug delivery system).

Initially at gastric medium (pH 1.2), very less release of core material (Thymoquinone) from microcapsules was found, but at pH 6.8 and pH 7.4, formulation showed burst release initially and then tend to release at constant rate. As per the aim, formulation does not show release in gastric medium for desired period of time and releases the drug at pH 6.8 and 7.4, which is the pH of colon and their allied areas; the prepared microcapsules proved to be good candidate for site-specific drug delivery.

Determination of the *in vivo* targeting efficiency concluded that the formulation is able to target colonic region effectively.

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REFERENCES

1. Jemal, A., Center, M. M., Ward, E., & Thun, M. J. (2009). Cancer occurrence. *Cancer Epidemiology*, 3-29.
2. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., & Thun, M. J. (2008). Cancer statistics, 2008. *CA: a cancer journal for clinicians*, *58*(2), 71-96.
3. Benson, A. L. (2007). Epidemiology, disease progression, and economic burden of colorectal cancer. *Journal of managed care pharmacy*, *13*(6 Supp C), 5-18.
4. Maciosek, M. V., Solberg, L. I., Coffield, A. B., Edwards, N. M., & Goodman, M. J. (2006). Colorectal cancer screening: health impact and cost effectiveness. *American journal of preventive medicine*, *31*(1), 80-89.
5. Gupta, V. K. (2012). A review article on colonic targeted drug delivery system. *The Pharma Innovation*, *1*(7).
6. Qureshi, A. M., Momin, M., Rathod, S., Dev, A., & Kute, C. (2013). Colon targeted drug delivery system: A review on current approaches.
7. Gali-Muhtasib, H., Diab-Assaf, M., Boltze, C., Al-Hmaira, J., Hartig, R., Roessner, A., & Schneider-Stock, R. (2004). Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. *International journal of oncology*, *25*(4), 857-866.
8. Sharma, M. K., Kavitha, K., Singh, S. D. J., & Kumar, M. R. (2016). Design and Evaluation of Microcapsules of Flurbiprofen for Colon Specific Drug Delivery. *Research Journal of Pharmaceutical, Biological and Chemical Sciences July–August 2016 RJPBCS 7*(4).
9. Chaudhary, A., & Kaundal, A. (2014). A review on microcapsules based colon targeted drug delivery system.
10. Phillip, A. K., Phillip, B. (2010). Colon targeted drug delivery system. *OMJ*, *25*, 70-78.
11. Asghar, L. F. A., & Chandran, S. (2006). Multiparticulate formulation approach to colon specific drug delivery: current perspectives. *J Pharm Pharm Sci*, *9*(3), 327-338.
12. Jose, S., Dhanya, K., Cinu, T., Litty, J., & Chacko, A. (2009). Colon targeted drug delivery: Different approaches. *Journal of Young Pharmacists*, *1*(1), 13.

13. Aswar, P. B., Khadabadi, S. S., Kuchekar, B. S., Wane, T. P., & Matake, N. (2009). Development and in-vitro evaluation of colon-specific formulations for orally administered diclofenac sodium. *Arch Pharm Sci & Res*, *1*(1), 48-53.
14. Pandya, H. V. (2012). Formulation and evaluation of microcapsules of trihexyphenidyle hydrochloride by solvent evaporation method. *International Journal of Pharmaceutical Sciences and Research*, *3*(2), 539.
15. Kandelwal, K. R. (2009). Practical Pharmacognosy Techniques and Experiments, Tenth edition, Nirali Prakashan Pune, 149-156.
16. Sharma, M. K., Kavitha, K., Singh, S. D. J., & Kumar, M. R. Design and Evaluation of Microcapsules of Flurbiprofen for Colon Specific Drug Delivery. *Research Journal of Pharmaceutical, Biological and Chemical Sciences July–August 2016 RJPBCS 7*(4), 1811.
17. Singh, V., & Chaudhary, A. K. (2011). Preparation of Eudragit E100 microspheres by modified solvent evaporation method. *Acta poloniae pharmaceutica*, *68*(6), 975-80.
18. Olayemi, O., & Jacob, O. (2011). Preliminary evaluation of Brachystegia eurycoma seed mucilage as tablet binder. *International Journal of Pharmaceutical Research and Innovation*, *3*, 1-6.
19. Bhaskaran, S. (2006). Micromeritics. In: Physical pharmaceutics. Birla Publications, Delhi. pp. 97-109.
20. Manjule, D. B., Shaikh, G., Surwase, U., & Kadam, B. (2011). Isolation and characterization of hibiscus rosa sinensis linn (shoe flowers plant). *International Journal Of Pharmaceutical And Chemical Sciences*, *1*(3), 593-598.
21. Kaus, L. C., Fell, J. T., Sharma, H., & Taylor, D. C. (1984). On the intestinal transit of a single non-disintegrating object. *International journal of pharmaceutics*, *20*(3), 315-323.
22. Cummings, J. H., Bingham, S. A., Heaton, K. W., & Eastwood, M. A. (1992). Fecal weight, colon cancer risk, and dietary intake of nonstarch polysaccharides (dietary fiber). *Gastroenterology*, *103*(6), 1783-1789.
23. Metcalf, A. M., Phillips, S. F., Zinsmeister, A. R., MacCarty, R. L., Beart, R. W., & Wolff, B. G. (1987). Simplified Assessment of Segmental Colonic Transit. *Gastroenterology*, *92*(1), 40-47.
24. Kala, D., & Nair, C. S. In Vivo Evaluation of Budesonide Microspheres for Colon Specific Drug Delivery.