International Journal of Research in Pharmaceutical sciences and Technology

Formulation and evaluation of biodegradable polymer triple layer coated multiparticulate system for colon targeting

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ABSTRACT

Abstract: In this present research work, the aim was to develop extended on targeted triple layer coated pellets filled capsule system of Curcumin for chronotherapeutic treatment of colon cancer. Materials and Methods: So triple layer coated pellets of curcumin were prepared using pH sensitive polymers by using fluidized bed dryer which was further filled into an empty gelatin capsule. The compatibility was assessed using FT-IR, DSC and SEM studies for pure drug, polymers and their physical mixtures. Results and Discussion: The prepared batches were subjected to physicochemical studies, drug content estimation, in-vitro drug release and stability studies. When FT-IR, DSC and SEM studies were performed, it was found that there was no interaction between curcumin and polymers used. The physicochemical properties of the entire prepared triple layer coated pellets batches were found to be in limits. The drug content percentage in the optimized formulation C9 was found to be 99.86± 0.02%. The optimized triple layer coated pellets-filled-capsule formulation C9 releases curcumin after a lag time of 3.14 ± 1.58, 17.48 ±0.03%, 68.72 ± 0.86%, and 98.46± 0.34% at the end of 2, 5, 9, and 12 h respectively. Conclusion: Thus, a novel colon targeted delivery system of curcumin was successfully developed by filling triple layer coated pellets into an empty gelatin capsule shell for targeting colon cancer treatment.

Keywords: *Curcumin; multiparticulate system; colonic drug delivery; in-vitro study.*

ISSN: 2581-9143
Research Article

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Article Info

Received on: 12-11-2022
Revised on: 06-12-2022
Accepted on: 15-12-2022

DOI: https://doi.org/10.33974/ijrpst.v3i3.336

INTRODUCTION

Colon cancer is the third leading cause of cancer death in the USA.[1] More than 66,000 cases of colon cancer are reported to occur every year in India. Current chemotherapeutic agents used for the treatment of cancers are associated with toxicities. Thus, the use of naturally occurring dietary substances, which are nontoxic are preferred over synthetic agents. Curcumin is one such naturally occurring dietary compound, which demonstrated promise to treat colon cancer. [2-5]However, some problems have to be solved before it can come to the practical clinical utility. Several groups all around the world are working on this problem. The works aimed at increasing its aqueous solubility, reducing its stomach and intestinal degradation and achieving-site specific delivery after oral administration. [6-8] A colon-targeted delivery system of curcumin with aforementioned specific advantages is beneficial. The various strategies for colon targeting include coating with pH-dependent polymers, formulation of timed release systems, use of prodrugs, exploitation of carriers that degrade specifically by colonic bacteria, microspheres, nanospheres, and bioadhesive systems.[9-13]

Pharmaceutical inventions is singly stressing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects. Oral drug delivery system represents one of the frontier areas of drug delivery systems. Such a dosage form manages common concern which exists in area of cost-efficient treatment, patient compliance, optimum drug delivery and bioavailability. [14] The site specific delivery of the drugs to the target sites has the potential to reduce the side effects and improved pharmacological response.

Drug targeting is the delivery of drugs to receptors or organ or any other specific part of the body to which one wishes to deliver the drug exclusively. Colon

specific drug delivery systems have gained increasing attention for the treatment of diseases such as Cancer, crohn's disease, ulcerative colitis and inflammatory bowel syndrome. This approach will localize the drug only at target site & minimize the drug-induced toxicity.[15]

EXPERIMENTAL SECTION

Materials: Neutral pellets were obtained as a gift sample from Murli krishnan Pharmaceuticals Pvt. Ltd, Pune, India. Curcumin was obtained as gift sample from HiMedia Laboratories Pvt. Ltd. Mumbai, India, and *Konjac* Glucomannan was obtained from Dalian Ruishengda International Trade Co., Ltd., Western Hills Village Shahekou District Dalian, China. Zein (MW 220,000) was procured from Central Drug House, New Delhi, India. Other excipients used were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Methods

Preparation of Coating Solution for Drug Loading:

Preparation of drug-layered pellets: Drug loaded pellets were prepared by spraying drug solution over non-pareil seeds by fluidized bed coating technique. Curcumin was homogeneously dispersed in an organic solution of PVP K30 and HPMC E5 as plasticizer while stirring with a magnetic stirrer. The drug dispersion was passed through a 100 mesh sieve.

Coating of *Konjac* Glucomannan over drug layered pellets (Protective coating/Sub-coating): In order to bring the rupture of the outer functional coat, a layer of swelling agent *Konjac* glucomannan was applied over the drug layered pellets by fluidized bed coating technique. *Konjac* glucomannan coating solution was prepared by mixing required amount of PVP K30 and PEG 400 as plasticizer in aqueous medium.

Application of outer enteric functional coat of Zein (Enteric coating): Zein coating solution preparation requires addition of Zein to the mixture of solvents acetone, isopropyl alcohol and purified water which is mixed together properly stirring with a magnetic stirrer. This was followed by the addition of stated amount of triethyl citrate as plasticizer and stirred the solution for few minutes.

Characterization of pellets: Based on the dissolution studies performed on all the batches of pellets, some of the optimized formulations were selected and further investigated for FT-IR, DSC and SEM studies.

Fourier Transform Infrared spectroscopy (FTIR): FTIR spectra of drug and optimized formulation were obtained. Sample about 5 mg was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in holder in IR spectrophotometer and the IR spectrum was recorded from 4000 cm-1 to 625 cm-1 in a scan time of 12 minutes. The resultant spectra were compared for any spectral changes. The FT-IR graphs of fluidized bed triple layer coated pellets given in Figure 1, Figure 2 and Figure 3.

Differential Scanning Calorimetry (DSC): A differential scanning calorimeter (DSC 60, Shimadzu) was used to obtain the DSC curves of curcumin pellets prepared by fluidized bed coating. About 3.052mg of sample was weighed in a standard open aluminum pan, and scanned from 30-450^{o}C, at a heating rate of 10 ^{o}C/minute while being purged with dry nitrogen. Results were observed in the Figure 4, Figure 5 and Figure 6 respectively.

Scanning Electron Microscopy (SEM): The samples were coated with a thin gold layer by sputter coater unit (SPI, Sputter, USA). Then, the SEM photographs were taken by a scanning electron microscope (Scanning electron microscope JSM-6390, Japan) operated at an accelerated voltage of 10000 Volt. The SEM photographs of fluidized bed coated pellets given in Figure 7.

RESULTS AND DISCUSSION

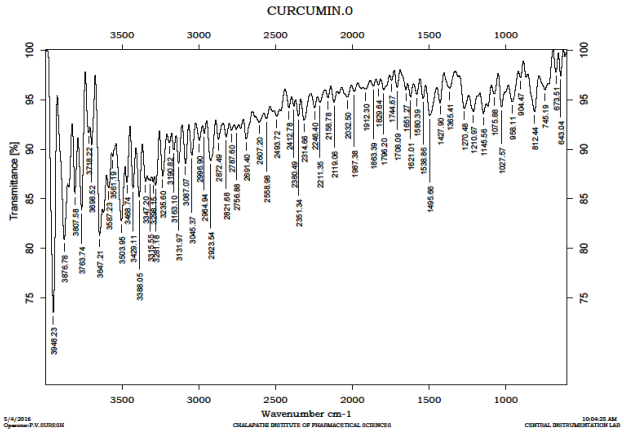

Figure 1: FT-IR graph of Curcumin

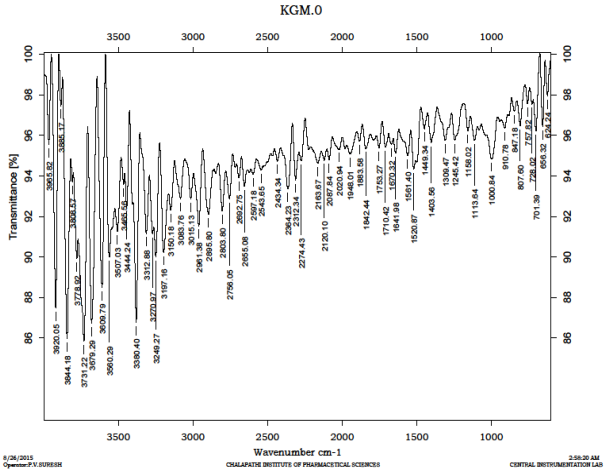

Figure 2: FT-IR graph of KGM

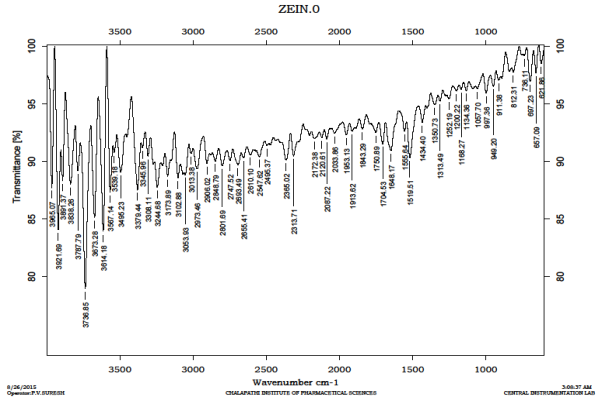

Figure 3: FT-IR graph of Zein

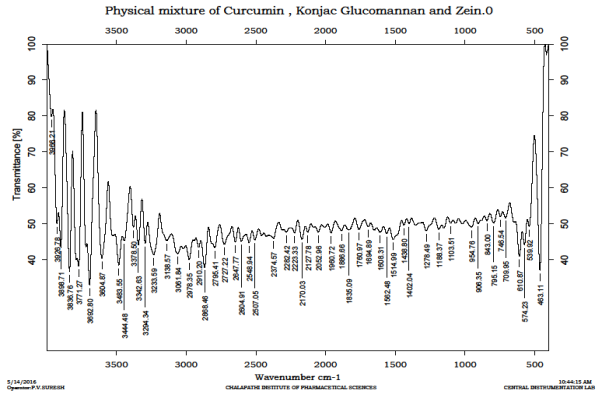

Figure 4: FT-IR graph of physical mixture of Curcumin, *Konjac* Glucomannan and Zein

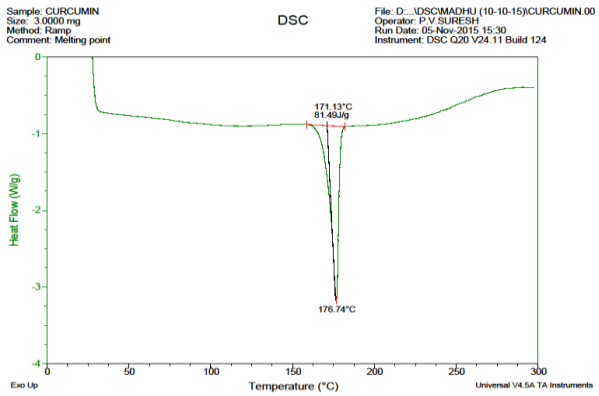

Figure 5: DSC thermogram of Curcumin

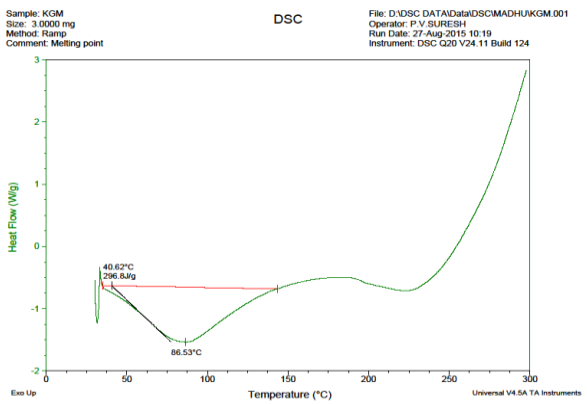

Figure 6: DSC thermogram of KGM

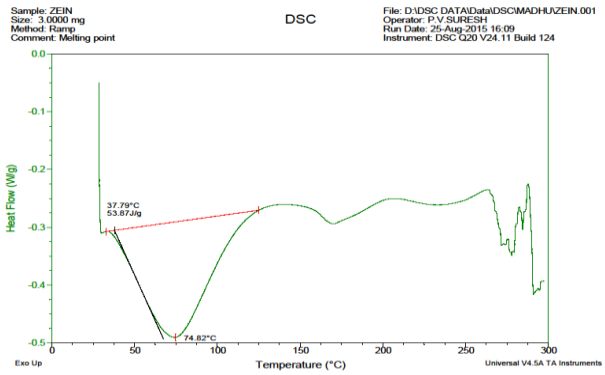

Figure 7: DSC thermogram of Zein

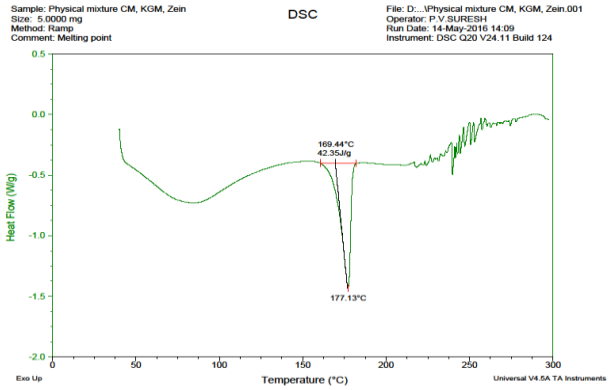

Figure 8: DSC thermogram of Physical mixture of Curcumin, *Konjac* Glucomannan and Zein

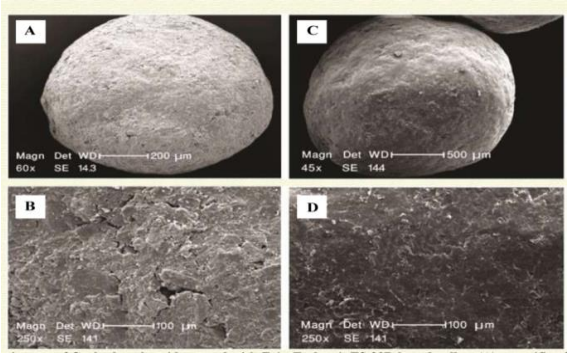

Figure 9: Scanning Electron Microscopy (SEM) analysis figures of optimized formulation

Evaluation Parameters

Physical properties: The values of bulk density for the prepared formulations were found to be in the range of 0.54 gm/cm^3 to 0.69 gm/cm^3 and tapped density from 0.63 gm/cm^3 to 0.76 gm/cm^3. The values of hausner's ratio were found to be in range 1.08 to 1.12. The angle of repose values were found to be in the range from 26.0 ^{0} to 30.1^0. This indicates good flow property of the blend. The details of coated pellets parameters are tabulated in Table 3. The % friability was less than 1% in all formulations ensuring that the pellets were mechanically stable. The percentage of drug content was in the range of 97.9% to 101.5% and found to be within acceptable limits. The values of coated pellets parameters were shown in Table 3.

Pharmacokinetic studies

The kinetic data of all formulations C1 to C12 could be best expressed by zero order equation as the plots showed highest linearity (R2: 0.974 to 1.012), than first order release kinetics (R2: 0.971 to 1.002). The 'n' values obtained from KorsmeyerPeppas plots range from (0.415 to 0.573) indicate that mechanism of release of formulations C5 to C10 was Quasi-Fickian diffusion, whereas the mechanism of drug release of formulations C2 to C5 and C11, C12 was Anomalous (non-fickian) diffusion. The mechanism of drug release from the formulation C4 was found to be fickian diffusion. All the in-vitro dissolution parameters evaluated for the various batches of pellet formulations were given in the Table 4.

FT-IR analysis: FTIR spectrum of Curcumin showed in scan at Figure 1 FTIR spectra of polymers like Konjac Glucomannan and zein are shown in Figure 2, Figure 3). The characteristic peaks of the drug were observed in the spectra of mixture of drug and polymer mixture, however the intensity of the peaks were reduced this might be due to very low concentration of drug in the mixture this indicates that there is no interaction between the drug and polymer mixtures. The FT-IR of pure drug was characterized by N-H stretching at 3131.97 cm^{-1} and C = O stretching at 1708.09 cm^{-1}, indicating the presence of –CONH group, asymmetric C-H stretching at 2923.54 cm^{-1}, symmetric C-H stretching at 2821.68 cm^{-1}, N-H deformation at 1621.01 cm^{-1}, aromatic C-H stretching at 3045.37 cm^{-1} and C = C at 1621.01 cm^{-1} shown in the Figure 1. FTIR Characterization of optimized formulation is shown in Figure 17. The peaks observed at 2964.94 cm^{-1} reveals asymmetric C-H stretching and peak observed at 2821.68 cm^{-1} reveals symmetric C-H stretching and peak observed at 1641.98 cm^{-1} reveals C=C stretching were observed in Figure 3 and Figure 4. Hence these release retarding materials were selected for formulation of targeted drug release pellets.

DSC analysis: The DSC thermogram for Curcumin was observed at 176.74° C and the melting peak of polymers i.e *Konjac* glucomannan and zein are at 86.53°C and 74.82°C were observed in the Figure 5, Figure 6 and Figure 7 respectively. This shows that there is no interaction between drug and optimized formulation. DSC studies revealed that there was no much shift in the melting point of the drug in the physical mixture compared to the pure drug; this indicates that there is no interaction between drug and other excipients. For the physical mixture of pure drug and the polymers, the DSC curve shows characteristic endothermic peak (up) at 177.13° C was showed in the Figure 8 and the temperature cycle was maintained at 500 C/min. Thus both the DSC curves are exhibiting the characteristic endothermic peak at the same temperature which infers that there is no interaction between the drug and the polymers used.

SEM analysis: SEM analysis was performed for the pellets prepared by triple layering technique. From the SEM images it was observed that the prepared pellets were having wide pores on its surface. The SEM images of powdered curcumin pellets taken at 20 min, 35min, 6th and 10th hours were shown in the Figure 9.

***In-vitro* drug release studies**

The *in-vitro* cumulative drug release profile of formulations C1, C2 and C3 containing 0.5% KGM and 1%Zein showed 42.38±1.71, 38.03±1.47 and 46.04±1.46% respectively in 8 hrs Figure: 10. The formulations C1-C3 does not have the desired extended drug release up to 6 hrs. The *in-vitro* cumulative drug release profile of formulations C4, C5 and C6 containing 1%KGM and 2% Zein showed 41.05±1.55, 38.42±2.36 and 46.02±2.78 respectively in 8 hrs Figure 11. The formulations C4-C6 have extended drug release up to 8 hrs, but the percentage drug release was less. The *in-vitro* cumulative drug release profile of formulations C7, C8 and C9 containing 2% KGM and 3% zein showed 54.10±1.05, 53.07±1.13 and 57.46±1.92 within 8 hrs respectively showed in Figure 12. The formulations C10, C11 and C12 containing 2.5% KGM and 4%zein showed 27.05±2.69, 35.03±1.28 and 32.35±1.54 within 8 hrs Figure 13. Among these twelve formulations, C9 has desired highest percentage drug release i.e. 98.46±0.34% at the end of 12 hrs and was selected as the best formulation.

DISCUSSION

The triple layer coated pellets of curcumin were prepared using KGM as sub coating/protective /primary polymer and in other enteric coating with secondary polymer like zein by fluidized bed processor technique/method. IR spectroscopic studies, DSC and SEM analysis studies indicated that there was no drug-polymer and polymer-polymer interaction. All the prepared/coated pellets were in acceptable range of tapped density, bulk density, angle of repose, haunser's ratio, moisture content and drug content as per the pharmacopoeia specifications.

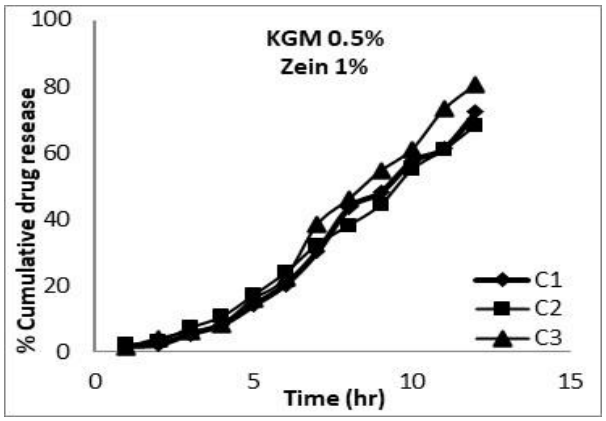

Figure 10: In-vitro release profile of formulations C1, C2 & C3

Table 1: Formula for pellets coating

Ingredients	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
Sugar spheres (20/25#)	200	200	200	200	200	200	200	200	200	200	200	200
Drug loading												
Curcumin	16	16	16	16	16	16	16	16	16	16	16	16
PVP K30	3	3	3	3	3	3	3	3	3	3	3	3
HPMC E5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Talc	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85
Isopropyl alcohol	150	150	150	150	150	150	150	150	150	150	150	150
Amaranth red	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Barrier coating/protective coating												
KGM (1%)	2	2	2									
KGM (1.5%)				3	3	3						
KGM (2.0%)							4	4	4			
KGM (2.5%)										5	5	5
PEG 400	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Talc	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Methanol	100	100	100	100	100	100	100	100	100	100	100	100
Distilled water	50	50	50	50	50	50	50	50	50	50	50	50
Sunset yellow	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Enteric coating												
Zein (1%)	2	2	2									
Zein (2%)				4	4	4						
Zein (3%)							6	6	6			
Zein (4%)										8	8	8
PEG 400	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Talc	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
triethyl citrate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Acetone	20	20	20	20	20	20	20	20	20	20	20	20
Isopropyl alcohol	100	100	100	100	100	100	100	100	100	100	100	100
purified water	50	50	50	50	50	50	50	50	50	50	50	50

Table 2: Various process parameters of Fluidized bed processor

S.NO	Process Parameters	Drug layering	Swellable Polymer coating	Enteric coating
1	Batch size (g)	200	200	200
2	Spray rate (g/min)	30-60	20-55	30-50
3	Coating nozzle diameter	1mm	1mm	1mm
4	Atomizing air pressure (bar)	0.5-1.0	1.5-2.0	1.0-1.5
5	Air inlet temperature	55-58°C	60-65°C	60-65°C
6	Air outlet temperature	50-56°C	52-57°C	50-55°C
7	Curing in Fluid bed	15 min at 45 °C	20 min at 35 °C	30 min at 35 °C
8	Coating efficiency	82.00 to 85.00%	50.00 to 60.00%	80.00 to 75.00%
9	Processing time	2-3 hours	3-4 hours	2-3 hours

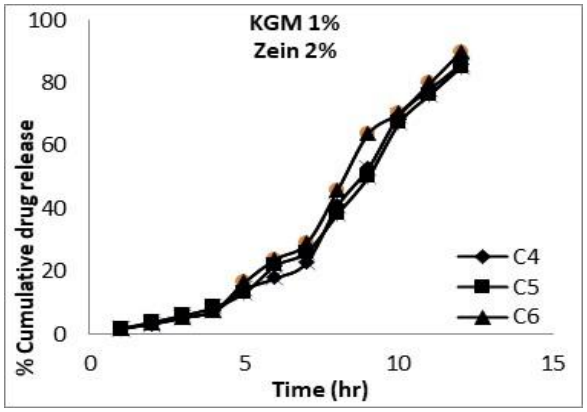

Figure 11: In-vitro release profile of formulations C4, C5 & C6

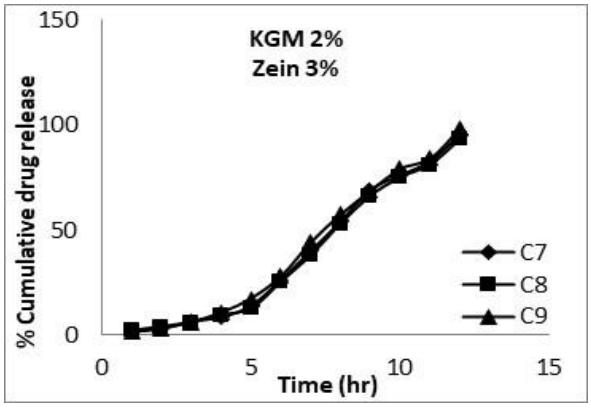

Figure 12: In-vitro release profile of formulations C7, C8 & C9

Table 3: Physical and chemical parameters of curcumin colon targeted drug release pellets

Parameters	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
Particle Size (±SD)	1745±14	1679±32	1764±18	1732±24	1722±27	1789±33	1748±53	1749±44	1782±37	1795±15	1786±38	1787±31
Angle of repose (θ) ± SD*	25.02 ± 0.321	25.85 ± 0.321	24.28 ± 0.124	25.04 ± 0.083	24.64 ± 0.314	25.13 ± 0.765	26.02 ± 0.243	25.28 ± 0.368	26.53 ± 0.534	25.41± 0.154	26.38± 0.432	25.23± 0.253
Bulk Density (D_B) (gm/cm)³ ± SD*	0.8185 ± 0.001	0.8246 ± 0.004	0.8147 ± 0.010	0.8234± 0.0011	0.8124 ± 0.009	0.8326 ± 0.008	0.8174 ± 0.005	0.8266± 0.001	0.8086± 0.010	0.8124± 0.011	0.8142± 0.013	0.8146± 0.008
Tapped Density (D_T) (gm/cm)³ ± SD*	0.8523 ± 0.012	0.8537± 0.018	0.8468 ± 0.012	0.8522± 0.013	0.8573± 0.007	0.8869 ± 0.018	0.8814 ± 0.017	0.8910 ± 0.010	0.8462 ± 0.15	0.9961 ± 0.24	0.9572 ± 0.076	0.8891 ± 0.045
Hausner Ratio (HR) ± SD*	1.05 ± 0.010	1.03 ± 0.041	1.04 ± 0.014	1.06 ± 0.016	1.05 ± 0.028	1.04 ± 0.017	1.06 ± 0.023	1.08 ± 0.025	1.07 ± 0.026	1.08 ± 0.072	1.06 ± 0.063	1.08 ± 0.054
Carr's Index (%CI) ± SD*	4.05 ± 0.023	5.17± 0.041	4.55 ± 0.020	4.07 ± 0.036	4.18 ± 0.015	4.79 ± 0.034	5.04 ± 0.039	5.46 ± 0.011	4.85 ± 0.014	5.06 ± 0.040	5.36 ± 0.028	4.78 ± 0.091
Moisture content (%) ± SD*	1.65± 0.011	1.22 ± 0.042	1.53 ± 0.072	1.44± 0.038	1.48± 0.024	1.75± 0.076	1.86± 0.038	1.82± 0.027	1.74± 0.086	1.45± 0.015	1.57± 0.039	1.48± 0.013
Assay (%) ± SD*	99.24± 0.010	99.31± 0.028	99.18± 0.034	101.25± 0.037	99.84± 0.046	99.66± 0.067	99.17± 0.086	99.53± 0.051	99.86± 0.026	99.89± 0.043	99.25± 0.014	99.71± 0.025

*n=3, all values are expressed as mean ± SD

Table 4: Pharmacokinetic parameters of curcumin colon drug release pellets

				Peppas-model	
Formulation code	Zero order R^2	First order R^2	Higuchi R^2	R^2	Slope n
C1	0.9985	1.002	0.9921	0.9812	0.4251
C2	1.0128	0.9824	0.9647	0.9934	0.5443
C3	0.9847	0.9764	0.9726	0.9831	0.3864
C4	0.9832	0.9852	0.9842	0.9817	0.4261
C5	0.9755	0.9714	0.9728	0.9845	0.3982
C6	0.9863	0.9723	0.9815	0.9735	0.4153
C7	0.9815	0.9831	0.9732	0.9726	0.4654
C8	0.9658	0.9913	0.9863	0.9816	0.5674
C9	0.9863	0.9845	0.9924	0.9834	0.5732
C10	0.9745	0.9683	0.9847	0.9736	0.4845
C11	0.9752	0.9842	0.9810	0.9826	0.4591
C12	0.9831	0.9723	0.9756	0.9814	0.4352

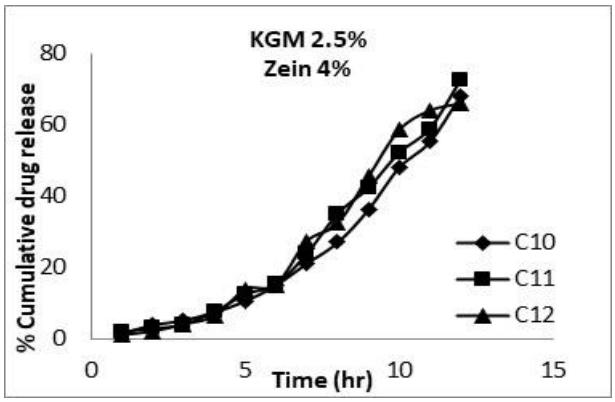

Figure 13: In-vitro release profile of formulations C10, C11 and C12

Colon targeted drug delivery triple layer coated pellets were performed with different phosphate buffers. The in-vitro release of pellets coating curcumin was mainly affected by drug polymer ratio, nature and amount of polymer. The triple layer pellets coated curcumin containing 0.5% KGM and 1% zein showed initially release of the drug followed by 45% release up to 8 hrs. The addition of sub-coating and enteric coating polymers i.e 1% KGM and 2% Zein in formulations C4-C6 prolong the release of curcumin from 5 to 8 hrs.

The triple layer coated pellets containing 2% KGM and 3% Zein showed a maximum release 95.43±2.58, 93.27±1.83 and 98.46±0.34 i.e formulations C7, C8 and C9. According to *Konjac* Glucomannan polysaccharide has hydrophilic gel forming matrix which was used as a release retardant. The coated pellets containg 3% zein showed >90% of extended release up to 12 hrs due to fact that zein get rapidly swelled and form complex with KGM as it is an anionic polymer. Among all formulations C9 was selected as best formulation showing 98.46% of drug release up to 12 hrs. The further increasing of KGM (2.5) and zein (4%) significantly decreases the cumulative drug release.

Hence, the triple layer pellets of curcumin can be prepared with extended therapeutic effect to relieve colon cancer and associated symptoms of cancer. The study conducted so far reveals a promising result suggesting scope for pharmacodynamic and pharmacokinetic evaluations.

CONCLUSION

The multiunit dosage form, pellets that were formulated by drug layering technique showed optimized extended release of curcumin for a prolonged period of time**.** The present study concludes that the curcumin pH dependent pulsatile burst release could be successful option for colon targeting by achieving the desired lag time. Lag time and quick release of drug after lag time was achieved with proper selection of extent of zein coating and *Konjac* glucomannan layering over drug layered pellets. Thus, the designed formulation can be considered as one of the promising formulation technique for preparing a colon targeted drug delivery system in management of cancer.

AKNOLEDGEMENT

This work was supported by Research grant (Endt. No F1-17.1/2012-13/UGC-2012-13-SC AND-34273) from University Grants Commission (UGC), New Delhi (India). The author also sincerely thankful to Prof. Rama Rao Nadendla, Principal, Chalapathi Institute of Pharmaceutical Sciences (CIPS) and management of Chalapathi Institutions, Guntur for providing infrastructure facilities to carry out this research work.

REFERENCES

1. Davis, S. S. (1990). Overcoming barriers to the oral administration of peptide drugs. Trends in Pharmacological Sciences, 11(9), 353-355.
2. Van den Mooter, G., & Kinget, R. (1995). Oral colon-specific drug delivery: a review. Drug delivery, 2(2), 81-93.
3. Ashford, M., & Fell, J. (1994). Targeting drugs to the colon: delivery systems for oral administration. Journal of drug targeting, 2(3), 241-257.
4. Pozzi, F., Furlani, P., Gazzaniga, A., Davis, S. S., & Wilding, I. R. (1994). The time clock system: a new oral dosage form for fast and complete release of drug after a predetermined lag time. Journal of controlled release, 31(1), 99-108.
5. Gupta, V. K., Beckert, T. E., & Price, J. C. (2001). A novel pH-and time-based multi-unit potential colonic drug delivery system. I. Development. International journal of pharmaceutics, 213(1-2), 83-91.
6. Krishnaiah, Y. S. R., Satyanarayana, S., Prasad, Y. R., & Rao, S. N. (1998). Evaluation of guar gum as a compression coat for drug targeting to colon. International journal of pharmaceutics, 171(2), 137-146.
7. Krishnaiah, Y. S. R., Raju, P. V., Kumar, B. D., Bhaskar, P., & Satyanarayana, V. (2001). Development of colon targeted drug delivery systems for mebendazole. Journal of controlled Release, 77(1-2), 87-95.
8. Krishnaiah, Y. S. R., Satyanarayana, V., Kumar, B. D., & Karthikeyan, R. S. (2002). In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil. European journal of pharmaceutical sciences, 16(3), 185-192.
9. Lane, M. E. (2003). Modified-release drug delivery technology (Vol. 1). M. J. Rathbone, J. Hadgraft, & M. S. Roberts (Eds.). New York: Marcel Dekker.
10. Rubinstein, A., Radai, R., Ezra, M., Pathak, S., & Rokem, J. S. (1993). In vitro evaluation of calcium pectinate: a potential colon-specific drug delivery carrier. Pharmaceutical research, 10, 258-263.

11. Milojevic, S., Newton, J. M., Cummings, J. H., Gibson, G. R., Botham, R. L., Ring, S. G., ... & Allwood, M. C. (1996). Amylose as a coating for drug delivery to the colon: Preparation and in vitro evaluation using 5-aminosalicylic acid pellets. Journal of controlled release, 38(1), 75-84.
12. Tozaki, H., Komoike, J., Tada, C., Maruyama, T., Terabe, A., Suzuki, T., ... & Muranishi, S. (1997). Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. Journal of pharmaceutical sciences, 86(9), 1016-1021.
13. Sinha, V. R., & Kumria, R. (2001). Polysaccharides in colon-specific drug delivery. International journal of pharmaceutics, 224(1-2), 19-38.
14. Vijaya, R., Prabhakaran, L., & Purushothaman, M. (2010). Colon targeted drug delivery system-an overview. Pharma infonet, 8.
15. Vyas, S. P., & Khar, R. K. (2002). Controlled drug delivery concepts and advances. vallabh prakashan, 1, 411-47.
16. Dorożyński, P., Jachowicz, R., Kulinowski, P., Kwieciński, S., Szybiński, K., Skorka, T., & Jasiński, A. (2004). The macromolecular polymers for the preparation of hydrodynamically balanced systems—methods of evaluation. Drug development and industrial pharmacy, 30(9), 947-957.