DEVELOPMENT AND IN-VITRO EVALUATION OF ORLISTAT MICROCAPSULES BY IONIC GELATION METHOD

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ABSTRACT

The microcapsules of orlistat, an anti-hyperlipidemic drug, were developed utilizing the concept of controlled release and to obtain a unique drug delivery systems which could remain in body and control the drug release for longer period of time. Orlistat microcapsules with a coat consisting of varying combinations of alginate, HPMC and carbopol-940 were prepared by ionic-gelation method. The prepared orlistat microcapsules were evaluated in different rheological behaviour and release studies. It was found that the microcapsules were discrete, nearly spherical and free flowing as revealed by Scanning electron microscope. The size of microcapsules was found in the range of 637±1.73 to 679±2.64 µm where as percentage yield varies within range of 69.33 ± 0.44 to 76.21 ± 0.21%. The Carr's index, Hausner's ratio and angle of repose indicated the excellent flowing properties. Sharp endothermic peaks were found from the microcapsules formulated with polymers. FTIR analysis indicated the compatibility between the drug and the polymers which was also confirmed by DSC studies. The entrapment efficiency was found in the range of 58.55 ± 0.12 to 77.37 ± 0.09 % and the drug release from microcapsules was found slow, followed Higuchi model with non-fickian release mechanism. These microcapsules proved to be suitable for oral controlled release of orlistat.

Keywords: Microcapsules, orlistat, ionic-gelation method, HPMC, carbopol-940.

INTRODUCTION

Oral drug delivery system is often more susceptible route of administration than other routes because physiology of GI tract offers more flexibility in dosage regimen (Gupta and Robinson, 1992). Controlled drug delivery systems help in slow dissolution and release of drug with respect to time. The controlled drug delivery systems alter the pharmacology of active moiety by modifying the molecular structure or physiological parameters using novel drug delivery system in a prescribed route (Robinson and Vincent, 1987). Hence to provide a sustained therapeutic effect over a prolonged period of time, there is a need of delivering a therapeutic dose which gradually and continuously releases the medication, so for this purpose we design a sustained release formulation to deliver a therapeutically effective and non-toxic medication in order to achieve a desired steady state blood or tissue level over a extended duration of time resulting in optimization of dosage regimen.

Obesity is marked by excess body fats or Hyperlipidemia. In this condition the fat level is elevated to the extent which may cause adverse effects on human health and lead to excessive health problems (Haslam and James, 2005). Orlistat is the mainstream product for the treatment of obesity. The biological half life of orlistat is less than 2 hours (Keating and Jarvis, 2001) and is lipophilic in nature, so it is not much absorbed into general circulation. Therefore, orlistat is appropriate entrant for its formulation into sustained release formulation.

The present study is marked by the microencapsulation of orlistat by polymers as HPMC, hydrophilic biodegradable polymer and Carbopol-940, acrylic acid derivative, using ionic gelation method. The microcapsules were characterized by physical parameters and statistical procedures. Rheological properties, entrapment efficiency, fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) were used for characterization. Scanning electron microscopy was used to determine microcapsules shape and surface characteristics. In vitro drug release studies were used for further evaluation of microcapsules.

MATERIALS AND METHODS
MATERIALS

Orlistat was gifted by Pharm Evo Pvt. Ltd Karachi, Pakistan. Polymers like Sodium alginate (BDH Lab.), HPMC K-100 (Riedel-deHaen), Carbopol-940 (Uni-Chem) and other chemicals like Chloroform (Riedel-deHaen), Acetonitrile (HPLC Grade - RCI Labscan), Hydrochloric acid (Merck), Calcium Chloride (Merck), Sodium lauryl sulphate (Merck), Sodium Chloride (Merck), Phosphoric acid (HPLC Grade – RCI Labscan), Methanol (HPLC Grade – RCI Labscan), n- Octanol (Merck) are all of analytical grade.

METHOD
METHOD FOR PREPARATION OF MICROCAPSULES

Calcium alginate microcapsules were prepared by ionic gelation method (George and Abraham, 2006). Sodium alginate was added in 100ml distilled water which is then dissolved by magnetic stirrer (Velp Scientifica). Orlistat was dissolved in chloroform (100 ml) in volumetric flask. A mixture of Orlistat and sodium alginate was prepared (2: 1.75) at speed of 1000 rpm. This homogenous blend was dropped through needle size of 26G from a hypodermic syringe into a 2 % w/v solution of $CaCl_2$. The resulting microcapsules were poured in gelling bath for half an hour to harden and finally filtered and washed. These were dried at room temperature and stored in vacuum desiccator until constant weight was achieved.

Microcapsules using HPMC and Carbopol-940 were prepared by same method as described above and ratios of different formulation are shown in table 1.

Table 1: Composition of formulations of microcapsules

Batch code	Drug (%w/v)	Sodium alginate (%w/v)	HPMC (%w/v)	Carbopol-940 (%w/v)
ORL 1	2	1.75	-	-
ORL 2	2	1.75	0.5	-
ORL 3	2	1.75	0.75	-
ORL 4	2	1.75	1	-
ORL 5	2	1.75	-	0.5

ORL 6	2	1.75	-	0.75
ORL 7	2	1.75	-	1
ORL 8	2	1.75	1	0.5

CHARACTERIZATION AND EVALUATION OF MICROCAPSULES
PERCENTAGE YIELD (W/W)

Percentage yield of different batches of dried microcapsules was determined by their weight and was calculated as (Ziyaur et al., 2006):

$$\text{Percentage yield} = \frac{\text{Weight of dried microcapsule}}{\text{Theoretical weight of drug and polymer}} \times 100$$

PARTICLE SIZE ANALYSIS

Sieving method is most widely used procedure to assess the particle size (Loyd et al., 2000). In this test sieves ranging from 10-40-sieve number are used, which are either shaken mechanically or by hand. The sample is introduced in the sieve on top, which has largest screen opening while lower sieves have smaller opening than the above, after completion of shaking the weight of particles that are retained on each sieve is determined Then divide weight of the sample retained on each sieve by the total weight to give a percentage retained on each sieve. Mean particle size was calculated as:

$$\text{Mean particle size} = \frac{\Sigma\ (\text{mean particle size of fraction} \times \text{weight fraction})}{\Sigma\ (\text{weight fraction})}$$

FLOW PROPERTIES OF MICROCAPSULES
ANGLE OF REPOSE

Weighed quantity of microcapsules was passed through a funnel fixed on a stand at a specific height upon graph paper. A static heap of powder with only gravity acting upon it was tending to form a conical mound. The height of the heap (h) and radius (r) of lower part of cone were measured. The angle of repose was calculated using formula:

$$\theta = \tan^{-1} h/r$$

Where θ = angle of repose, h = height of cone and r = radius of cone base.
Angle of repose less than 30 shows excellent flow properties.

BULK DENSITY

Bulk density of all formulations was determined by following formula (Banker et al., 1987):

$$\text{Bulk density} = \frac{\text{Sample weight}}{\text{Sample volume}}$$

TAPPED DENSITY

Tapped density was calculated by conventional tapping method as 100 tapings were sufficient to calculate the result. Tapped density was calculated by the following formula (Shariff et al., 2007):

$$\text{Tapped density} = \frac{\text{Weight of microcapsules}}{\text{Volume of microcapsules after 100 tapings}}$$

CARR'S INDEX

The simple test evaluated the flowability of a powder by comparing the bulk density and tapped density of a powder. Carr's index was calculated as:

$$I = \frac{\rho_t - \rho_b}{\rho_t} \times 100$$

where ρ_b is bulk density and ρ_t is tapped density**.**
Ci<15% gives good flow characteristics and above 25% indicates poor flow characteristics (Taylor et al., 2000).

HAUSNER RATIO

Hausner ratio was calculated using formula (Shariff et al., 2007):

$$\text{Hausner ratio} = \frac{\rho t}{\rho d}$$

Where, ρt is tapped density and ρd is bulk density**.**
Hausner's ratio value between 1.00-1.11 shows excellent flowability.

SHAPE AND SURFACE MORPHOLOGY

The external morphology of microcapsules was analyzed by scanning electron microscope (Model S3400N). For scanning electron microscopy samples were prepared by sprinkling microcapsule powder on a double adhesive tape, which is pounded on aluminum stub. The stubs were coated with gold (thickness of 150–200 Å) using a fine coat ion sputter. The microcapsules were examined under scanning electron microscope.

FOURIER-TRANSFORMED INFRARED (FTIR) SPECTROSCOPY

FTIR spectroscopy was carried out for any possible drug–polymer interaction and for the identification of characteristic functional groups. FTIR analysis was performed on Pristige-21 (Shimadzu- Japan) using KBr disc (2 mg sample in 200 mg KBr). The scanning range was 4,000–400 cm^{-1} and the resolution was 2 cm^{-1}.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC analysis of pure drug and drug-loaded microcapsules were carried out to evaluate any possible drug-polymer interaction.

Thermal analysis system (SDT Q 600, Shimadzu, Japan) was used and orlistat and drug-loaded microcapsules were triturated separately to get a finely divided powder and heated in sealed aluminium pans at a rate of 10 ^{o}C /min from 0 ^{o}C to 120^{o}C temperature range under a nitrogen flow of 40 ml/min. Reproducibility was checked by running the sample in triplicate (Tayade and Kale, 2004).

DETERMINATION OF PERCENTAGE ENCAPSULATION EFFICIENCY

Percentage encapsulation efficiency is percentage of drug encapsulated in the microcapsules relating to initial quantity used. Appropriate amount of microcapsules containing 100 mg of orlistat in 100 ml of methanol were placed in an ultrasonic bath. Then it was filtered through 0.45 μm filter paper. 1 ml of aliquot of this solution was taken into a syringe and diluted to 10

ml with methanol (100µg/ml). Take 6ml of this solution and make volume 10ml with mobile phase containing acetonitrile, water and phosphoric acid in the ratio of 85:15:0.5 (v/v/v). The peak of orlistat was measured at 205 nm using a HPLC (Sreekanth et al., 2010). The measured peak area was then converted to the amount of orlistat by using standard calibration curve. Percentage encapsulation efficiency was calculated as follows:

$$\text{Encapsulation efficiency \%} = \frac{\text{Estimated amount of drug per gm microcapsule}}{\text{Theoretical amount of drug per gm microcapsule}} \times 100$$

DISSOLUTION STUDIES

For dissolution studies, paddle apparatus (Pharmatest) was used in order to study the release behavior of microcapsules. The microcapsules were enclosed in hard gelatin capsules in quantity equivalent to 100mg of drug. The dissolution study was carried out for 2 hours in 900 ml of simulated gastric fluid pH 1.2 at 37˚C ± 0.5˚ C and rotation speed was 75 rpm. 5ml of aliquots of dissolution medium was withdrawn at definite interval and analyzed by HPLC for orlistat contents at wavelength 205 nm, the dissolution medium was kept constant by adding the same volume of fresh dissolution medium after each withdrawal.

After 2 hours these microcapsules were shifted to dissolution medium of 3% sodium lauryl sulphate and 0.5% sodium chloride in water. To each 10 liters of media, add 1–2 drops of n-octanol, and adjust with phosphoric acid to a pH of 6.0; make final volume 900 ml (Anthony and Marques, 2007) and proceed under similar conditions as described above.

The dissolution studies were evaluated by fitting data in Zero order (Xu and Sunada, 1995), first order (Singla and Medirata, 1988), Higuchi model (Higuchi, 1963), Korsmeyer-Peppas model (Ritger and Peppas, 1987) and Hixson-Crowell model (Hixson and Crowell, 1931) to determine the release rate and mechanism from microcapsules.

RESULT AND DISCUSSION
FLOW PROPERTIES OF MICROCAPSULES

The present study was conducted to formulate the controlled release microcapsules of Orlistat. The Orlistat which is an oral anti-obesity drug, having short half life, was encapsulated by ionic gelation method.

The particle size of orlistat microcapsules was within range of 637±1.73 to 679±2.64 µm, which showed increase in size of microcapsules by increasing polymer concentration. Percentage yield varies within range of 69.33 ± 0.44 to 76.21 ± 0.21% in different formulations. Result of angle of repose of microcapsules was below 30^{o}. Carr's index values which were below 10% and Hausner's ratios which were less than 1.11 showed that microcapsules had excellent flow behavior. Assay of microcapsules showed that percentage entrapment efficiencies of microcapsules were in range of 58.55 ± 0.12 to 77.37 ± 0.09 % as shown in table 2.

Table 2: Physical Characteristics of Microcapsules

Formulation	Mean particle size (μm)	% Age Yield (%)	Angle of Repose	Carr's Index	Hausner's Ratio	% Age Entrapment
ORL 1	637 ± 1.73	73.33 ± 0.53	27.33 ± 0.18	7.39 ± 0.80	1.08	58.55 ± 0.12
ORL 2	649 ± 2.64	72.47 ± 0.47	26.29 ± 0.35	9.59 ± 0.95	1.10	71.40 ± 0.07
ORL 3	651 ± 1.0	73.55 ± 0.22	25.16 ± 0.01	9.89 ± 0.58	1.10	73.34 ± 0.08
ORL 4	657 ± 1.73	76.21 ± 0.21	24.29 ± 0.30	6.99 ± 0.86	1.07	74.16 ± 0.10
ORL 5	653 ± 3.0	69.41 ± 0.70	25.56 ± 0.32	7.36 ± 0.64	1.08	65.85 ± 0.08
ORL 6	657 ± 2.0	69.33 ± 0.44	24.44 ± 0.01	8.96 ± 0.99	1.09	66.30 ± 0.08
ORL 7	662 ± 2.0	70.73 ± 0.21	23.51 ± 0.32	8.24 ± 0.33	1.09	68.47 ± 0.08
ORL 8	679 ± 2.64	75.04 ± 0.38	26.08 ± 0.12	7.31 ± 0.84	1.08	77.37 ± 0.09

All values are represented as mean ± A.M
* n =3

SCANNING ELECTRON MICROSCOPY (SEM)

Microcapsules of different formulations were analyzed for external morphology and surface properties. The microcapsules prepared with sodium alginate showed the presence of drug on the surface because of which it showed less retarding efficiency whereas the microcapsules prepared from HPMC and Carbopol-940 showed uniform distribution of drug within microcapsules as shown in Fig. 1. All microcapsules were nearly spherical in shape and showed good retarding properties which was further confirmed by dissolution studies.

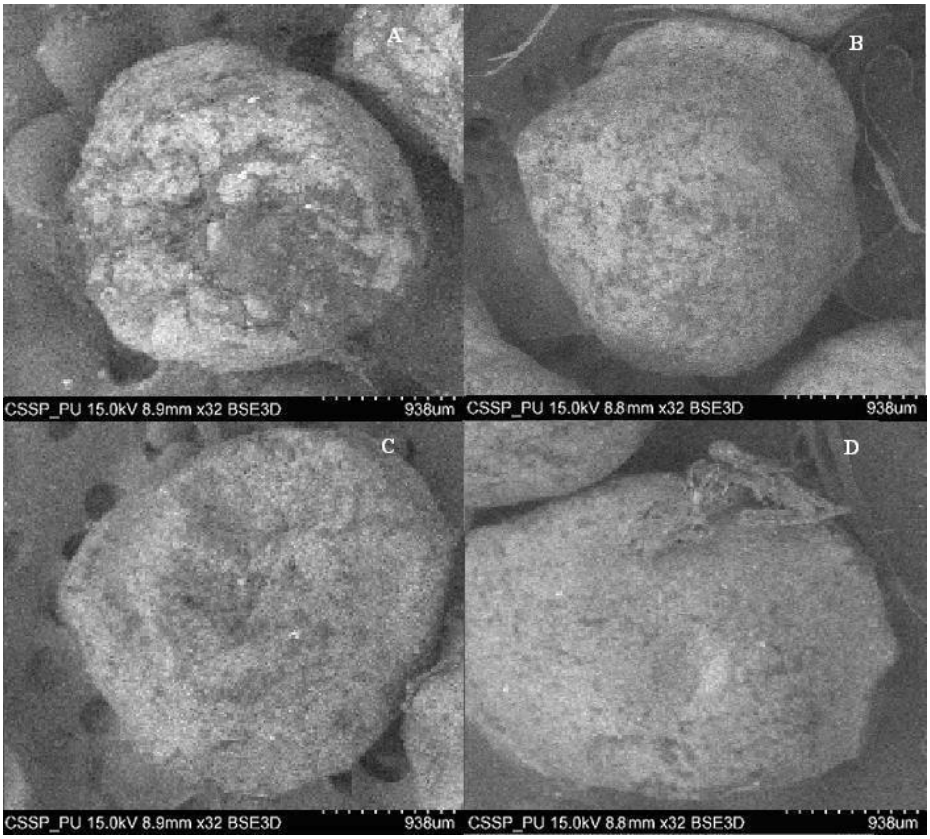

Figure: 1. SEM Photographs of Orlistat loaded microcapsules where A: ORL-1, B: ORL-2, C: ORL-5, D: ORL-8

FOURIER TRANSFORM INFRARED SPECTROSCOPY:

FTIR of the pure drug, polymers and their physical mixtures were done to determine the drug and polymers incompatibilities. FTIR of orlistat showed the principle peaks at the wave number 1203-1519 cm^{-1} justifying the presence of carboxyl, carboxylate groups, and carbonyl at 1683 cm^{-1}, C-H stretching between at 2744-2933 cm^{-1}. C=O vibration at 1722 cm^{-1} and N-H stretching appeared at 3319 cm^{-1} as shown in Fig. 2.

The principle peaks corresponding to orlistat were appeared with less intensity in the microcapsule formulations which attributes to the fine dispersion of drug in polymers and increase drug polymer ratio. Also there were no new peaks appeared indicating the absence of interaction.

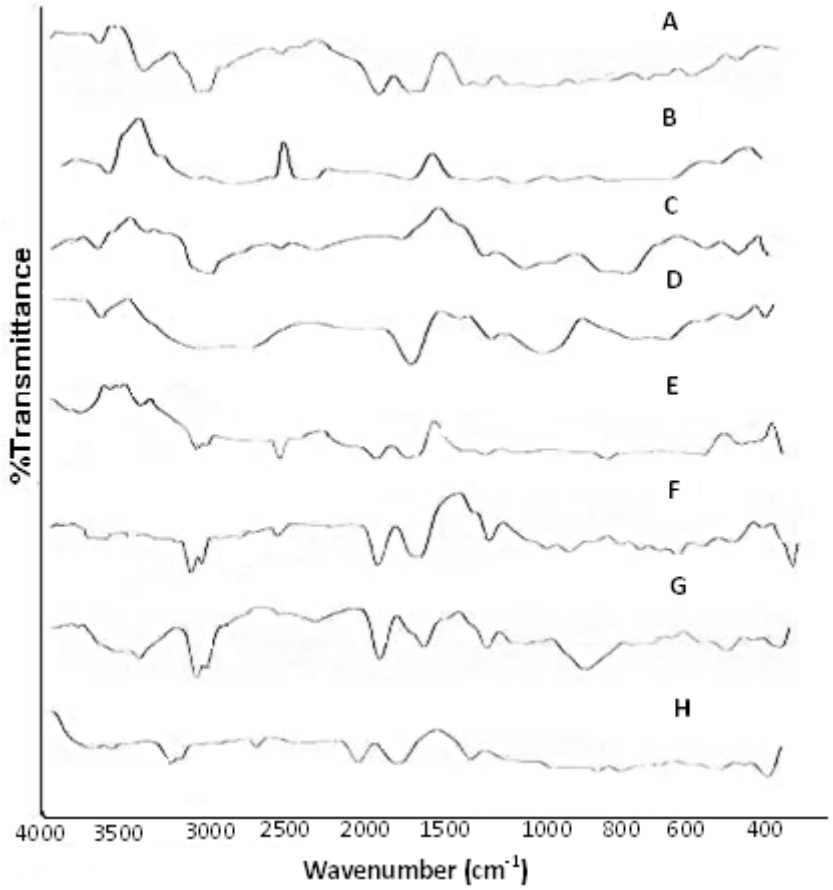

Figure: 2. FTIR Spectra of components and microcapsules where A: Orlistat, B: Sodium Alginate, C: HPMC, D: Carbopol-940, E: ORL-1, F: ORL-2, G: ORL-5, H: ORL-8

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC studies were carried out to measure the thermal profile of drug and to confirm any possible interaction of drug and polymer in the formulations. The thermal curves of pure orlistat and drug-polymer microcapsules are presented in Fig. 3. A sharp endothermic peak corresponding to the melting point of orlistat was observed (Melting point of orlistat = 40-48^{o}C). In the case of sodium alginate, HPMC and carbopol-940, peaks were observed in the temperature range of >300 ^{o}C, 260 ^{o}C and 106 ^{o}C respectively. The characteristic, well recognizable thermal profile of the drug at the temperature corresponding to its melting point was also observed in drug-polymers microcapsules indicating absence of any possible drug-polymers interactions. It appeared that there was a significant reduction of drug crystallinity in the microcapsules because thermal peak of drug had lost its sharp appearance in microcapsules.

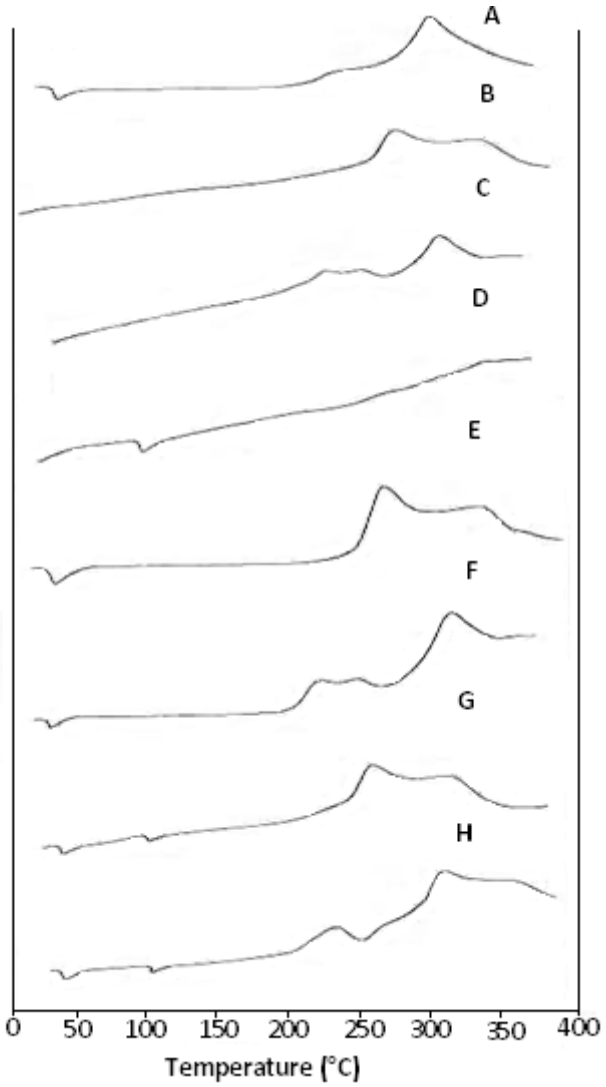

Figure 3: DSC Thermograms of components and microcapsules where A: Orlistat, B: Sodium Alginate, C: HPMC, D: Carbopol-940, E: ORL-1, F: ORL-2, G: ORL-5, H: ORL-8

IN VITRO DRUG RELEASE STUDIES

Dissolution studies of microcapsules were carried out in order to study drug release behavior of polymer matrix. Orlistat release from microcapsules was studied in SGF pH 1.2 and buffer pH 6.0 for 12 hours using USP type II dissolution apparatus.

Dissolution studies of all formulations showed that, the increase in concentration of polymer resulted in retarded drug release. Orlistat release from the microcapsules was found slow which was controlled over extended period and release was found to be dependent on the composition of the coat as shown in cumulative release, Fig. 4. Formulation ORL 2 had provided better sustained release profile because of low permeability to water, thus proved to be the best formulation.

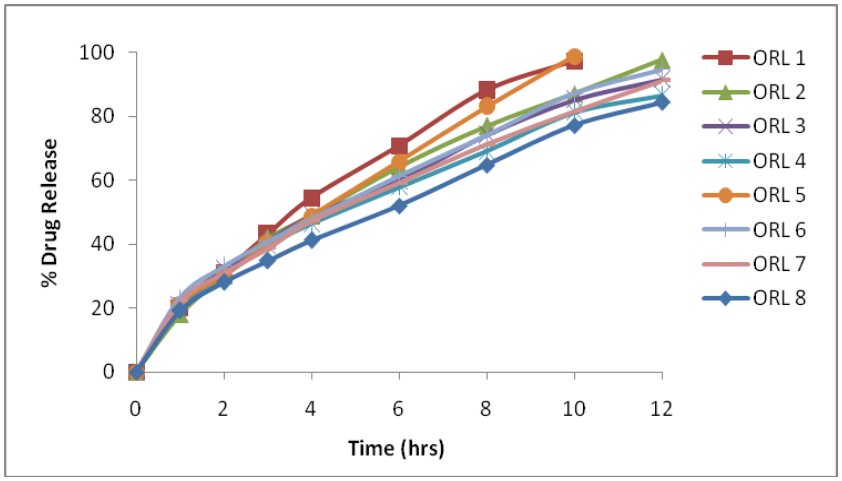

Figure 4. Cumulative dissolution profile of various formulations of Orlistat

To find out the mechanism of drug release from these formulations, dissolution data obtained from in-vitro studies of different formulations was fitted in Zero order, first order, Higuchi Model, Korsmeyer-Peppas Model and Hixson–Crowell Model. The model that best fits the release data was evaluated by correlation coefficient (R^2) showed in table 3. In most of the formulated microcapsules drug release followed Higuchi Model. In all trials, drug release mechanism was studied by applying Korsmeyer–Peppas model in which 'n' values range between 0.574 and 0.704, indicating non-fickian release. As the n value indicated anomalous transport in which the drug release was controlled by swelling followed by diffusion. The release of drug from polymer matrix by the process of diffusion might be due to the presence of hydrophilic polymer, HPMC, which swelled upon hydration and released drug by diffusion.

Table 3: Values of correlation coefficient for the fit of various kinetic models

Formulation	Zero order Model		First order Model		Higuchi Model		Korsmeyer-Peppas Model			Hixson-Crowell Model	
	k_o	R^2	K_1	R^2	k_{HC}	R^2	k_p	n	R^2	k_H	R^2
ORL 1	8.717	0.983	0.357	0.920	37.22	0.996	0.198	0.704	0.997	0.317	0.988
ORL 2	7.040	0.979	0.282	0.887	32.41	0.999	0.189	0.673	0.997	0.275	0.975
ORL 3	6.366	0.982	0.197	0.973	29.23	0.997	0.212	0.595	0.998	0.248	0.984
ORL 4	6.020	0.981	0.161	0.986	27.66	0.997	0.201	0.594	0.998	0.243	0.984
ORL 5	8.613	0.998	0.401	0.803	36.29	0.985	0.198	0.677	0.993	0.309	0.997
ORL 6	6.515	0.991	0.228	0.940	29.72	0.994	0.223	0.574	0.996	0.245	0.991
ORL 7	6.225	0.990	0.186	0.960	28.45	0.996	0.212	0.579	0.997	0.243	0.993
ORL 8	5.924	0.993	0.146	0.977	26.96	0.990	0.185	0.600	0.995	0.244	0.992

CONCLUSIONS

Ionic gelation method was employed for the preparation of microcapsules which was simple, reproducible and produced microcapsules of regular shape and size. During study, it was concluded that the variation observed in entrapment efficiency, percentage yield, mean particle size and drug release behavior among formulations was due to the drug polymer ratio. The DSC and FTIR studies indicated that there was no interaction between drug and polymer. Mostly the microcapsules were of nearly spherical shape, discrete and free flowing as cleared in SEM studies. In-vitro release studies indicated that there was a slow and controlled release of drug for all the formulations. The formulation ORL 2, which contains orlistat and HPMC (2:0.5) was found to be the best in order to achieve the objective of this study for novel formulation design and successful development of orlistat microcapsules. Drug release was non-fickian i.e. both diffusion and dissolution controlled which followed Higuchi Model. These results clearly indicate that this formulation is gastric friendly and also offers patient compliance.

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