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Design and Evaluation of a Controlled Release Drug Delivery System for Management of Rheumatism

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Abstract

The present research was aimed to design, formulate and evaluate Mucoadhesive colon targeted microspheres of Ketoprofen for many advantages especially increased bioavailability and reduction in dosing frequency etc. Ketoprofen is a NSAID, like other drugs in this group reduces pain, inflammation and stiffness in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. In this study an attempt was made to prepare mucoadhesive microspheres of Ketoprofen using the natural polymers designed for oral controlled release.

Ketoprofen microspheres were prepared following 32 full factorial designs with varying concentrations of the polymers using sodium alginate and natural polymer such guar gum, xanthan gum by orifice-ionic gelation method. The prepared ketoprofen microspheres were evaluated for surface morphology and particle shape, rheological studies. Micro encapsulation efficiency, swelling index and *in vitro* drug release studies were done, and compatibility studies. The microspheres were found discrete, spherical and free flowing.

The percentage yield ranged from 88% to 96% and encapsulation efficiency was from 86.23% to 94.46%. The particle size was found to be between 400-550 µm. From the *in vitro* drug release studies the (KPN5) showed 92.12% drug release in 12 hrs and showed better control of drug release. The in vitro release data was treated with mathematical equations, and was concluded that ketoprofen followed zero order release from the microspheres and Peppas model with non-Fickian diffusion Super Case II transport. The results indicate that Enteric coated mucoadhesive colon targeted microspheres of ketoprofen containing xanthan gum; guar gum provides a better option for controlled release action and improved bioavailability.

Keywords: Microencapsulation; Mucoadhesion; Colon targeted; Enteric coating; Natural gums; Factorial design; Controlled release

Introduction

To address the short comings of the molecules and thereby alter the pharmacokinetic and pharmacodynamic properties, release of the drug from polymers are being of immense importance, Polymeric materials provide the most important avenues for delivery technology, primarily because of their ease of processing and the ability of researchers to readily control their chemical and physical properties via molecular synthesis. Basically, two broad categories of polymer systems, both known as "Microspheres" because of their size and shape, have been studied: reservoir devices and matrix devices. The former involves the encapsulation of a pharmaceutical product within a polymer shell, whereas the latter describes a system in which a drug is physically entrapped within a polymer network.

Microencapsulation is a useful method for prolonging drug release from dosage forms and reducing adverse effects recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres and microspheres (having a core of the drug) of 1–1000 µm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. Bioadhesive/non bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to their high surface to volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [1-7].

Rheumatic diseases and conditions primarily affect joints, tendons, ligaments, bones, and muscles. Rheumatic diseases are characterized by the signs of inflammation-redness, heat, swelling, and pain [7-12].

Among 30% of ill elderly people are suffering from rheumatism in which medication should be used for prolonged period, which may extend up to some months also. While therapy if medicine as conventional dosage form is given twice or thrice in a day, that elevates lot of inconvenience and fluctuations in therapy with some adverse effects also. To overcome demerits of a conventional dosage form a suitable controlled drug delivery system should be developed. Microencapsulation is a technique to deliver the medicament at controlled rate with more advantages over conventional formulations.

The Present aim of the study is to Design and Evaluation of a controlled release drug delivery system for management of Rheumatism, Development of dosage form as colon targeted mucoadhesive microspheres with natural gums (Gum Xanthan & Gum Guar) as mucoadhesive polymers followed by enteric coating and to evaluate various parameters for the prepared microspheres (Figures 1-5).

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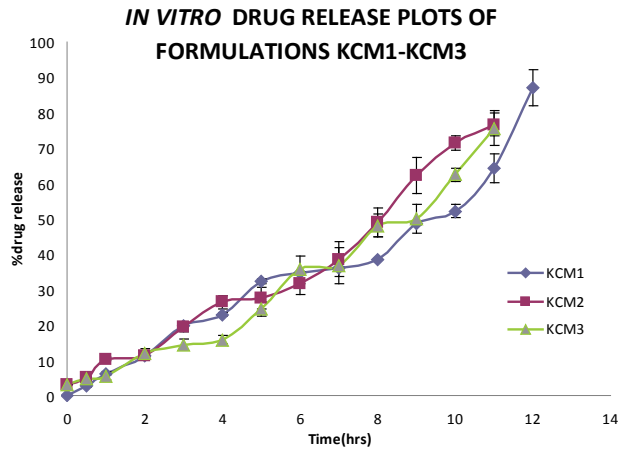

Figure 1: *In vitro* drug release plots of formulations KPN1-KPN3.

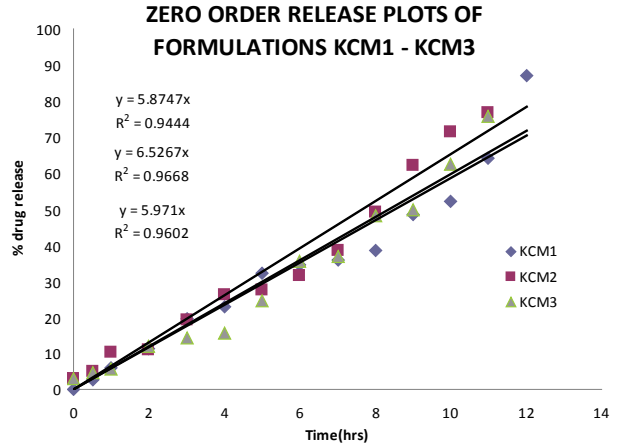

Figure 2: Zero order release of plots of KCM1 & KCM3 formulations.

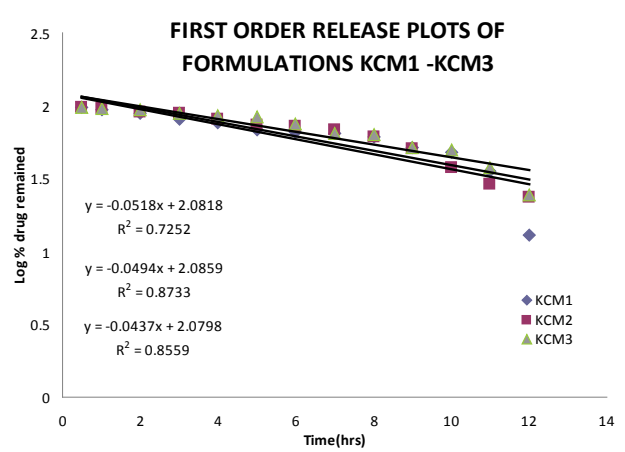

Figure 3: First order release of plots of KCM1 & KCM3 formulations.

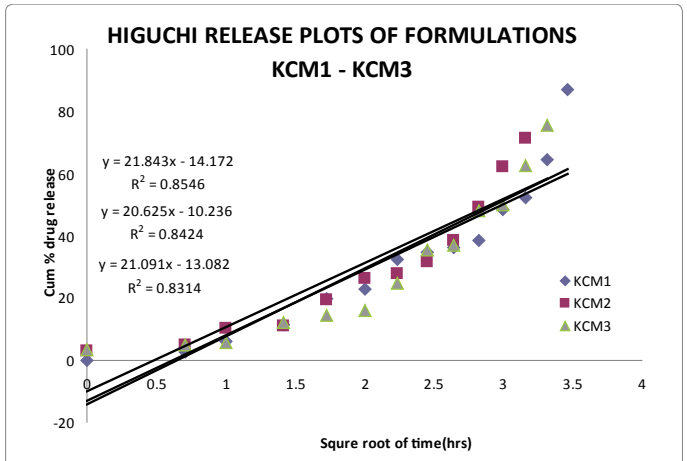

Figure 4: Higuchi release of plots of KCM1 & KCM3 formulations.

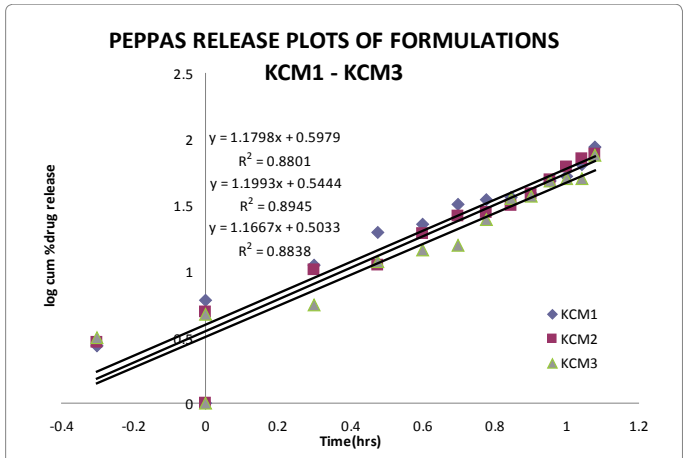

Figure 5: Peppas release of plots of KCM1 & KCM3 formulations.

Materials and Methods

Preformulation studies

Preformulation activities range from supporting discovery's identification of new active agents to characterizing physical properties necessary for the design of dosage form. Critical information provided during Preformulation can enhance the rapid and successful introduction of new therapeutics entities for humans. The overall objective of preformulation testing is to generate information useful [13-22] in developing the formulation which is stable and enhancing bioavailability. Further the use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product. The physicochemical properties of the bulk drug like physical appearance, solubility, bulk density, tapped density, compressibility index, angle repose, sieve analysis were studied (Tables 1-3).

Formulation and preparation of ketoprofen microspheres with different polymers and different ratios:

Formulation and preparing microspheres of ketoprofen using polymers Guar gum, Xanthan by using ionotropic gelation technique

Preparation of ketoprofen microspheres: Ketoprofen microspheres were prepared by Ionic orifice gelation technique by using different concentrations of polymers (1:1:0.25, 1:1:0.5, 1:1:0.75, 1:1:1) according to the formula generated by 32 full factorial design, and the pure drug & sodium alginate were mixed thoroughly in a de-mineralized water in order to prepare a clear solution. To this the required quantity of the polymer was added by adding the required amount of de-mineralized water until it [23-32]

found free flowing & with out stringing in nature. Drop wisely this solution was poured in to 15% $CaCl_2$ solution by using 22# needle stirring it continuously at 500 rpm over a magnetic stirrer. The microspheres thus formed are allowed for curing for 30 min then decanted and air dried.

Drug entrapment efficiency and % drug content

Accurately weighed 50 mg of drug-loaded microspheres were suspended in 100 ml of simulated intestinal fluid of pH 7.4 PB. The resulting solution was kept for 24 hrs. Next day it was stirred for 5 min and filtered. After suitable dilution, ketoprofen content in the filtrate was analyzed spectrophotometrically at 259 nm using a UV-Vis spectrophotometer. The drug entrapment efficiency was determined using following formula.

% DEE=Actual drug content/Theoritical drug content×100 (1)

FT-IR spectra

Fourier Transform Infrared Analysis (FT-IR) measurements of pure drug, carrier and drug-loaded microspheres formulations were obtained using a Perkin-Elmer system 200 FT-IR spectrophotometer. The pellets were prepared on KBr-press under hydraulic pressure of 150 kg/cm^2; the spectra were scanned over the wave number range of 4000 to 400 cm^{-1} at the ambient temperature (Tables 4-8).

In vitro drug release studies

Release of ketoprofen form microspheres was studied phosphate buffer of pH 7.4 (900 ml) using USP XXIV six-station Dissolution Rate Test Apparatus with a basket stirrer at 50 rpm. A sample of microspheres equivalent to 100 mg of ketoprofen was used in each test. The rotational speed of the basket was set at 100 rpm at 37 ± 0.5°C. 5 ml of aliquots was withdrawn at predetermined time intervals by replacing a fresh sample of dissolution medium. The absorbance of the collected samples measured using UV-spectrophotometer at λmax 259 nm in phosphate buffer of pH 7.4 (Figures 5-9).

n	Mechanism
0.5	Fickian diffusion (Higuchi Matrix)
0.5<n<1	Non-Fickian diffusion
1	Case II transport

Table 1: Different release mechanisms.

S. No.	Microspheres code	Number of microspheres adhering to tissue at hour				
		Phosphate buffer pH 7.4				
		1	2	4	6	8
1	KCM1	30 ± 1	30 ± 1	28 ± 1	20 ± 1	15 ± 1
2	KCM2	30 ± 2	25 ± 2	22 ± 3	18 ± 3	15 ± 2
3	KCM3	30 ± 1	24 ± 2	21 ± 2	18 ± 2	16 ± 3
4	KCM4	30 ± 2	22 ± 3	20 ± 3	17 ± 2	15 ± 2
5	KCM5	30 ± 3	23 ± 3	18 ± 2	14 ± 3	10 ± 3
6	KCM6	30 ± 2	22 ± 3	20 ± 2	15 ± 2	15 ± 2
:7	KCM7	30 ± 2	23 ± 4	21 ± 3	16 ± 4	15 ± 3
8	KCM8	30 ± 2	23 ± 2	20 ± 3	18 ± 5	15 ± 4
9	KCM9	30 ± 2	27 ± 2	25 ± 2	23 ± 3	21 ± 2

Table 2: Mucoadhesion test results by using *in vitro* wash off test.

In vitro wash-off test for mucoadhesive property of microspheres

The mucoadhesive property of the microspheres was evaluated by an *in vitro* adhesion testing method known as wash-off method. A piece of intestinal mucous (2 × 2 cm) was mounted on to glass slides of (3 × 1 inch) with Cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microspheres were spread on to each wet tissue specimen and there after the support was hung on to the arm of a USP tablet disintegrating test machine. The disintegration machine containing tissue specimen was adjusted at slow, regular up and down moment in a test fluid at 37°C taken in a beaker. At the end of 30 min, 1 hr and later at hourly intervals up to 8 hrs, the machine was stopped and the number of microspheres still adhering on to the tissue was counted. The test was performed in phosphate buffer of pH 7.4.

Determination of release kinetic data

First Order Kinetics: A first order release would be predicated by the following equation:

$$\text{Log } C = \text{Log } C_o - \left[\frac{K}{2.303}\right] \quad (2)$$

where C=Amount of drug remained at time 't'.

Co=Initial amount of drug.

K=First order rate constant (Hr-1).

When, the data was plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Higuchi's model: Drug released from the matrix devices by diffusion has been described by Higuchi's classical diffusion equation:

$$Q = \left[\frac{D\epsilon}{\phi}(2A - Cst)\right] \quad (3)$$

where Q=Amount of drug release at time 't'.

D=Diffusion coefficient of the drug in the matrix.

A=Total amount of drug in unit volume of matrix.

S. No.	Formulation Code	Angle of Repose	Bulk Density (g/cm3)	Carr's Index	Hausner's ratio	True density (g/cm^3)	Average particle size
1	KCM1	24.4 ± 0.61	0.66 ± 0.03	96.46 ± 0.31	0.04	0.90 ± 0.2	490 ± 10
2	KCM2	27.3 ± 0.72	0.63 ± 0.04	96.35 ± 0.46	0.04	0.96 ± 0.4	510 ± 5
3	KCM3	26.5 ± 0.81	0.56 ± 0.05	96.68 ± 0.56	0.03	0.92 ± 0.5	530 ± 8
4	KCM4	24.3 ± 0.73	0.64 ± 0.06	96.28 ± 0.51	0.04	0.94 ± 0.3	528 ± 5
5	KCM5	24.6 ± 0.91	0.60 ± 0.03	96.63 ± 0.49	0.03	0.83 ± 0.5	510 ± 10
6	KCM6	26.2 ± 0.63	0.55 ± 0.02	96.39 ± 0.59	0.04	0.91 ± 0.2	525 ± 5
7	KCM7	25.8 ± 0.74	0.57 ± 0.09	96.25 ± 0.41	0.04	0.93 ± 0.3	528 ± 8
8	KCM8	24.3 ± 0.91	0.62 ± 0.06	96.34 ± 0.51	0.03	0.89 ± 0.1	531 ± 6
9	KCM9	27.7 ± .0.84	0.61 ± 0.04	96.93 ± 0.41	0.04	0.79 ± 0.4	500 ± 10

Table 3: Physical properties of ketoprofen microspheres formulations KCM1-KCM9.

Time	log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.301	0.7071	0.248	3.0244	1	2.721951	2.721951	2.721951	97.27805	0.43488	1.988015
1	0	1	0.272	3.3171	2	5.970732	5.985854	5.985854	94.01415	0.777126	1.973193
2	0.301	1.4142	0.51	6.2195	2	11.19512	11.22683	11.22683	88.77317	1.050257	1.948282
3	0.4771	1.7321	0.891	10.866	2	19.55854	19.62134	19.62134	80.37866	1.292729	1.905141
4	0.6021	2	0.692	8.439	3	22.78537	22.8904	22.8904	77.1096	1.359653	1.887108
5	0.699	2.2361	0.732	8.9268	4	32.13659	32.29591	32.29591	67.70409	1.509148	1.830615
6	0.7782	2.4495	0.784	9.561	4	34.41951	34.57884	34.57884	65.42116	1.53881	1.815718
7	0.8451	2.6458	0.652	7.9512	5	35.78049	36.00116	36.00116	63.99884	1.556316	1.806172
8	0.9031	2.8284	0.697	8.5	5	38.25	38.54152	38.54152	61.45848	1.585929	1.788582
9	0.9542	3	0.732	8.9268	6	48.20488	48.5389	48.5389	51.4611	1.68609	1.711479
10	1	3.1623	0.787	9.5976	6	51.82683	52.20549	52.20549	47.79451	1.717716	1.679378
11	1.0414	3.3166	0.831	10.134	7	63.84512	64.27177	64.27177	35.72823	1.80802	1.553012
12	1.0792	3.4641	0.986	12.024	8	86.57561	87.05293	87.05293	12.94707	1.939783	1.112172

Table 4: *In vitro* drug release data of formulation KCM1.

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.265	3.231707	1	2.908537	2.908537	2.908537	97.09146	0.463675	1.987181
1	0	1	0.449	5.47561	1	4.928049	4.944207	4.949207	95.05079	0.694536	1.977956
2	0.30103	1.414214	0.467	5.695122	2	10.25122	10.29476	10.30476	89.69524	1.013038	1.952769
3	0.477121	1.732051	0.501	6.109756	2	10.99756	11.06957	11.08957	88.91043	1.044915	1.948953
4	0.60206	2	0.582	7.097561	3	19.16341	19.26598	19.29598	80.70402	1.285467	1.906895
5	0.69897	2.236068	0.597	7.280488	4	26.20976	26.3478	26.3928	73.6072	1.421486	1.86692
6	0.778151	2.44949	0.623	7.597561	4	27.35122	27.52567	27.59067	72.40933	1.440762	1.859795
7	0.845098	2.645751	0.71	8.658537	4	31.17073	31.38317	31.46817	68.53183	1.497871	1.835892
8	0.90309	2.828427	0.696	8.487805	5	38.19512	38.45085	38.55585	61.44415	1.58609	1.788481
9	0.954243	3	0.738	9	6	48.6	48.89817	49.02817	50.97183	1.690446	1.70733
10	1	3.162278	0.803	9.792683	7	61.6939	62.04378	62.23378	37.76622	1.794026	1.577104
11	1.041393	3.316625	0.921	11.23171	7	70.75976	71.15189	71.34689	28.65311	1.853375	1.457172
12	1.079181	3.464102	0.989	12.06098	7	75.98415	76.43244	76.66244	23.33756	1.884583	1.368055

Table 5: *In vitro* drug release data of formulation KCM2.

TIME	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.286	3.487805	1	3.139024	3.139024	3.139024	96.86098	0.496795	1.986149
1	0	1	0.432	5.268293	1	4.741463	4.758902	4.763902	95.2361	0.677963	1.978802
2	0.30103	1.414214	0.506	6.170732	1	5.553659	5.597439	5.607439	94.39256	0.748765	1.974938
3	0.477121	1.732051	0.539	6.573171	2	11.83171	11.90634	11.92134	88.07866	1.076325	1.944871
4	0.60206	2	0.652	7.95122	2	14.3122	14.4197	14.4447	85.5553	1.159708	1.932247
5	0.69897	2.236068	0.709	8.646341	2	15.56341	15.71067	15.74567	84.25433	1.197161	1.925592
6	0.778151	2.44949	0.739	9.012195	3	24.33293	24.52341	24.56841	75.43159	1.390377	1.877553
7	0.845098	2.645751	0.803	9.792683	4	35.25366	35.48921	35.54921	64.45079	1.55083	1.809228
8	0.90309	2.828427	0.829	10.10976	4	36.39512	36.67963	36.75963	63.24037	1.565371	1.800994
9	0.954243	3	0.869	10.59756	5	47.68902	48.02409	48.12409	51.87591	1.682362	1.714966
10	1	3.162278	0.901	10.9878	5	49.44512	49.83317	49.95817	50.04183	1.698607	1.699333
11	1.041393	3.316625	0.939	11.45122	6	61.83659	62.27957	62.42957	37.57043	1.698607	1.574846
12	1.079181	3.464102	0.976	11.90244	7	74.98537	75.48561	75.66561	24.33439	1.878899	1.38622

Table 6: *In vitro* drug release data of formulation KCM3.

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.389	4.743902	1	4.269512	4.269512	4.269512	95.73049	0.630378	1.98105
1	0	1	0.409	4.987805	1	4.489024	4.512744	4.517744	95.48226	0.654922	1.979923
2	0.30103	1.414214	0.45	5.487805	2	9.878049	9.926707	9.936707	90.06329	0.997242	1.954548
3	0.477121	1.732051	0.506	6.170732	3	16.66098	16.73707	16.75707	83.24293	1.224198	1.920347
4	0.60206	2	0.573	6.987805	4	25.1561	25.26305	25.29805	74.70195	1.403087	1.873332
5	0.69897	2.236068	0.657	8.012195	5	36.05488	36.19677	36.25177	63.74823	1.559329	1.804468
6	0.778151	2.44949	0.712	8.682927	5	39.07317	39.25512	39.33512	60.66488	1.594781	1.782937
7	0.845098	2.645751	0.769	9.378049	5	42.20122	42.42659	42.53159	57.46841	1.628712	1.759429
8	0.90309	2.828427	0.811	9.890244	5	44.5061	44.77835	44.90835	55.09165	1.652327	1.741086
9	0.954243	3	0.892	10.87805	5	48.95122	49.27293	49.42793	50.57207	1.693972	1.703911
10	1	3.162278	0.899	10.96341	6	59.20244	59.57854	59.75854	40.24146	1.7764	1.604674
11	1.041393	3.316625	0.945	11.52439	7	72.60366	73.03457	73.24457	26.75543	1.7764	1.427412
12	1.079181	3.464102	0.993	12.10976	8	87.19024	87.67878	87.92378	12.07622	1.944106	1.081931

Table 7: *In vitro* drug release data of formulation KCM4.

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.443	5.402439	1	4.862195	4.862195	4.862195	95.1378	0.686832	1.978353
1	0	1	0.576	7.02439	1	6.321951	6.348963	6.353963	93.64604	0.803045	1.971489
2	0.30103	1.414214	0.675	8.231707	2	14.81707	14.87921	14.88921	85.11079	1.172872	1.929985
3	0.477121	1.732051	0.727	8.865854	2	15.95854	16.06183	16.08183	83.91817	1.206335	1.923856
4	0.60206	2	0.779	9.5	3	25.65	25.79762	25.82762	74.17238	1.412084	1.870242
5	0.69897	2.236068	0.811	9.890244	3	26.70366	26.89878	26.94378	73.05622	1.430459	1.863657
6	0.778151	2.44949	0.834	10.17073	4	36.61463	36.85921	36.91921	63.08079	1.567252	1.799897
7	0.845098	2.645751	0.957	11.67073	4	42.01463	42.31006	42.39006	57.60994	1.627264	1.760497
8	0.90309	2.828427	0.984	12	5	54	54.35378	54.45378	45.54622	1.736028	1.658452
9	0.954243	3	0.997	12.15854	5	54.71341	55.1272	55.2522	44.7478	1.74235	1.650772
10	1	3.162278	0.836	10.19512	7	64.22927	64.70384	64.85384	35.14616	1.811936	1.545878
11	1.041393	3.316625	0.779	9.5	8	68.4	68.92555	69.11055	30.88945	1.811936	1.48981
12	1.079181	3.464102	0.933	11.37805	9	92.1622	92.73524	92.96024	7.039756	1.968297	0.847558

Table 8: *In vitro* drug release data of formulation KCM5.

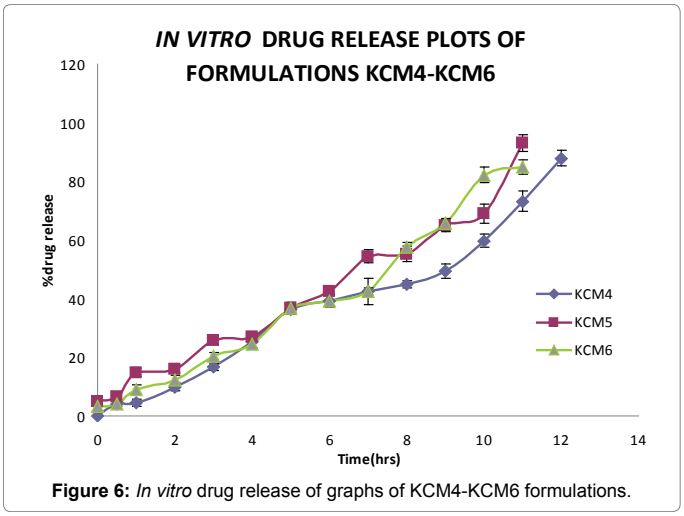

Figure 6: *In vitro* drug release of graphs of KCM4-KCM6 formulations.

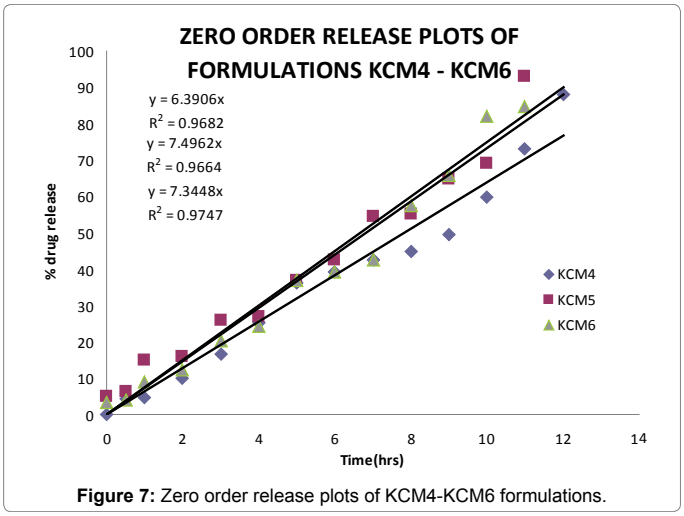

Figure 7: Zero order release plots of KCM4-KCM6 formulations.

Cs=the solubility of the drug in the matrix.

∈=Porosity of the matrix.

τ= Tortuosity.

t=Time (hours).

The above equation may be simplified, if one assumes that D, ∈, τ, Cs and A are constant. Then the equation 15 becomes:

$$Q = KT^{1/2}$$

When the data is plotted, according to Higuchi's equation 16 ($Q=KT^{1/2}$), cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Peppas release model: The release rate data were fitted to the following equation 17, Mt /M∝=K.tn.

$$\left[\frac{Mt}{M}\right] = K.tn \quad (4)$$

Where Mt /M∝ is the fraction of drug released, 'K' is the release constant,'t' is the release time. 'n' is diffusion exponent, if n is equal to 0.89, the release is zero order. If n is equal to 0.45 the release is best explained by Fickian diffusion, and if 0.45<n<0.89 then the release is through anomalous diffusion or nonfickian diffusion (Swellable & Cylindrical Matrix). In this model, a plot of log (Mt/M∝) versus log (time) is linear.

Mucoadhesive colon targeted microspheres were prepared and [33-38] evaluated for their use as colon targeting drug delivery systems to increase its local action and bioavailability.

In the present work total nine formulations were prepared and complete composition of all batches shown in Table 9. The microspheres were then characterized for various physico-chemical parameters.

Standard calibration curve

Standard calibration curve of ketoprofen was drawn by plotting absorbance vs. concentration. The λmax of ketoprofen in 7.4 pH phosphate buffer was determined to be 259 nm.

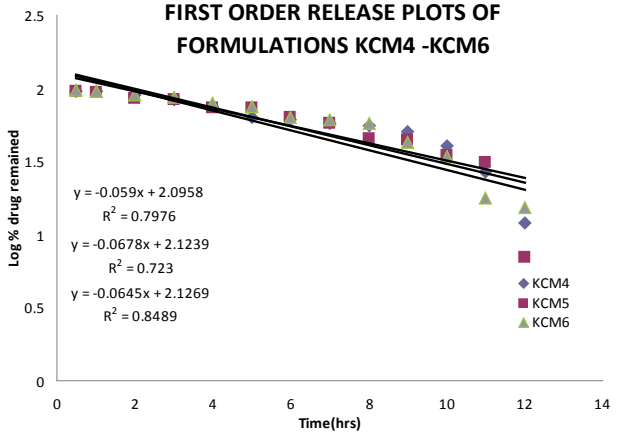

Figure 8: First order release plots of KCM4-KCM6 formulations.

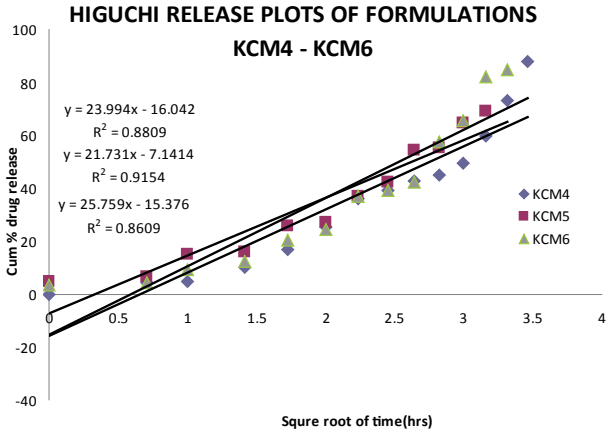

Figure 9: Higuchi release plots of KCM4-KCM6 formulations.

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.303	3.695122	1	3.32561	3.32561	3.32561	96.67439	0.521871	1.985311
1	0	1	0.371	4.52439	1	4.071951	4.090427	4.095427	95.90457	0.612299	1.981839
2	0.30103	1.414214	0.41	5	2	9	9.041098	9.051098	90.9489	0.956701	1.958797
3	0.477121	1.732051	0.563	6.865854	2	12.35854	12.42463	12.44463	87.55537	1.094982	1.942283
4	0.60206	2	0.615	7.5	3	20.25	20.35043	20.38043	79.61957	1.309213	1.90102
5	0.69897	2.236068	0.733	8.939024	3	24.13537	24.27329	24.31829	75.68171	1.385933	1.878991
6	0.778151	2.44949	0.834	10.17073	4	36.61463	36.79726	36.85726	63.14274	1.566523	1.800323
7	0.845098	2.645751	0.889	10.84146	4	39.02927	39.26274	39.34274	60.65726	1.594865	1.782883
8	0.90309	2.828427	0.959	11.69512	4	42.10244	42.39012	42.49012	57.50988	1.628288	1.759742
9	0.954243	3	0.867	10.57317	6	57.09512	57.44128	57.56128	42.43872	1.76013	1.627762
10	1	3.162278	0.989	12.06098	6	65.12927	65.52829	65.67829	34.32171	1.817422	1.535569
11	1.041393	3.316625	0.929	11.32927	8	81.57073	82.03006	82.21006	17.78994	1.817422	1.250174
12	1.079181	3.464102	0.957	11.67073	8	84.02927	84.54524	84.76524	15.23476	1.928218	1.182836

Table 9: *In vitro* drug release data of formulation KCM6.

Compatibility study

Drug excipients compatibility status was determined by IR (infrared) spectroscopy where the spectra of pure drug were clearly matched with the spectra of formulations. The desired peaks of functional groups which were identified in pure drug are also found in formulation spectra without changing. The extra peaks which are found in formulations indicate the presence of polymers and other excipients.

In IR spectra of ketoprofen pure drug the peaks were found prominently at different wave numbers indicating the presence of functional groups and substituents like peaks at 1655 cm^{-1}, 1598 cm^{-1}, 1457 cm^{-1} wave number due to C=C stretching inside the benzyl ring. Prominent peaks at 1697 cm^{-1} due to C=O stretching, and at 1228 cm^{-1} due to C-O stretching in carboxylic group. Prominent peaks appeared at 2978 cm^{-1} wave number are due to C-H asymmetric and symmetric stretching, in methyl group indicates the presence of methyl groups in the structure, peak 717 cm^{-1}, 703 cm^{-1} wave number are due to C-H bending indicates the benzene ring. Peaks observed in between 1420 cm^{-1} wave number is due to C-O-H stretching in plane which clearly indicates the presence of carboxylic group and peak at 3076 cm^{-1} and 3455 cm^{-1} wave numbers indicates the presence of Aromatic -H stretching and O-H stretching out plane. And all these peaks were appeared unchanged in IR spectra of combinations of ketoprofen with natural polymers. The data [39-44] clearly states that there is no interaction between the pure drug ketoprofen and other excipients. The peaks obtained in the spectra of each formulation correlates with the peaks of drug spectrum. The spectrum for pure drug was is compatible with the formulation components (Tables 10-12).

Evaluation Results of Mucoadhesive Colon Targeted Microspheres Formulations

Particle size

The prepared microspheres were analyzed by sieve analysis and the particle size range between 490-540 µm and the data depicted in Table 13. And low particle size (490 ± 10 µm) was found in KP and high particle size (530 ± 8 µm) was found in KPN8. This data depicted

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.248	3.02439	1	2.721951	2.721951	2.721951	97.27805	0.43488	1.988015
1	0	1	0.277	3.378049	1	3.040244	3.055366	3.060366	96.93963	0.485773	1.986501
2	0.30103	1.414214	0.51	6.219512	1	5.597561	5.629573	5.639573	94.36043	0.751246	1.97479
3	0.477121	1.732051	0.891	10.86585	1	9.779268	9.842378	9.857378	90.14262	0.993761	1.95493
4	0.60206	2	0.684	8.341463	2	15.01463	15.13207	15.15207	84.84793	1.180472	1.928641
5	0.69897	2.236068	0.692	8.439024	2	15.19024	15.34939	15.37939	84.62061	1.186939	1.927476
6	0.778151	2.44949	0.652	7.95122	3	21.46829	21.66963	21.70963	78.29037	1.336653	1.893708
7	0.845098	2.645751	0.697	8.5	3	22.95	23.1911	23.2461	76.7539	1.36635	1.8851
8	0.90309	2.828427	0.932	11.36585	3	30.6878	30.9714	31.0414	68.9586	1.491941	1.838588
9	0.954243	3	0.787	9.597561	4	34.55122	34.89165	34.97665	65.02335	1.543778	1.813069
10	1	3.162278	0.831	10.13415	5	45.60366	45.99207	46.09707	53.90293	1.663673	1.731612
11	1.041393	3.316625	0.986	12.02439	5	54.10976	54.54884	54.67884	45.32116	1.663673	1.656301
12	1.079181	3.464102	0.997	12.15854	7	76.59878	77.09799	77.25299	22.74701	1.887915	1.356924

Table 10: *In vitro* drug release data of formulation KCM7.

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.253	3.085366	1	2.776829	2.776829	2.776829	97.22317	0.443549	1.98777
1	0	1	0.301	3.670732	1	3.303659	3.319085	3.324085	96.67591	0.521672	1.985318
2	0.30103	1.414214	0.432	5.268293	2	9.482927	9.516707	9.526707	90.47329	0.978943	1.95652
3	0.477121	1.732051	0.489	5.963415	2	10.73415	10.79427	10.81427	89.18573	1.033997	1.950295
4	0.60206	2	0.523	6.378049	2	11.48049	11.57043	11.60043	88.39957	1.064474	1.94645
5	0.69897	2.236068	0.567	6.914634	3	18.66951	18.79134	18.83134	81.16866	1.274881	1.909388
6	0.778151	2.44949	0.598	7.292683	3	19.69024	19.84665	19.90165	80.09835	1.298889	1.903624
7	0.845098	2.645751	0.645	7.865854	4	28.31707	28.50994	28.57994	71.42006	1.456061	1.85382
8	0.90309	2.828427	0.702	8.560976	4	30.81951	31.05171	31.14171	68.85829	1.493342	1.837956
9	0.954243	3	0.789	9.621951	5	43.29878	43.57378	43.68378	56.31622	1.64032	1.750633
10	1	3.162278	0.843	10.28049	5	46.2622	46.5853	46.7203	53.2797	1.669506	1.726562
11	1.041393	3.316625	0.879	10.71951	6	57.88537	58.25988	58.41988	41.58012	1.669506	1.618886
12	1.079181	3.464102	0.977	11.91463	8	85.78537	86.21348	86.40348	13.59652	1.936531	1.133428

Table 11: *In vitro* drug release data of formulation KCM8.

Time	Log T	SQRT	Abs	Con	DF	ADR	CDR	%CDR	%CDRM	L%CDR	L%CDRM
0	#NUM!	0	0	0	0	0	0	0	100	#NUM!	2
0.5	-0.30103	0.707107	0.112	1.365854	1	1.229268	1.229268	1.229268	98.77073	0.089647	1.994628
1	0	1	0.198	2.414634	1	2.173171	2.18	2.185	97.815	0.339451	1.990405
2	0.30103	1.414214	0.254	3.097561	1	2.787805	2.806707	2.816707	97.18329	0.449742	1.987592
3	0.477121	1.732051	0.299	3.646341	1	3.281707	3.316098	3.331098	96.6689	0.522587	1.985287
4	0.60206	2	0.334	4.073171	2	7.331707	7.384329	7.404329	92.59567	0.869486	1.966591
5	0.69897	2.236068	0.412	5.02439	2	9.043902	9.11689	9.14689	90.85311	0.961273	1.95834
6	0.778151	2.44949	0.489	5.963415	2	10.73415	10.83226	10.87226	89.12774	1.03632	1.950013
7	0.845098	2.645751	0.532	6.487805	3	17.51707	17.645	17.695	82.305	1.247851	1.915426
8	0.90309	2.828427	0.591	7.207317	3	19.45976	19.62012	19.68512	80.31488	1.294138	1.904796
9	0.954243	3	0.667	8.134146	4	29.28293	29.47933	29.55933	70.44067	1.470695	1.847823
10	1	3.162278	0.721	8.792683	5	39.56707	39.80415	39.90415	60.09585	1.601018	1.778845
11	1.041393	3.316625	0.823	10.03659	5	45.16463	45.44567	45.57067	54.42933	1.601018	1.735833
12	1.079181	3.464102	0.875	10.67073	7	67.22561	67.55683	67.70683	32.29317	1.830632	1.509111

Table 12: *In vitro* drug release data of formulation KCM9.

in Scanning Electron Microscopy (SEM) analysis was done and the pictograms revealed spherical and uniform shaped microspheres (Figure 10).

Flow property

The flow property of the prepared formulations was checked by the method, angle of repose. The values obtained for angle of repose for all the formulations are tabulated in Table 13. The values were found to be in the range from 24.3 to 27.7. This indicates good flow property of the microspheres.

Hausner's ratio of microspheres was found in the range of 0.03-0.04 and Carr's index of microspheres was found in the range of 96.25 ± 0.41 to 96.93 ± 0.41 and data shown in the Table 13.

Mucoadhesion test by using in vitro wash of test

The results of Mucoadhesive microspheres are presented in Table 12 and showed fairly good Mucoadhesive property of microspheres in all the cases. The best mucoadhesive property was observed in KPN9 formulation and least mucoadhesion property was observed in KPN5.

In vitro dissolution studies

The dissolution rate studies were performed by using USP-XXIV dissolution apparatus employing rotating Basket at a speed of 50 rpm in the dissolution medium of 7.4 pH Buffer study was continued up to 12 hrs at suitable time intervals, samples of 5 ml were withdrawn by means of pipette and it was immediately replaced with fresh dissolution medium. The withdrawn samples were analyzed for the drug content after appropriate dilutions by measuring the absorbance at 259 nm (7.4 pH buffer) with UV spectrophotometer.

The in vitro drug release profiles of microspheres from each batch (KPN1 to KPN9) were carried in 7.4 pH buffer for 12 hrs by using ring mesh device and the values are shown in Tables 14-20. The plot of % Cumulative drug release vs. time (hr) was plotted and depicted (Figures 11-14).

Curve fitting analysis

The results of dissolution data fitted to various drug release kinetic equations. Peppas model was found to be the best fitted in all dissolution profile having higher correlation coefficient (r-value) followed by

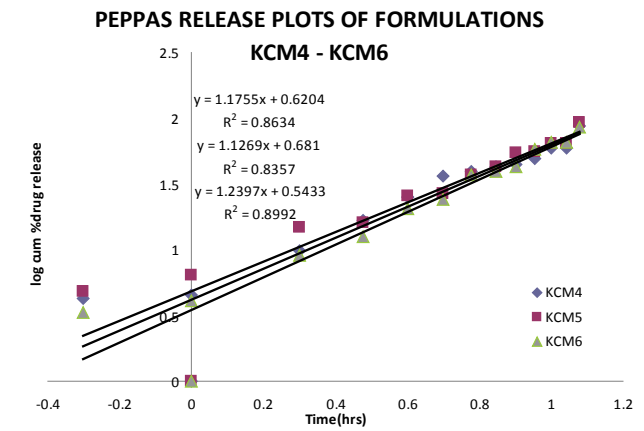

Figure 10: Peppas release plots of KCM4-KCM6 formulations.

Formulation	Zero order	First order	Higuchi	Peppas	
	R2	R2	R2	R2	n (Slope)
KCM1	0.944	0.725	0.842	0.880	1.1798
KCM2	0.966	0.873	0.854	0.894	1.1993
KCM3	0.960	0.855	0.831	0.883	1.1667
KCM4	0.961	0.804	0.880	0.863	1.1755
KCM5	0.968	0.73	0.887	0.899	1.1269
KCM6	0.974	0.830	0.860	0.835	1.2397
KCM7	0.880	0.729	0.753	0.905	1.1773
KCM8	0.810	0.741	0.738	0.892	1.1714
KCM9	0.828	0.659	0.667	0.917	1.2842

Table 13: Curve fitting data for formulations KCM1-KCM9.

Higuchi model and First order release equation. Korsemeyer-Peppas model indicates that release mechanism is not well known or more than one type of release phenomena could be involved. The 'n' value could be used to characterize different release mechanisms as:

The results are reported in Table 20 and in the present study 'n' values of all the formulations was found to be greater than 1(n>1) so, it can be concluded that all the formulations followed super case II transport.

Response	1	% DR 5 hrs	Transform	None		
Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob>F**	
Mean vs Total	4050.898	1	4050.898	-	-	-
Linear vs Mean	348.2115	2	174.1058	6.461529	0.0319	Suggested
2FI vs Linear	20.5209	1	20.5209	0.726923	0.4328	-
Quadratic vs 2FI	20.79584	2	10.39792	0.259185	0.7874	-
Cubic vs Quadratic	58.31127	2	29.15563	0.469935	0.718	Aliased
Residual	62.04188	1	62.04188	-	-	-
Total	4560.78	9	506.7533	-	-	-
I+"Sequential Model Sum of Squares [Type I]"0+: Select the highest order polynomial where the additional terms are significant and the model is not aliased						
Model Summary Statistics						
	Std.		**Adjusted**	**Predicted**		
Source	**Dev.**	**R-Squared**	**R-Squared**	**R-Squared**	**PRESS**	
Linear	5.190856	0.682926	0.577235	0.32319	345.093	Suggested
2FI	5.313172	0.723173	0.557077	0.112447	452.5469	
Quadratic	6.333855	0.763959	0.370556	-1.29937	1172.407	
Cubic	7.876667	0.878321	0.026568	-21.176	11307.13	Aliased
I+"Model Summary Statistics"0+: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared"						

Table 14: Factorial analysis data of response % Drug release at time 5 hrs.

Response	1	%DR 5 hr				
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares-Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**Df**	**Square**	**Value**	**Prob>F**	
Model	389.5283	5	77.90566	1.941927	0.3103	Not significant
A-A	3.776267	1	3.776267	0.09413	0.7791	
B-B	344.4353	1	344.4353	8.585615	0.0610	
AB	20.5209	1	20.5209	0.511517	0.5261	
A^2	5.962756	1	5.962756	0.148631	0.7256	
B^2	14.83309	1	14.83309	0.369739	0.5861	
Residual	120.3531	3	40.11771			
Cor Total	509.8814	8				
The "Model F-value" of 1.94 implies the model is not significant relative to the noise. There is a 31.03 % chance that a "Model F-value" this large could occur due to noise						
Values of "Prob>F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms Values greater than 0.1000 indicate the model terms are not significant If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model						
Std. Dev.	6.333855		R-Squared	0.763959		
Mean	21.21556		Adj R-Squared	0.370556		
C.V. %	29.85477		Pred R-Squared	-1.29937		
PRESS	1172.407		Adeq Precision	3.855373		
A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model.						
"Adeq Precision" measures the signal to noise ratio. A ratio of 3.86 indicates an inadequate signal and we should not use this model to navigate the design space						
	Coefficient		**Standard**	**95% CI**	**95% CI**	**VIF**
Factor	**Estimate**	**Df**	**Error**	**Low**	**High**	
Intercept	20.55111	1	4.720977	5.526844	35.57538	
A-A	-0.79333	1	2.585785	-9.02246	7.435797	1
B-B	-7.57667	1	2.585785	-15.8058	0.652464	1
AB	-2.265	1	3.166927	-12.3436	7.813585	1
A^2	-1.72667	1	4.478712	-15.9799	12.52661	1
B^2	2.723333	1	4.478712	-11.5299	16.97661	1

Table 15: Factorial analysis data of response % Drug release at time 5 hrs.

Response	1	%DR 10hr	Transform	None		
Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob>F**	
Mean vs. Total	26136.11	1	26136.11			Suggested
Linear vs. Mean	327.6617	2	163.8308	2.81281	0.1375	Suggested
2FI vs. Linear	26.5225	1	26.5225	0.410635	0.5499	
Quadratic vs. 2FI	32.26944	2	16.13472	0.166523	0.8539	
Cubic vs. Quadratic	244.2083	2	122.1042	2.627764	0.3998	Aliased
Residual	46.46694	1	46.46694			
Total	26813.24	9	2979.249			
I+"Sequential Model Sum of Squares [Type I]"0+: Select the highest order polynomial where the additional terms are significant and the model is not aliased						
Model Summary Statistics						
Source	**Std. Dev.**	**R-Squared**	**Adjusted R-squared**	**Predicted R-squared**	**Press**	
Linear	7.631811	0.483899	0.311865	-0.13842	770.8554	Suggested
2FI	8.036725	0.523068	0.236908	-0.93354	1309.254	-
Quadratic	9.843361	0.570724	-0.14474	-3.90731	3322.881	-
Cubic	6.816667	0.931377	0.451012	-11.5066	8468.601	Aliased
I+"Model Summary Statistics"0+: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared"						

Table 16: Factorial analysis data of response % Drug release at time 10 hrs.

Response	1	%DR 10 hr			
ANOVA for response surface quadratic model					
Analysis of variance table [Partial sum of squares-Type III]					Not significant
Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob>F**
Model	386.4536	5	77.29072	0.797702	0.6161
A-A	3.526667	1	3.526667	0.036398	0.8609
B-B	324.135	1	324.135	3.345331	0.1648
AB	26.5225	1	26.5225	0.273733	0.6370
A^2	20.90889	1	20.90889	0.215796	0.6739
B^2	11.36056	1	11.36056	0.11725	0.7546
Residual	290.6753	3	96.89176		
Cor Total	677.1289	8			
The "Model F-value" of 0.80 implies the model is not significant relative to the noise. There is a 61.61 % chance that a "Model F-value" this large could occur due to noise.					
Values of "Prob>F" less than 0.0500 indicate model terms are significant In this case there are no significant model terms Values greater than 0.1000 indicate the model terms are not significant If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model					
Std. Dev.	9.843361		R-Squared	0.570724	
Mean	53.88889		Adj R-Squared	-0.14474	
C.V. %	18.26603		Pred R-Squared	-3.90731	
PRESS	3322.881		Adeq Precision	2.647109	
A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model					
"Adeq Precision" measures the signal to noise ratio. A ratio of 2.65 indicates an inadequate signal and we should not use this model to navigate the design space					
Coefficient			**Standard**	**95% CI**	**95% CI**
Factor	**Estimate**	**Df**	**Error**	**Low**	**High**	**VIF**
Intercept	50.14444	1	7.336808	26.79543	73.49346	-
A-A	0.766667	1	4.018535	-12.0221	13.55545	1
B-B	-7.35	1	4.018535	-20.1388	5.438784	1
AB	-2.575	1	4.921681	-18.238	13.088	1
A^2	3.233333	1	6.960307	-18.9175	25.38416	1
B^2	2.383333	1	6.960307	-19.7675	24.53416	1

Table 17: Factorial analysis data of response % Drug release at time 10 hrs.

Response	1	Diffusion exponent	Transform	None		
*** WARNING: The Cubic Model is Aliased! ***						
Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob>F**	
Mean vs. Total	328.0554	1	328.0554			Suggested
Linear vs. Mean	3.246755	2	1.623377	1.60855	0.2758	Suggested
2FI vs. Linear	0.099856	1	0.099856	0.083836	0.7838	
Quadratic vs. 2FI	0.645859	2	0.32293	0.18246	0.8418	
Cubic vs. Quadratic	3.436251	2	1.718126	0.917146	0.5940	Aliased
Residual	1.87334	1	1.87334			
Total	337.3575	9	37.48416			
I+"Sequential Model Sum of Squares [Type I]"0+: Select the highest order polynomial where the additional terms are significant and the model is not aliased						
Model Summary Statistics						
Source	**Std. Dev.**	**R-squared**	**Adjusted R-squared**	**Predicted R-squared**	**Press**	
Linear	1.004598	0.349036	0.132048	-0.29168	12.01526	Suggested
2FI	1.091371	0.359771	-0.02437	-1.17692	20.24984	
Quadratic	1.330362	0.429203	-0.52213	-5.00166	55.82781	
Cubic	1.3687	0.79861	-0.61112	-35.7033	341.4162	Aliased
I+"Model Summary Statistics"0+: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared"						

Table 18: Factorial analysis data of response final diffusion exponent.

Response	1	Diffusion exponent			
ANOVA for Response Surface Mean Model					
Analysis of variance table [Partial sum of squares-Type III]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	0	0	-	-	-
Residual	9.302061	8	1.162758	-	-
Cor Total	9.302061	8	-	-	-
Values of "Prob>F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.					
Std. Dev.	1.078312		R-Squared	0	
Mean	6.037433		Adj R-Squared	0	
C.V. %	17.86044		Pred R-Squared	-0.26563	
PRESS	11.77292		Adeq Precision		
A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model.					
	Coefficient		**Standard**	**95% CI**	**95% CI**
Factor	**Estimate**	**df**	**Error**	**Low**	**High**
Intercept	6.037433	1	0.359437	5.208569	6.866298
Final Equation in Terms of Coded Factors					
Diffusion exponent=6.037433					
Final Equation in Terms of Actual Factors					
Diffusion exponent=6.037433					

Table 19: Factorial analysis data of response diffusion exponent.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding	
A	A	5.88	5	15	0	Actual	
B	B	14.69	5	15	0	Actual	
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
%DR 5hr	14.80589	3.783439	2.765286	26.84649	7.377813	-8.67363	38.2854
%DR 10hr	48.90098	7.253834	25.81602	71.98593	12.22742	9.987826	87.81413
Diffusion exponent	6.037433	0.359437	5.208569	6.866298	1.136641	3.416334	8.658532

Table 20: Factorial prediction data for responses % DR 5 hr, %DR 10 hr, diffusion exponent.

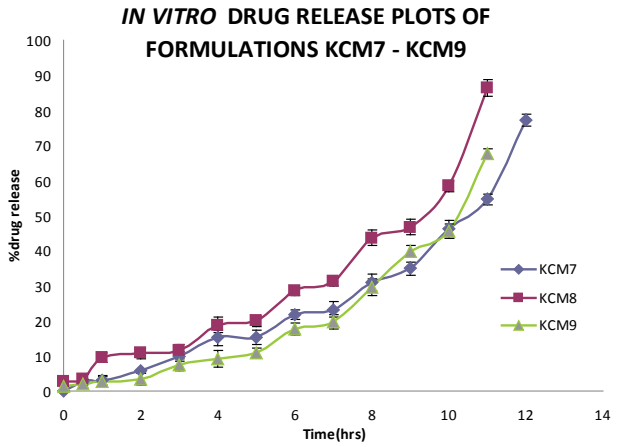

Figure 11: *In vitro* drug release of graphs of KCM7-KCM9 formulations.

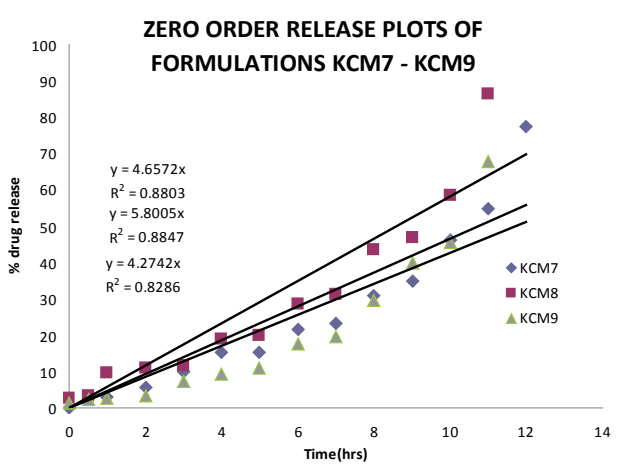

Figure 12: Zero order release plots of KCM7-KCM9 formulations.

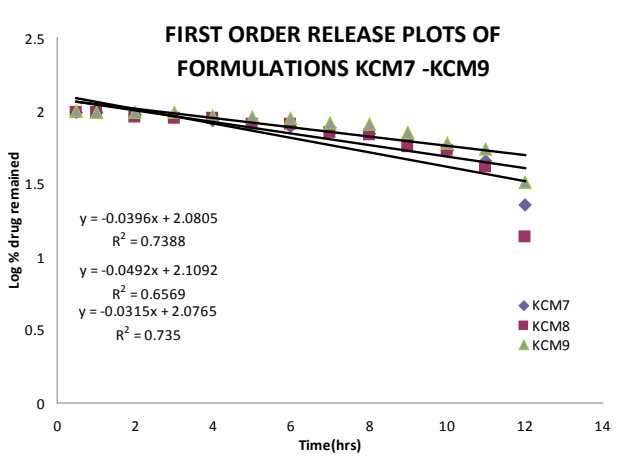

Figure 13: First order release plots of KCM7-KCM9 formulations.

Maximum regression was found in zero order release from the kinetic data from the data it can be concluded that KPN5 is the best formulation that showed a release of 92.23% in 12 hrs with guar gum and xanthan gum polymers in the ratio of 5% and 5% (Figures 15-20).

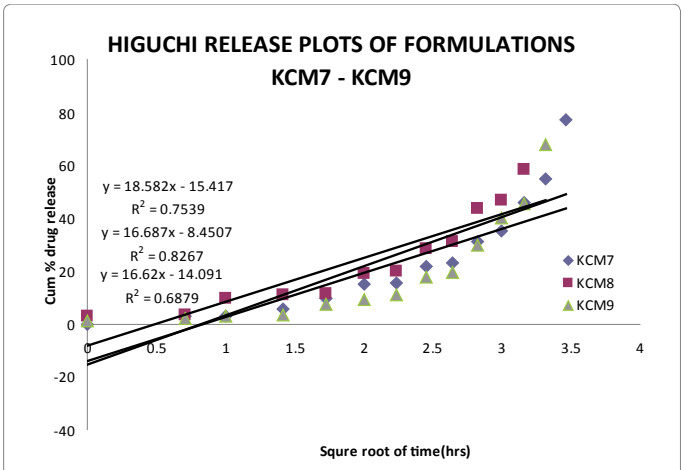

Figure 14: Higuchi release plots of KCM7-KCM9 formulations.

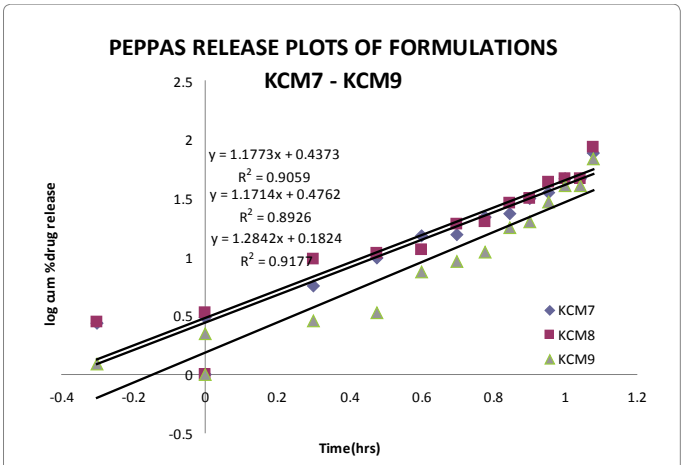

Figure 15: Peppas release plots of KCM7-KCM9 formulations.

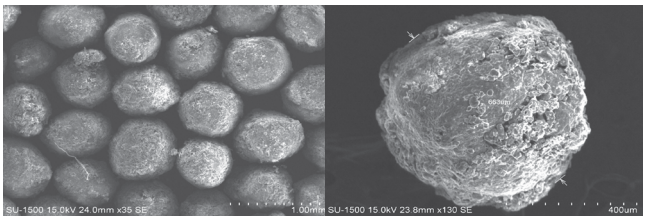

Figure 16: SEM pictograms depicting the size and shape of microspheres.

Discussion

The prepared ketoprofen formulations were found with desirable physical properties and, release parameters are also found in acceptable range where the release followed zero order case II super transport. Data of statistical analysis stated that the formulations with much percentage of polymer retarded and even the mucoadhesive property was also found more, that to with Xanthan gum (Figures 21-24).

The ANOVA analysis for %CDR 5 hrs revealed that in linear vs. mean the coefficient was found to be suggested with an F value of

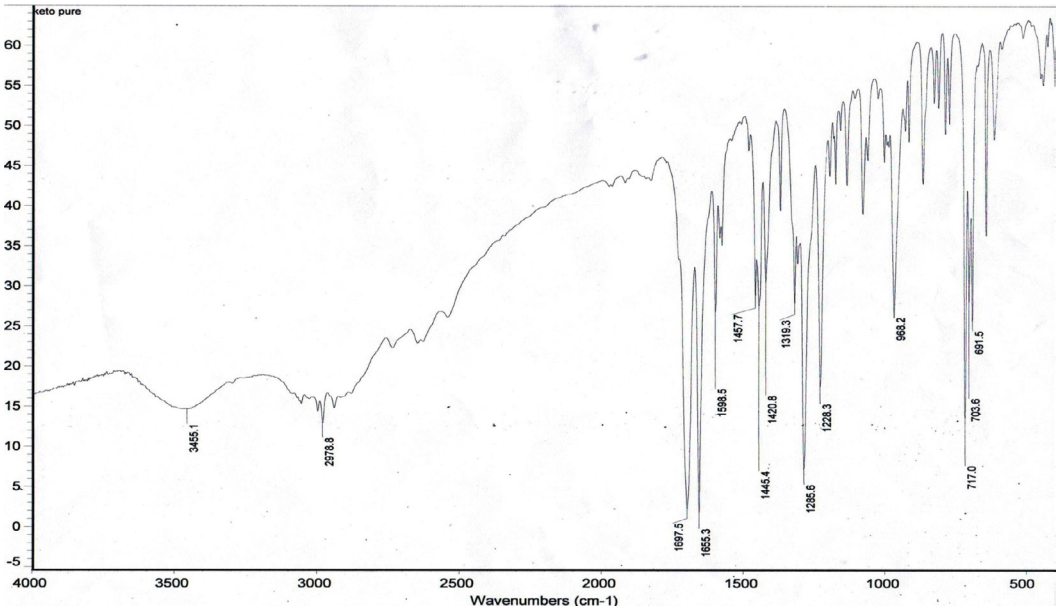

Figure 17: IR Spectra of pure drug Ketoprofen.

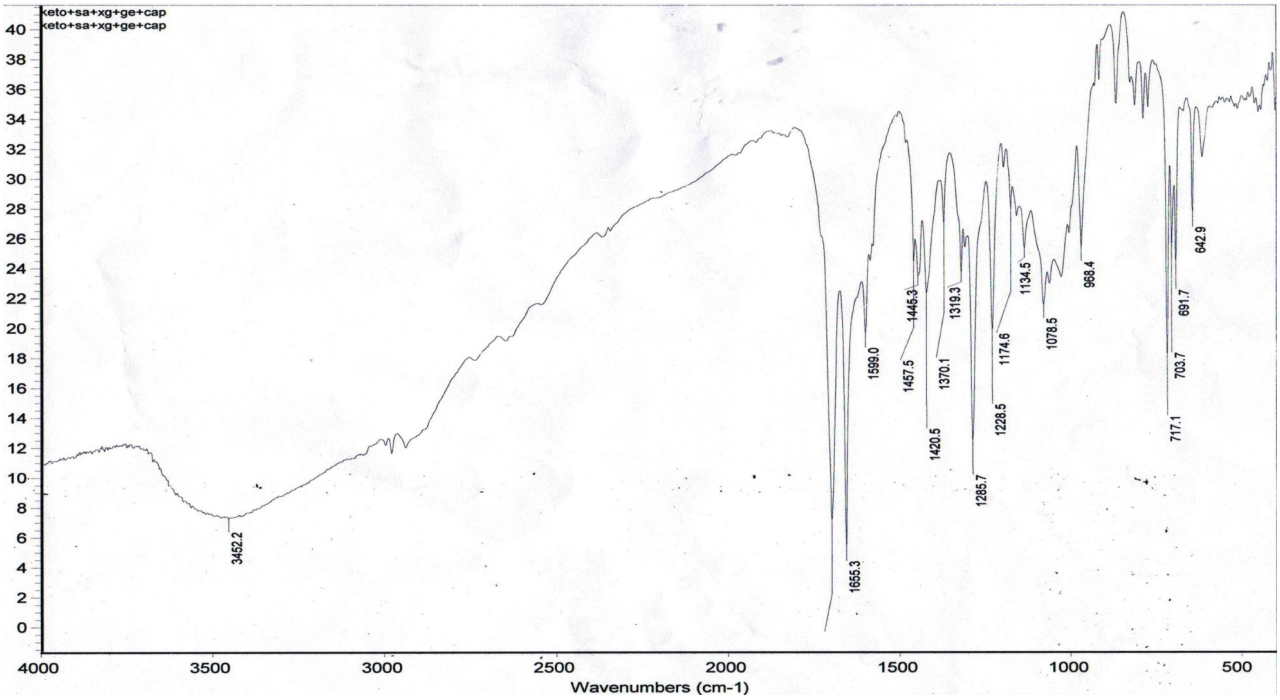

Figure 18: IR spectra of ketoprofen formulation.

6.46159 ($p<0.0319$), in model [45-50] found not significant F value 1.941927 ($p=0.03103$) and % CDR 10 hrs revealed a suggested F value of 2.81281 ($p<0.1375$), in model F value 0.797702 ($p<0.6161$) was found not significant. Positive effect was also observed in release rate constant with increase in Xanthan gum and guar gum and showed linear vs. mean coefficient was d with an suggested F value of 1.60855 ($p<0.0.2758$) and in model F value 9.0162 ($p>0.050$) found to aliased and insignificant

Conclusion

In the present investigation Mucoadhesive colon targeted microspheres were prepared with guar gum and Xanthan gum. Ketoprofen may cause gastric irritation so this was developed as mucoadhesive colon targeted system hence these systems are useful in the improving the absorption and bioavailability of the drug. From the findings obtained, it can be concluded that:

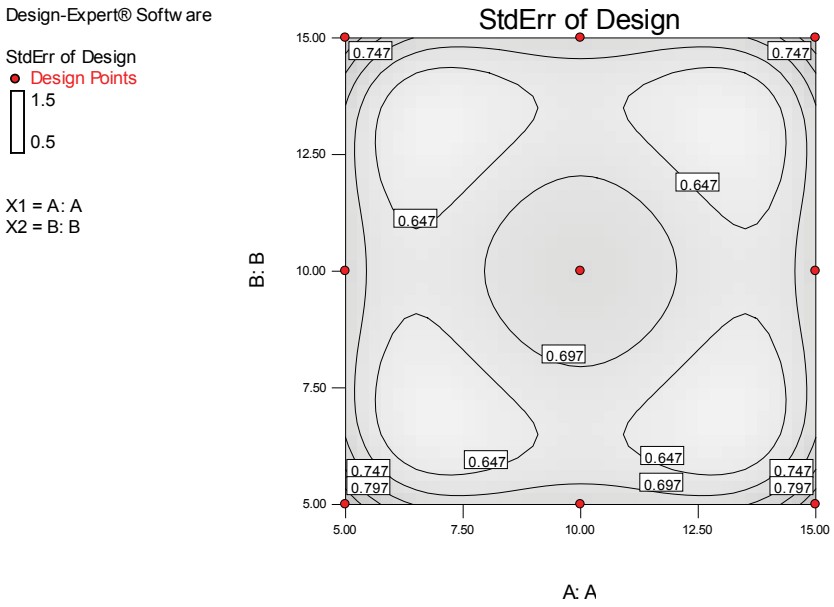

Figure 19: Contour graph of std. error of design (3^2 factorial).

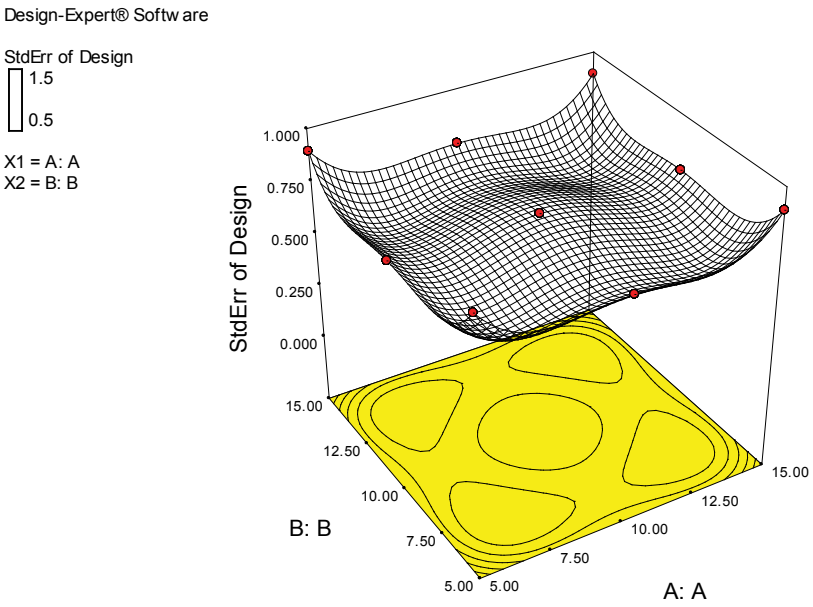

Figure 20: Response surface plot for influence of polymers on % drug release at time 5 hrs.

Mucoadhesive colon targeted microspheres of ketoprofen could be formulated as an approach to improve its bioavailability.

- The flow properties of the polymers and drug were determined and found acceptable.
- Formulated microspheres gave satisfactory results for various physicochemical evaluations for flow property, bulk density and tapped density, *in vitro* wash off test found in acceptable range.
- FT-IR studies revealed that there was no chemical interaction between ketoprofen and the polymers used in the study.
- The dissolution profiles for ketoprofen made with guar gum, xanthan gum, showed that the use of these polymers permit efficient control of the release of the drug.
- The microspheres made with lower polymer content have faster dissolution rates, thus increasing the dissolution of drug.
- The formulation KPN5 showed 92.23% in 12 hrs. So from the *in vitro* dissolution profile it can be concluded that KPN5 is best formulation.
- From the kinetic data maximum regression was observed in zero order.

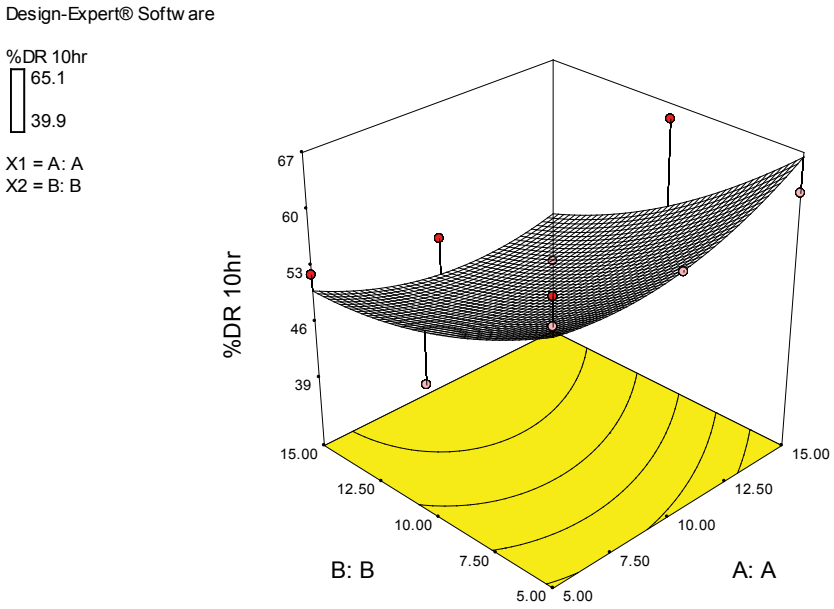

Figure 21: Response Surface plot for influence of polymers on % drug release at time 10 hrs.

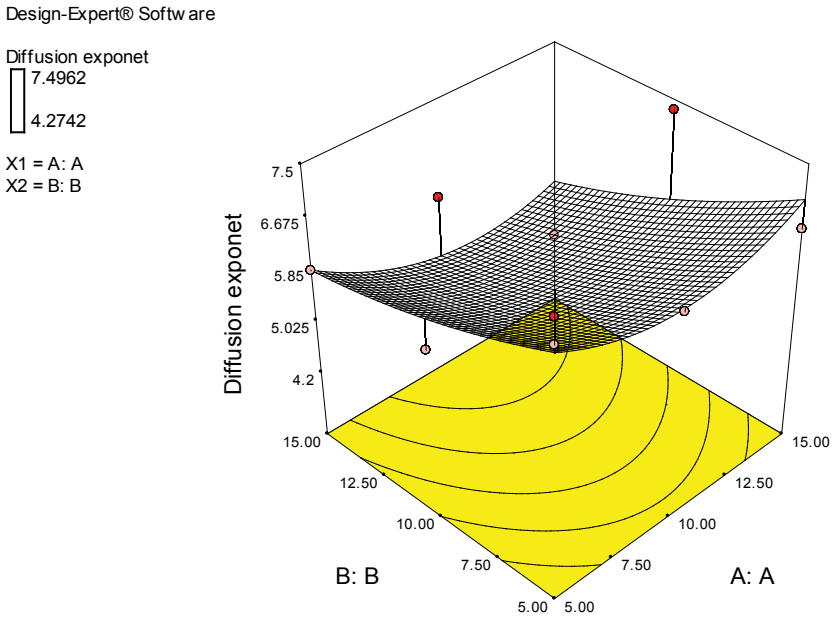

Figure 22: Response surface plot for influence of polymers on final diffusion coefficient.

- In Peppas release 'n' is greater than >1 so, it can be concluded that the mechanism followed is super case II transport.
- The ANOVA analysis for %CDR 5 hrs revealed that in linear vs. mean the coefficient was found to be suggested with an F value of 6.46159 ($p<0.0319$), in model found not significant F value 1.941927 ($p=0.03103$) and % CDR 10 hrs revealed a suggested F value of 2.81281 ($p<0.1375$), in model F value 0.797702 ($p<0.6161$) was found not significant.

Positive effect was also observed in release rate constant with increase in Xanthan gum and guar gum and showed linear vs. mean coefficient was d with an suggested F value of 1.60855 ($p<0.0.2758$) and in model F value 9.0162 ($p>0.050$) found to aliased and insignificant.

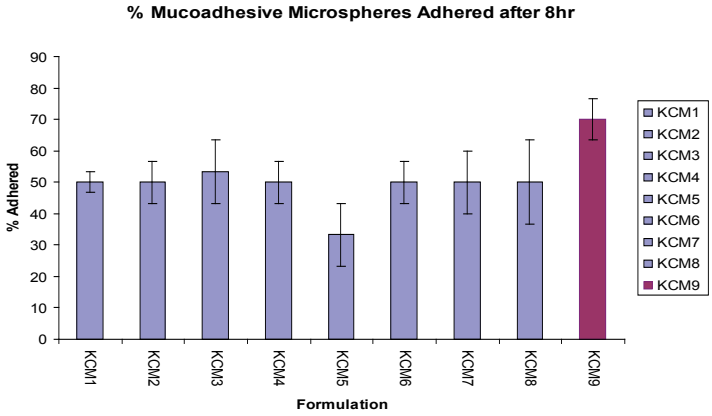

Figure 23: *In vitro* mucoadhesive test results of formulations KCM_1-KCM_9.

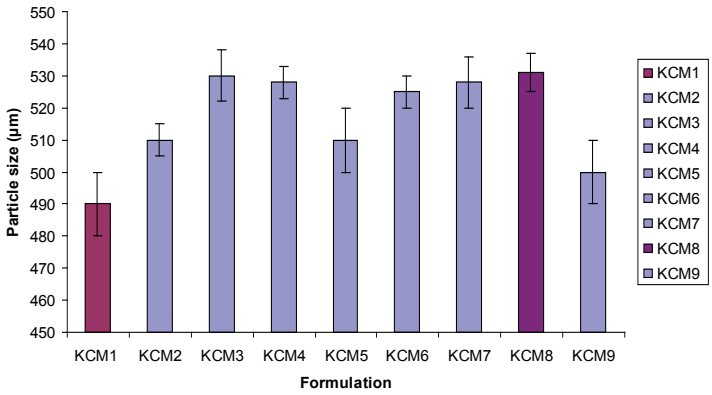

Figure 24: Average particle size for formulations KCM_1-KCM_9.

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