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FORMULATION AND IN-VITRO EVALUATION OF 4-(Dipropylsulfamoyl) benzoic acid INTO EXTENDED RELEASE MICROBEADS.

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ABSTRACT In the present research work, drug PRBENECID an anti-gout drug called as a prototypical URICOSURIC agent was selected for preparation of controlled release microspheres. Polymers sodium alginate and Methocel K100 was used as release retarding agents and to prolong the release of drug in a predetermined time rate of release. To attain the objectives of present work an attempt of formulation was made from trial T1-T5 using sodium alginate and from trial T6-T10 using Methocel K100. Micrometric properties bulk density, tapped density, angle of repose and Hausner's ratio was found to be satisfactory in trial T5 with sodium alginate and more in T10 with Methocel K100 as T5-1.35, 0.76, 29.46 and 1.03: T10- 1.83, 0.59, 35.65 and 1.21. Comparative drug release was performed from T1-T5 and T6-T10 of which trial T10 was optimized based on drug release and rate of release of the drug from the microspheres were determined by placing the values in various kinetic models and the rate of release was conformed based on the regression coefficient R2 value of various kinetic models as to be followed in the order of 0.969 KORESMEYER PEPPAS PLOT>0.887 HIGUCHIS MODEL>0.886 FIRST ORDER>0.796 ZERO ORDER. Base on the R2 value the release of PROBENECID was following KORESMEYER PEPPAS PLOT MODEL with R2 0.969.

KEYWORDS : Probenecid, Uricosuric, Methocel K100

Introduction:

Oral drug release is for the most part ideal and appropriate preference since the oral means offer highest active surface area amongst the entire drug release method for administration of a range of drugs. The magnetism of these dosage forms is owing to knowledge to toxicity and uselessness of drugs once administered by oral common technique in the type of pills as well as capsules. Generally usual dosage form produces extensive sort of difference in drug concentration within the blood flow in addition to tissues by resulting unwanted toxicity and poor efficacy.[1]

More than the last 30 years, since the outlay and complications involved in marketing new drug entities have improved, with instantaneous recognition of the therapeutic advantages of controlled drug delivery, larger consideration is being given on progress of oral controlled discharge drug release methods. The purpose in making controlled discharge drug release method is to lessen the dependability of the dose, dropping the dose and providing smooth drug delivery. Subsequently, controlled discharge dosage type is a dosage type that releases single or many drugs constantly in preset model for a predetermined phase of time, moreover systemically or locally to particular target organ. Controlled discharge dosage type offer improved management of plasma drug levels, fewer dosage frequency, a reduced amount of side effects increased effectiveness and steady delivery.[2]

Controlled release method means any drug delivery system that maintains sufficient and preferred discharge of drug above an extensive phase of time. Hydrophilic polymer matrix is broadly used in support of formulating a controlled dosage form. The function of perfect drug delivery method is to offer correct quantity of drug at expected time period as well as at exact site of action to prolong therapeutic range of drug inside blood plasma.[3]

Microbeads (or) Microspheres Definition:[4]

"Microbeads or Microspheres are defined as firm sphere-shaped elements containing dispersed drug in either solution or micro-crystalline type".

Micro beads or Microspheres are tiny sphere-shaped elements, with diameters within the micrometer range (on average 1 µm to 1000 µm). Microspheres are at times referred to micro- particles and micro beads. Microspheres are typically literately flowing powders containing proteins or artificial polymers. The series of methods for making microspheres present a range of chances to manage characteristics of drug administration as well as boost the therapeutic effectiveness of a specified drug. Microspheres have played an extremely crucial part in the progress of controlled/ sustained discharge drug release systems.

Material and Methods:

Active Pharmaceutical ingredient PROBENCIDE was supplied from INTAS Pharma limited as a gift sample. All the other inactive ingredients were purchased locally form SD fine chemicals, Hyderabad.

Methodology:

Preformulation studies:

Determination of Melting Point:[5]

Melting point of the Probenced was determined by using open capillary tube technique in digital melting point apparatus.

Method: In this method, the capillary tube is closed by gently heating from one end. Then the little amount of the drug Probenced was filled into the sealed capillary tube. Then this tube was tied to the tube having the oil phase in such that the sealed part of the capillary containing the drug was dipped into the oil. Gently the oil bath was heated. When powder starts melting, the heating was stopped and the temperature is noted down at which the drug melts starts melting.

Determination of Partition Coefficient:[6]

The partition coefficient of the drug Probenced was known by using equal volumes of 1-octanol and aqueous solution in a separating funnel.

For water soluble drugs, drug solution was prepared in distilled water, and for water insoluble drugs, drug solution was prepared using 1-octanol.

1-octanol (100 ml) is added to the equal volume of the drug solution

prepared in separating funnel by using distilled water and the solutions were allowed to separate with shaking at irregular intervals. Then the drug solution was separated and assayed for drug content.

$$Partition\ Coefficient = \frac{Concentration\ of\ drug\ in\ organic\ phase}{Concentration\ of\ drug\ in\ aqueous\ phase}$$

Determination of Drug Excipients Compatibility:[7]

During the preparation of patch formulation, drug and polymers interact when they in contact with each other, which may cause instability of the drug.

FT-IR spectroscopy is employed to confirm the compatibility between the polymer and Probenced. The pure drug and drug with all the excipients are scanned separately.

KBr Pellet method is used and the samples were mixed with dry powder KBr crystals. The blend was compacted to make a disc. This disc was kept in FTIR and spectrum was recorded.

Chemical contact among drug and polymers was found by using the FT-IR spectra.

UV SPECTROSCOPIC METHOD: 8

Preparation of stock solution:

From the stock course of action B, advance weakening were made with methanol in 10ml test tube to get the game plans in the extent of 2-10µg/ml center and absorbance was recorded at 247nm against sensible clear using UV-Spectrophotometer. Modification twist of absorbance against obsession was plotted.

Table no 1: concentration and absorbance of probenced

S. No.	Concentration	Absorbance
1	0	0
2	2	0.193
3	4	0.394
4	6	0.631
5	8	0.841

Formulation of Probenced Microspheres:

Table no 2: Formulation microspheres from T1-T5

Ingredients	F1	F2	F3	F4	F5
Sodium alginate	400	375	350	325	300
Drug	40	40	40	40	40
Water	20	20	20	20	20
$CaCl_2$%	5	5	5	5	5
Total Weight	460	435	410	385	360

Table no 3: Formulation Microspheres from T6-T10

Ingredients	F6	F7	F8	F9	F10
Sodium alginate	400	375	350	325	300
Drug	40	40	40	40	40
Methocel k 100	15	30	45	60	75
Water	20	20	20	20	20
$CaCl_2$ %	5	5	5	5	5
Total Weight	475	465	455	445	435

In-Vitro Evaluation Parameters:[9]

Bulk density- It is the ratio of the overall mass of powder to the majority quantity of powder. It's far measured by way of pouring the weighed powder in measuring cylinder and initial weight was stated. This preliminary volume is called as the majority quantity.

Db = M/Vb
In which M = mass of powder
Vb = bulk extent of powder.

The angle of repose
It's far the maximum perspective viable among the floor of the pile of powder and the horizontal aircraft. The microspheres have been allowed to float thru the funnel constant to a stand at specific peak. The attitude of repose becomes then calculated by using measuring the height and radius of the heap of microspheres shaped. Care turned into taken to see that the microspheres align and roll over each different via the edges of the funnel.

It is given through - Tan= h/r

o= 〖tan〗 ^(-1) h/r.
Where o = angle of repose;
h=height in cm and r = radius in cm

Content uniformity

Microspheres with pre determined weight from each batch were taken and weight equivalent to10mg & transfer to a 250 ml volumetric hip flask with 0.1N HCl. The quantity was then set up to the blotch with 0.1N HCl. The solution was filtered and the filtrate was sufficiently diluted and the absorbance was recorded against the blank at 247 nm. The drug content of the Standard containing the drug powder was also determined.

In-Vitro Drug Release Studies

The release rate of (Probenced) drug from the polymeric microspheres was determined using The United States Pharmacopoeia (USP) XXIV dissolution testing apparatus II (paddle method). The dissolution test was performed using 900 ml of 0.1 N HCl, at 37 ± 0.5°C with 50 rpm. An appetizer (5 ml) of the solution is introverted from the dissolution apparatus hourly for 8 hours, and the samples were replaced with fresh dissolution medium. The sample is diluted to an appropriate concentration by 0.1N HCl. Absorbance of the following solutions is calculated at 247 nm by means of a UV-Visible spectrophotometer. Increasing percentage of drug release was considered using the equation obtained from a standard curve.

Determination of release rate kinetics:

Drug release from optimized trial was interpreted into to various kinetic models, Zero order, first order, Higuchis plot and Koresmeyer Peppas plot for determination of rate release kinetics.

Stability studies:

Optimized trial will be allowed to keep in force degradation studies or accelerated stability studies at 60oC and 60%RH, for period of one month.

Results and Discussion:

Analytical method for probenced:

Calibration curve:

Different concentrations of probenced in 0.1N HCL was prepared and absorbance's were determined at 247 nm calibrations curve was drawn with absorbance values on y-axis and concentrations on x-axis where R2 value was found to be 0.998.

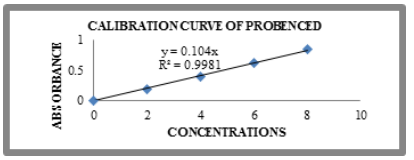

Figure no 1: Calibration curve of probenced in 0.1N HCL

FTIR STUDIES:

FTIR of PROBENECID:

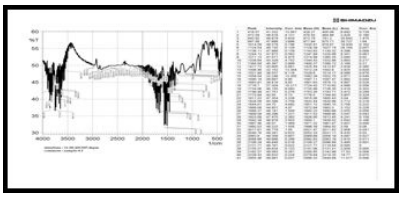

Figure no 2: FTIR Spectra of pure Probenecid

FTIR of spectra of sodium alginate:

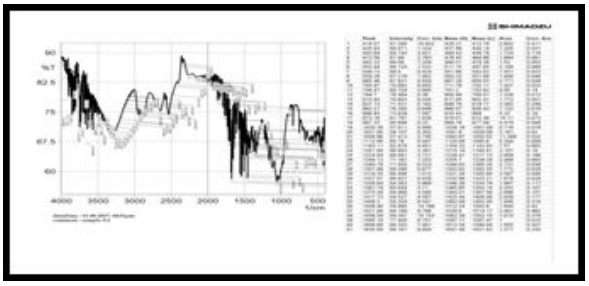

Figure no 4: FTIR Spectra of Methocel K 100

FTIR Spectra optimized trial T10:

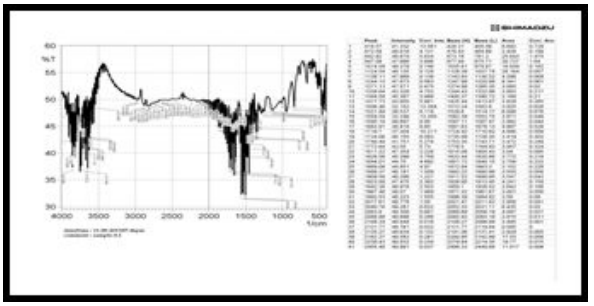

Figure no 5: FTIR Spectra of optimized formulation trial T10

Micromeritic properties:

The properties like compressibility index, angle of repose and Hauser ratio were calculated.

Micromeritic properties of microspheres:

Table no 4: physical characteristic of microspheres form trial T-1 to T-5

Trials	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Compress ability index	Hausner's ratio
T-1	1.14	0.15	[illegible]	43.29	2.19
T-2	1.62	0.53	41.46	34.43	2.34
T-3	0.76	0.48	39.84	31.45	2.18
T-4	1.76	0.68	33.29	36.54	1.39
T-5	1.35	0.76	29.46	26.49	1.03

Table no 5: physical characteristic of microspheres form trial T-6 to T-10

Trials	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Compress Ability index	Hausner's ratio
T-6	1.67	0.37	41.28	35.93	1.38
T -7	1.32	0.65	32.38	37.31	1.43
T-8	1.53	0.66	37.38	29.03	2.48
T-9	1.85	0.65	35.43	36.76	2.15
T-10	1.83	0.59	35.65	34.37	1.21

Graphical representation:

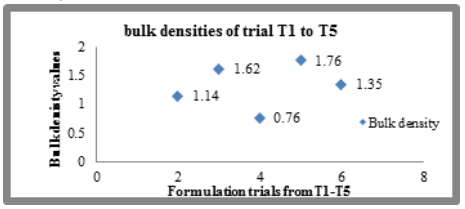

Figure no 6: bulk densities from trial T1-T5

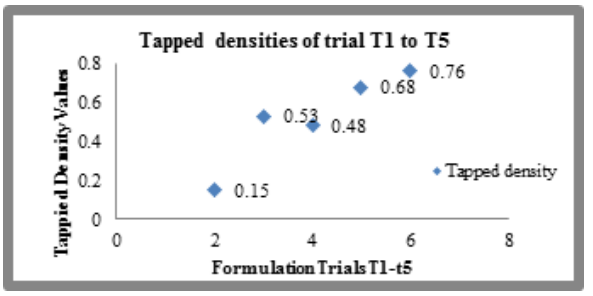

Figure no 7: Tapped densities from trial T1-T5

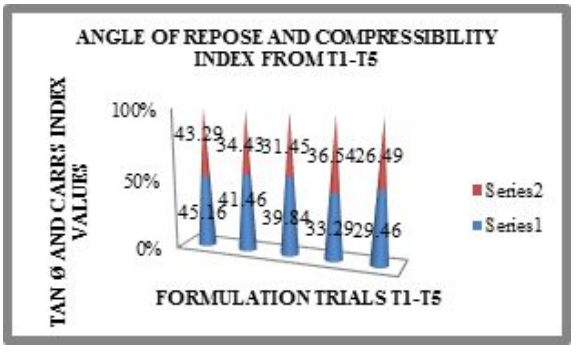

Figure no 8: angle of repose and cars index of trial T1-T10

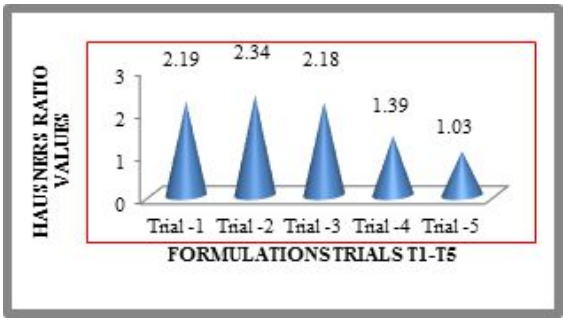

Figure no 9: Hausner's values from trial T1-T5

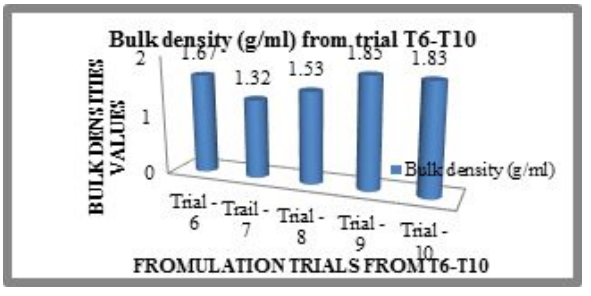

Figure no 10: Bulk densities from trial T6-T10

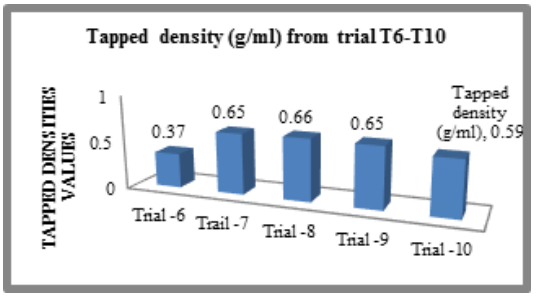

Figure no 11: Tapped densities from trial T6-10

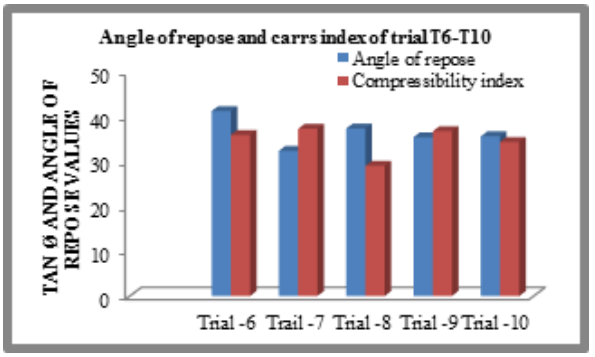

Figure no 12: Angle of repose and cars index T6-T10

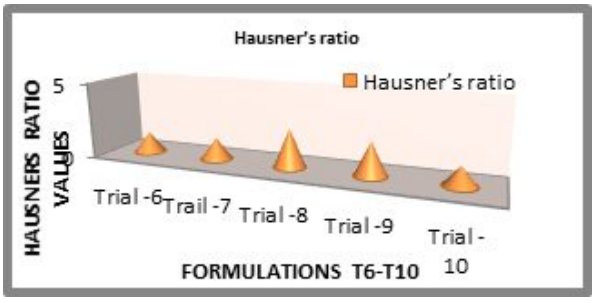

Figure no 13: Hausner's values from trial T6-T10

Practical yield:

Table no 6: Percentage yield from trials T1-T5

Trials	F1	F2	F3	F4	F5
Theoretical Weight	460	435	410	385	360
Practical Weight	480	447	421	411	381
Percent Yield	95.83	97.31	97.38	93.67	94.48

Table no 7: Percentage yield from trials T6-T10

Trials	F6	F7	F8	F9	F10
Theoretical Weight	475	465	455	445	435
Practical Weight	494	488	472	482	452
Percent Yield	96.15	95.28	96.39	92.32	96.23

Content uniformity:

Table no 8: Content uniformity from trials T1-T10

TRIALS	TCU % yield with practical yield	practical yield Mg.	CU in half practical yield	CU in total yield
F1	41.74	240	15.49	29.69
F2	41.10	223.5	19.01	37.01
F3	41.07	210.5	17.61	34.29
F4	42.70	205.5	19.01	35.62
F5	42.33	190.5	21.48	40.59
F6	41.60	247	17.25	33.18
F7	41.98	244	12.32	23.49
F8	41.49	236	12.68	24.44
F9	43.33	241	17.25	31.86
F10	41.56	226	21.13	40.66

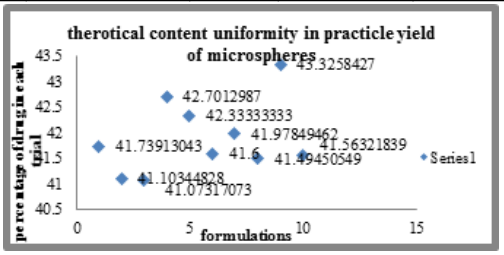

Figure no 14: theoretical content uniformity in practical yield of microspheres form trial T1-T10

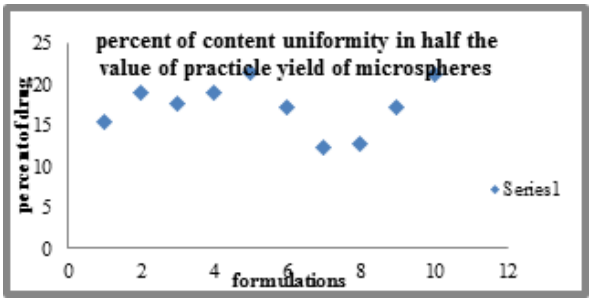

Figure no 15: Percent of drug in 50 percent of each trials form T1-T10

In-Vitro Drug Release Studies:

Probenecid Release Form Trial T1-T5:

Table no 9: Drug release profile from trial T1-T10

TIME-M	T-1	T-2	T-3	T-4	T-5
0	0	0	0	0	0
30	8.86	3.52	4.70	2.53	1.71
60	9.94	4.36	5.36	3.21	3.08
120	10.37	14.75	9.73	15.35	9.28
180	9.07	16.26	13.33	6.41	10.29
240	30.24	23.47	18.29	2.96	11.50
300	20.52	15.92	18.95	7.95	13.26
360	2.48	1.93	19.60	18.50	14.77
420	2.92	2.26	6.40	10.97	14.02

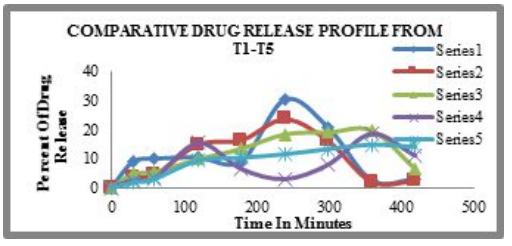

Figure no 16: Comparative drug release form trial T1-T5

PROBENCED RELEASE FORM TRIAL T6-T10:

Table no 10: Drug release profile form trials T6-T10

TIME-M	T-6	T-7	T-8	T-9	T-10
0	0	0	0	0	0
30	2.43	1.95	1.64	1.58	3.43
60	8.18	6.59	5.55	11.90	7.55
120	10.51	10.09	8.50	12.75	11.99
180	11.52	10.90	9.18	13.41	13.06
240	14.95	12.04	10.14	14.13	14.72
300	16.47	13.26	11.17	10.71	15.15
360	19.50	15.70	13.22	12.69	15.68
420	7.88	6.34	5.34	5.13	16.10

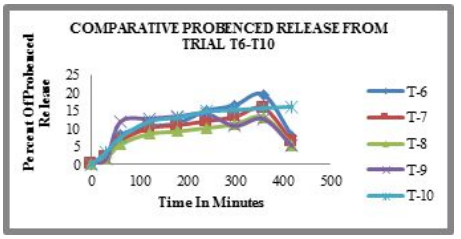

Figure no 17: Comparative release profile from trial T6-T10

Optimization and one month stability studies of formulation

trial T10:

Based on the Micromeritic properties, drug content uniformity and in-vitro release rate studies formulation trial T10 was optimized accelerated stability studies for formulation trial T10 was carried out and the in-vitro results are given below.

In-vitro drug release studies:

Table no 11: release of drug from optimized trial T10

Time in minutes	Percent of drug release
0	0
30	3.343922
60	7.367078
120	11.70373
180	12.7487
240	14.36842
300	14.78641
360	15.30889
420	15.57014

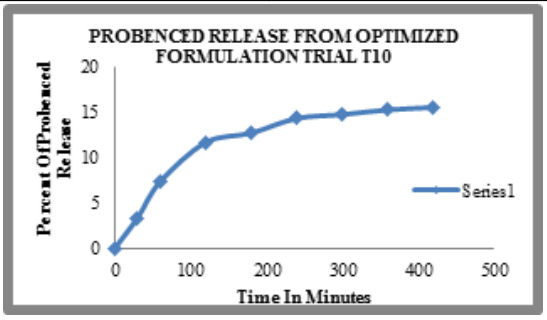

Figure no 18: Graphical representation of probenced release from optimized trial T10

Determination of Release Rate Kinetics:

Table no 12: Drug release rate kinetic parameters for optimized trial T10

ZERO ORDER		FIRST ORDER		HIGUCHIS MODEL		KORESMEYER PEPPAS PLOT	
Time	% drug undissolved	time	log 100-Q	sq. time	mean % drug dissolved	log time	log cumulative % drug dissolved
0	100	0	2	0	0	0	0
30	96.57	30	1.98	5.48	3.43	1.48	0.53
60	92.45	60	1.97	7.75	7.55	1.78	0.88
120	88.01	120	1.94	10.95	11.99	2.08	1.08
180	86.94	180	1.94	13.42	13.06	2.26	1.12
240	85.28	240	1.93	15.49	14.72	2.38	1.17
300	84.85	300	1.93	17.32	15.15	2.48	1.18
360	84.32	360	1.93	18.97	15.68	2.56	1.20
420	83.9	420	1.92	20.49	16.10	2.62	1.21

Graphical Representation:

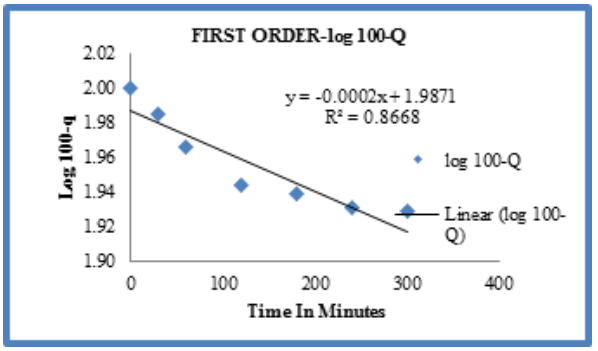

Figure no 19: First order rate kinetics for T10

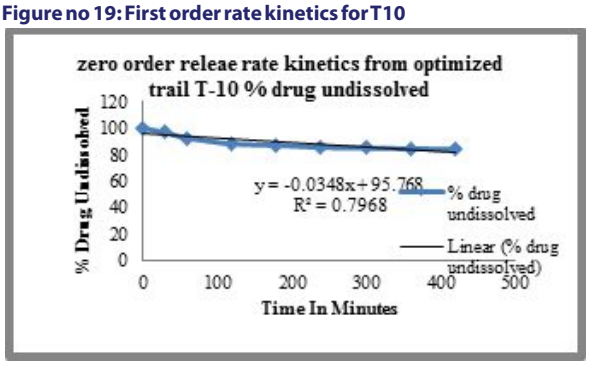

Figure no 20: Zero order kinetics of T10

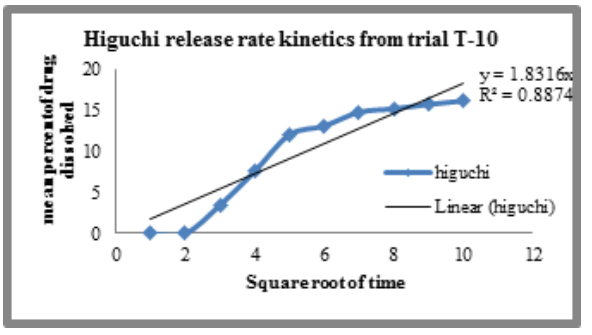

Figure no 21: Higuchis plot for trial T10

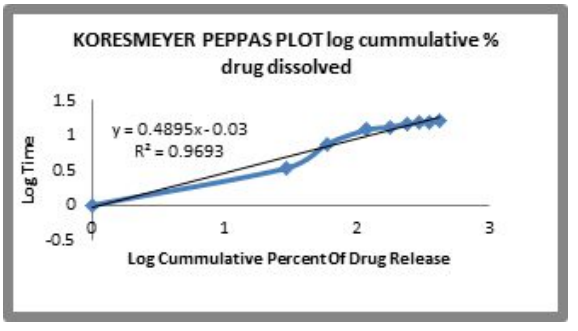

Figure no 22: Koresmeyer Peppas plot for trial T10

Based on the review of literature and FTIR compatibility studies API, sodium alginate and Methocel K100 was taken for formulation of PROBENCED MICBEADS/MICROSPHERES.

Using sodium alginate formulation trials from T1 to T5 were performed, from trial T1 to T5 the percent practical yield, content uniformity are given in table no 6 and similarly in table 7 using Methocel K100 formulation trials from T6-T10 were carried out by varying the concentration of polymers based on the drug entrapment and drug release studies, and the results of percent practical yield and content uniformity are given in respective table 8 Release rate kinetics were also determined to formulation trial T10 and the release rate of the drug was determined based on the regression coefficient R2value and release of PROBENCED was to be followed in the order of 0.969 KORESMEYER PEPPAS PLOT>0.887 HIGUCHIS MODEL>0.886 FIRST ORDER>0.796 ZERO ORDER.

Conclusion:

In the present investigation, preparation, characterization and drug release studies were performed using sodium alginate and Methocel K100.

Formulation trial T5 was optimized using sodium alginate and formulation trial T10 was optimized using Methocel K100,

Formulation trial T10 with Methocel was taken for optimization and

stability studies as it has given better results in percent of practical yield, content uniformity and constant and extended drug release studies and the results after one month stability studies were proven to be satisfactory.

Determination of release rate kinetics were also performed and based on the regression coefficient R2value, probenced release rate fallows KORESMEYER-PEPPAS PLOT (R2= 0.969)

Acknowledgement:
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