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PREPARATION, CHARACTERIZATION AND IN-VITRO EVALUATION OF PROBENECID: A PROTOTYPICAL URICOSURIC AGENT IN TO EXTENDED RELEASE MICROSPHERES

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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 R^2 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 R^2 value the release of PROBENECID was following KORESMEYER PEPPAS PLOT MODEL with R^2 0.969.

Key Words: ***Probenecid, Uricosuric, Methocel K100***

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INTRODUCTION [1]:

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.

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.

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 [2].

Advantages:

1. Reduced dosing frequency. ·
2. Dose reduction.
3. Improved patient compliance.
4. Constant intensity of drug concentration within blood plasma.
5. Reduced toxicity owing to overdose.
6. Reduces the changeability of peak valley concentration.
7. Night time dosing is able to be avoided.

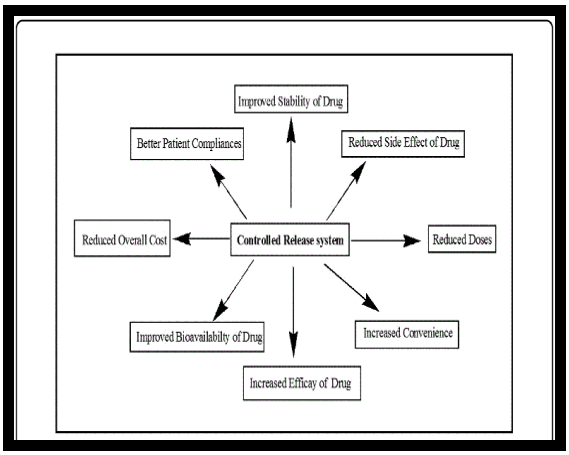


Fig 1: Advantages of CDDS

Factors disturbing the Formulation of Oral controlled discharge Drug release system: [3]

Physicochemical factors

Aqueous Solubility

The majority of the drugs are weak acids or weak bases. Drugs with little aqueous solubility will be complicated to include into the sustained discharge method. In favor of a drug with elevated solubility and quick dissolution rate, it is often quite complex to delay its dissolution rate. A drug of high water solubility can disintegrate in water or gastrointestinal fluid readily and tends to release its dosage form in a split open and as a result is absorbed rapidly leading to a quick raise within the blood drug concentration compared to a lesser amount of soluble drug. It is often not easy to include a extremely aqueous soluble drug in the dosage form and delay the drug release especially when the dose is high.

Partition coefficient (P(o/w))

Partition coefficient is known as the portion of drug in an oil phase to that of an adjoining aqueous phase. Drugs that passes all the way through biological membrane, if partition co-efficient of drug shows extremely a lot bioavailability for the reason that lipophilic character of biological membrane. Drugs that have lesser partition coefficient are not appropriate for oral CR drug liberation method and drugs that have elevated partition co-efficient are as well not appropriate for oral CR drug release system as they will not separate out of the lipid casing one time it gets in the membrane.

Drug pKa and ionization at physiological pH

Drugs presented for the most part in ionized form are deprived candidates for oral controlled discharge

drug release method. Absorption of the unionized drugs are fine while penetration of ionized drug is insignificant as the absorption time of ionized drug is 3-4 times not as much as that of the unionized drug. The pKa variety for acidic drug whose ionization is pH responsive is around 3.0-7.5 and pKa array for basic drug whose ionization is pH aware is approximately 7.0-11.0 are idyllic for most favorable optimistic absorption.

Drug stability:
Drugs experience both acid/base hydrolysis along with enzymatic degradation once administered by oral means. If the drug is in the solid condition, the degradation will take place in reduced rate, for the drugs that are unbalanced in stomach that extend delivery to the whole GI tract are advantageous. If drug is taken in extended discharge dosage type that are not stable in small intestine possibly will express decreased bioavailability.

Molecular size and diffusivity
Diffusivity depends on dimension and figure of the hollow spaces of the covering. The diffusion co-efficient of intermediary molecular mass drug is 100-400 Daltons; in the course of elastic polymer range is 10-6-10-9cm^2/sec. For drugs having molecular weight > 500 Daltons, the diffusion coefficient in a number of polymers are extremely fewer i.e. less than 10-12cm2/sec. The examples of drugs which are complicated to control discharge rate of medicament from dosage type are proteins and peptides.

Biological Factor
The absorption of a drug can influence its appropriateness as an extended discharge product. The intend of formulating controlled discharge product is to lay a control on the delivery method. It is necessary that the rate of release is a lot slower than the rate of absorption. If we presume the transit time of dosage type in the absorptive region of GI tract is 8-12 hours, the highest half-life for absorption be supposed to be approximately 3-4 hours or else the dosage form will surpass the absorptive area prior to drug discharge is over.
Half-life

The half-life of a drug is an sign of its habitation time in the body. If the drug has small half life (below 2 hours) the dosage type may possibly include a prohibitively great amount of the drug. In contrast, drug with elimination half-life of 8 hours or extra are adequately controlled within the body, when taken in usual dosage from and controlled discharge drug release system is in general not required in such cases. Preferably, the drug ought to have half-life of 3-4 hours for formulation of drug delivery system.

Therapeutic indicator
Medicines with small therapeutic index are inappropriate for inclusion in controlled discharge formulations. If the technique be unsuccessful in the body, dose dumping might take place, which leads to toxicity.

Size of dose
If the amount of a drug in the usual dosage type is high, then it is not as much of appropriate candidates for CRDDS. This is for the reason that the size of a unit dose controlled discharge oral formulation would happen to be too large to administer without trouble.

Absorption window
Several medicines when taken by mouth are absorbed just from a definite part of gastrointestinal tract. This part is known as the 'absorption window'. These nominees are as well not appropriate for CRDDS.

Plasma concentration response link
In general, plasma drug concentration is actually accountable for pharmacological activity relatively than dose. However the drug having pharmacological action free of plasma concentrations, are unfortunate nominee for oral CR drug release system.

Concentration dependence on shift of drug
Shift of drug from one compartment to other, if follow zero order kinetic procedure then those drugs are unfortunate nominee for oral CR delivery system. It ought to be of first order kinetics.

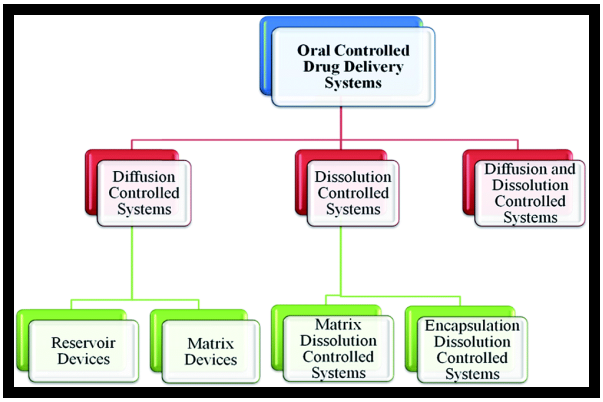


Fig 2: Classification of controlled drug delivery systems

A well planned controlled drug release method can rise above quite a lot of the troubles of conservative treatment as well as develop the therapeutic effectiveness of a known drug. To acquire utmost therapeutic usefulness, it is essential to carry the drug to the marked tissue in the most advantageous

quantity in the right phase of time there by causing small toxicity and negligible side effects. There are a variety of move towards delivering a therapeutic material to the intentional location in a sustained controlled discharge style. One such advance is by using microspheres as transporter for drugs. Microspheres are typically liberated flowing fine particles consisting of protiens or synthetic polymers which are biodegradable in character and preferably having a particle size not as much of as 200 µm.

Microspheres are spherical & free flowing particles in normal particle size of 1 to 50 microns which consist of proteins or artificial polymers. A variety of the troubles to rise above by producing control drug release method which increase the therapeutic effectiveness of a specified medicine. One such advance is by using microspheres as transporter for medicine. The mark location drug conveys with Specificity & keeps up the concentration at location of concern with no problematic effects. It will discover the vital place in original drug release. Drugs can be targeted to precise location in the body by means of microspheres.

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.

Materials used throughout the preparation of Microsphere [5]:

Microspheres are generally made of polymers. Polymers are of two categories:

1. Natural polymers

2. Synthetic polymers

1. Natural polymers are acquired from various sources like carbohydrates, proteins along with chemically modified Carbohydrates.

Carbohydrates: Agarose, Chitosan,Starch
Proteins: Albumin, Collagen and Gelatin

Chemically personalized carbohydrates: Poly dextran, Poly starch.
2. Synthetic polymers are of two kinds.

Biodegradable polymers
E.g. Lactides, Glycolides & their copolymers, Poly anhydrides, Poly alkyl cyano acrylates.

Non-biodegradable polymers
E.g. Poly methyl metha acrylate (PMMA), Glycidyl methacrylate, Acrolein, Epoxy polymers.

Synthetic polymers:
Poly alkyl cyano acrylates is a great medicine transporter for ophthalmic, oral as well as parenteral preparations. Poly lactic acid is a suitable carrier for continuous discharge of anti neoplastic medicines for instance cisplatin, cyclo phosphamide, doxorubicin etc. Co-polymer of poly lactic acid in addition to poly glycolic acid are employed for continuous discharge preparation for anti malarial remedy. They do not involve any opening step as the surface free aldehyde groups on the poly acrolein can counteract with Ammonia group of protein to make Schiff's base.
Natural polymers:

Albumin is extensively spread natural protein .This is known to be possible transporter of drugs or proteins (for their site specific localization). It is broadly utilize for the targeted drug deliverance to the tumour cells in cancer. Gelatin microspheres can be utilized as transporter system competent of conveying the medicines to phagocytes. Starch, is a polysaccharide and have many free hydroxyl groups. Due to these free hydroxyl groups many active agents can be included inside and on surface of microspheres.

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 [6]:
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 [7]:
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.

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

Determination of Drug Excipients Compatibility [8]:
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.

Brilliant perceptible (UV-self-evident) spectroscopy: [9]
Advancement of modification twist of model pharmaceutical by UV-evident spectroscopy:

Availability of standard stock plans
1. Probenced indistinguishable to 50mg was weighed and traded to 100ml volumetric container, separated in 50ml methanol. The occurred plan has the union of 1mg/ml(1000µg/ml) which was set apart as "stock course of action A".

2. From the stock game plan A, 1ml was pipette out in 10ml test tube and the last volume was made upto 10ml with methanol. The came to fruition game plan had the gathering of 0.1mg/ml (1000 stock game plan A, 1ml was pipette out in 10ml test tube and the last volume was made up to 10ml with methanol. The occurred course of action had the gathering of 0.1mg/ml (100µg/ml) which was named as "stock plan B". This stock course of action B is used as working stock response for moreover consider. Encourage weakening were set up from a comparable game plan.

Course of action of standard work plan:
From the stock course of action B, advance weakenings 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 1: concentration and absorbance of probenced Formulation of Probenced Microspheres:

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

Table 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 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

Method followed is phase separation emulsion technique:
1) Initially prepare a polymer solution i.e.,40mg sodium alginate in 20ml water taken in a 50ml beaker.
2) To this slowly add 20mg of drug (probenced).
3) Allow it for stirring, under a mechanical stirrer for at least 10 – 15min.
4) Due to excessive stirring, bubbles will be formed in the solution which can be removed by addition of 5mg S.L.S. under a digital ultra sonicator.
5) Then in another beaker take 5%Cacl2 (i.e.; 5gm in 100ml H2O) and mix it properly to figure a consistent solution.
6) Then with the aid of a needle, add the prepared polymer solution drop wise into the Cacl2 solution.
7) Transparent micro beads can be observed in the cacl2 solution, these micro beads are nothing but the microspheres.
8) Filter the solution to separate the microspheres.
9) The separated microspheres are kept a side till 15-20 min for drying in a tray drier at 60 degrees until all the moisture is evaporated.
10) Add Titanium dioxide to the above formulated microspheres to make them stable for a longer duration.
11) These microspheres are then evaluated and characterized for their quality and in-vitro dissolution studies.

***In-Vitro* Evaluation Parameters [10]:**

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.

Tapped density-

After carrying out the procedure as given in the dimension of bulk density, the cylinder containing the sample is tapped the use of an appropriate mechanical tapped density tester that provides one hundred drops in keeping with minute and this became repeated till distinction among succeeding size is much less than 2% after which tap volume changed into measured to the nearest graduated unit.
The tapped density is expressed in g/ml and is calculated the usage of system

$$D_{\_}(t) = M/Vt$$

Wherein M – mass of powder

Vt- tapped quantity 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

$$\Theta = [\![\ \tan]\!]\ \wedge\ (-1)\ h/r.$$

Where ɵ =angle of repose;

h=height in cm and r = radius in cm

Compressibility Index:

It indicates powder flow properties. It is expressed in % and is given by

$$\frac{D_t - D_b}{D_t} * 100$$

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:
As explained in methodology, different concentrations of probenced in 0.1N HCL was prepared and absorbances were determined at 247 nm calibrations curve was drawn with absorbance values on y-axis and concentrations on x-axis where R^2 value was found to be 0.998.

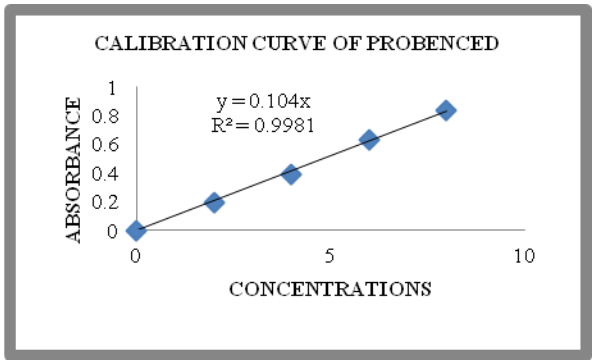
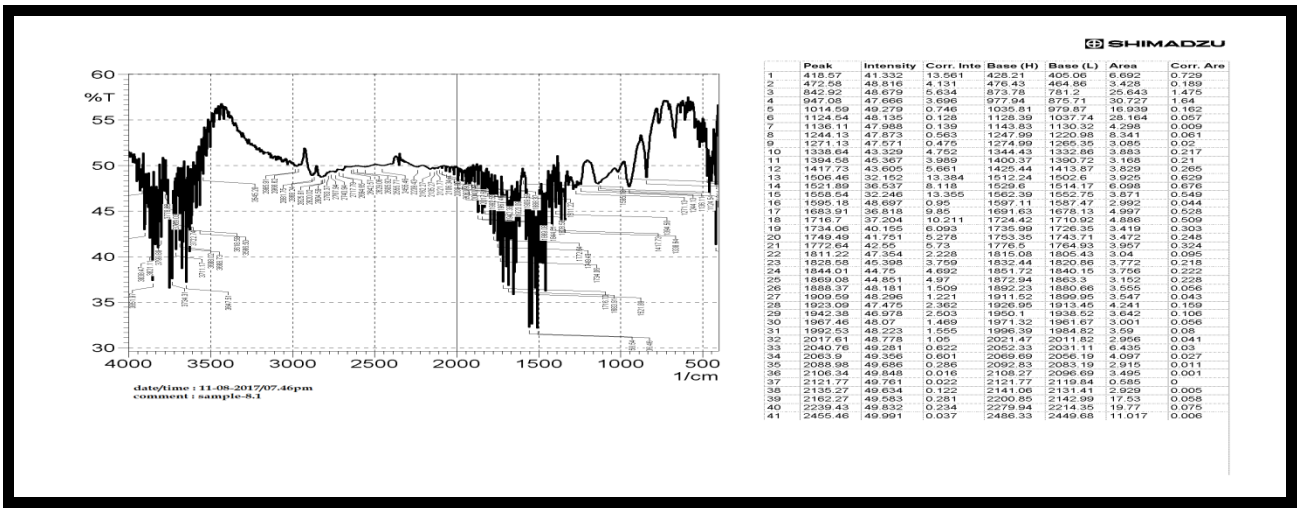


Fig 3: Calibration curve of probenced in 0.1N HCL

FTIR STUDIES:
FTIR compatibility studies were performed between API probenced and polymers like Methocel K100 and sodium alginate, and the compatibility indices were being to be seen ok and no interaction was been found between FTIR spectra of PROBENCED and METHOCEL K100, SODIUM ALGINATE.

The FTIR spectra of PRBENCED, METHOCEL K 100 and SODIUM ALGINATE were given below.

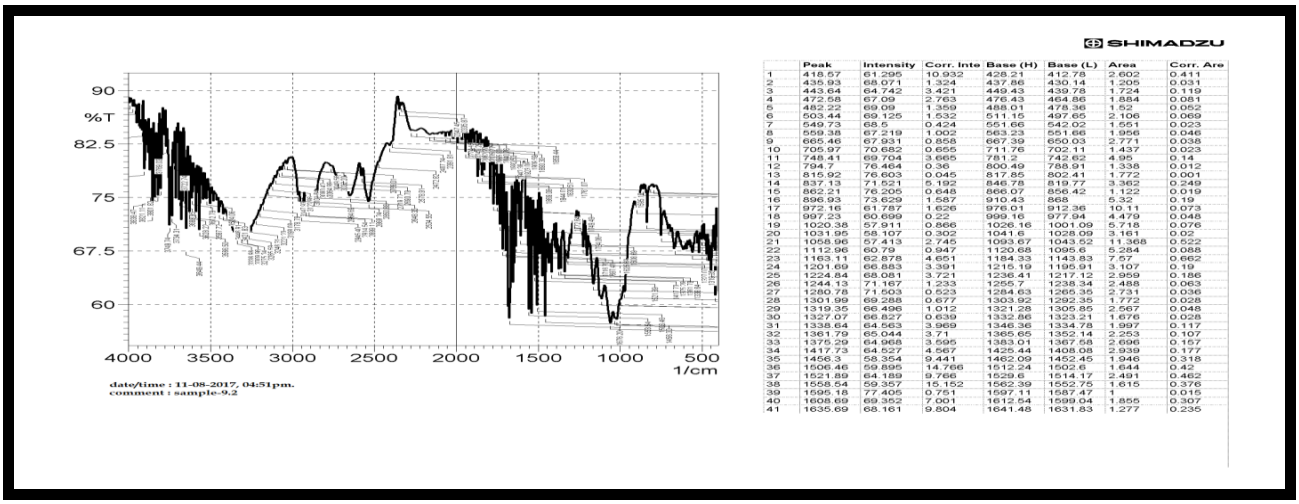
FTIR of PROBENECID:



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	418.57	41.332	13.561	428.21	405.06	6.692	0.729
2	472.58	48.816	4.151	476.43	464.86	3.428	0.189
3	842.92	48.679	5.634	873.78	781.2	25.643	1.475
4	947.08	47.666	3.696	977.94	875.71	30.727	1.64
5	1014.59	49.279	0.746	1035.81	979.87	16.939	0.162
6	1124.54	48.135	0.128	1128.39	1037.74	28.164	0.057
7	1136.11	47.988	0.139	1143.83	1130.32	4.298	0.009
8	1244.13	47.873	0.563	1247.99	1220.98	8.341	0.061
9	1271.13	47.571	0.475	1274.99	1265.35	3.085	0.02
10	1338.64	43.329	4.752	1344.43	1332.86	3.883	0.217
11	1394.58	45.367	3.989	1400.37	1390.72	3.168	0.21
12	1417.73	43.605	5.661	1425.44	1413.87	3.829	0.265
13	1506.46	32.152	13.384	1512.24	1502.6	3.925	0.629
14	1521.89	36.537	8.118	1529.6	1514.17	6.098	0.676
15	1558.54	32.246	13.355	1562.39	1552.75	3.871	0.549
16	1595.18	48.697	0.95	1597.11	1587.47	2.992	0.044
17	1683.91	36.818	9.85	1691.63	1678.13	4.997	0.528
18	1716.7	37.204	10.211	1724.42	1710.92	4.886	0.509
19	1734.06	40.155	6.093	1735.99	1726.35	3.419	0.303
20	1749.49	41.751	5.278	1753.35	1743.71	3.472	0.248
21	1772.64	42.55	5.73	1776.5	1764.93	3.957	0.324
22	1811.22	47.354	2.228	1815.08	1805.43	3.04	0.095
23	1828.58	45.398	3.759	1832.44	1820.86	3.772	0.218
24	1844.01	44.75	4.692	1851.72	1840.15	3.756	0.222
25	1869.08	44.851	4.97	1872.94	1863.3	3.152	0.228
26	1888.37	48.181	1.509	1892.23	1880.66	3.555	0.056
27	1909.59	48.296	1.221	1911.52	1899.95	3.547	0.043
28	1923.09	47.475	2.362	1926.95	1913.45	4.241	0.159
29	1942.38	46.978	2.503	1950.1	1938.52	3.642	0.106
30	1967.46	48.07	1.469	1971.32	1961.67	3.001	0.056
31	1992.53	48.223	1.555	1996.39	1984.82	3.59	0.08
32	2017.61	48.778	1.05	2021.47	2011.82	2.956	0.041
33	2040.76	49.281	0.622	2052.33	2031.11	6.435	0.03
34	2063.9	49.356	0.601	2069.69	2056.19	4.097	0.027
35	2088.98	49.686	0.286	2092.83	2083.19	2.915	0.011
36	2106.34	49.848	0.016	2108.27	2096.69	3.495	0.001
37	2121.77	49.761	0.022	2121.77	2119.84	0.585	0
38	2135.27	49.634	0.122	2141.06	2131.41	2.929	0.005
39	2162.27	49.583	0.281	2200.85	2142.99	17.53	0.058
40	2239.43	49.832	0.234	2279.94	2214.35	19.77	0.075
41	2455.46	49.991	0.037	2486.33	2449.68	11.017	0.006

Fig 4: FTIR Spectra of pure Probenecid

FTIR of spectra of sodium alginate:



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	418.57	61.295	10.932	428.21	412.78	2.602	0.411
2	435.93	68.071	1.324	437.86	430.14	1.205	0.031
3	443.64	64.742	3.421	449.43	439.78	1.724	0.119
4	472.58	67.09	2.763	476.43	464.86	1.884	0.081
5	482.22	69.09	1.359	488.01	478.36	1.53	0.052
6	503.44	69.125	1.532	511.15	497.65	2.106	0.069
7	549.73	68.5	0.424	551.66	542.02	1.551	0.023
8	559.38	67.219	1.002	563.23	551.66	1.956	0.046
9	665.46	67.931	0.858	667.39	650.03	2.771	0.038
10	705.97	70.682	0.655	711.76	702.11	1.437	0.023
11	748.41	69.704	3.665	781.2	742.62	4.95	0.14
12	794.7	76.464	0.36	800.49	788.91	1.338	0.012
13	815.92	75.603	0.045	817.85	802.41	1.772	0.001
14	837.13	71.521	5.192	846.78	819.77	3.362	0.249
15	862.21	76.205	0.648	866.07	856.42	1.122	0.019
16	896.93	73.629	1.587	910.43	868	5.32	0.19
17	972.16	61.787	1.626	976.01	912.36	10.11	0.073
18	997.23	60.699	0.22	999.16	977.94	4.479	0.048
19	1020.38	57.911	0.866	1026.16	1001.09	5.718	0.076
20	1031.95	58.107	0.302	1041.6	1028.09	3.161	0.02
21	1058.96	57.413	2.745	1093.67	1043.52	11.368	0.522
22	1112.96	60.79	0.947	1120.68	1095.6	5.284	0.088
23	1163.11	62.878	4.651	1184.33	1143.83	7.57	0.662
24	1201.69	66.883	3.391	1215.19	1195.91	3.107	0.19
25	1224.84	68.081	3.721	1236.41	1217.12	2.959	0.186
26	1244.13	71.167	1.233	1255.7	1238.34	2.488	0.063
27	1280.78	71.503	0.623	1284.63	1265.35	2.731	0.036
28	1301.99	69.288	0.677	1303.92	1292.35	1.772	0.028
29	1319.35	66.496	1.012	1321.28	1305.85	2.567	0.048
30	1327.07	66.827	0.639	1332.86	1323.21	1.676	0.028
31	1338.64	64.563	3.969	1346.36	1334.78	1.997	0.117
32	1361.79	65.044	3.71	1365.65	1352.14	2.253	0.107
33	1375.29	64.968	3.595	1383.01	1367.58	2.698	0.157
34	1417.73	64.527	4.567	1425.44	1408.08	2.939	0.177
35	1456.3	58.354	9.441	1462.09	1452.45	1.948	0.318
36	1506.46	59.895	14.766	1512.24	1502.6	1.644	0.42
37	1521.89	64.189	9.766	1529.6	1514.17	2.491	0.462
38	1558.54	59.357	15.152	1562.39	1552.75	1.615	0.376
39	1595.18	77.405	0.751	1597.11	1587.47	1	0.015
40	1608.69	69.352	7.001	1612.54	1599.04	1.855	0.307
41	1635.69	68.161	9.804	1641.48	1631.83	1.277	0.235

Fig 5: FTIR Spectra of sodium alginate

FTIR Spectra of METHOCEL K 100:

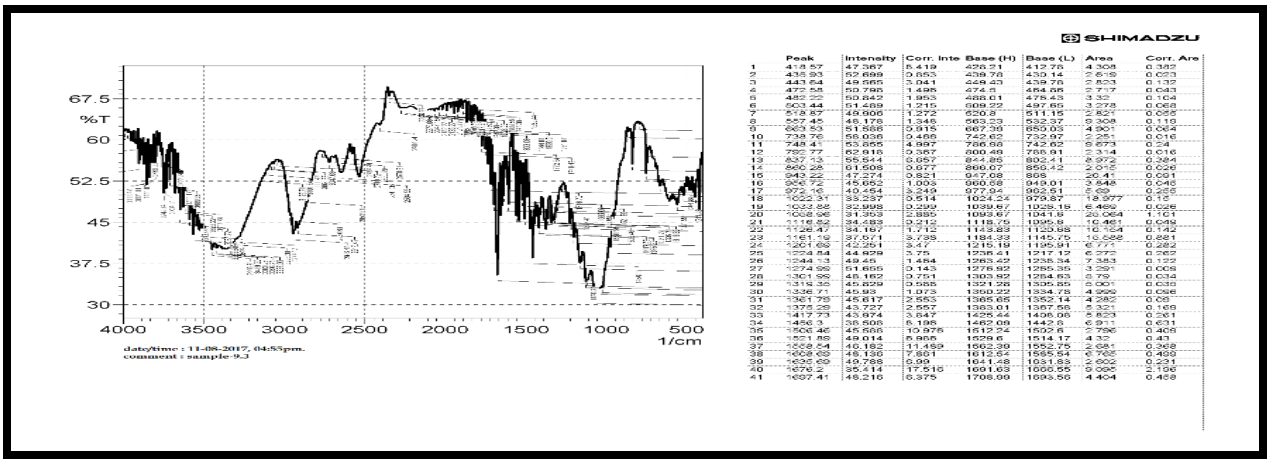
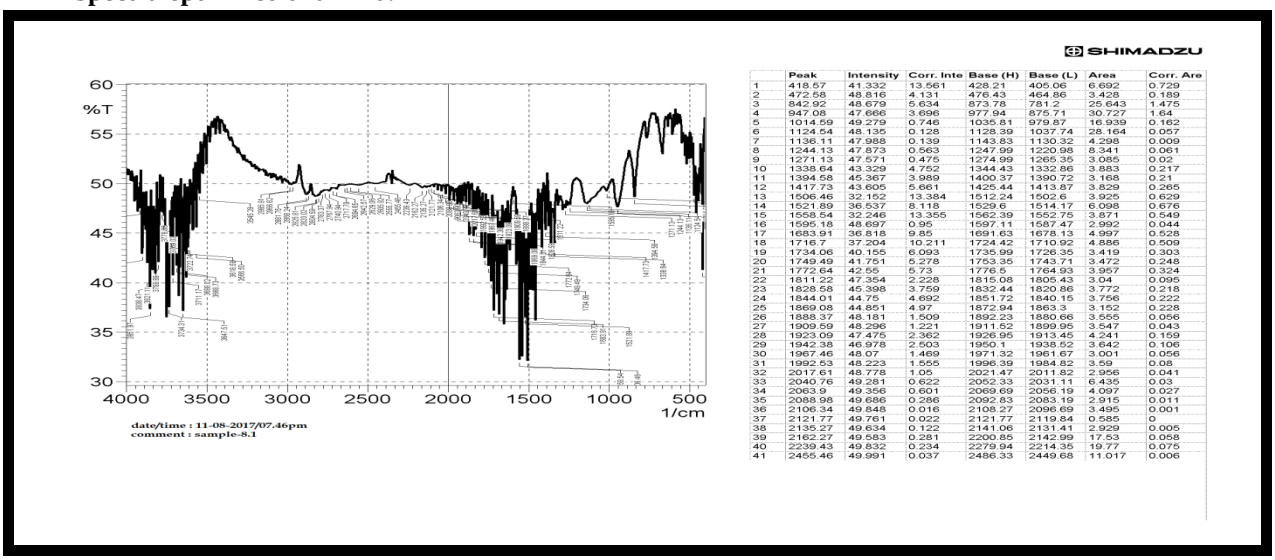


Fig 6: FTIR Spectra of Methocel K 100

FTIR Spectra optimized trial T10:



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	418.57	41.332	13.561	428.21	405.06	6.692	0.729
2	472.58	48.816	4.131	476.43	464.86	3.428	0.189
3	842.92	48.679	5.634	873.78	781.2	25.643	1.475
4	947.08	47.666	3.696	977.94	875.71	30.727	1.64
5	1014.59	49.279	0.746	1035.81	979.87	16.939	0.162
6	1124.54	48.135	10.128	1128.39	1037.74	28.164	0.057
7	1136.11	47.988	0.139	1143.83	1130.32	4.298	0.009
8	1244.13	47.873	0.563	1247.99	1220.98	8.341	0.061
9	1271.13	47.571	0.475	1274.99	1265.35	3.085	0.02
10	1338.64	43.329	4.752	1344.43	1332.86	3.883	0.217
11	1394.58	45.367	3.989	1400.37	1390.72	3.168	0.21
12	1417.73	43.605	5.661	1425.44	1413.87	3.829	0.265
13	1506.46	32.152	13.384	1512.24	1502.6	3.925	0.629
14	1521.89	36.537	8.118	1529.6	1514.17	6.098	0.676
15	1558.54	32.246	13.355	1562.39	1552.75	3.871	0.549
16	1595.18	48.697	0.95	1597.11	1587.47	2.992	0.044
17	1683.91	36.818	9.85	1691.63	1678.13	4.997	0.528
18	1716.7	37.268	10.211	1724.42	1710.92	4.886	0.509
19	1734.06	40.155	6.093	1735.99	1726.35	3.419	0.303
20	1749.49	41.751	5.278	1753.35	1743.71	3.472	0.248
21	1772.54	42.58	5.73	1776.5	1764.93	3.957	0.324
22	1811.22	47.354	2.228	1815.08	1805.43	3.04	0.095
23	1828.58	45.398	3.759	1832.44	1820.86	3.772	0.218
24	1844.01	44.75	4.692	1851.72	1840.15	3.756	0.222
25	1869.08	44.851	4.97	1872.94	1863.3	3.152	0.228
26	1888.37	48.181	1.509	1892.23	1880.66	3.555	0.056
27	1909.59	48.267	1.221	1911.52	1899.95	3.547	0.043
28	1923.09	47.475	2.362	1926.95	1913.45	4.241	0.159
29	1942.38	46.978	2.503	1950.1	1938.52	3.642	0.106
30	1967.48	48.07	1.469	1971.32	1961.67	3.001	0.056
31	1992.53	48.223	1.555	1996.39	1984.82	3.59	0.08
32	2017.61	48.778	1.05	2021.47	2011.82	2.956	0.041
33	2040.76	49.281	0.622	2052.33	2031.11	6.435	0.03
34	2063.9	49.356	0.601	2069.69	2056.19	4.097	0.027
35	2088.98	49.686	0.286	2092.83	2083.19	2.915	0.011
36	2106.34	49.848	0.016	2108.27	2096.69	3.495	0.001
37	2121.77	49.761	0.022	2121.77	2119.84	0.585	0
38	2135.27	49.634	0.122	2141.06	2131.41	2.929	0.005
39	2162.27	49.583	0.281	2200.85	2142.99	17.53	0.058
40	2239.43	49.832	0.234	2279.94	2214.35	19.77	0.075
41	2455.46	49.991	0.037	2486.33	2449.68	11.017	0.006

Fig 7: 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 4: physical characteristic of microspheres form trial T-1 to T-5

Trials	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Compressibility index	Hausner's ratio
Trial -1	1.14	0.15	45.16	43.29	2.19
Trial -2	1.62	0.53	41.46	34.43	2.34
Trial -3	0.76	0.48	39.84	31.45	2.18
Trial -4	1.76	0.68	33.29	36.54	1.39
Trial -5	1.35	0.76	29.46	26.49	1.03

Table 5: physical characteristic of microspheres form trial T-6 to T-10

Trials	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Compressibility index	Hausner's ratio
Trial -6	1.67	0.37	41.28	35.93	1.38
Trail -7	1.32	0.65	32.38	37.31	1.43
Trial -8	1.53	0.66	37.38	29.03	2.48
Trial -9	1.85	0.65	35.43	36.76	2.15
Trial -10	1.83	0.59	35.65	34.37	1.21

GRAPHICAL REPRESENTATION OF ICROMERTRIC PROPERTIES OF MICRBEADS

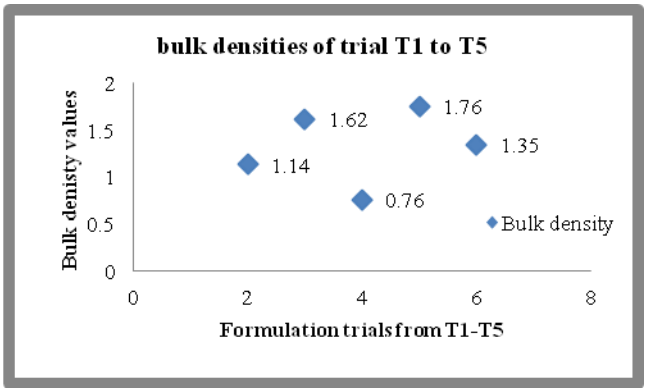


Fig 9: Tapped densities from trial T1-T5

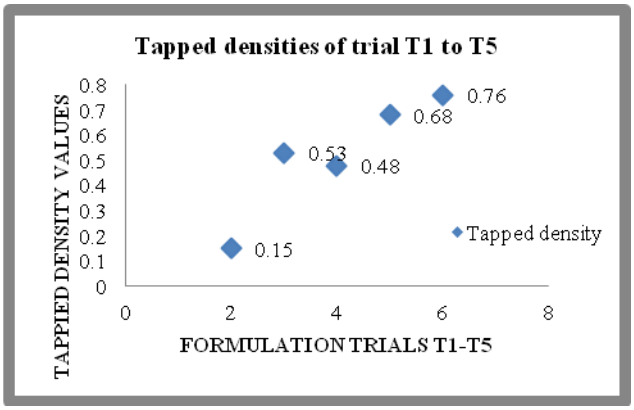


Fig 8: bulk densities from trial T1-T5

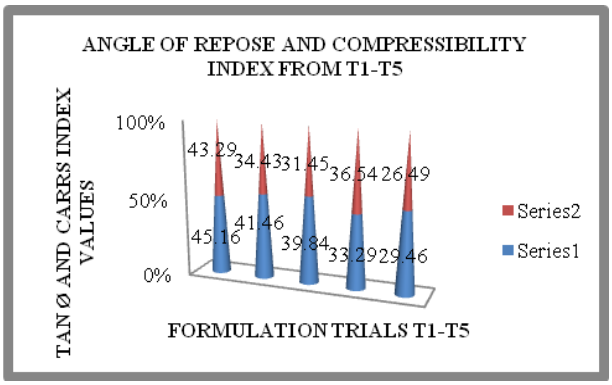


Fig 10: angle of repose and cars index of trial T1-T10

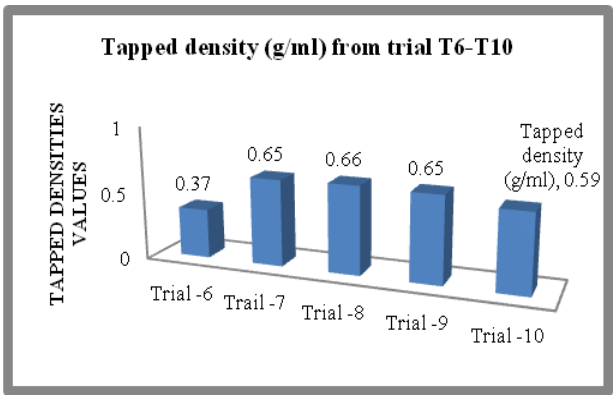


Fig 13: Tapped densities from trial T6-10

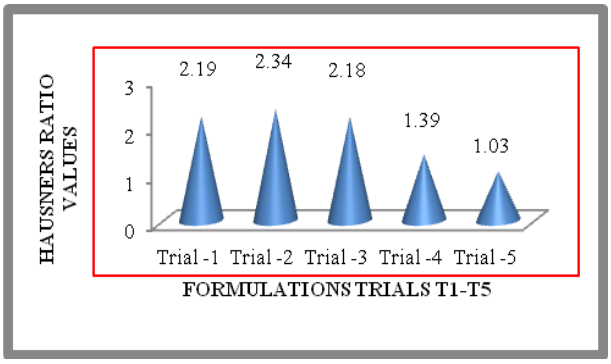


Fig 11: Hausner's values from trial T1-T5

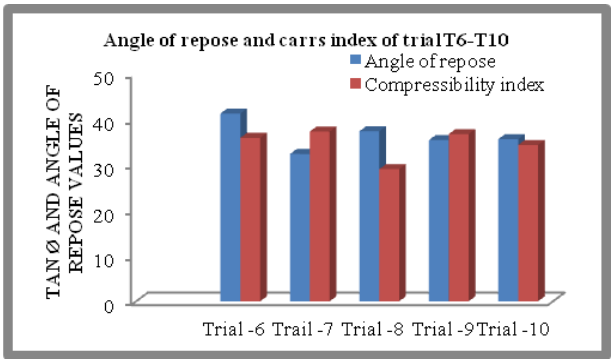


Fig 14: Angle of repose and cars index T6-T10

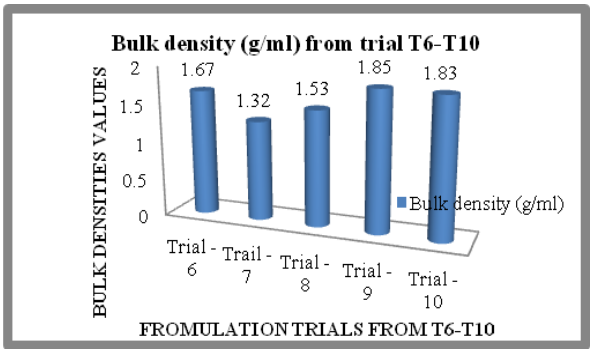


Fig 12: Bulk densities from trial T6-T10

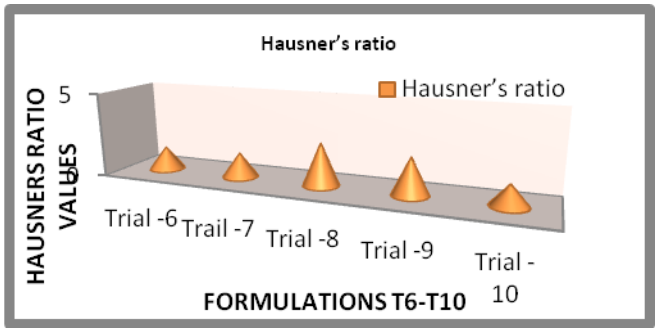


Fig 15: Hausner's values from trial T6-T10

Practical yield:

Table 6: Percentage yield of all trials T1-T10

Trials	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Theoretical Weight	460	435	410	385	360	475	465	455	445	435
Practical Weight	480	447	421	411	381	494	488	472	482	452
Percent Yield	95.83	97.31	97.38	93.67	94.48	96.15	95.28	96.39	92.32	96.23

Content uniformity:

Table 7: theoretical and estimated content uniformity from trials T1-T10

TRIALS	content uniformity % yield with practical yield	half of the practical yield microspheres-Mg.	content uniformity in half of the practical yield of microspheres	estimated content uniformity in yield practical weight of the microspheres
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

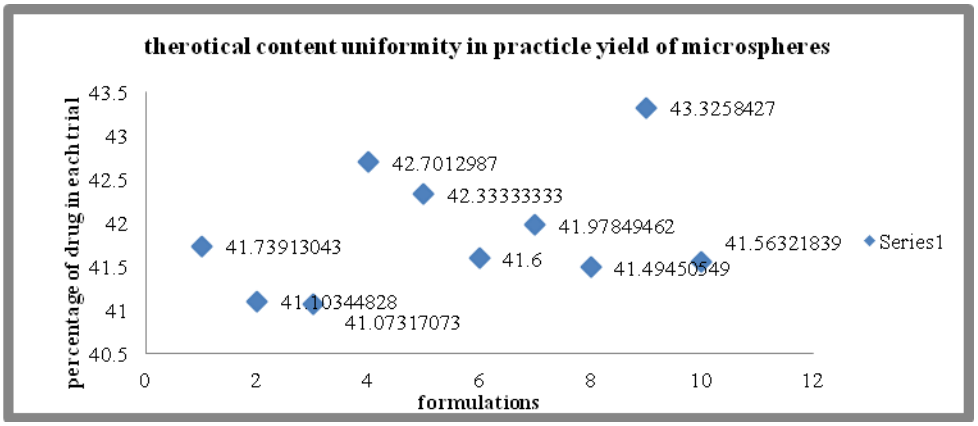


Fig 16: theoretical content uniformity in practical yield of microspheres form trial T1-T10

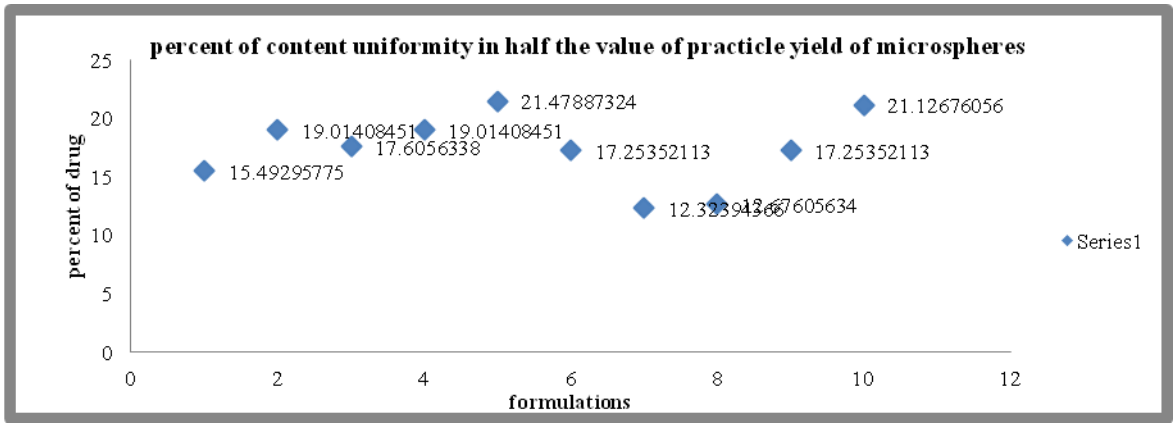


Fig 17: Percent of drug in 50 percent of each trials form T1-T10

***In-Vitro* Drug Release Studies:**
Probenced Release Form Trial T1-T5:

Table 8: Drug release profile from trial T1-T10

TIME IN MINUTES	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

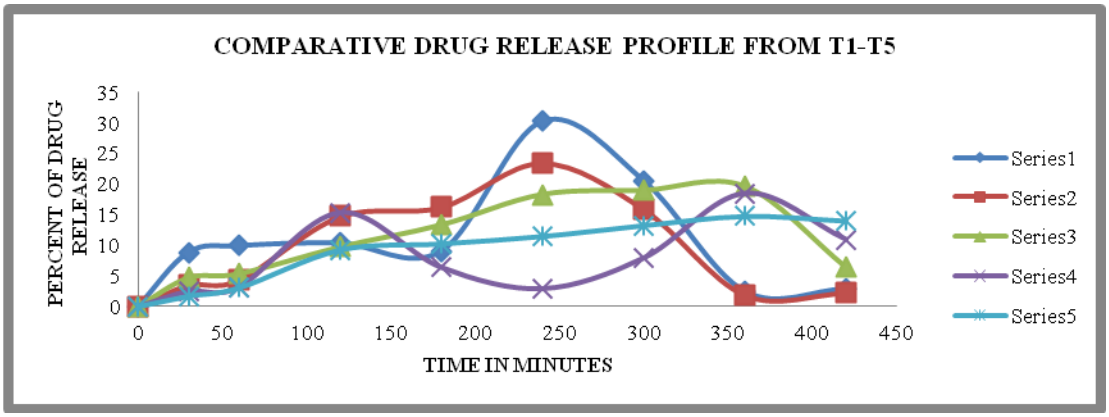


Fig 18: Comparative drug release form trial T1-T5

Probenced Release Form Trial T6-T10:

Table 9: Drug release profile form trials T6-T10

TIME IN MINUTES	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

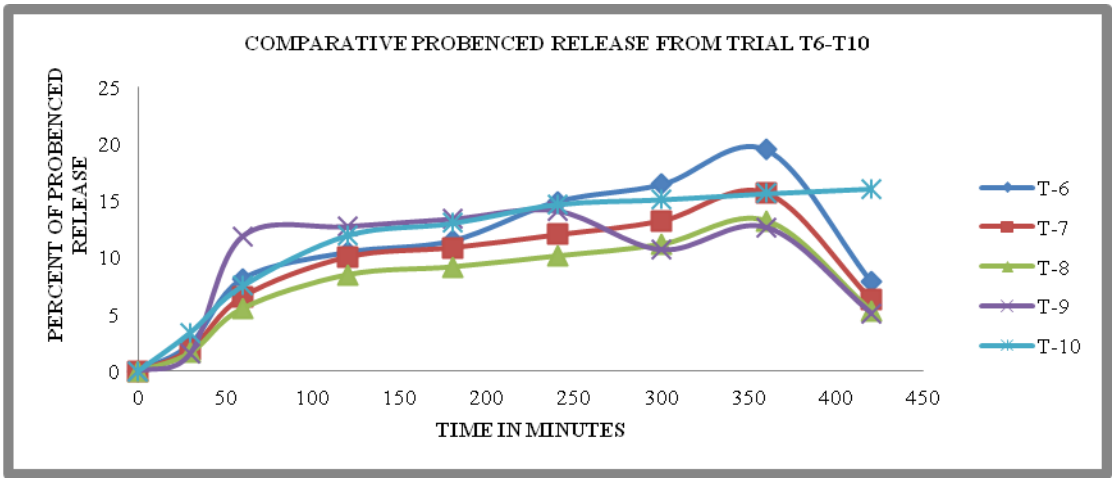


Fig 19: Comparative release profile from trial T6-T10

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.

Table 10: physical characteristic of microspheres from optimized trial T-10

Trials	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Compressibility index	Hausner's ratio
Trial -10	1.37	0.54	36.62	34.28	1.84

Content uniformity:

Table 11: percent yield and content uniformity form optimized trial T10

TRIAL	Theoretical weight	Practical yield	Percent yield	theoretical content uniformity practical yield	50% practical yield microspheres-Mg.	Content uniformity in 231 Mg. microspheres	Content uniformity in 462 Mg. microspheres	Percent of drug in 462 Mg.
F10	**435**	**462**	**94.15**	**42.78**	**231**	**19.13**	**38.26 Mg.**	**95.65%**

In-vitro drug release studies:

Table 12: release of drug from optimized trial T10

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

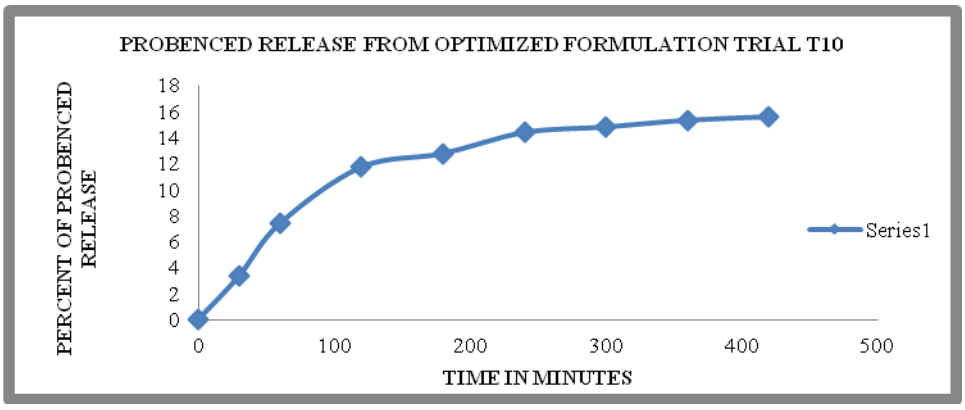


Fig 20: Graphical representation of probenced release from optimized trial T10

Determination of Release Rate Kinetics:

Table 13: Drug release rate kinetic parameters for optimized trial T10

ZERO ORDER		FIRST ORDER		HIGUCHIS ODEL		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

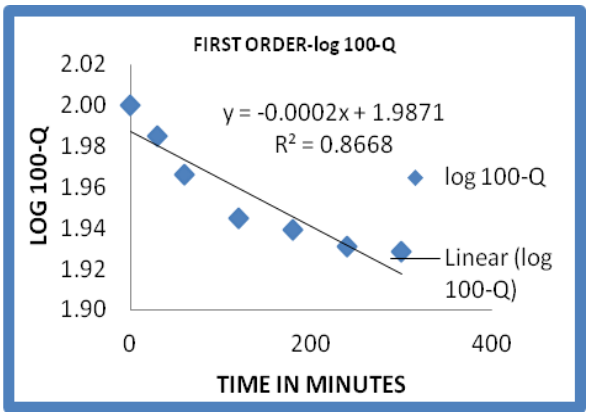


Fig 21: First order rate kinetics for T10

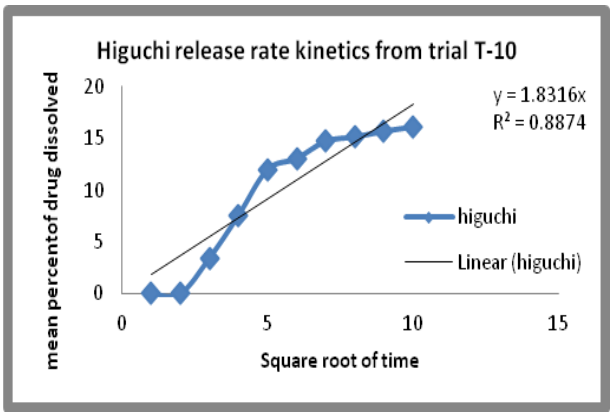


Fig 23: Higuchis plot for trial T10

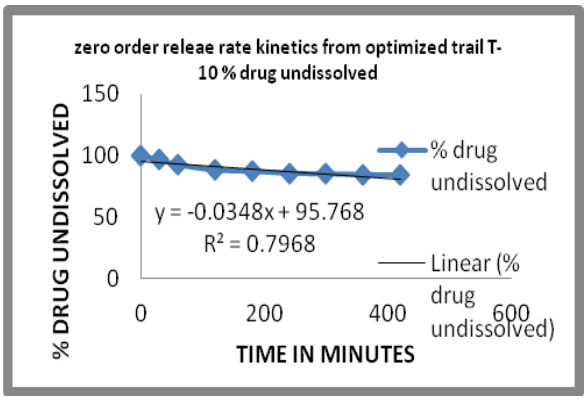


Fig 22: Zero order kinetics of T10

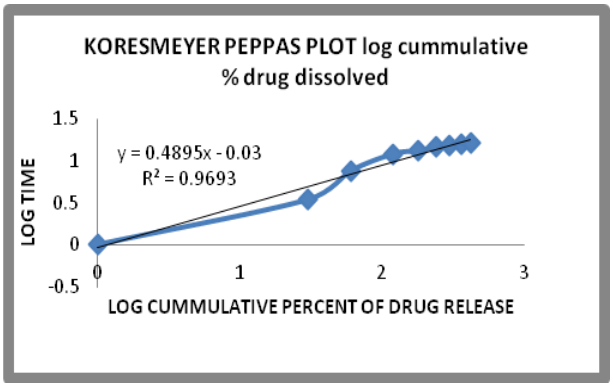


Fig 24: Koresmeyer Peppas plot for trial T10

DISCUSSION:

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 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 tables 7, 8,9.

Release rate kinetics were also determined to formulation trial T10 and the release rate of the drug was determined based on the regression coefficient R^2value 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 R^2value, probenced release rate fallows KORESMEYER-PEPPAS PLOT (R^2= 0.969)

ACKNOWLEDGEMENT:

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