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DESIGN AND CHARACTERIZATION OF HYDROGEL BEADS OF TERBUTALINE SULPHATE

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Abstract

The main aim of any drug therapy is to achieve a desire concentration of the drug in blood or tissue, which is therapeutically effective and non-toxic for an extended period of time. Hydrogel beads are the potential candidate for oral sustained release of drug. Hydrogel beads of Terbutaline sulphate were prepared by ionotropic gelation technique and different evaluation parameters were assessed, with a view to obtain oral control release of Terbutaline sulphate. The microparticles were found to be in the size range of 500-550 µm. It was observed that in these prepared Terbutaline sulphate Hydrogel beads, with the increase in alginate percentage the distribution of particle size shifts to the higher value due to increase in the initial viscosity of the medium. F-20 shows that more sustained release was observed with the increase in percentage of sodium alginate. The best formulation was observed as F-20, by the observation of all results of the nine formulations Terbutaline sulphate Hydrogel beads.

Key words: Terbutaline sulphate, Hydrogel beads, Ionotropic gelation technique.

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INTRODUCTION

Asthma is a heterogeneous common chronic condition characterized by endo-bronchial inflammation with consequent bronchial hyperesponsiveness. It may be classified as mild intermittent or mild, moderate, or severe persistent. This leads to variable airflow obstruction and typical symptoms such as cough, breathlessness, chest tightness, wheezing and reduced exercise tolerance. The precise a etiology of asthma remains uncertain, but genetic and environmental factors such as viruses, allergen exposure, early use of antibiotics, and the numbers of siblings have all been implicated in its inception and development.

Pathophysiology of asthma:

Airway inflammation is the primary problem in asthma. An initial event in asthma appears to be the release of inflammatory mediators (e.g., histamine, tryptase, leukotrienes and prostaglandins) triggered by exposure by exposure to allergens, irritants, cold air or exercise. Depending up on the inflammatory mediators activitiy there are two types of responses:

- Early-phase asthmatic response.
- Late-phase asthmatic response.

Varying airflow obstruction leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough. [1]

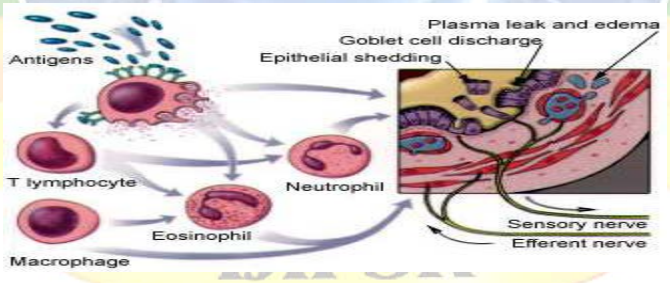


Fig. no. 1: Pathophysiology of asthma

It has been recognized that asthma is worse in night. NOCTURNAL ASTHMA is defined as a variable nighttime exacerbation of the underlying asthma condition associated with increase in symptoms and need for medication, increased airway responsiveness, and/or worsening of lung function. Approximately two-thirds of asthmatics suffer from nighttime symptoms. Lung function (e.g., peak expiratory flow rate or FEV1) is usually highest at 4 PM and lowest at 4 AM.

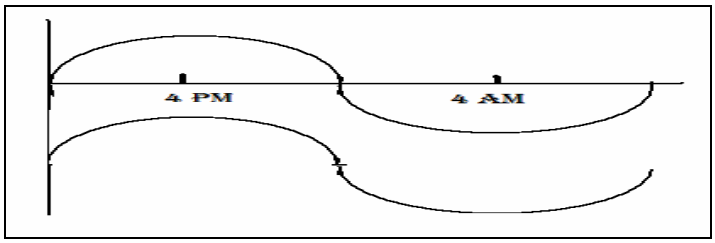


Fig. No. 2: Diurnal variations in lung function in healthy (top curve) and asthmatic (bottom curve) subjects. PEFR = peak expiratory flow rate; FEV 1 = forced volume in one second.

Management of Nocturnal Asthma: chronotherapy [2]

Chronotherapeutics is the synchronization of medication levels in time with reference to need, taking into account biologic rhythms in the pathophysiology of medical conditions, and/or rhythmodependencies in patient tolerance for given chemical interventions. It is based on importance of biologic rhythms in the pathophysiology of medical conditions and uses the timing of medication to provide maximal efficacy and minimal toxicity. Available therapy includes inhaled and oral corticosteroids, sustained release salbutamol sulphate, long-acting β-agonists, leukotriene-modifying agents and anticholinergic medication. Three chronotherapies have been proposed: [3]

- Inhaled corticosteroids administered at 5:30 PM rather than 8 AM.
- Time-release Theophylline formulation (Theo-24) dose ingested at 3 PM
- B2 agonists administered at 3 PM rather than 8 AM.

Hydrogels [4]**:**

Hydrogels form a specific class of polymeric biomaterials. Precise definition of this term is not obvious. Many years ago Dorothy Jordan Lloyd stated that "the colloidal condition, the gel, is one which is easier to recognize than to define" [5]. Since this time more accurate definitions have appeared. Nowadays, hydrogels are defined as two- or multicomponent systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Depending on the properties of the polymer (polymers) used, as well as on the nature and density of the network joints, such structures in an equilibrium can contain various amounts of water; typically in the swollen state the mass fraction of water in a hydrogel is much

higher than the mass fraction of polymer. Two general classes of hydrogels can be defined - physical gels (pseudogels), where the chains are connected by electrostatic forces, hydrogen bonds, hydrophobic interactions or chain entanglements (such gels are non-permanent and usually they can be converted to polymer solutions by heating) and chemical (true, permanent) hydrogels with covalent bonds linking the chains. In this paper we deal with the formation and applications of permanent polymer networks.

Properties of hydrogel

Hydrogels are water swollen polymer matrices, with a tendency to imbibe water when placed in aqueous environment. This ability to swell, under biological conditions, makes it an ideal material for use in drug delivery and immobilization of proteins, peptides, and other biological compounds. Due to their high water content, these gels resemble natural living tissue more than any other type of synthetic biomaterial. These networks, have a three dimensional structure, crosslinked together either physically (entanglements, crystallites), or chemically (tie-points, junctions). This insoluble crosslinked structure allows immobilization of active agents, biomolecules effectively, and allows for its release in well-defined specific manner. Thus the hydrogels biocompatibility and crosslinked structure are responsible for its varied applications.

MATERIALS AND METHODS

Materials used:

Terbutaline Sulfate, Sodium Alginate, Metlose SR, Eudragit RS 100, Celkol, Chitosan, Poly Vinyl Alcohol, Calcium Carbonate.

Physicochemical Properties

Colour, odour, taste and appearance:

The color, odor and taste of the drug were recorded using descriptive terminology.

Melting point determination:

Melting point of the drug sample was determined by capillary method by using melting point apparatus.

Determination of solubility:

The solubility of the Terbutaline sulphate was determined by adding excess amount of drug in the solvent and equilibrium solubility was determined by taking supernatant and analyzing it on

Lab India, double beam spectrophotometer. The reported and observed melting point is shown in Table 11.

% solubility = sample absorbance /standard absorbance × dilution factor ×100

Ultraviolet Visible (UV-visible) spectroscopy:

Construction of Calibration Curve:

Preparation of Stock Solution: 100 mg of Terbutaline sulphate was taken in a 100 ml volumetric flask. To that 5 ml of methanol was added and shaken well to dissolve the drug. The solution was made up to the mark with different buffer solutions i.e. 0.1N HCl and 6.8 PH phosphate buffer.From the above solution 1 ml is diluted to 10 ml with, buffer solutions to give 100 µg /ml concentration. From the above solution 1 ml is diluted to 10 ml with, buffer solutions to give 10 µg /ml concentration. The prepared solution i.e., 10 µg/ml concentration was scanned for λ_{max} from 200-400 nm in UV/Visible spectrophotometer.

Formulation development

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
Terbutaline Sulphate	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
Sodium Alginate	250	125	125	125	125	500	250	250	250	250	750	375	375	375	375	1000	500	500	500	500
celkol		125					250					375					500			
PVA			125					250					375					500		
Metalose SR				125					250					375					500	
Chitosan					125					250					375					500
Eudragit S100	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Calcium Chloride	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%
Hardning time	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr

Table no. 2: Composition of Drug loaded Hydrogel beads

Method of Preparation of drug loaded Hydrogel beads:

The beads were prepared by the ionotropic gelation technique. Sodium alginate solution was prepared by dissolving in deionized water and heated at 60°. The drug was dissolved uniformly in 50 ml of polymer solution below 40° under continuous stirring. The resultant homogeneous bubble free slurry dispersion was dropped through a 23G syringe needle into 100 ml of calcium

chloride solution containing different concentrations of polymeric solutions (chitosan, celkol, PVA, Metlose SR and Eudragit S100 used combination) which was kept under stirring to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. Immediate formation of small sodium alginate beads took place; after 5 min of curing time, the formed beads were collected by filtration and dried at 40°.

FTIR Studies

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of Terbutaline sulphate, polymer and physical mixtures of samples were weighed and mixed properly with Potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a thin semi transparent pellet by applying pressure. The IR spectrum of the pellet from 450-4000cm^1 was recorded taking air as the reference and compared to study any interference.

Yield of production

The yields of production of micro beads of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of micro beads and percent production yields were calculated as per the Formula mentioned below.

Percentage yield = (practical yield/theoretical yield) ×100

Estimation of Terbutaline sulphate (Drug content)

About 25 mg of Hydrogel beads were weighed and added to 50 ml of phosphate buffer (pH 7.4). The resulting mixture was agitated on mechanical shaker for 24 hrs, then solution was filtered and the drug content was estimated at 282 nm spectrophotometrically after suitable dilution.

***In vitro* release studies of Terbutaline sulphate from Hydrogel beads:**

In vitro release studies of prepared Hydrogel beads were carried out using USP XXIV dissolution (basket) apparatus at 100 rpm. Dissolution was carried out for a total period of 8 hr using 0.1 N HCl (pH 1.2) for first 2 hrs, phosphate buffer (pH 6.8) for three hours and 7.4 pH for the rest of the period maintained at a temperature of 37±1°C. At periodic time intervals, 5 ml of sample withdrawn suitably diluted and absorbance was measured at 282 nm. Five milliliters of fresh dissolution media was added each time to maintain the sink conditions.

Kinetic-models:

In order to describe the DS release kinetics from individual tablet formulations, the corresponding dissolution data were fitted in various kinetic dissolution models like Zero order, first order, Higuchi and Korsmeyer Peppas respectively.

Stability studies:

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^0C/60% RH analyzed every month for period of three months.
2. 30^0C/75% RH analyzed every month for period of three months.
3. 40^0C/75% RH analyzed every month for period of three months**.**

RESULTS AND DISCUSSION

Preformulation:

Table no. 5: Preformulation Parameters for the Hydrogel beads

S.no	API characterisation	Results
1	Physical appearance	It is a white to gray-white crystalline powder
2	Melting point	120°c
3	Solubility	it is soluble in water and in 0.1n hydrochloric acid, slightly soluble in methanol, and insoluble in chloroform.
4	Bulk density	0.28 gm/ml
5	Tapped density	0.41 gm/ml
6	Carr's index/compressibility index	31.71
7	Hausner's ratio	1.46

The value of compressibility index above 25%, 15-25%, less than 15% indicates poor flowability, optimum flowability and high flowability respectively. As Terbutaline sulphate value is more than 25% it exhibits good flow

Table no. 6: Standard Calibration Curve of API in 0.1N HCl

S.NO	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.068
3	4	0.157
4	6	0.232
5	8	0.305
6	10	0.386

Fig. no. 4: Standard Calibration Curve of API in 0.1N HCl

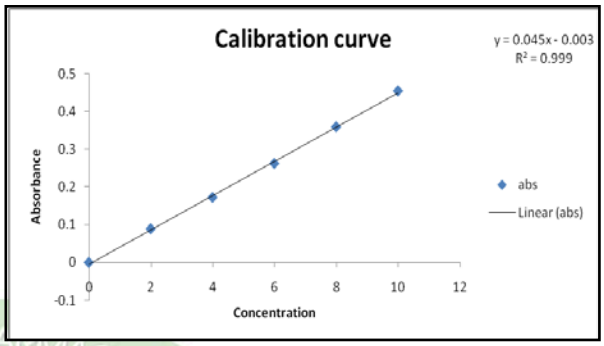


Table 7: Standard Calibration Curve of API in 6.8 pH Phosphate buffer

S.NO	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.038
3	20	0.078
4	30	0.123
5	40	0.163
6	50	0.207

Fig. 5: Standard Calibration Curve of API in 6.8 pH Phosphate buffer

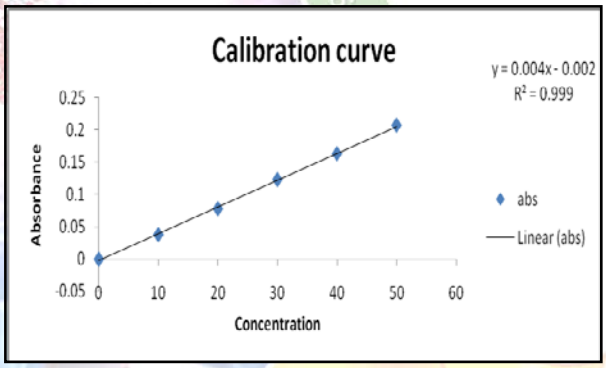
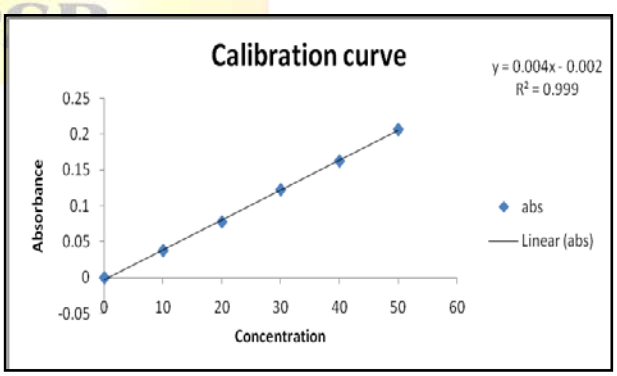


Table 8: Standard Calibration Curve of API in 7.4 pH Phosphate buffer

S.NO	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.072
3	20	0.151
4	30	0.221
5	40	0.294
6	50	0.38

Fig 6: Standard Calibration Curve of API in 7.4 pH Phosphate buffer



FTIR Studies

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of Terbutaline sulphate, polymer and physical mixtures of samples were weighed and mixed properly with Potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a thin semi transparent pellet by applying pressure. The IR spectrum of the pellet from 450-4000cm^{1} was recorded taking air as the reference and compared to study any interference.

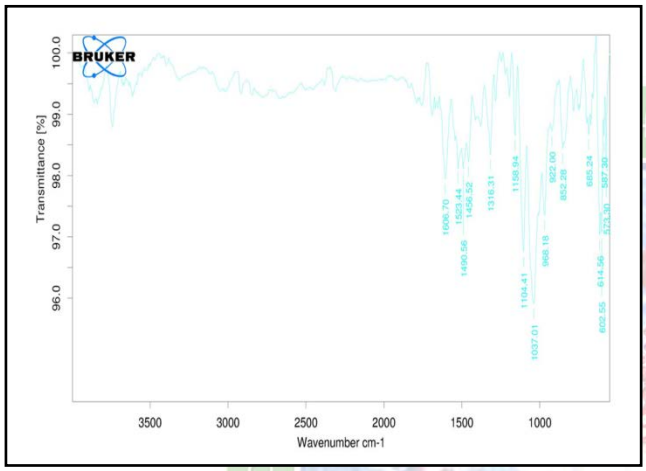


Fig. 7: FT-IR spectra of Terbutaline sulphate

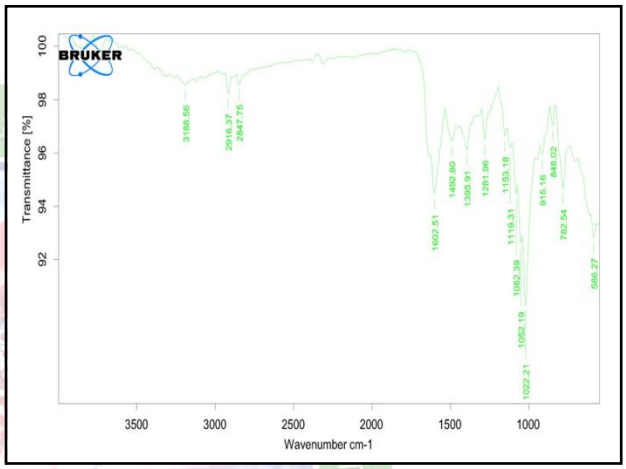


Fig. 8: FT-IR spectra of Optimised formula

Scanning electron Microscopy analysis (SEM)

The shape and surface characteristics were determined by scanning electron Microscopy (model-JSM, 35CF, jeol, Japan) using gold sputter technique. The particles were Vacuum dried, coated to 200 A^{o} thicknesses with gold palladium using prior to Microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15 kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.

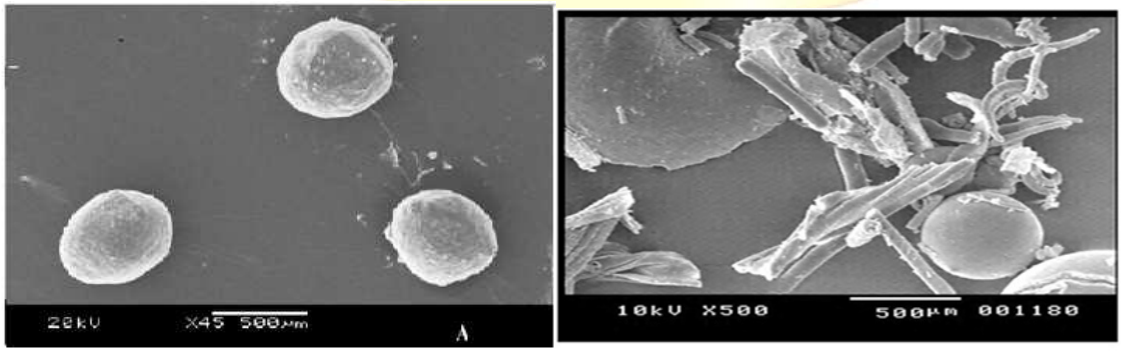


Fig. no. 9: Scanning electron Microscopy analysis (SEM) for Optimized formula

Evaluation of hydrogel beads

Table no. 9: Determination of drug entrapments efficiency, drug loading, and yield

Formulation code	Particle size (µm)	% yield	Swelling ratio in 1.2 ph	Swelling ratio in 6.8 ph	Swelling ratio in 7.4 ph	Entrapment efficiency	Drug content
F1	586.5±43.8	56	3.18	16.33	17.00	73.53+1.54	75.56%
F2	587.9±40.01	73	3.20	16.22	17.18	83.23+1.84	84.52%
F3	590.9±49.02	76	3.24	16.29	17.16	86.97+1.2	76.71%
F4	579.6±33.9	80	3.22	16.34	17.21	90.57+0.58	84.31%
F5	586.3±34.3	83	3.26	16.55	17.28	90.76+0.33	79.88%
F6	587.52±29.5	70	3.60	17.00	17.32	95.49+1.05	84.39%
F7	585.67±40.02	75	3.65	17.16	17.34	87.18+0.1	87.37%
F8	590.71±34.08	79	3.72	17.12	17.39	82.73+0.43	79.19%
F9	588.90±48.64	81	3.70	17.18	17.42	81.46+0.31	78.58%
F10	587.2±38.20	84	3.66	17.25	17.45	90.57+0.58	79.58 %
F11	595.8±35.9	76	3.87	17.32	17.50	86.97+1.2	86.71%
F12	583.14±38.26	80	3.84	17.29	17.56	90.57+0.58	87.31%
F13	592.67±42.2	83	3.80	17.34	17.62	90.76+0.33	74.88%
F14	588±28.9	70	3.89	17.38	17.69	95.49+1.05	76.39%
F15	591.90±31.52	75	3.89	17.55	17.72	87.18+0.1	86.37%
F16	590.19±38.76	79	4.42	17.60	17.98	82.73+0.43	76.19%
F17	586.62±38.89	89	4.46	17.66	18.12	95.49+1.05	79.58%
F18	588.14±23.89	75	4.45	17.72	18.18	82.73+0.43	78.44%
F19	599.52±35.65	78	4.48	17.69	18.23	81.46+0.31	84.54 %
F20	580.90±33.42	92	4.50	17.82	18.28	98.49+1.05	98.89%

Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity; this influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. And F-20 formulation shows good results when compared to other formulations.

***In vitro*-Dissolution profiles**

Table 10: Dissolution Parameters

Dissolution media	pH 1.2 1N HCl, pH 6.8 Phosphate buffers and pH 7.4 Phosphate buffer
Volume	900 ml
Apparatus	Paddle
Speed	100 rpm
Time	0, 1, 2, 3, 4, 5, 6, 7 and 8 hrs
Wave length	282 nm

Table 11: Dissolution profile of prepared Formulations (F1-F5)

Time (Hrs)	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	8.99	9.21	11.25	7.48	6.89
2	12.49	15.38	22.87	11.48	10.75
3	76.41	73.81	78.59	64.81	62.37
4	92.63	89.23	94.56	87.53	84.21
5	85	86	89	75	78
6	--	--	--	87	77
7	--	--	--	--	--
8	--	--	--	--	--

Fig. 10: Dissolution profiles of prepared Formulations F1 to F5

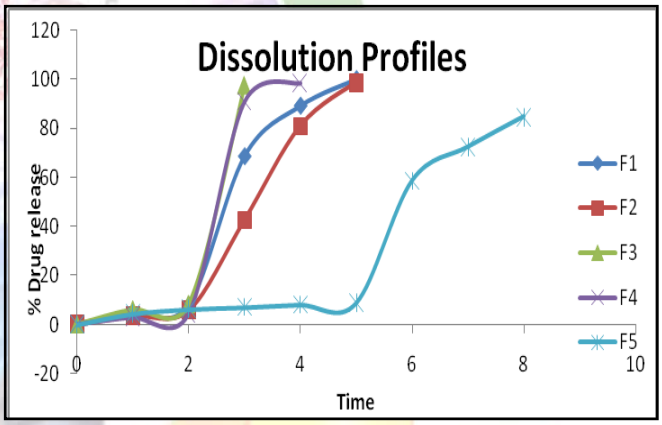


Table 12: Dissolution profile of prepared Formulations F6 to F10

Time (Hrs)	F6	F7	F8	F9	F10
0	0	0	0	0	0
1	4.67	3.6	6.1	2.95	4.43
2	7.82	5.84	8.34	4.31	6.31
3	68.7	42.7	35.50	84	7.1
4	89.4	81.1	--	85.50	8.2
5	84	82.50	--	--	8.91
6	--	--	--	--	58.7
7	--	--	--	--	72.5
8	--	--	--	--	84.8

Fig. 11: Dissolution profiles of prepared Formulations F6 to F10

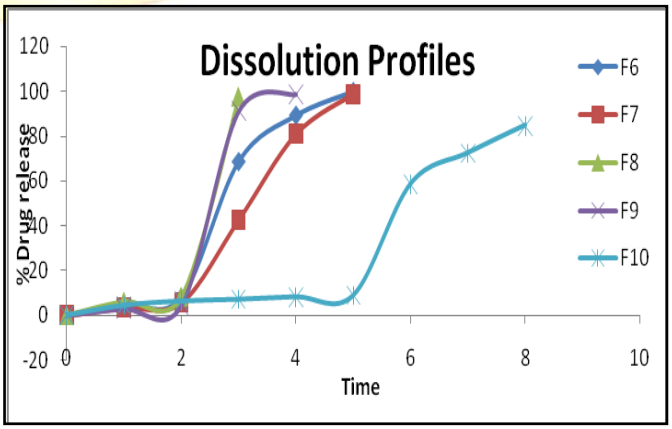


Table13: Dissolution profile of prepared Formulations F11 to F15

Time (Hrs)	F11	F12	F13	F14	F15
0	0	0	0	0	0
1	4.2	2.6	5.44	2.54	4.3
2	6.6	4.88	7.22	4.02	6.74
3	64.7	38.9	40.8	78.7	7.62
4	85.3	71.1	84.2	78.6	9.13
5	84.7	89.9	--	84.7	9.18
6	--	87.6	--	--	60.5
7	--	--	--	--	76.5
8	--	--	--	--	78.7

Fig. 12: Dissolution profiles of prepared Formulations F11 to F15

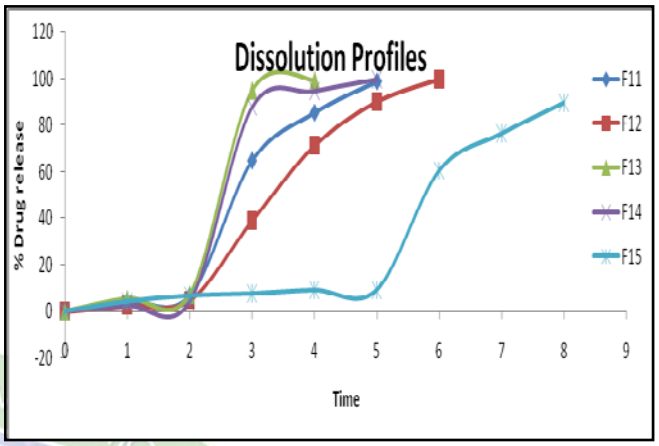
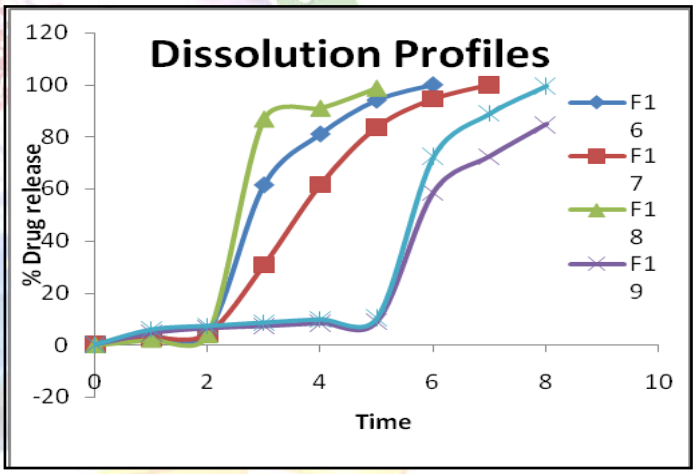


Table 14: Dissolution profile of prepared Formulations F16 to F20

Time(hrs)	F16	F17	F18	F19	F20
0	0	0	0	0	0
1	3.2	2.48	2.13	4.43	5.83
2	4.83	4.3	3.96	6.31	7.21
3	61.4	30.7	86.9	7.1	8.5
4	81	61.5	91.1	8.2	9.72
5	94	83.6	98.8	8.91	10.3
6	99.9	94.5	--	58.7	72.4
7	--	100	--	72.5	89
8	--	--	--	84.8	99.6

Fig. 13: Dissolution profiles of prepared Formulations F16 to F20



Above graph indicates that %Drug release of F20 formulation shows better drug release when compared with other formulations.

KINETIC MODELS:

Dissolution data of above two methods was fitted in Zero order, First order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation.

Table 14: Drug Release of Optimised Formulation

S.NO	time	log T	Square root of Time	%CR	%Drug remaining	log %CR	LOG% DRUG RETAINED	cube root of %drug remaining
0	0	0	0	0	100	0	2	4.641589
1	1	0	1	5.83	94.17	0.765669	1.973913	4.549575
2	2	0.30103	1.414214	7.21	92.79	0.857935	1.967501	4.527242
3	3	0.477121	1.732051	8.5	91.5	0.929419	1.961421	4.506164
4	4	0.60206	2	9.72	90.28	0.987666	1.955592	4.486047
5	5	0.69897	2.236068	10.3	89.7	1.012837	1.952792	4.47642
6	6	0.778151	2.44949	72.4	27.6	1.859739	1.440909	3.02206
7	7	0.845098	2.645751	89	11	1.94939	1.041393	2.22398
8	8	0.90309	2.828427	99.6	0.4	1.998259	-0.39794	0.736806

Fig. 14: Zero order plot

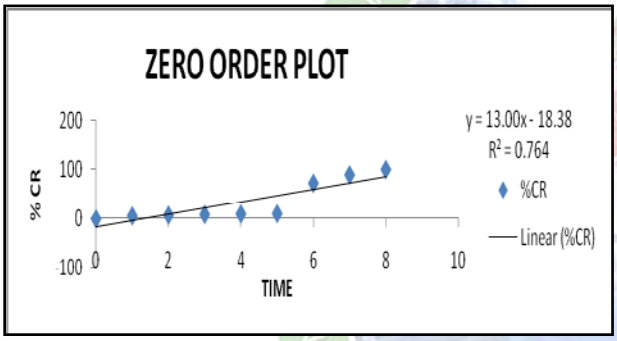


Fig. 15: first order plot

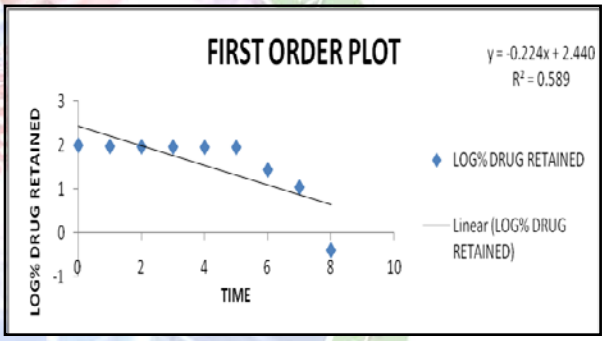


Fig. 16: Higuchi plot

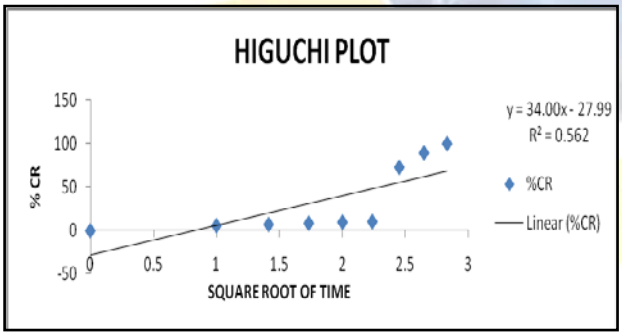


Fig. 17: Korsmeyer peppas

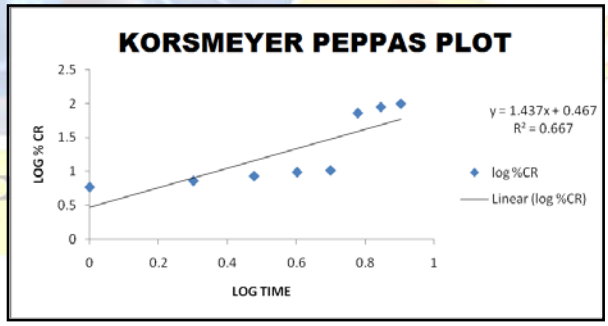
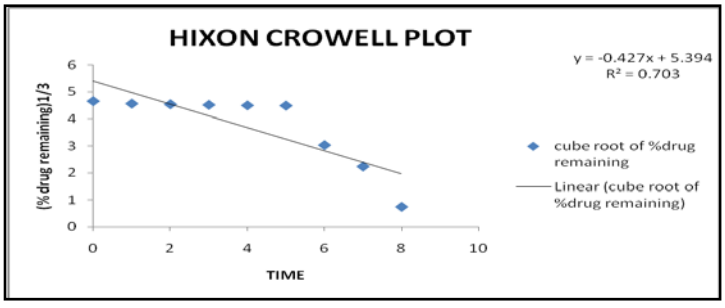


Fig. 16: Hixon crowell



Stability Study

There was no significant change in physical and chemical properties of the tablets of formulation F-20 after 3 Months. Parameters quantified at various time intervals were shown;

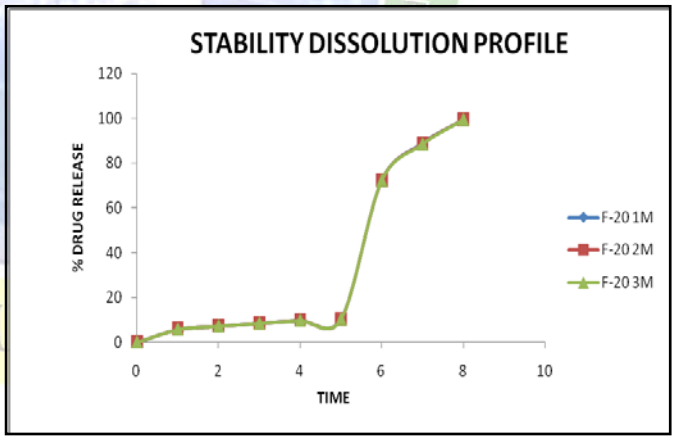
Table no. 15: Results of stability studies of optimized formulation F-20

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-20	25^0C/60%RH % Release	99.6	99.55	99.53	99.51	Not less than 85 %
F-20	30^0C/75% RH % Release	99.57	99.54	99.52	99.50	Not less than 85 %
F-20	40^0C/75% RH % Release	99.57	99.56	99.55	99.54	Not less than 85 %
F-20	25^0C/60% RH Assay Value	99.89	99.87	99.84	99.83	Not less than 90 % Not more than 110 %
F-20	30^0C/75% RH Assay Value	99.89	99.86	99.84	99.84	Not less than 90 % Not more than 110 %
F-20	40^0C/75% RH Assay Value	99.89	99.85	99.85	99.84	Not less than 90 % Not more than 110 %

Table 16: Stability studies of F-20 for 1st, 2nd & 3rd months

TIME (Hrs)	F-20 1M	F-20 2M	F-20 3M
0	0	0	0
1	5.83	5.80	5.78
2	7.21	7.20	7.20
3	8.5	8.48	8.45
4	9.72	9.70	9.65
5	10.3	10.27	10.24
6	72.4	72.36	72.32
7	89	88.8	88.5
8	99.55	99.53	99.51

Fig. 17: Stability studies of F-20 for 1st, 2nd & 3rd months



SUMMARY & CONCLUSION: Hydrogel beads of Terbutaline sulphate were prepared by ionotropic gelation technique and different evaluation parameters were assessed, with a view to obtain oral control release of Terbutaline sulphate. In the drug entrapment efficiency study, decrease in initial alginate concentration decreased DEE at a given curing time (1hr.) and CaCl2 concentration (2%). Decrease in initial alginate concentration provides lesser number of binding

sites of alginate for Ca2+ ions resulting in the formulation of a less compact gel membrane which, in turn, increases influx of Ca2+ ions leading to decrease in DEE.

The microparticles were found to be in the size range of 500-550 µm. It was observed that in these prepared Terbutaline sulphate Hydrogel beads, with the increase in alginate percentage the distribution of particle size shifts to the higher value due to increase in the initial viscosity of the medium. In order to find the effect of cross linking on the release rates of Terbutaline sulphate from the matrix, swelling was studied in terms of percentage of water uptake by the beads. However the swelling property of the beads was measured in terms of percentage of water uptake by the beads at a particular time interval.

The higher the amount of sodium alginate in the beads, the lower the swelling rate. The in-vitro drug release studies of different formulations cumulative percentage drug release was mentioned in the Table.

F-20 shows that more sustained release was observed with the increase in percentage of sodium alginate. The best formulation was observed as F-20, by the observation of all results of the nine formulations Terbutaline sulphate Hydrogel beads.

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