DESIGN, CHARACTERIZATION AND EVALUATION OF A SUSTAINED RELEASE FORMULATION OF ASHWAGANDHA

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Abstract
The objective of the research was to design, characterize and evaluate the sustained release formulation of Ashwagandha (*Withania somnifera*). In the study, a sustained release and a immediate release dosage form of Ashwagandha Hydro-Alcoholic dry powdered Root extract and Bhavana form of Ashwagandha Raw Herb (root) and to compare the Physico-chemical properties and release profiles of the two dosage forms. The tablets were prepared by wet granulation method by using HPMC (for Sustained release formulation), used as a hydrophilic polymer for sustained action, and SSG (for Immediate release formulation), used as Super Disintegrant. Ashwagandha is the most useful herbal drug, consisting of Withanolide, Withaferin, Withanone, Withanoside IV, ashwagandhanolide, steroids, flavonoids, nitrogen containing compounds, alkaloids etc. A very unique process used in Ayurveda is the process known as "Bhavana" (Impregnation). Bhavana is a process of wet grinding in which materials are ground with specific liquid media for a particular period to bring minute particles of the material in contact with the liquid media, transformation of the coarse powder to finer state, for physico-chemical changes. The prepared tablets were evaluated for different parameters.

KEYWORDS:
Aswagandha, Bhavana, Sustained Release Dosage Form, Immediate Release Dosage Form, Polymers and Methods for Preparation, Results and Discussion.

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INTRODUCTION:

Ayurveda is the most ancient science of life having a holistic health approach. The preparation of medicines i.e. pharmacy is an integral part of this science, and evolved from a very rudimentary form. Ayurvedic science is dynamic and progressive. It give importance to therapeutic strategy. The Ayurvedic pharmaceutical preparations were evolved gradually from a simpler form to more complex forms based on plants and plant-mineral combinations. Plants are very important commercial source of chemical compounds including primary and secondary metabolites. Ayurveda practitioner employ these plant to cure swelling, poultices lesions, tubercular ulcers, scabies, ophthalmia, muscular pain, dropsy, rheumatism, diabetes and even cancer. These properties are mainly because of its primary and secondary metabolites of drugs. This drug is naturally present in the parts like leaf, stem, root, seed or some times in the whole plant.

The term "AYURVEDA" meaning "the knowledge of life" comprises of two Sanskrit words viz. "AYU" meaning "Life" and "Veda" meaning "Knowledge" or "Science". Ayurvedic pharmaceutical science can be broadly considered under two major heads 1. *"Dravya Guna"*(Ayurvedic pharmacology) and 2. *"Bhaisajya Kalpana"* (Ayurvedic Pharmaceutics). The basic dosage forms of plant drugs are known as *"Pancavidha Kasaya Kalpana"* (five dosage forms), are *"Svarasa"* (expressed juice),*"kalka"* (paste), *"kvatha"* (decoction), *"Phanta"* (hot infusion) and *"Hima"* (cold infusion). Many more dosage forms such as *"Curna"* (powder), *"Vati"* (pill), *"Asava-Arista"*(medicated-fermented preparations),*"Lehya"*(linctus), aqueous extracts etc. derived with modern technology are also in practice. The methods of preparation of

these dosage forms are fairly simple, because of which the Ayurvedic practitioners prefer to prepare the required drugs on their own. However preparation of *"Rasausadhi"* (mineral and metal drugs) is a complicated procedure. Minerals and metals are generally known to be potentially harmful to the human body if not processed properly. These minerals and metals are 5 subjected to complex and meticulous processing to make them therapeutically useful and safe to the body in prescribed doses. The final product of mineral / metal drugs made with incineration generally known as *"Bhasma"* (calcined material) and others as *"Rasausadhi"*. (Ayurveda- The Science of Life; Department of AYUSH).

***Withania somnifera* Dunal (Ashwagandha):** Ashwagandha consists of dried mature roots of *Withania somnifera* Dunal. (Fam. Solanaceae), a perennial shrub, found in waste land, cultivated field and open grounds throughout India, widely cultivated in certain areas of Madhya Pradesh and Rajasthan, roots collected in winter, washed and cut into short pieces. (The Ayurvedic Pharmacopoeia of India; part-I, volume-I)

Classification: (Christian. Monika): Kingdom: Plantae; Division: Angiosperma; Class: Dicotyledoneae; Order: Tubiflorae; Family: Solanaceae; Genus: *Withania*; Species: *somnifera* Dunal.

Description: (The Ayurvedic Pharmacopoeia of India; part-I, volume-I)

Macroscopic: Roots straight, unbranched, thickness varying with age. Roots bear fiber-like secondary roots, outer surface buff to grey-yellow with longitudinal wrinkles, crown consists of 2-6 remains of stem base, stem bases variously thickened, nodes prominent only on the side from where petiole arises, cylindrical, green with longitudinal wrinkles, fracture, short and uneven, odour, characteristic, taste, bitter and acrid.

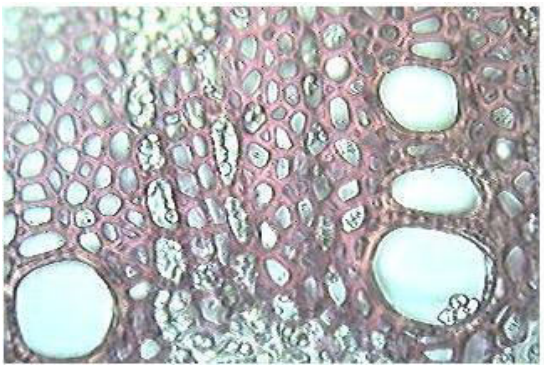
Withania somnifera leaves

Fig. 1: Ashwagandha leaves;

ASHWAGANDHA DRIED ROOTS

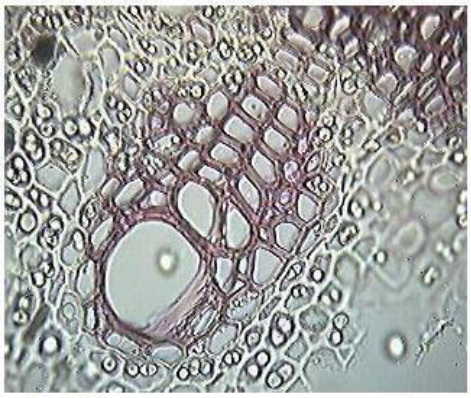
Fig. 2: Ashwagandha roots

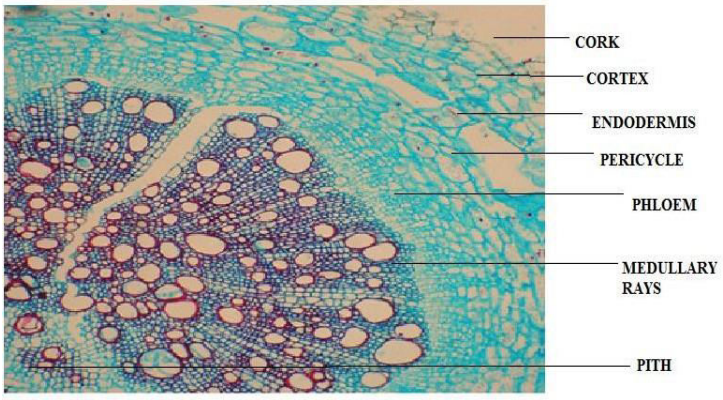
Microscopic: Transverse section of root shows cork exfoliated or crushed, when present isodiamatric and non-lignified, cork cambium of 2-4 diffused rows of cells, secondary cortex about twenty layers of compact parenchymatous cells, phloem consists of sieve tubes, companion cells, phloem parenchyma,



XYLEM

cambium 4-5 rows of tangentially elongated cells, secondary xylem hard forming a closed vascular ring separated by multiseriate medullary rays, a few xylem parenchyma.





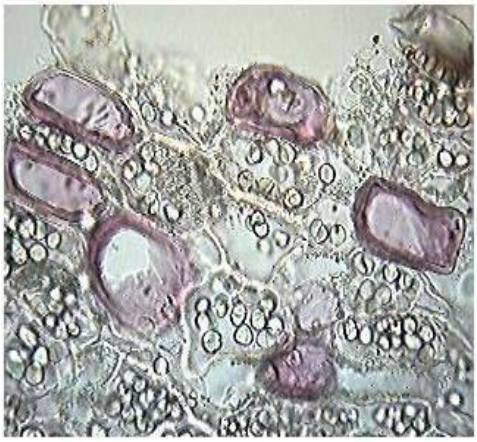
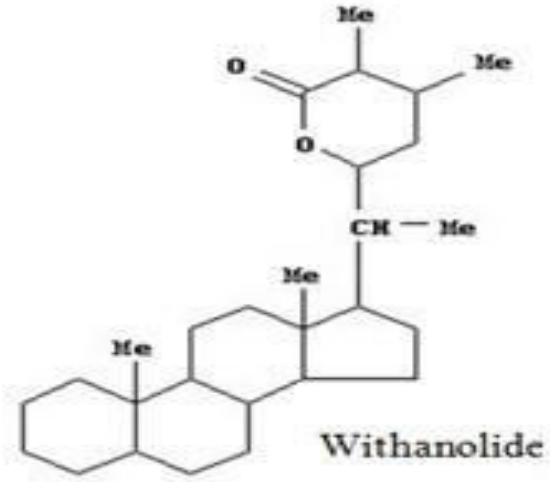


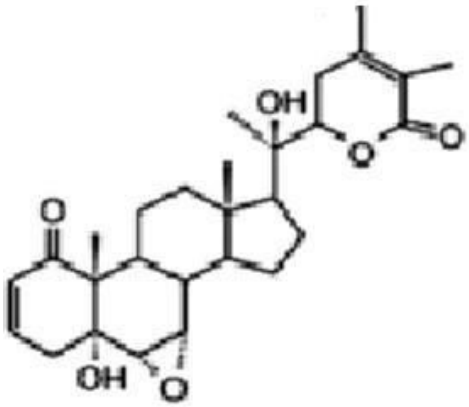
Fig.3: Xylem; Fig. 4: Microscopy; Fig. 5: Starch Grains; Fig.6: Cortex

API reference standards: API Withaferin A RS, Withanoside IV RS and Withanolide A RS.
Active Constituents: Alkaloids and Withanolides. (The Ayurvedic Pharmacopoeia of India; part-I, volume-I page-20) *Known chemical constituents: Alkaloids:* Withanine, Withaninine, Somniferine, Tropeltigloate, Somniferinine, Somninine, Nicotine, Visamine, Withasomine. *Salts:* Cuscohygrine, Anahygrine, Tropine, Pseudotropine, Anaferine. *Steroidal Lactones:* Withaferin-A, Withanone, WS-1, Withanolide E ($C_{28}H_{38}O_7$), Withanolide F ($C_{28}H_{38}O_6$), Withanolide G ($C_{28}H_{36}O_4$), Withanolide H ($C_{28}H_{36}O_5$), Withanolide I ($C_{28}H_{36}O_5$), Withanolide J ($C_{28}H_{36}O_5$), Withanolide K ($C_{28}H_{36}O_5$), Withanolide L ($C_{28}H_{36}O_5$), Withanolide M (C28H36O6). *Nitrogen containing compounds:* Withanol ($C_{25}H_{34}O_5$), Somnisol ($C_{32}H_{46}O$), Somnitol (C33H46O7). *Steroids:* Cholesterol, β-sitosterol, Stigmasterol, Diosgenin, Stigmastadien, Sitoinosides VII, Sitoinosides VIII, Sitoinosides IX, Sitoinosides X. *Flavonoids:* Kaempferol, Quercetin (Christian. Monika)
The main constituents of Ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, Withanine is the main constituent. The other alkaloids are Somniferine, Somnine, Somnifericinine, Withananine, Pseudo-Withanine, Tropine, Pseudo-Tropine, 3-α- gloyloxytropane, choline, Cuscohygrine, isopelletierine, Anaferine and anahydrine. Two acyl steryl glucosides viz. sitoindoside VII and sitoindoside VIII have been isolated from roots. The leaves contain steroidal lactones, which are commonly called as "withanolides". The withanolides have C_{28} steroidal nucleus with C_9 side chain, having six membered lactones ring. (Kokate C.K. et al.). The withanolides have been obtained from *W. somnifera* chemotype I. another series of steroidal lactones viz. withanolide E to M have been obtained from chemotype III. The drug

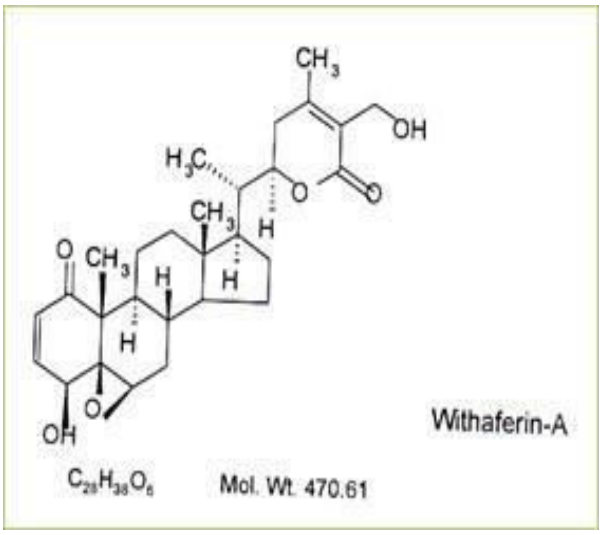
also contains two monohydric alcohols called Somnitol and somnirol; withanic acid; a phytosterol and ipuranol; and a mixture of fatty acids containing cerotic acid, oleic acid, palmitic acid and stearic acid. (Kokate C.K. et al.)



Withanolide

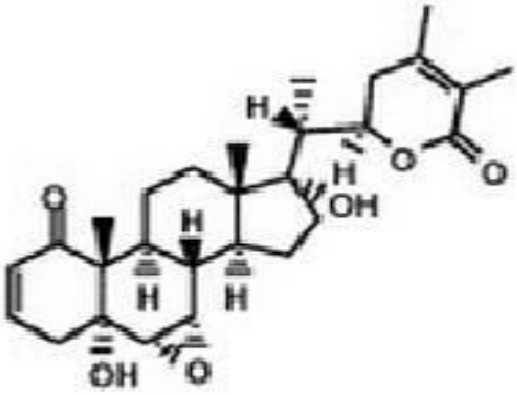


Withanolide A



Withaferin-A

$C_{28}H_{38}O_6$ Mol. Wt. 470.61



Withanone

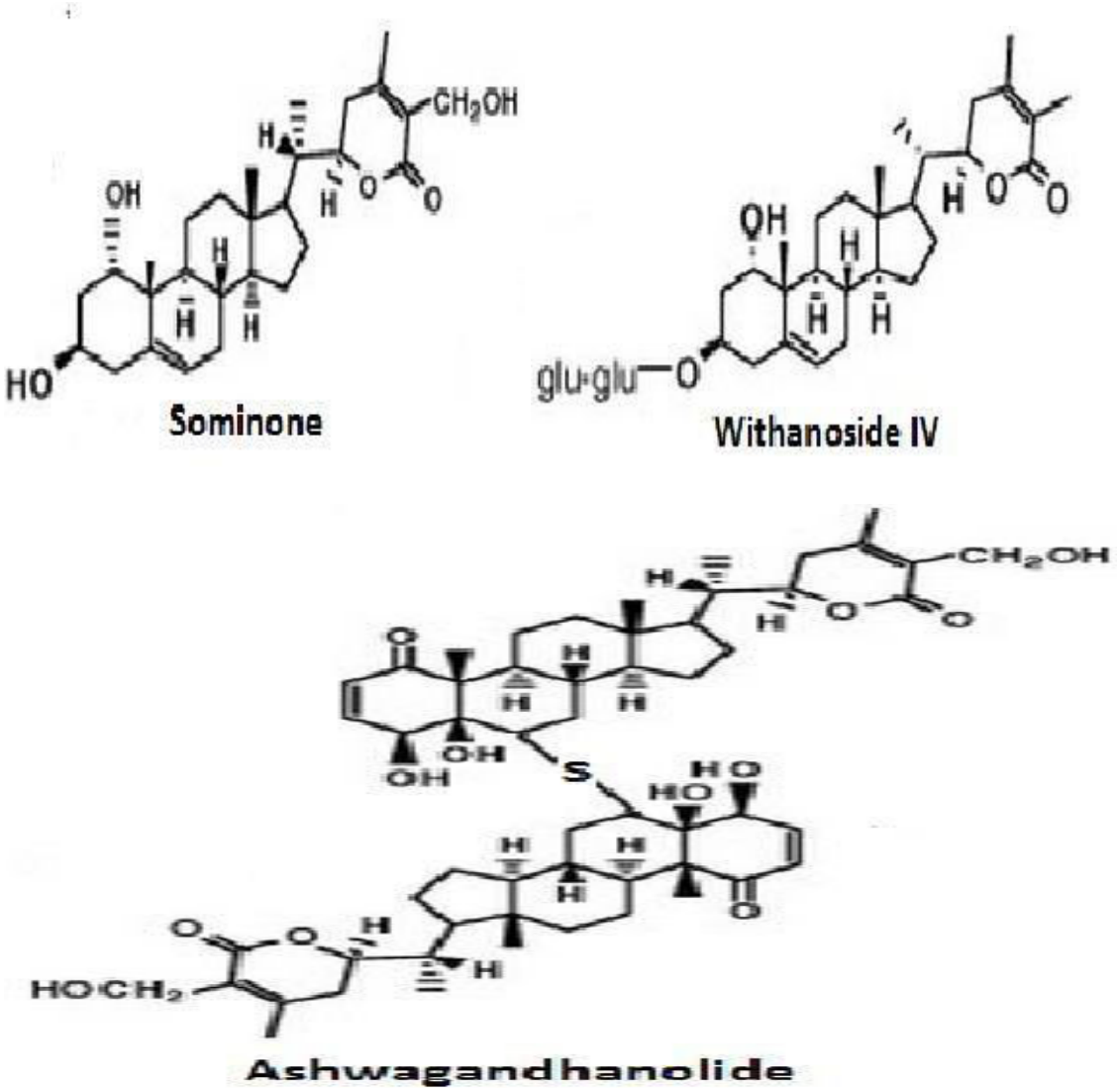


Fig. 7: Withanolide; Fig. 8: Withanolide A; Fig. 9: Withaferin A; Fig. 10: Withanone; Fig. 11: Sominone; Fig. 12: Withanoside IV; Fig. 13: Ashwagandhanolide.

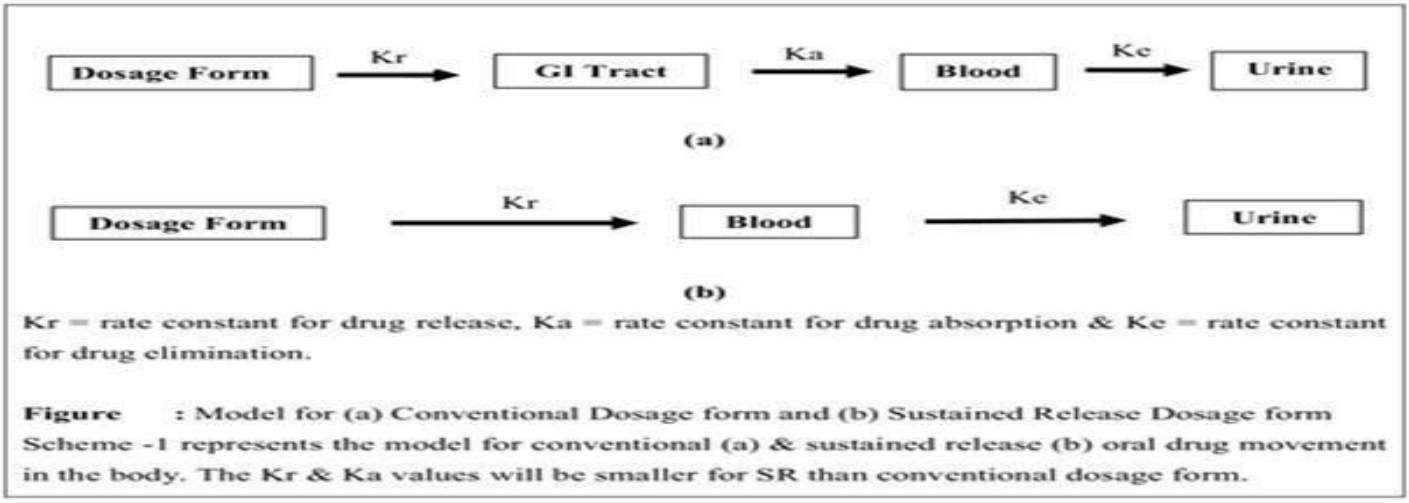
BHAVANA

Ayurveda is a science which deals with various herbal (Kashtha) and mineral (Ras) as well as Herbomineral preparations to combat diseases. A very unique process used in Ayurveda is the process known as "Bhavana" (Impregnation). In this process, a drug or mixture of drugs in powdered form is triturated with a liquid till all liquid portions are absorbed completely. This unique process exclusively mentioned in Ayurveda mixes the drug completely, breaks the complicated chemical molecules into easily absorbable simpler ones thus augmenting the potency of medicines to many folds. (Verma Dilip *et al.* 2011)

Bhavana is a process of wet grinding in which materials are ground with specific liquid media for a particular period to bring minute particles of the material in contact with the liquid media, transformation of the coarse powder to finer state, impregnation of properties of the media to the material which leads to unique and suitable physic-chemical changes i.e. induction of trace elements from herbal juices, to potentiate the efficacy of the material. Required amount of substances and liquid media are levigated smoothly for specific period and is shaped as requirement, often in flat shape and if pressed between finger tips it should be soft to touch, this is considered as indication of proper completion of process.(Chaudhary Anand *et al.*, 2010)

Immediate Release Dosage Form: Immediate Release Dosage Form are those tablets which are designed to disintegrate and release their medication with no special rate controlling features, such as special coatings and other techniques. Immediate release and fast dispersing drug delivery system may offer a solution to these problems. Recently immediate release tablets have started gaining popularity and acceptance as a drug delivery system, mainly because they are easy to administer, has quick onset of action is economical and lead to better patient compliance. They are also a tool for expanding markets, extending product life cycles and generating opportunities. (Akula Nikhil Prashant et al; ISSN: 0974- 2115)

Sustained-Release Dosage Form:



Kr = rate constant for drug release, Ka = rate constant for drug absorption & Ke = rate constant for drug elimination.

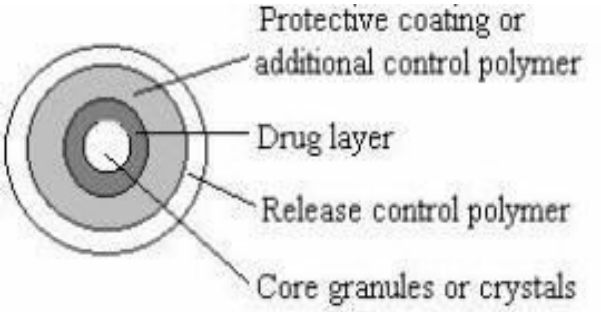
Figure : Model for (a) Conventional Dosage form and (b) Sustained Release Dosage form Scheme -1 represents the model for conventional (a) & sustained release (b) oral drug movement in the body. The Kr & Ka values will be smaller for SR than conventional dosage form.

Factors influencing the design of sustained-release dosage form: The basic rationale of a sustained-release dosage form is to optimize the- i) bio-pharmaceutics; ii) pharmacokinetic; iii) pharmacodynamic properties of a drug.

Serial No.	Properties Of The Candidate Drug
A.	Biopharmaceutic properties
1.	Aqueous solubility of the drug
2.	drug dissociation constant (pK_a) and ionization at physiological pH
3.	Stability in GI milieu
4.	Ionization at physiological pH
5.	Absorption mechanism
B.	Pharmacokinetic properties
1.	Absorption rate constant
2.	Metabolism rate
3.	Dosage form index
C.	Pharmacodynamic properties

1.	Drug dose
2.	Therapeutic range
3.	Therapeutic index
4.	PK/PD relationship

Table No. 1: Factors influencing the design of sustained-release dosage form



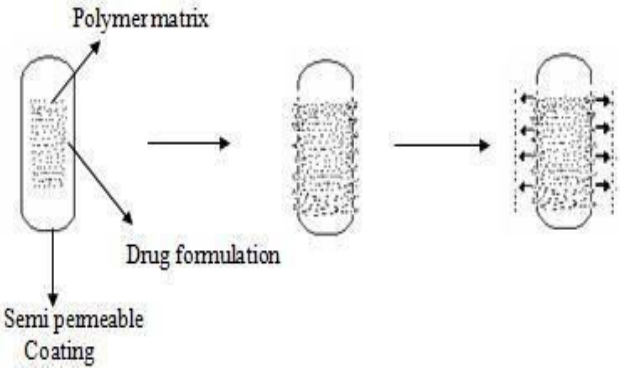


Figure-14: Sustained Release Mechanism; Figure-15: Sustained release Mechanism.

Materials used for the experiments-

i)Ashwagandha hydro-alcoholic extract powder & Ashwagandha root- gift sample by Emami Ltd.; ii) Methanol- for analysis (Merk); iii) Ethyl Acetate- for analysis (Merk); iii) Toluene- GR grade, for proanalysis (Merk); iv) Glacial Acetic Acid- for proanalysis (Merk); v) Chloroform- for analysis (Merk); vi)Hydrochloric Acid- for analysis (Merk); vii) Potassium Dihydrogen Phosphate- for proanalysis GR grade (Merk); viii) Disodium Hydrogen Phosphate- for proanalysis GR grade (Merk); ix) Sodium Sulphite Anhydrous (IP); x) Ethanol- absolute, analytical reagent, for Laboratory use only; xi) Sodium Lauryl sulphate IP; xii) Ammonia IP; xiii) Anisaldehyde Sulphuric Acid Reagent; xiv) TLC Plate- silica gel $60F_{254}$ (Merk), xv) Liebermann Buerchard reagent, xvi) Filter Paper- Whatmann (1) 125 mm qualitative circles, Cat No. 1001-125 and Whatmann (41) Ashless Circles 125mm Cat No. 1441-125.

Excipients used in formulating Tablets:

Hydroxypropyl Methyl Cellulose (HPMC) – HPMC is mixed alkyl-hydroxyl alkyl cellulose ether containing methoxy Hydroxypropyl groups.

Poly-vinyl pyrrolidone (PVP) - PVP (K-30) is a synthetic polymer that may be used as an adhesive in either an aqueous solution or alcohol. It also has some capabilities as a dry binder. Main application is its function as a binder in wet granulation (Raymond et al.).

Sodium Starch Glycolate (SSG) - SSG is used as a Super Disintegrants.

Microcrystalline cellulose (MCC) - MCC is used as a diluents.

Magnesium Stearate- Magnesium Stearate is used as a Compression Lubricants.

Talc- Talc is used as a Glidants.

Development of Standard Curve of Ashwagandha Hydro-Alcoholic dry Extract powder Preparation of phosphate buffer at pH 6.8 – Dissolve 28.80gm of disodium hydrogen phosphate and 11.45gm of potassium dihydrogen phosphate in sufficient water to produce 1000ml. [IP 2007]

Process–

i)0.1gm of drug (Ashwagandha Dry Extract powder) was added with 100ml of phosphate buffer solution (pH 6.8) in 100ml volumetric flask.

ii) Then the solution was filtered in an another volumetric flask.

iii)From this solution, 10ml solution was withdrawn and kept to other 100ml volumetric flask.

iv)The volume of the solution was made up to 100ml i.e. diluted to 100ml. [that was the stock solution; concentration 100µg/ml].

v) Then 1.0ml, 2.0ml, 3.0ml, 4.0ml, 5.0ml, 6.0ml, 7.0ml, 8.0ml, 9.0ml & 10.0ml were withdrawn from the stock solution to other 10ml of 10 volumetric flasks and marked respectively.

vi) Those were diluted to 10 ml individually.

vii) So, the final 10 concentrations would be 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml, 90µg/ml and 100µg/ml.

viii) The UV readings of absorbance were taken at **280 nm** and absorbance was plotted against concentration graphically.

Dilution Scheme-

100 ml of phosphate buffer with drug (1mg/ml) → 10ml

↓

100ml stock solution (100 µg/ml)

↓

1ml (10µg/ml) 2ml (20µg/ml) 3ml (30µg/ml) 4ml (40µg/ml) 5ml (50µg/ml) 6ml (60µg/ml) 7ml (70 µg/ml) 8ml (80 µg/ml) 9ml (90µg/ml) 10ml (100µg/ml) → 100µg/ml

Formulation

SERIAL NO.	NAME OF THE INGREDIENTS	IMMEDIATE RELEASE TABLETS			SUSTAINED RELEASE TABLETS		
		F1	F2	F3(BHAVANA)	F4	F5	F6 (BHAVANA)
1.	Ashwagandha Hydro-Alcoholic Extract	450mg	450mg	450mg	450mg	450mg	450mg
2.	Micro- Crystalline Cellulose (MCC)	131.4 mg	107.4mg	107.4mg	71.4mg	41.4mg	41.4mg
3.	Poly-Vinyl Pyrrolidone (PVP K-30)	9mg	9mg	9mg	9mg	9mg	9mg
4.	Sodium Starch Glycolate (SSG)	-	24 mg	24mg	-	-	-
5.	Hydroxy Propyl Methyl Cellulose (HPMC)	-	-	-	60mg	90mg	90mg
6.	Talc	6mg	6mg	6mg	6mg	6mg	6mg
7.	Magnesium Stearate	3.6 mg	3.6 mg	3.6mg	3.6mg	3.6mg	3.6mg
8.	Total Weight	600mg	600mg	600mg	600mg	600mg	600mg

Table No. 2: Formulation of Ashwagandha Hydro-Alcoholic Extract Powder & Bhavana

Preparation of tablets- The preparation of the tablets (at all batches) was done in wet granulation technique.

Immediate Release Tablets-

a) The Ashwagandha hydro-alcoholic extract powder and all the excipients were passed through the sieve no. #45 mesh (354 micron) to remove the lump and foreign particles and accurately weighed as per the formula.
b) The dispensed quantity of powder extract and MCC were mixed as per the required amount.
c) PVP K-30 and SSG (for F2 and F3) were added and mixed properly.
d) LOD of the mixture was checked and noted.
e) The mixture was passed through the sieve no. #30 mesh (600micron).
f) The mixture was subjected for wet granulation process by adding required amount of water and mixed properly.
g) The formulation was passed through the sieve no. #12 mesh (1680 micron).
h) It was allowed for dry in the tray dryer at 60°c for 15 minutes to achieve the target LOD.
i) The final LOD was checked and noted.
j) After drying, the granules was sized and passed through the sieve no. # 16 mesh (1190 micron).
k) Required amount of talc and magnesium stearate were passed through the sieve no. #60 mesh (250 micron) and blended to the granules for lubricated blend.
l) The precompression parameters of lubricated powder blends (granules) were evaluated.
m) The granules were allowed for compression to the single rotary mini press 8 stations tablet compression

machine.

Sustained Release Tablets-

a) The Ashwagandha hydro-alcoholic extract powder and the excipients were passed through the sieve no. #45 mesh (354 micron) to remove the lump and foreign particles and accurately weighed as per the formula.
b) The dispensed quantity of powder extract and MCC were mixed as per the required amount.
c) PVP K-30 and HPMC were added and mixed properly.
d) LOD of the mixture was checked and noted.
e) The mixture was passed through the sieve no. #30 mesh (600micron).
f) The mixture was subjected for wet granulation process by adding required amount of water and mixed properly.
g) The formulation was passed through the sieve no. #12 mesh (1680 micron).
h) It was allowed for dry in the tray dryer at 60°c for 15 minutes to achieve the target LOD.
i) The final LOD was checked and noted.
j) After drying, the granules were sized and passed through the sieve no. # 16 mesh (1190 micron).
k) Required amount of talc and magnesium stearate were passed through the sieve no. #60 mesh (250 micron) and blended to the granules for lubricated blend.
l) The precompression parameters of lubricated powder blends (granules) were evaluated.
m) The granules were allowed for compression to the single rotary mini press 8 stations tablet compression machine.

In-vitro Dissolution Study:

DILUTION SCHEME-

i)F1, F2, F4, F5- 10ml from dissolution basket → filter→2 ml→10ml

↓

Absorbance at 280nm

ii)F3 & F6- 20 ml from dissolution basket → filter → Absorbance at 280nm

Parameters of Dissolution Studies

Parameters	**Immediate Release Tablets**	**Sustained Release Tablets**
USP Type Apparatus	Type II- Paddle Type Apparatus	Type I- Basket Type Apparatus
Medium	6.8 pH Phosphate Buffer	6.8 pH Phosphate Buffer
Volume	900 ml	900 ml
Temperature	37˚C (± 0.5˚C)	37˚C (± 0.5˚C)
Speed	50 rpm	75 rpm
Time Interval	15 minutes [F1] & 10 minutes [F2 & F3]	1 hour
Wavelength	280 nm	280 nm
Withdrawn Volume	i)10 ml [F1 & F2]; ii) 20 ml [F3]	i)10 ml [F4 & F5]; ii) 20 ml [F6]

Table No. 3: Parameters of Dissolution Study

Experimental Findings, Results

Quantitative Parameters of Ashwagandha dry root powder and Ashwagandha Hydro- alcoholic dry extract powder:

Serial No.	Tests	Ashwagandha hydro-alcoholic dry extract powder			Ashwagandha dry root powder		
		Results	Mean	Standard deviation	Results	Mean	Standard deviation
1.	Foreign matter test	No Foreign matter present	-	-	0.5%	-	-
	API Specification	-			NMT 2%		
2.	Total Ash	7.131%; 5.91%; 6.592%	6.54%	±0.612	3.94%; 3.68%; 3.85%	3.823%	±0.132
	API Specification	NMT 16%			NMT 7%		
3.	Acid-insoluble Ash	1.986%; 1.97%; 1.939%	1.965%	±0.0239	0.37%; 0.3%; 0.31%	0.327%	±0.038
	API Specification	NMT 2%			NMT 1%		
4.	pH	4.98 (at 30.7°c)	-	-	7.12 (at 28.2°c)	-	-
	API Specification	4.5-5.5			-		
5.	Water Soluble Extractives	75.622%; 77.069%	76.35%	±1.02	30.62%; 37.38%	34%	±4.78
	API Specification	NLT 75%			NLT 22%		
6.	Loss on Drying(105° C)	3.19%; 3.14%	3.165%	±0.035	6.81%; 6.21%	6.51%	±0.424
	API Specification	NMT 5%			-		
7.	Total Soluble Solids	94.52%; 92%	93.26%	±1.782	-	-	-
	API Specification	NLT 90%			-		
8.	Alkaloid Content	0.100%; 0.120%	0.110%	±0.01414	0.25%; 0.27%	0.26%	±0.0141
	API Specification	-			NLT 0.2%		

Table No. 4: Quantitative Parameters of Ashwagandha dry root powder and Ashwagandha Hydro-alcoholic dry extract powder

Solubility study for Ashwagandha Hydro-Alcoholic Dry Extract Powder:

Test	pH	As Such	Adding Sodium Lauryl Sulphate
pH depended solubility study	pH 1	93.89 %	97.401 %
	pH4.5	79.12 %	98.898 %
	pH 6.8	76.123 %	98.503 %

Table No. 5: Table of Special Test for Ashwagandha Hydro-Alcoholic Dry Extract Powder

Other Quantitative Parameters of Ashwagandha dry root powder and Ashwagandha Hydro-alcoholic dry extract powder:

Tests	Ashwagandha hydro-alcoholic dry extract powder		Ashwagandha dry root powder	
	Results	API Specification	Results	API Specification
Total microbial Count	70 cfu/gm	NMT 10,000 cfu/gm	2400 cfu/gm	Complies with the prescribed limits
Presence of Microorganisms				
E.Coli	Absent	Absent	Absent	Absent
Salmonella sp.	Absent	Absent	Absent	Absent
S. aureus	Absent	Absent	Absent	Absent
P. aeruginosa	Absent	Absent	Absent	Absent

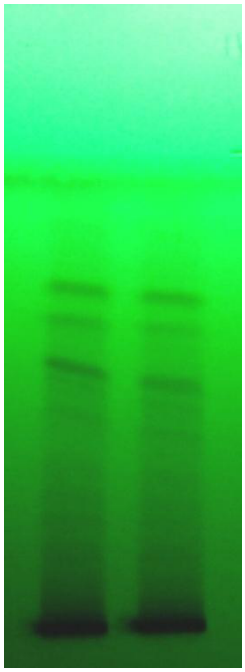
Table No. 6: Table of Microbiological Test of Ashwagandha dry root powder and Ashwagandha Hydro-alcoholic dry extract powder

Chromatographic Tests:

Tests	Ashwagandha hydro-alcoholic dry extract powder (track 2)	Ashwagandha dry root powder (track 1)
	Results	Results
Thin Layer Chromatography	R_f Value = 0.45	R_f Value = 0.55
API Specification	R_f Value= 0.43 Withaferin A	R_f Value= 0.5 (approximately) Withaferin A
High Performance Liquid Chromatography	Total Withanolides- 5.14%	Withaferrin- 0.216% Withanolides- 2%
API Specification	NLT 1% W/W (Withanolides)	NMT 2% (Withanolides)

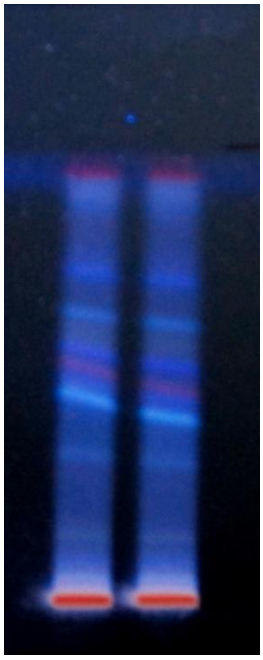
Table No. 7: Table of TLC & HPLC Techniques of Ashwagandha dry root powder and Ashwagandha Hydro-alcoholic dry extract powder

- **Pictures of Thin Layer Chromatography (TLC):**



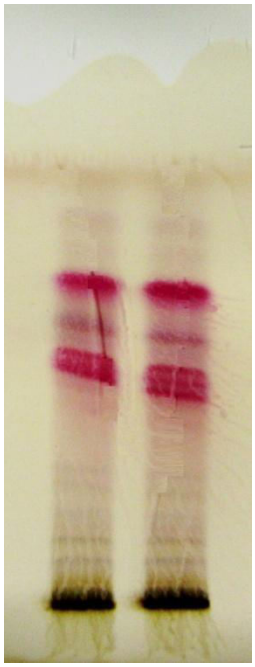
1 2

Fig.16: Under UV (254nm)



1 2

Fig. 17: Fluroscence light (366 nm)



1 2

Fig.18: **Anisaldehyde sulphuric acid**

- **High Performance Thin Layer Chromatography (HPTLC):**

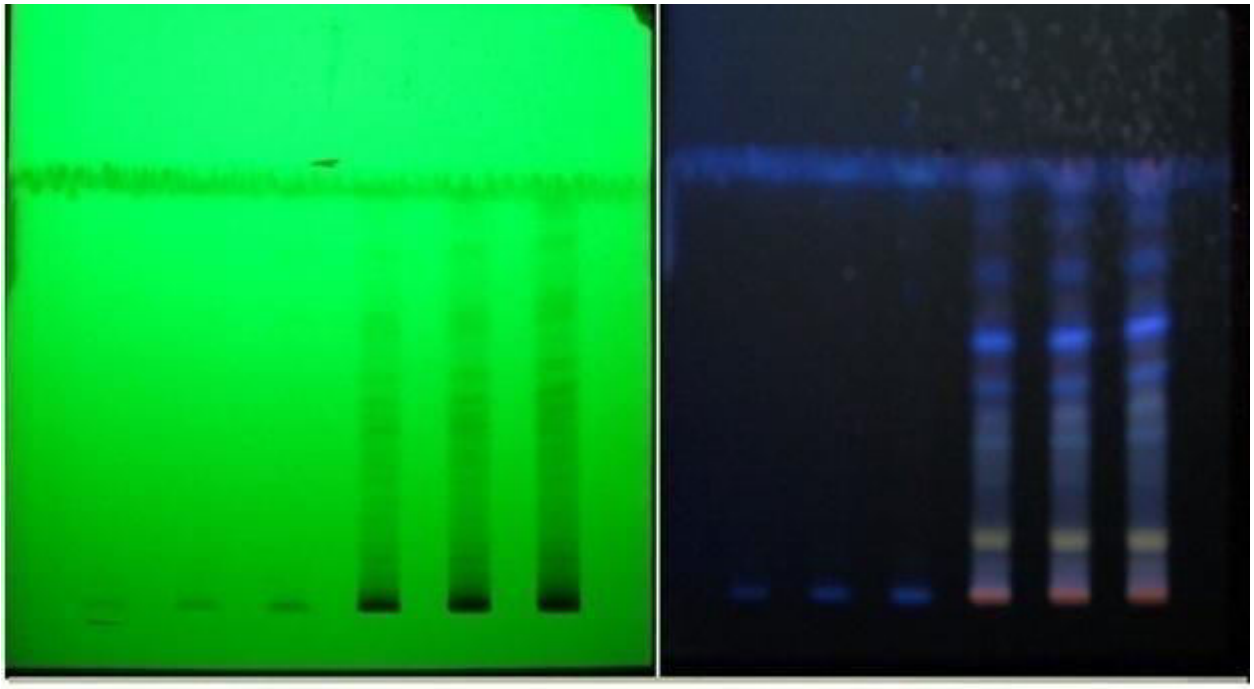
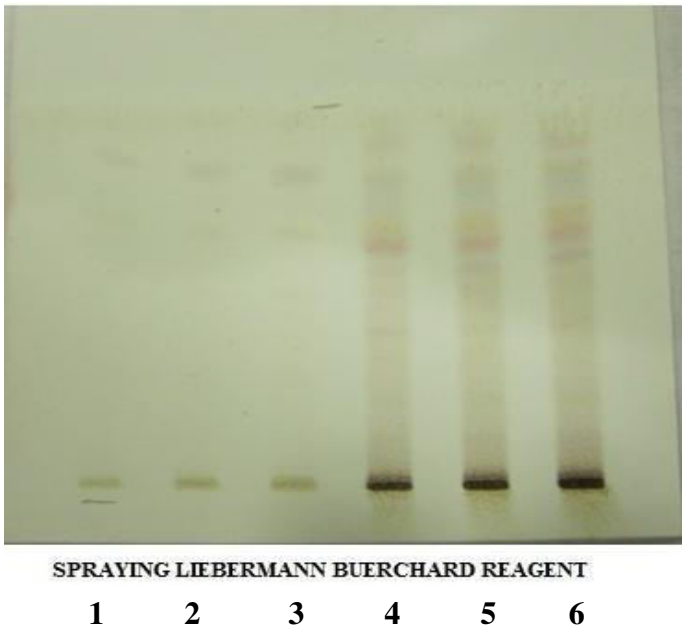


Fig.19 **Fig. 20**



Track 1,2,3= Ashwagandha dry root powder

Track 4,5,6 = Ashwagandha hydro- alcoholic dry extract powder

Fig. 21

Analysis of Bhavana:

Test	Absorbance	Concentration	Chromophore present in 100ml
Analysis of Bhavana	0.046	23µg/ml	11.5 mg/ 100ml

Table No. 8: Table of Analysis of Bhavana

Micromeritics of Ashwagandha Hydro-Alcoholic Extract Powder:

Tests	Results
Bulk Density	0.478
Tapped Density	0.579
Hausner Ratio	1.21
Percent Compressibility	17.44%
Angle of Repose (α=Degree)	26.565

Table No. 9: Table of Micromeritics of Ashwagandha Hydro-Alcoholic Extract Powder

Compatibility Study:

Compatibility Study– Physical Evaluation								
Drug + excipients	**Ratio**	**Initial color**	**Condition (30°C -35°C)**			**Condition (4°C)**		
			7 days	**14 days**	**30days**	**7days**	**14 days**	**30 days**
Ashwagandha hydro-alcoholic extract powder + MCC	1:1	BROWNISH POWDER	No Change	No Change	No Change	No Change	No Change	No Change
Ashwagandha hydro-alcoholic extract powder +PVP	1:1		No Change	No Change	No Change	No Change	No Change	No Change
Ashwagandha hydro-alcoholic extract powder +HPMC	1:1		No Change	No Change	No Change	No Change	No Change	No Change
Ashwagandha hydro-alcoholic extract powder +Talc	1:0.5		No Change	No Change	No Change	No Change	No Change	No Change
Ashwagandha hydro-alcoholic extract powder +Magnesium stearate	1:0.5		No Change	No Change	No Change	No Change	No Change	No Change
Ashwagandha hydro-alcoholic extract powder + All Excipients	1:1		No Change	No Change	No Change	No Change	No Change	No Change

Table No. 10: Table of Compatibility Study of Ashwagandha Hydro-Alcoholic Extract Powder

Observation - Drug and excipients are compatible.

Estimation By UV Spectrophotometric method:
Determination of λ_{max} of Ashwagandha hydro-alcoholic dry extract powder

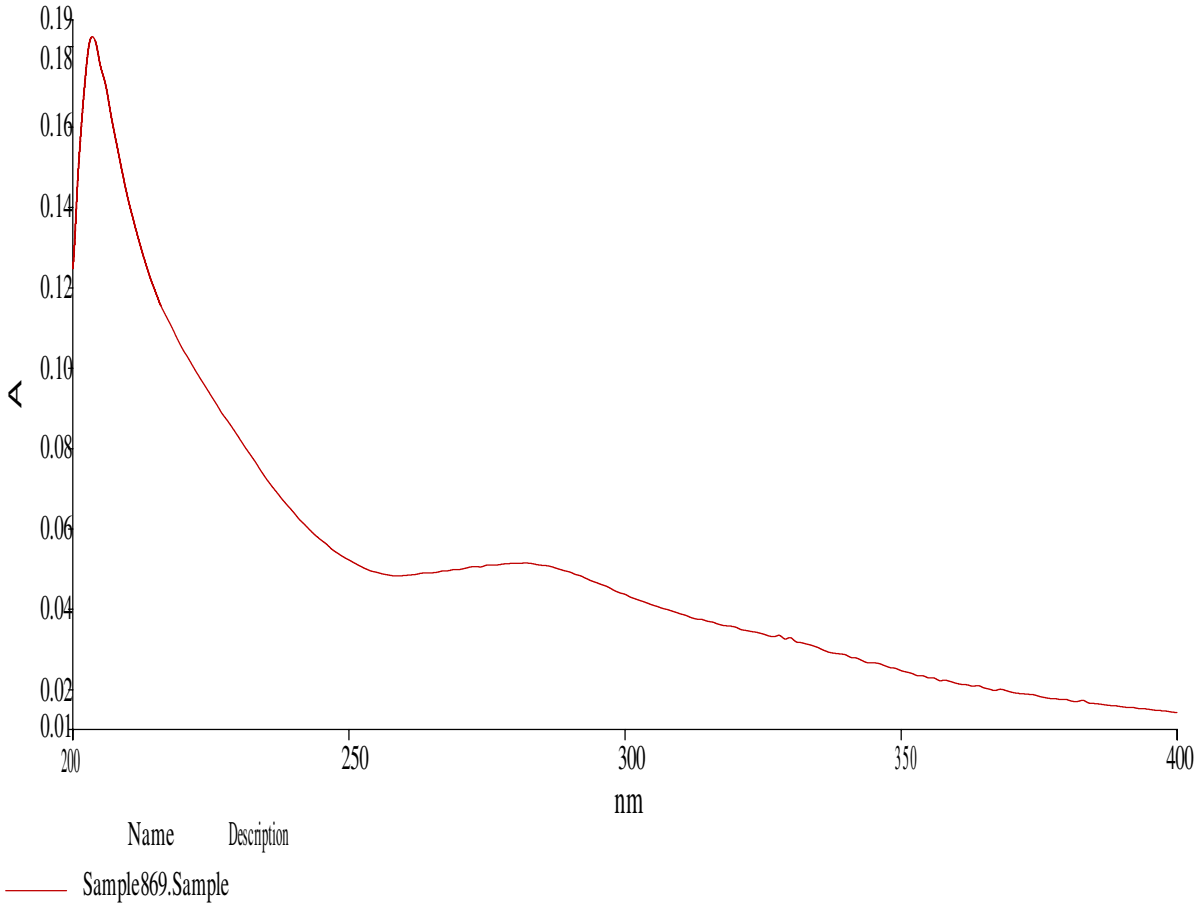


Fig. 22: **Maximum Wavelength (λ_{max})**

The maximum wavelength (λ_{max}) Ashwagandha hydro-alcoholic extract was found to be **280nm**.

Development of standard curve of Ashwagandha hydro-alcoholic extract in pH 6.8 phosphate buffer at 280 nm:

Concentration (µg/ml)	Absorbance at 280 nm
10	0.021
20	0.043
30	0.064
40	0.082
50	0.099
60	0.123
70	0.142
80	0.163
90	0.181
100	0.203

Table No. 11: Table of Standard Curve of Ashwagandha Hydro-Alcoholic Extract Powder

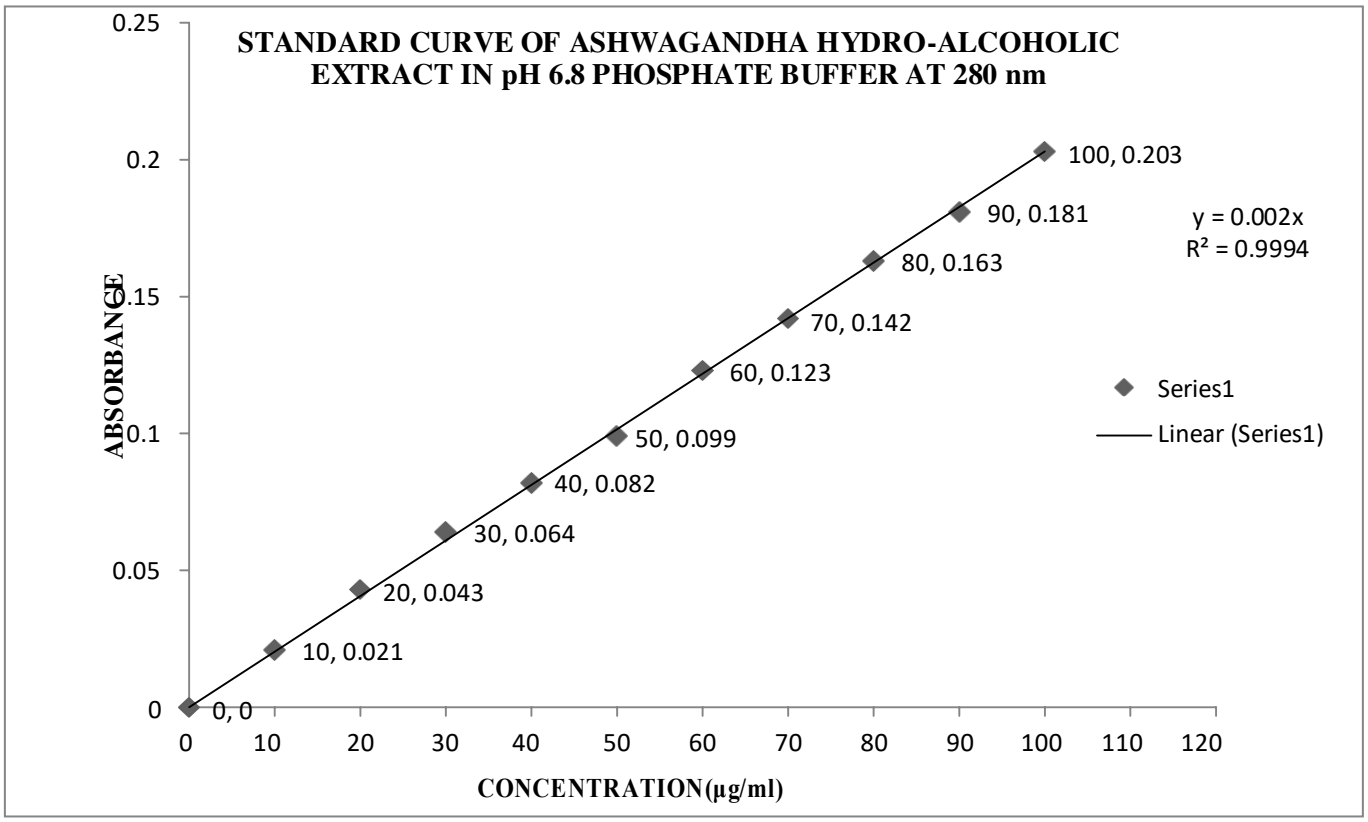


Fig. 23: Standard Curve

Experimental Findings and Results of Formulated Batches

Characterization of lubricated powder blends:

Results of the pre-compression parameters performed on the blend for batch F1 to F6 are enlisted here. The bulk density, tapped density, Carr"s Index, Hausner Ratio for all the formulations were varied from 0.385 to 0.476; 0.435 to 0.556; 11.49% to 14.39%; 1.129 to 1.168 respectively. Angle of repose of all the formulations were found to be less than 30º which indicates a good flow property the powders. The values were found to be in the range of 23.96 to 29.1. The Loss on Drying (LOD) was checked for all the formulations and found to be in the range of 4.14% to 4.99%.

		Immediate release tablets			Sustained release tablets		
Serial No.	**Batch No.**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
1.	**Bulk density(gm/ml)**	0.476	0.435	0.4	0.455	0.385	0.417
2.	**Tapped density (gm/ml)**	0.556	0.5	0.455	0.526	0.435	0.476
3.	**Angle of Repose**	27.7	26.57	23.2	29.1	28.37	23.96
4.	**Carr's Index (%)**	14.39%	13.0%	12.088%	13.498%	11.49%	12.39%
5.	**Hausner's Ratio**	1.168	1.149	1.137	1.156	1.129	1.141
6.	**Loss on Drying at 105°c**	4.93%	4.24%	4.85%	4.26%	4.14%	4.99%

Table No. 12: Table of Characterization of Lubricated Powder Blends

Physico-chemical evaluation of compressed tablets: The formulated tablets were subjected for post compressional evaluation such as thickness, hardness, weight variation, friability, drug-content, disintegration time, in-vitro dissolution studies.

BATCH NO.	F1	F2	F3	F4	F5	F6
Weight Variation (mg)	From 597mg to 609.7mg	From 598 to 606.4	From 599.1 to 601	From 598.9 to 601	From 594 to 607.6	From 595.6 to 605
Mean & Standard Deviation	Mean= 602.7 Std. dev= ±4.14	Mean= 600.8 Std. dev= ±2.997	Mean= 599.96 Std. dev= ±0.568	Mean= 600.01 Std. dev= ±0.734	Mean= 600.03 Std. dev= ±3.95	Mean= 600.69 Std. dev= ±3.27
Thickness (mm)	From 5.80 to 5.88	From 5.73 to 5.77	From 5.96 to 6.07	From 5.92 to 5.96	From 5.67 to 5.69	From 6.15 to 6.48
Mean & standard Deviation	Mean= 5.85 Std. dev= ±0.044	Mean= 5.753 Std. dev= ±0.021	Mean= 6.003 Std. dev= ±0.059	Mean= 5.933 Std. dev= ±0.023	Mean= 5.68 Std. dev= ±0.01	Mean= 6.31 Std. dev= ±0.165
Hardness (kg/cm^2)	From 4.5 to 5.0	From 4.7 to 5.0	From 4.6 to 4.8	From 4.4 to 4.7	From 4.8 to 5.1	From 4.8 to 5.0
Disintegration time	35min 10sec	10min	08min	*	*	*
Friability	0%	0%	0%	0%	0%	0%
Drug content estimation(Assay)	97.33%	98.67%	95.192%	98.67%	97.33%	98.077%

Table No. 13: Table of Physico-chemical evaluation of compressed tablets

***In-vitro* Dissolution Studies of formulation F1 (Immediate Release Tablets):**

Time (min)	Absorbance at 280nm	Concentration (µg/ml)	Drug Release (mg)	Cummulative % Drug Release
15	0.047	23.5	105.75	23.5%
30	0.084	42	189.00	42%
45	0.129	64.5	290.25	64.5%
60	0.169	84.5	380.25	84.5%
75	0.197	98.5	443.25	98.5%

Table No. 14: *in-vitro* Release Profile of Formulation F1

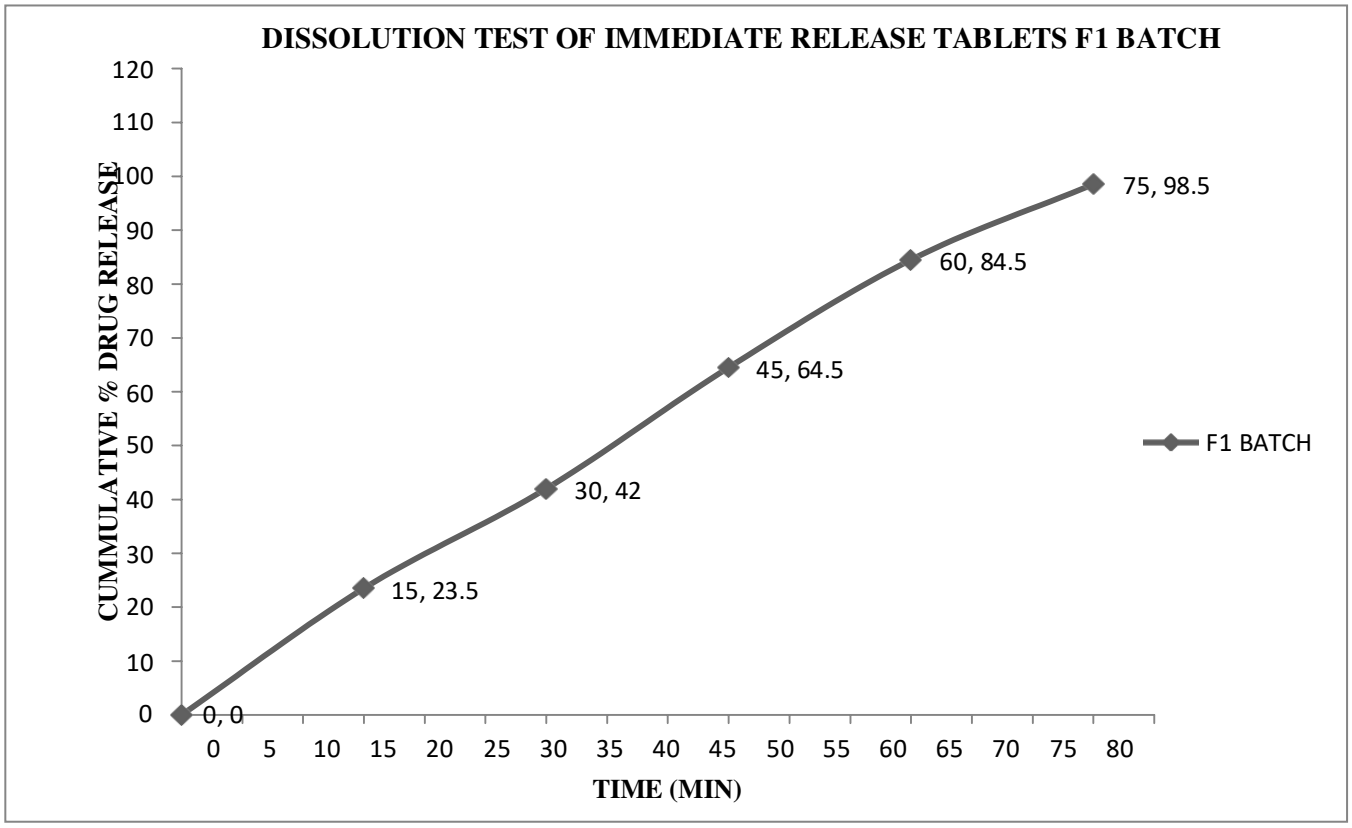


Fig. 24

***In-vitro* Dissolution Studies of formulation F2 (Immediate Release Tablets):**

Time (min)	Absorbance at 280 nm	Concentration (µg/ml)	Drug Release (mg)	Cummulative % Drug Release
10	0.088	44	198	44%
20	0.139	69.5	312.75	69.5%
30	0.189	94.5	425.25	94.5%

Table No. 15: *in-vitro* Release Profile of Formulation F2

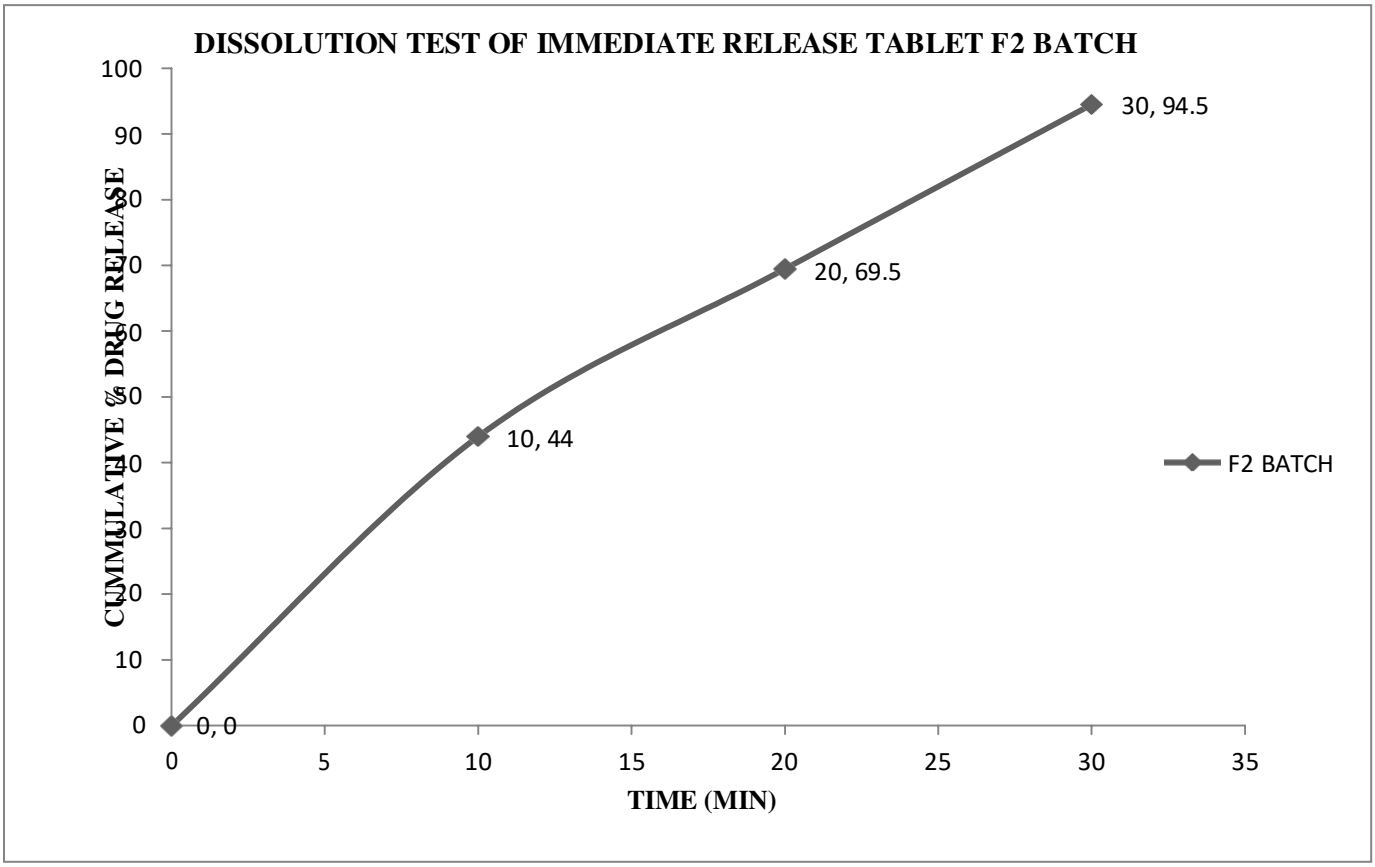


Fig. 25

***In-vitro* Dissolution Studies of formulation F3 (Immediate Release Bhavana Tablets):**

Time (min)	Absorbance at 280 nm	Concentration (µg/ml)	Drug Release (mg)	Cummulative % Drug Release
10	0.044	22	19.8	38.08%
20	0.075	37.5	33.75	64.90%
30	0.109	54.5	49.05	94.33%

Table No. 16: *in-vitro* Release Profile of Formulation F3

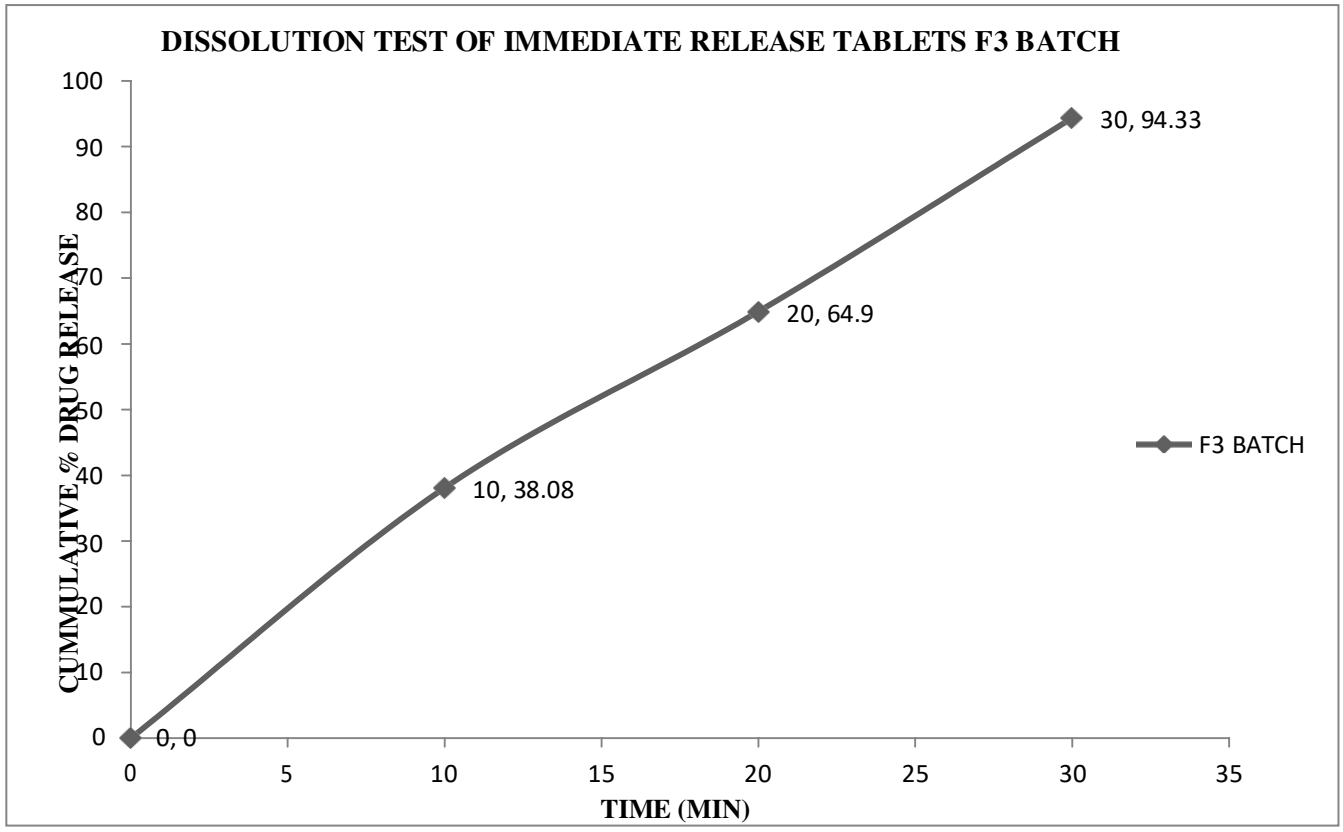


Fig. 26

***In-vitro* Dissolution Studies of formulation F4 (Sustained Release Tablets):**

Time (hour)	Absorbance at 280 nm	Concentration (µg/ml)	Drug Release (mg)	Cummulative % Drug Release
1	0.019	9.5	42.75	9.5%
2	0.045	22.5	101.25	22.5%
3	0.084	42	189	42%
4	0.126	63	283.5	63%
5	0.153	76.5	344.25	76.5%
6	0.177	88.5	398.25	88.5%
7	0.191	95.5	429.75	95.5%
8	0.197	98.5	443.25	98.5%

Table No. 17: *in-vitro* Release Profile of Formulation F4

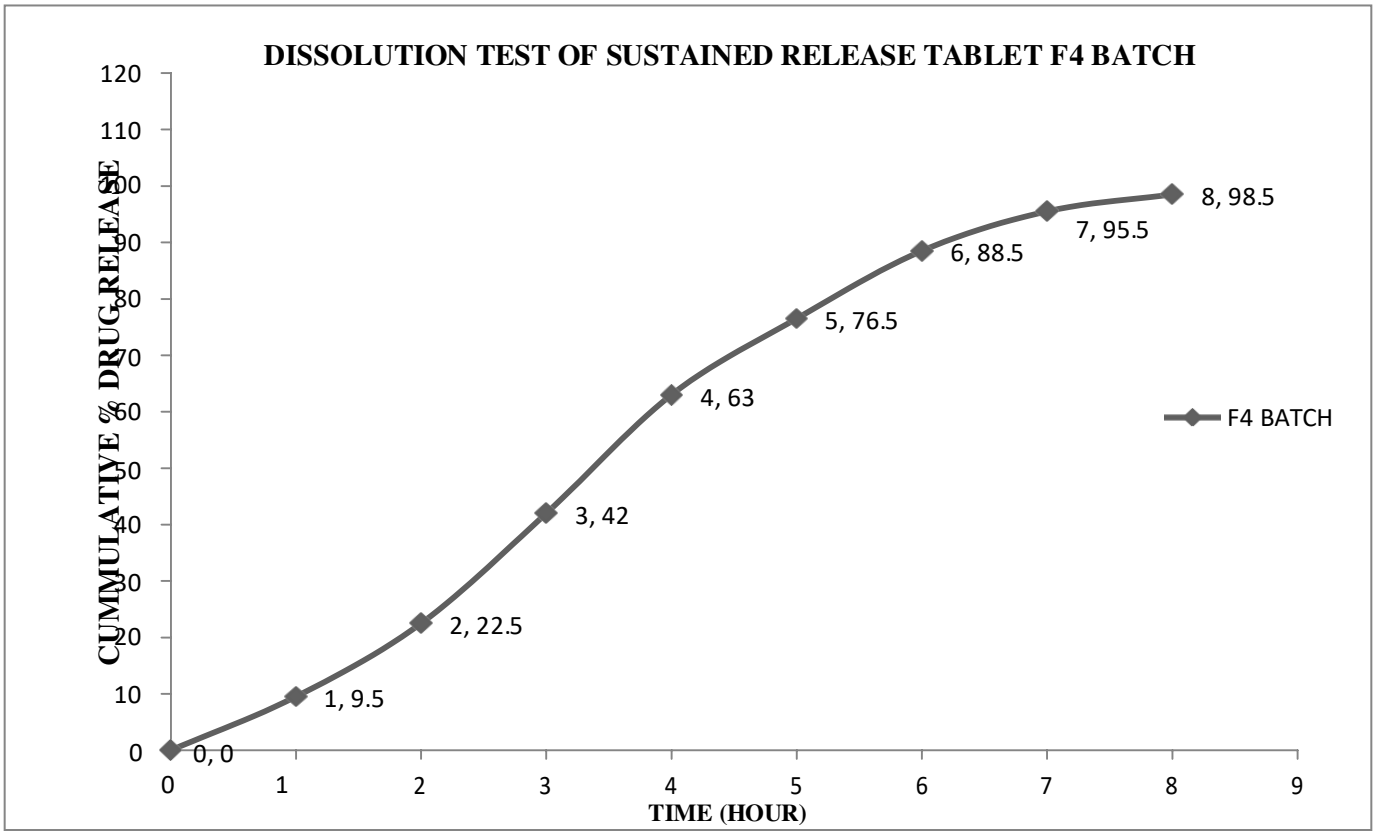


Fig. 27

***In-vitro* Dissolution Studies of formulation F5 (Sustained Release Tablets):**

Time (hour)	Absorbance at 280 nm	Concentration (µg/ml)	Drug Release (mg)	Cummulative % Drug Release
1	0.017	8.5	38.25	8.5 %
2	0.037	18.5	83.25	18.5 %
3	0.076	38	171.00	38 %
4	0.103	51.5	231.75	51.5%
5	0.127	63.5	285.75	63.5%
6	0.145	72.5	326.25	72.5%
7	0.156	78	351	78%
8	0.161	80.5	362.25	80.5%
9	0.165	82.5	371.25	82.5%
10	0.170	85	382.5	85%

Table No. 18: *in-vitro* Release Profile of Formulation F5

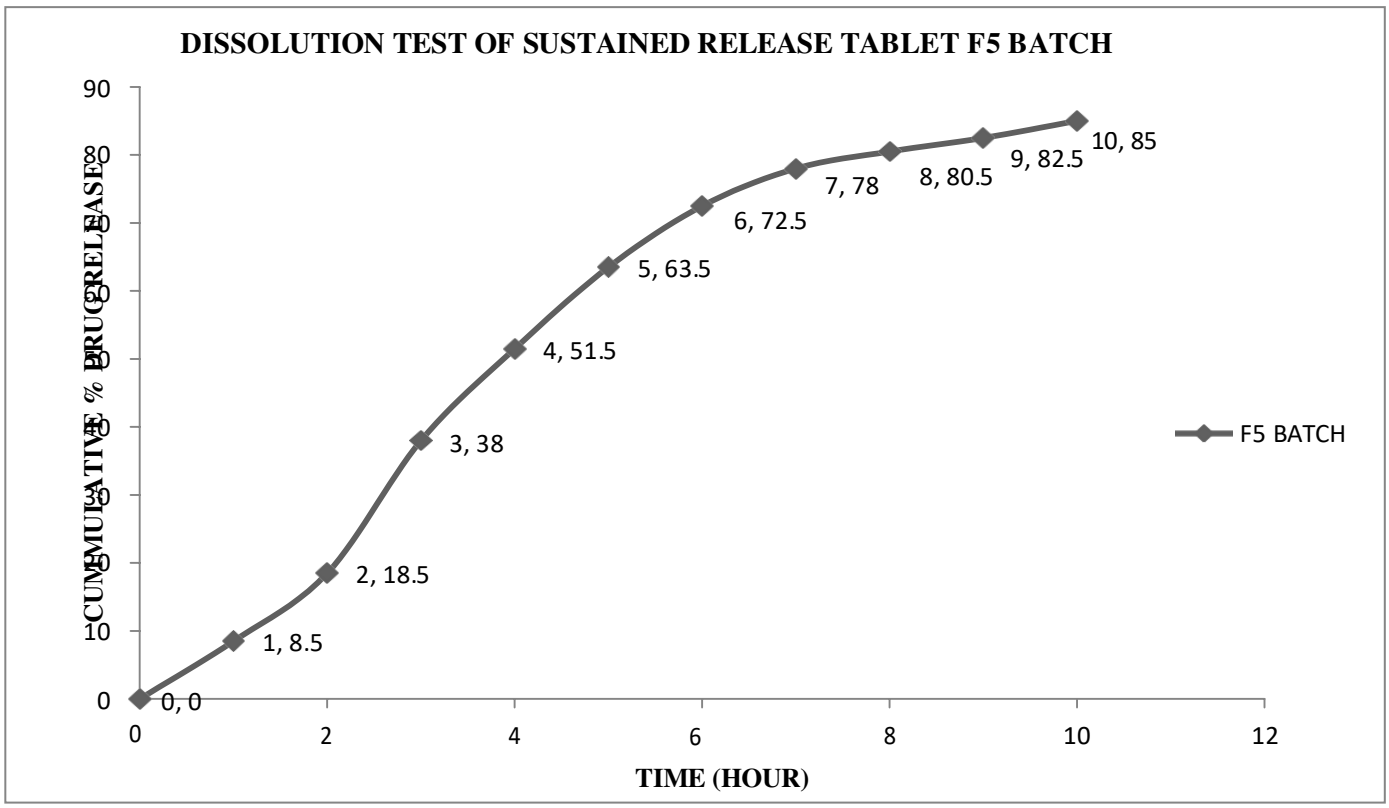


Fig. 28

***In-vitro* Dissolution Studies of formulation F6 (Sustained Release Bhavana Tablets):**

Time (hour)	Absorbance at 280 nm	Concentration (µg/mL)	Drug Release (mg)	Cumulative % Drug Release
1	0.008	4.00	3.600	6.923%
2	0.019	9.5	8.55	16.44%
3	0.041	20.5	18.45	35.48%
4	0.058	29	26.1	50.19%
5	0.070	35	31.5	60.58%
6	0.083	41.5	37.35	71.83%
7	0.088	44	39.6	76.15%
8	0.091	45.5	40.95	78.75%
9	0.094	47	42.30	81.35%
10	0.096	48	43.2	83.08%

Table No. 19: *in-vitro* Release Profile of Formulation F6

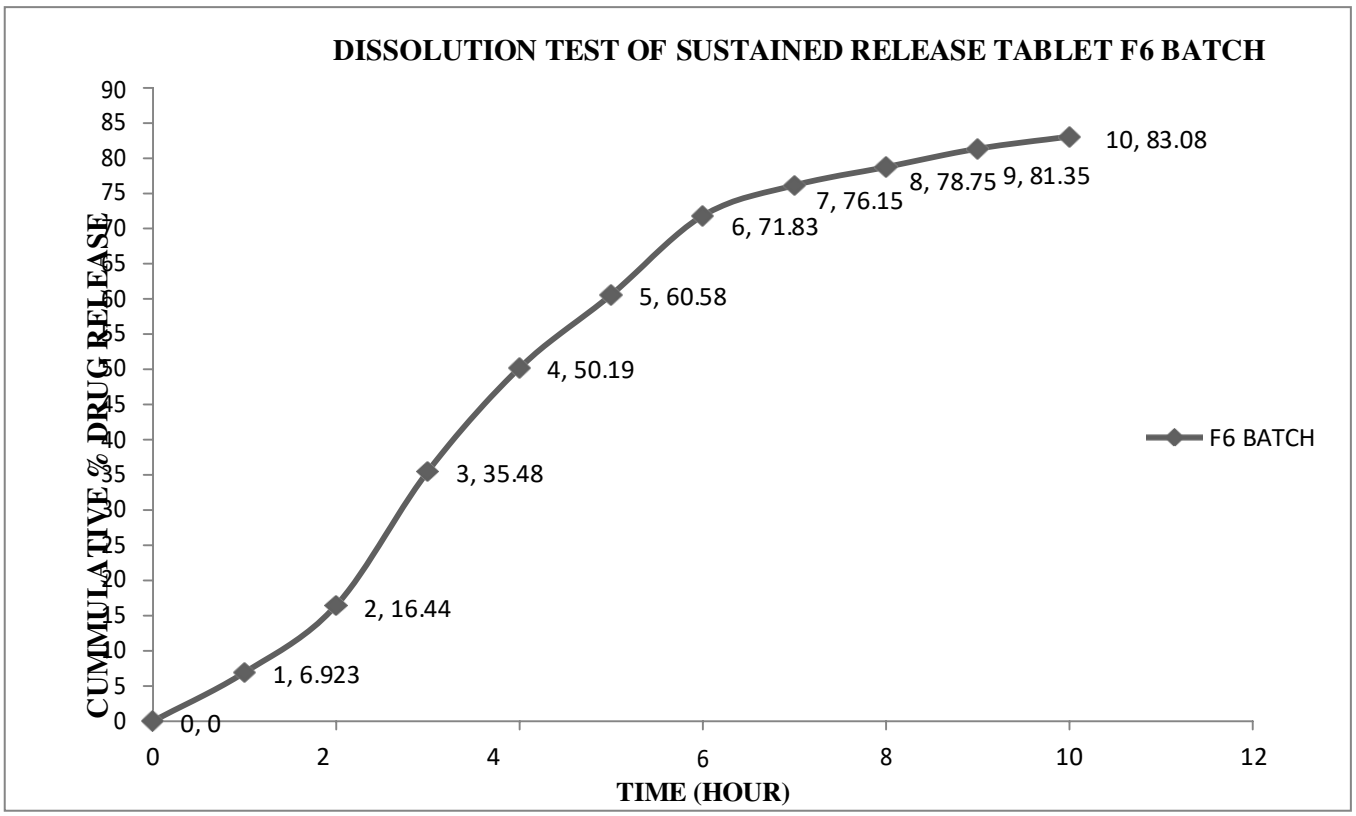


Fig. 29

***In-vitro* Dissolution Studies of Formulation F4, F5 and F6:**

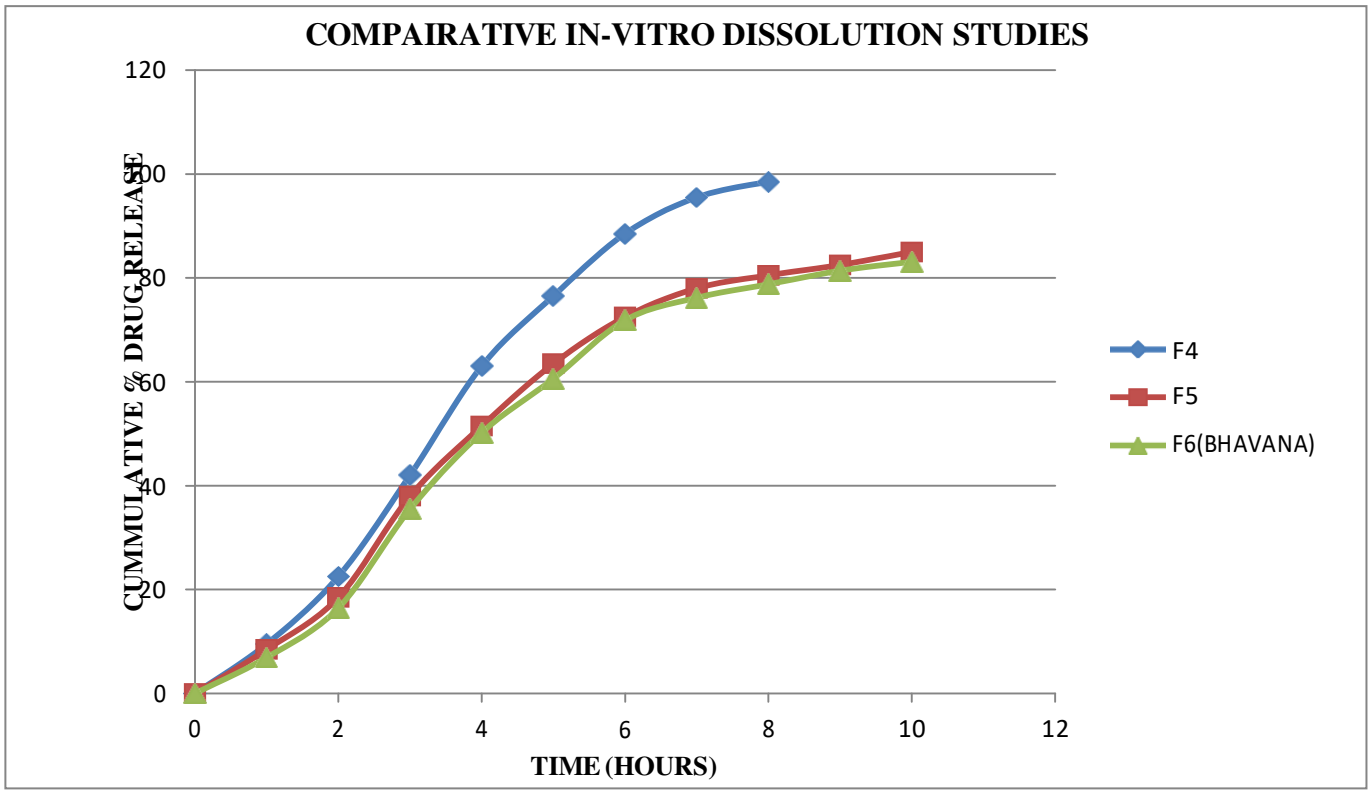


Fig. 30

Summary: Findings of Percent Drug Release of Different Sustained Release Formulations

Batch No.	HPMC	% CR at 2 hrs	% CR at 4 hrs	% CR at 6 hrs	%CR at 8 hrs	%CR at 10 hrs
F4	60 mg	22.5%	63%	88.5%	98.5%	-
F5	90 mg	18.5%	51.5%	72.5%	80.5%	85%
F6 (Bhavana)	90 mg	16.44%	50.19%	71.83%	78.75%	83.08%

Table No. 20: Summary: Findings of Different Formulations

Release Kinetics of the Sustained Release Dosage Forms of Different Formulations Batch-F4

Time (hr)	% Cummulative Drug Release	Square Root Of Time (√T)	Log (% Drug Remain To Be Released)	Log T	Fraction Drug Release	Log (Fraction Drug Release)
1	9.5%	1	1.9566	0	0.095	-1.022
2	22.5%	1.414	1.8893	0.301	0.225	-0.6478
3	42%	1.732	1.7634	0.477	0.42	-0.3768
4	63%	2	1.5682	0.602	0.63	-02007
5	76.5%	2.236	1.3711	0.699	0.765	-0.116
6	88.5%	2.449	1.06	0.778	0.885	-0.053
7	95.5%	2.646	0.6532	0.845	0.955	-0.019997
8	98.5%	2.828	0.176	0.903	0.985	-0.006564

Table No. 21: Release Kinetics data of Batch-F4

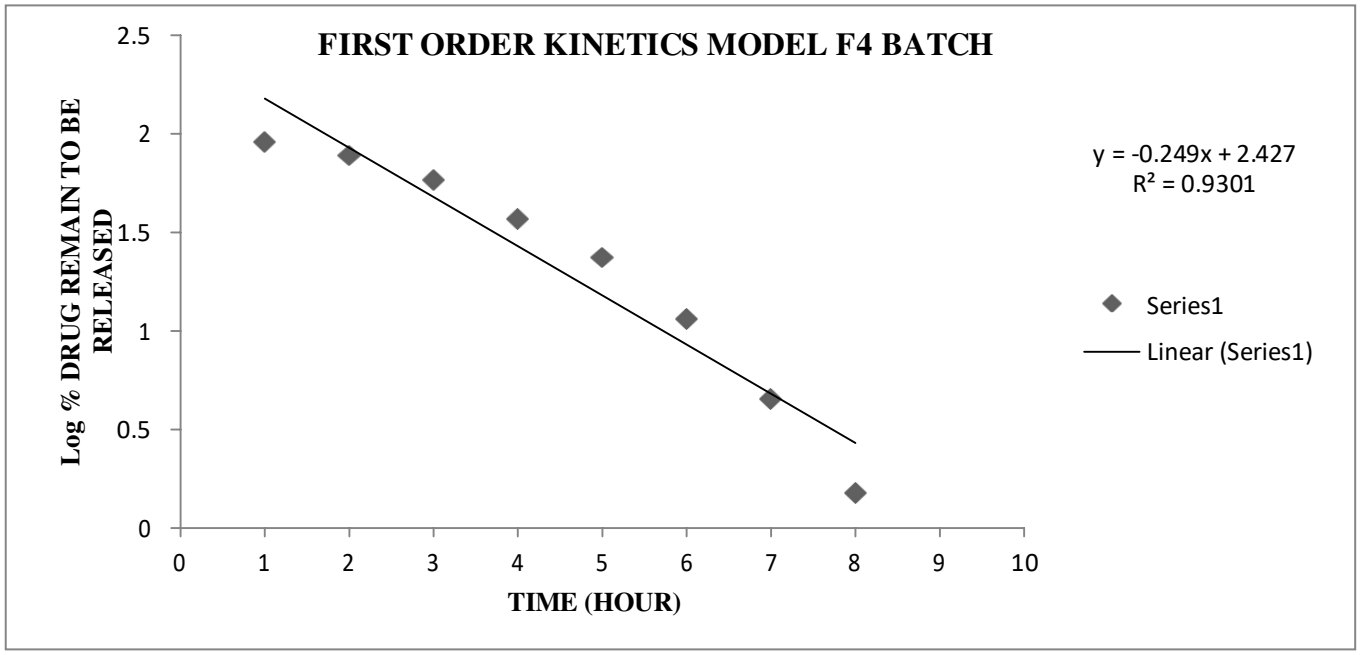


Fig. 31

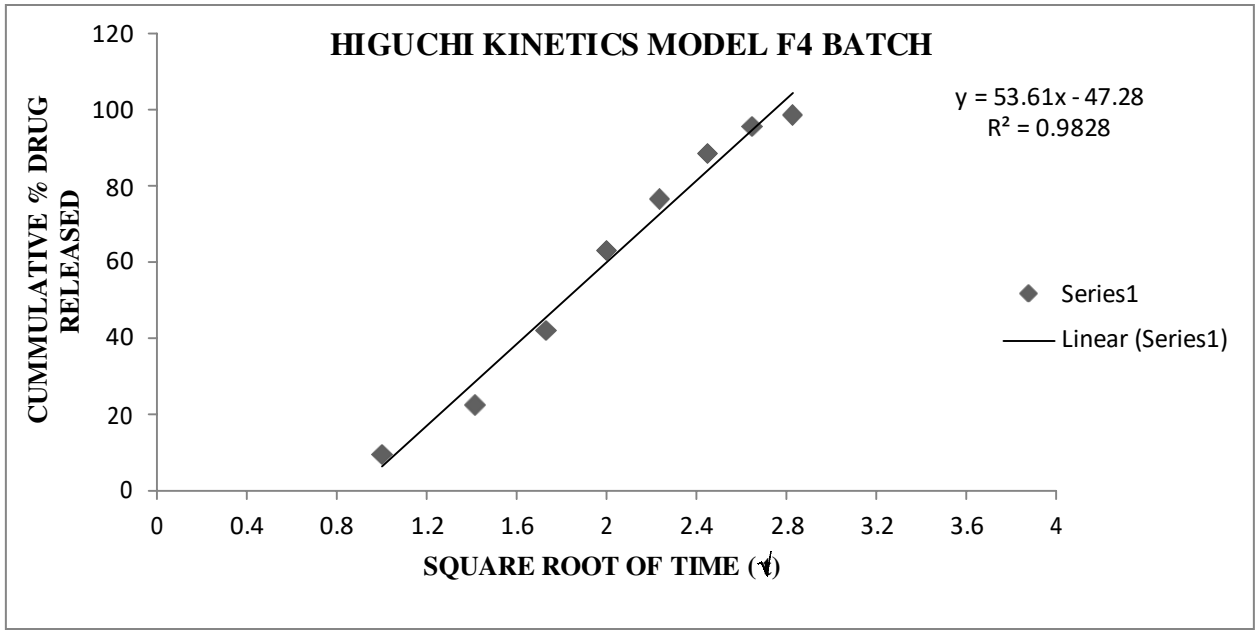

HIGUCHI KINETICS MODEL F4 BATCH
y = 53.61x - 47.28
R² = 0.9828
CUMMULATIVE % DRUG RELEASED
SQUARE ROOT OF TIME (√t)
Series1
Linear (Series1)
0
20
40
60
80
100
120
0
0.4
0.8
1.2
1.6
2
2.4
2.8
3.2
3.6
4

Fig. 32

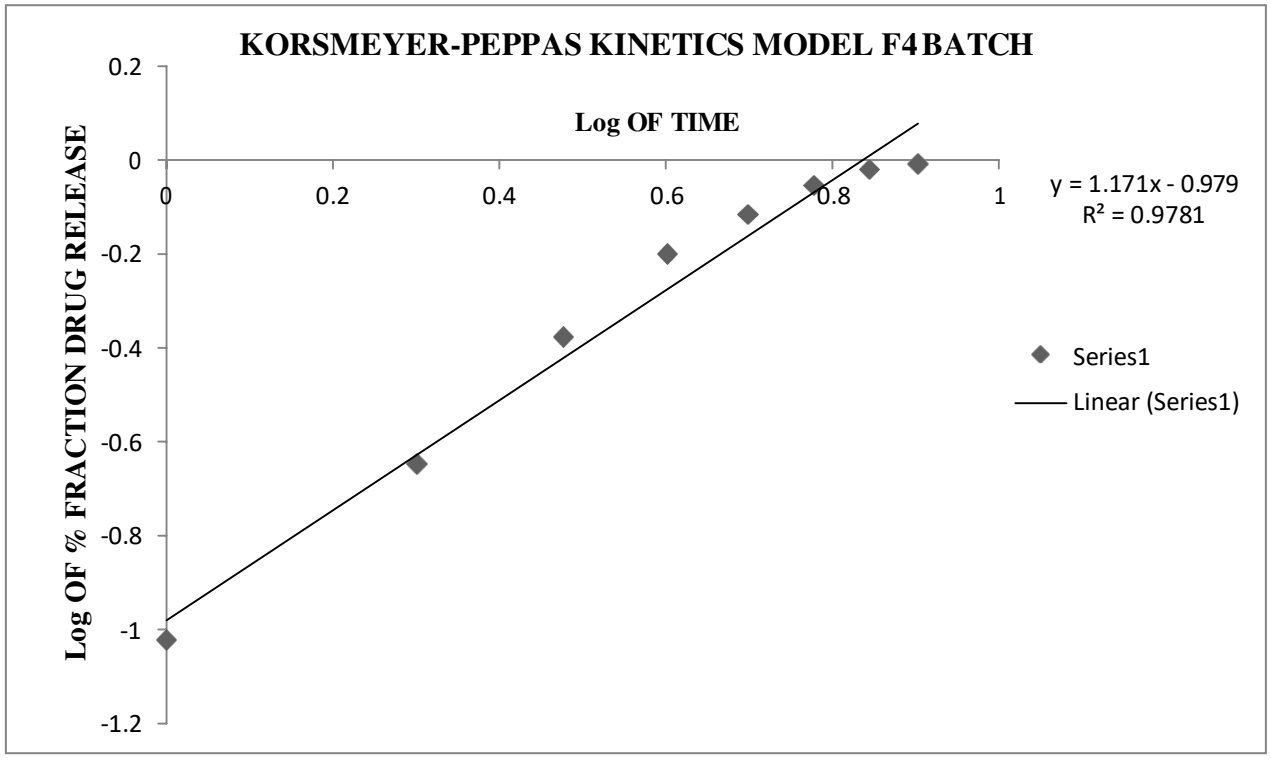

KORSMEYER-PEPPAS KINETICS MODEL F4 BATCH
y = 1.171x - 0.979
R² = 0.9781
Log OF % FRACTION DRUG RELEASE
Log OF TIME
Series1
Linear (Series1)
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
0
0.2
0.4
0.6
0.8
1

Fig. 33

Batch-F5

Time (hr)	%Drug Release	Square Root Of Time (√T)	Log (% Drug Remain To Be Released)	Log T	Fraction Drug Release	Log (Fraction Drug Release)
1	8.5	1	1.961	0	0.085	-1.0706
2	18.5	1.414	1.911	0.301	0.185	-0.73283
3	38	1.732	1.792	0.477	0.38	-0.4202
4	51.5	2	1.686	0.602	0.515	-0.2882
5	63.5	2.236	1.5623	0.699	0.635	-0.19723
6	72.5	2.449	1.4393	0.778	0.725	-0.1397
7	78	2.646	1.3424	0.845	0.78	-0.1079
8	80.5	2.828	1.29	0.903	0.805	-0.0942
9	82.5	3	1.24	0.954	0.825	-0.0835
10	85	3.162	1.17609	1	0.85	-0.0706

Table No. 22: Release Kinetics data of Batch-F5

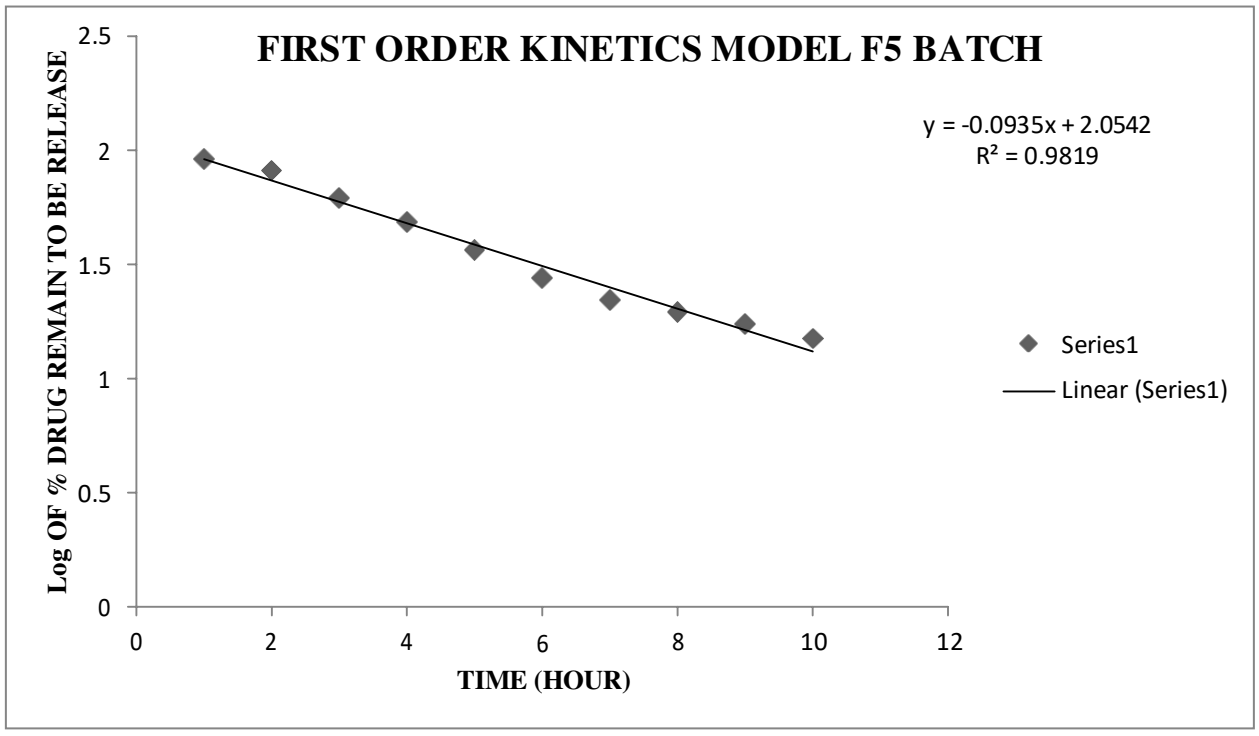


Fig. 34

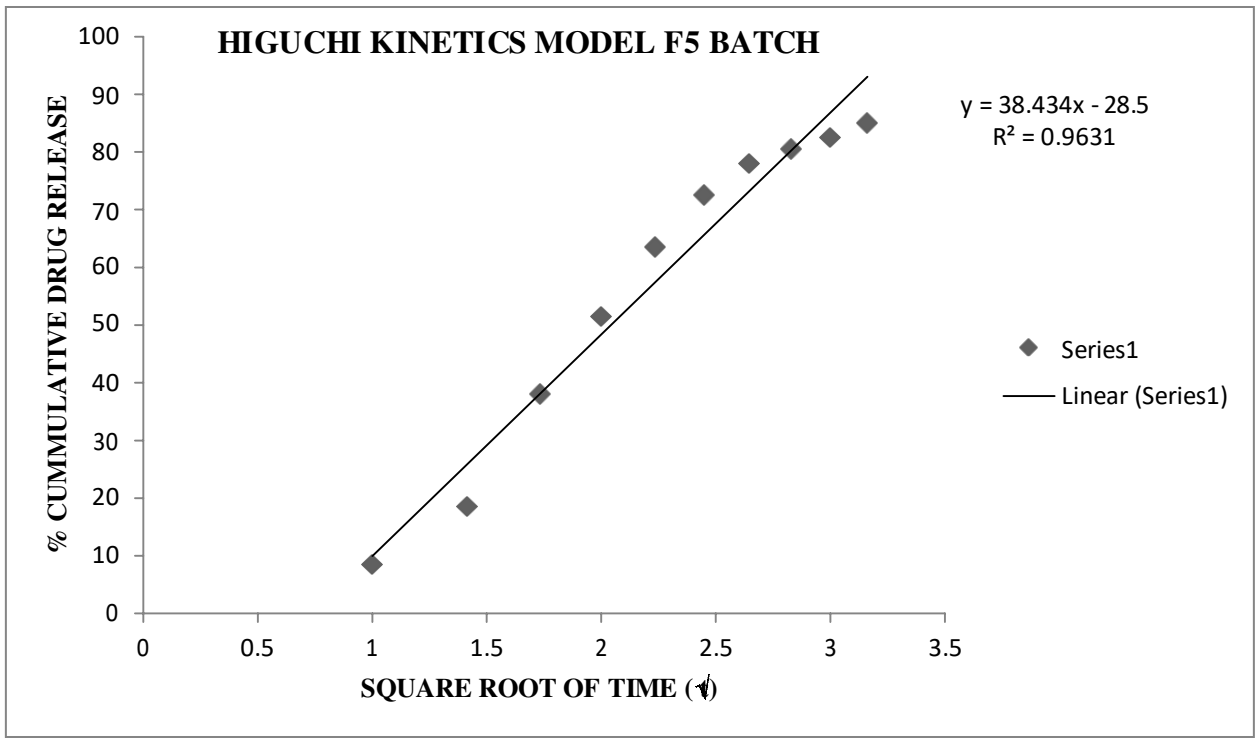

HIGUCHI KINETICS MODEL F5 BATCH
% CUMMULATIVE DRUG RELEASE
SQUARE ROOT OF TIME (√t)
y = 38.434x - 28.5
R² = 0.9631
Series1
Linear (Series1)
0
10
20
30
40
50
60
70
80
90
100
0
0.5
1
1.5
2
2.5
3
3.5

Fig. 35

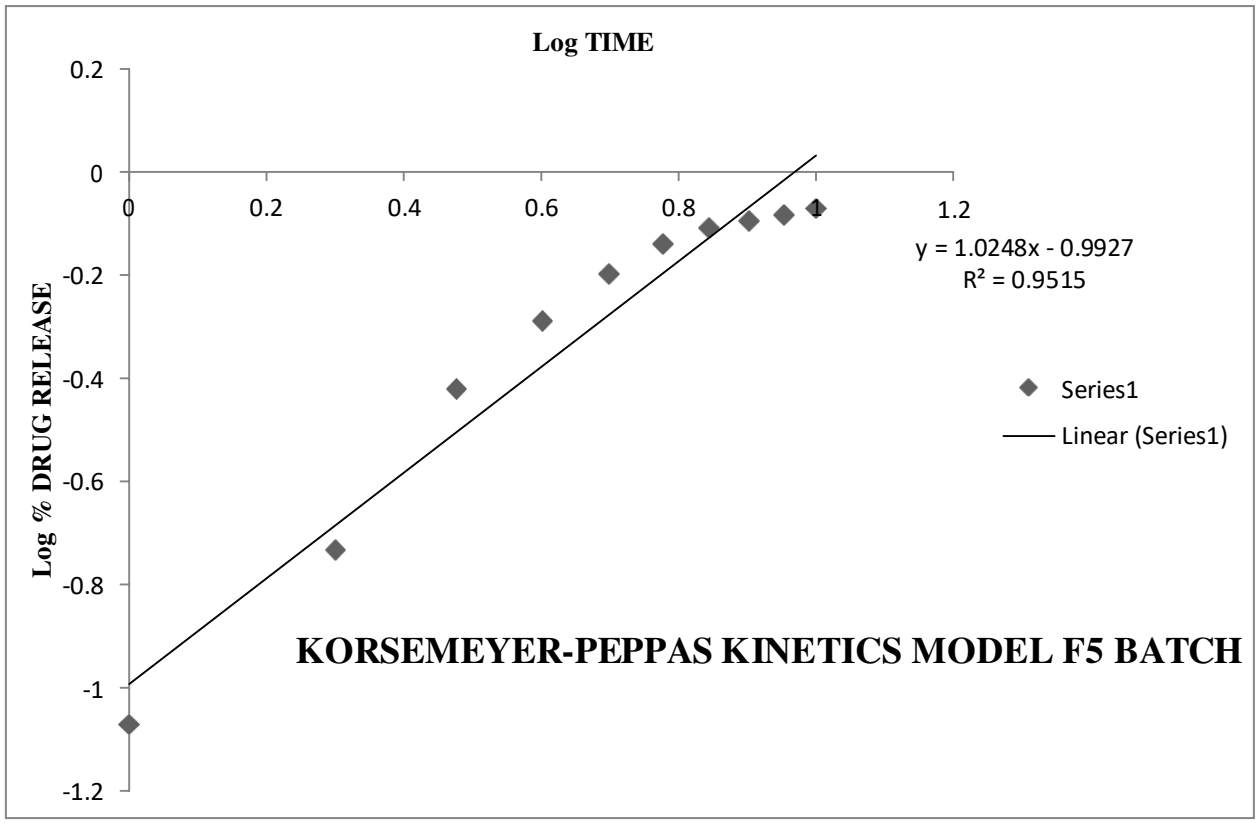

Log TIME
Log % DRUG RELEASE
y = 1.0248x - 0.9927
R² = 0.9515
Series1
Linear (Series1)
KORSEMEYER-PEPPAS KINETICS MODEL F5 BATCH
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
0
0.2
0.4
0.6
0.8
1
1.2

Fig. 36

Bhavana F6 Batch

Time (hr)	%Drug Release	Square Root Of Time (√T)	Log (% Drug Remain To Be Released)	Log T	Fraction Drug Release	Log (Fraction Drug Release)
1	6.923	1	1.9688	0	0.06923	-1.1597
2	16.44	1.414	1.922	0.301	0.1644	-0.7841
3	35.48	1.732	1.80969	0.477	0.3548	-0.450
4	50.19	2	1.6973	0.602	0.5019	-0.2994
5	60.58	2.236	1.5957	0.699	0.6058	-0.2177
6	71.83	2.449	1.4498	0.778	0.7183	-0.1437
7	76.15	2.646	1.3775	0.845	0.7615	-0.11833
8	78.75	2.828	1.3274	0.903	0.7875	-0.10375
9	81.35	3	1.2707	0.954	0.8135	-0.0896
10	83.08	3.162	1.228	1	0.8308	-0.0805

Table No. 23: Release Kinetics data of Batch-F6

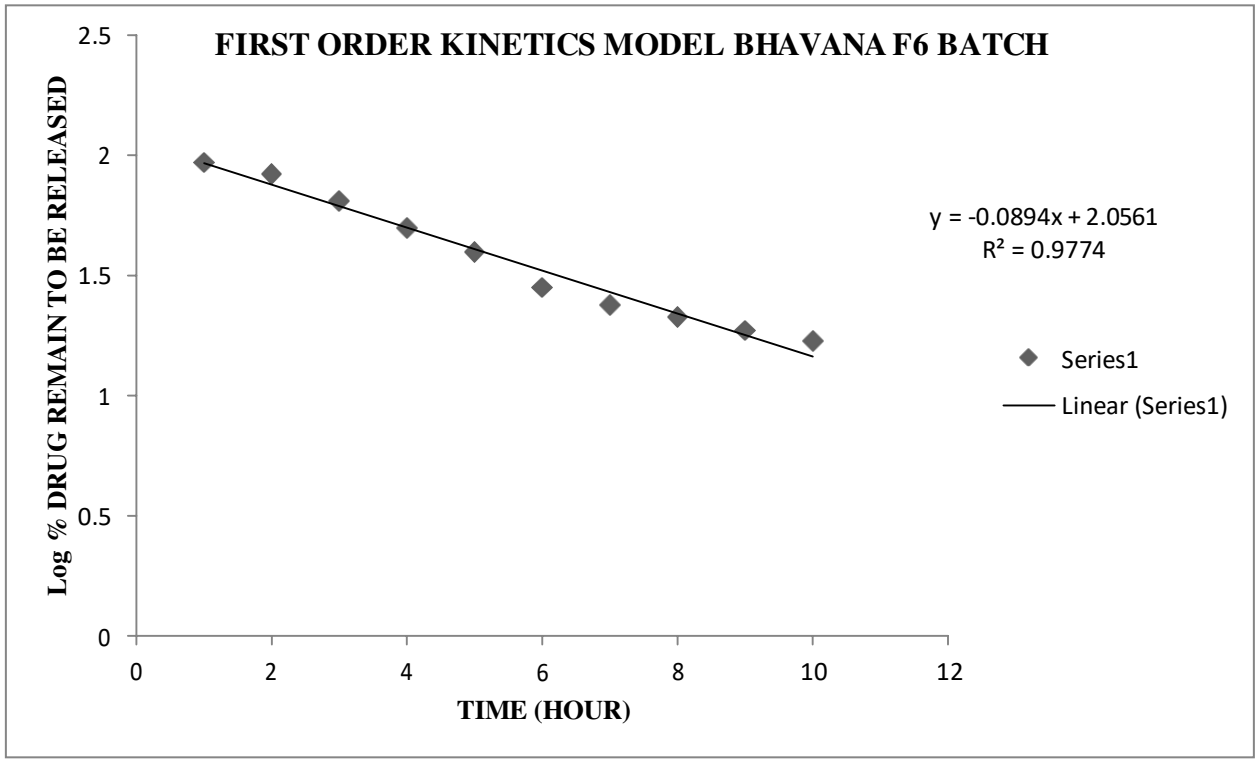


Fig. 37

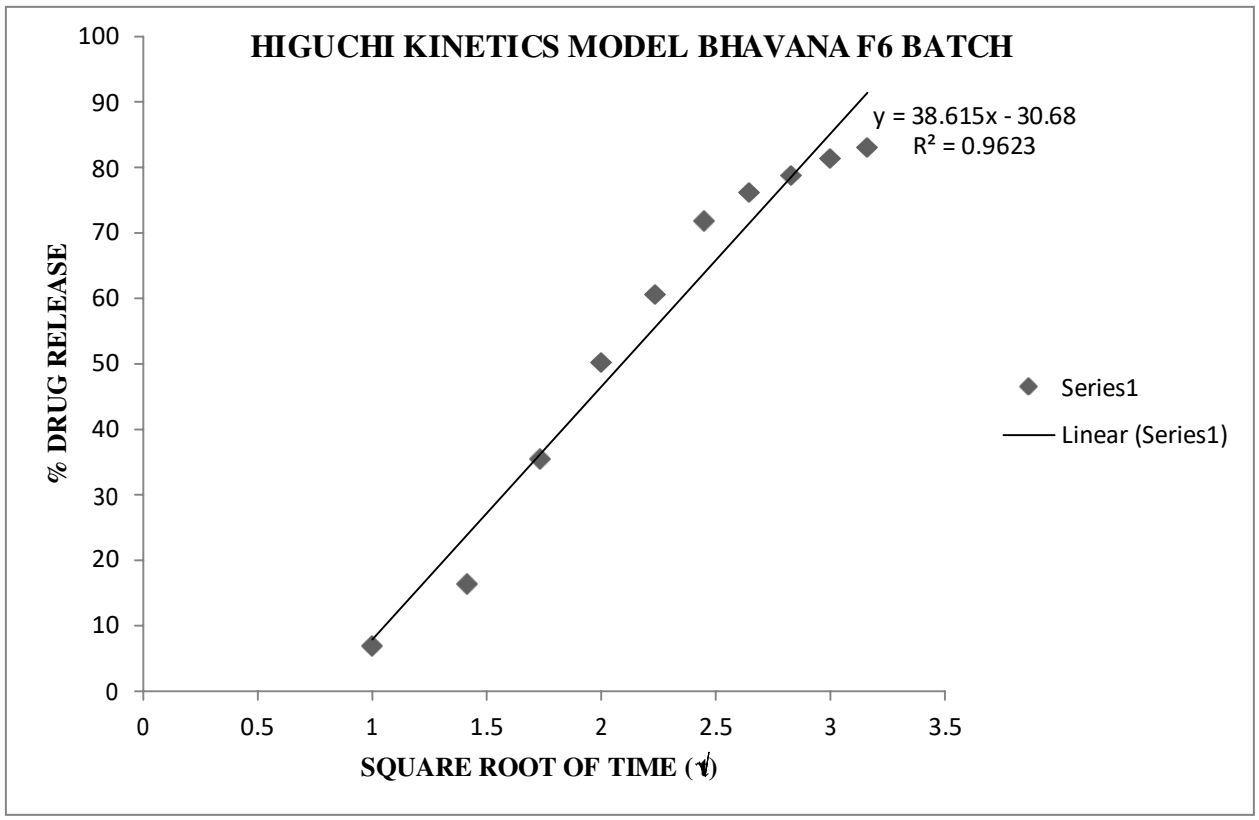

HIGUCHI KINETICS MODEL BHAVANA F6 BATCH
y = 38.615x - 30.68
R² = 0.9623
% DRUG RELEASE
SQUARE ROOT OF TIME (√t)
Series1
Linear (Series1)
0
10
20
30
40
50
60
70
80
90
100
0
0.5
1
1.5
2
2.5
3
3.5

Fig. 38

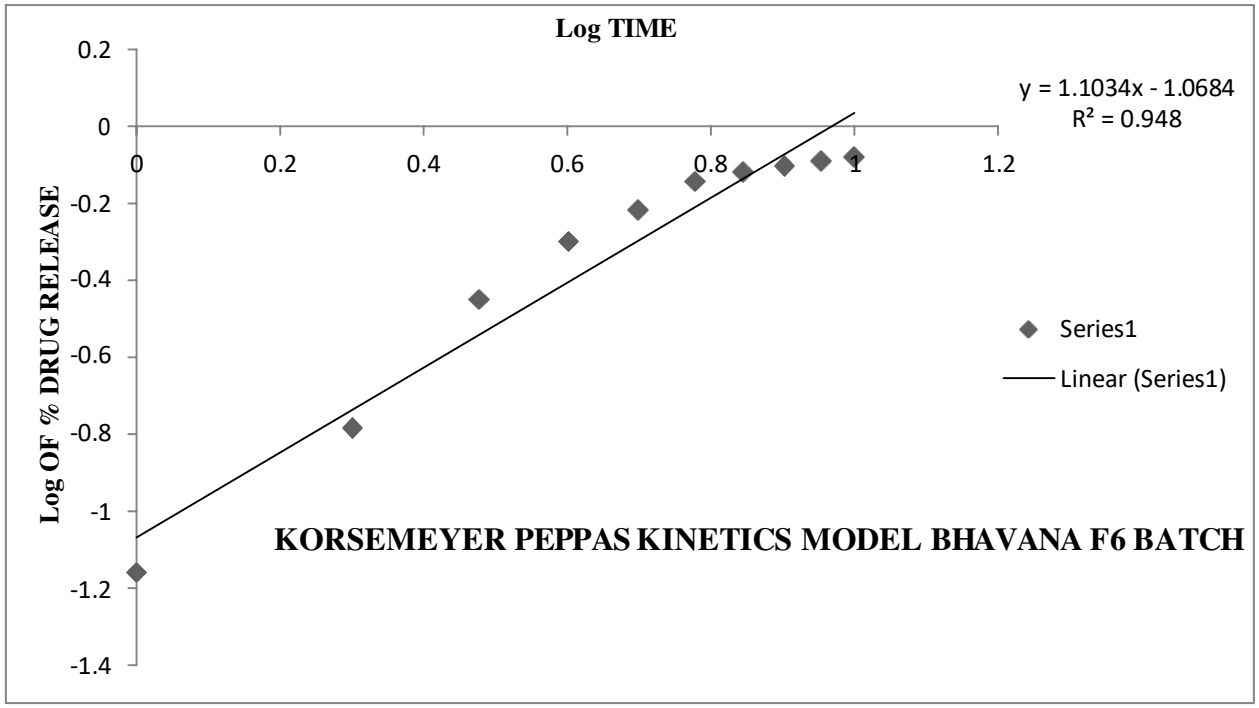

Log TIME
y = 1.1034x - 1.0684
R² = 0.948
Log OF % DRUG RELEASE
KORSEMEYER PEPPAS KINETICS MODEL BHAVANA F6 BATCH
Series1
Linear (Series1)
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
0
0.2
0.4
0.6
0.8
1
1.2

Fig. 39

Release Kinetics Data

Batch No.	Zero Order Kinetics (R^2)	First Order Kinetics (R^2)	Higuchi Kinetics (R^2	Korsmeyer-Peppas Kinetics (R^2)
F4	0.970	0.930	0.982	0.978
F5	0.911	0.981	0.963	0.951
F6 (Bhavana)	0.918	0.977	0.962	0.948

Table No. 24: Release Kinetics Study of different sustained release formulations

The data obtained from *in vitro* dissolution studies were fitted to Zero-order, First-order, Higuchi and Korsmeyer-Peppas equations. The dissolution data obtained were plotted as Time against Cummulative Percent Drug Release as Zero order, Time against log Cummulative percent drug remaining to be released as First order Kinetics, Square Root of Time against Cummulative percent drug released as Higuchi Equation and log Time against log Cummulative percent drug released as per Korsmeyer-Peppas equation. One common method uses the coefficient of determination, *R* , to assess the fit of a model equation. However, usually, this value tends to get greater with the addition of more model parameters, irrespective of the significance of the variable added to the model. For the same number of parameters, however, the coefficient of determination can be used to determine the best of this subset of model equations. When the data were plotted according to the first-order equation for formulations F4 to F6 showed a linear equation regression (R^2) values between 0.930 to 0.977. When the regression coefficient values of zero-order were plotted it was observed that the R^2 values of zero order plots were in the range of 0.918 to 0.970. The linear regressions (R^2) values for Higuchi Plots were found to be from 0.962 to 0.982. When the *in vitro* dissolution data was fitted to exponential model, the R^2 values were found to be in the range of 0.948 to 0.978 in most of the formulation, indicating the data fits the exponential model well. So, the assessment of the fit of a model equation is the „First Order kinetics" for F5 & F6 formulation.

Discussion

The Quantitative Parameters of Ashwagandha dry root powder and Ashwagandha Hydro- alcoholic dry extract powder were done such as Ash Content, Acid-insoluble Ash, Loss on Drying, pH, Water-soluble extractive value, Total Soluble solids, Alkaloid Content, Microbiological Test, Chromatographic Technique (TLC, HPTLC, HPLC), pH dependent solubility study and showed the most promising results. The results of Quantitative Parameters confirm the specifications of Ayurvedic Pharmacopoeia Of India. Bhavana material was prepared from Ashwagandha powdered root by the decoction of ashwagandha root. The Bhavana material was analyzed to determine or assay the active constituents. The Ashwagandha hydro- alcoholic extract powder was estimated by UV-Spectrophotometric method. Then the extract powder was allowed for Preformulation study such as Bulk Density, Tapped Density, Hausner Ratio, Percent Compressibility, Angle of Repose; which showed the free-flowing powder, and Compatibility Study. After that the extract powder and the Bhavana Material were allowed for the Formulation study of three immediate release and three sustained release dosage forms batches. In case of Immediate release formulation, sodium starch glycolate was introduced. At same time for Sustained release formulation, HPMC is used and to get the better cotrolled released of the drug, HPMC concentration was increased. The evaluation study of lubricated powder blends (granules) in each batch was done such as Bulk Density, Tapped Density, Hausner Ratio, Loss on Drying, Angle of Repose was done. The Physico-Chemical Properties of the formulated tablets were done such as average weight, friability, disintegration test, thickness, hardness, drug content as per Indian Pharmacopoeia 2007. The dissolution study at different time intervals and drug release kinetics of sustained release formulations were done and kinetics was evaluated by One common method uses the coefficient of determination, *R*, to assess the fit of a model equation.

Conclusion: The present study was carried out to design, characterization and evaluation of sustained released formulation of Ashawagandha. The quantitative parameters, chromatographic techniques, development of Bhavana process, preformulation, formulation and evaluation were done. From the results of the different batches, it was concluded that F2 and F3 batch was better immediate action than F1 while introducing the sodium starch glycolate and F5 and F6 batch was better controlled action than F4 while increasing the concentration of HPMC. But F3 and F6 batch of Bhavana was not optimized compairitively than the F2 and F5 batch. The F2 and F5 batch was showing better results than the total six batches. Thus it was also concluded that an effective immediate action and controlled action can be designed by using sodium starch glycolate, as a super disintegrants, and HPMC, as a hydrophilic polymer for sustained action. Further study would help in developing the better activity of sustained release formulation of Ashwagandha hydro-alcoholic extract powder and Bhavana of Ashwagandha powdered root. Such the study will definitely enrich, equip and empower the clinical world for the challenges ahead.

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