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FORMULATION AND EVALUATION OF PERINDOPRIL ORAL DISINTEGRATING TABLETS

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Abstract:
Difficulty in swallowing is common among all age groups especially elderly and pediatrics. Oral disintegrating tablets may constitute and innovative dosage form that overcome the problem of swallowing and provide a quick onset of action. This study was aimed to formulate and evaluate an orally disintegrate tablet (ODT) containing Perindopril while using Superdisintegrants. Oral disintegrate tablets were prepared by direct compression by using Superdisintegrants Croscarmellose Sodium, Crospovidone and Sodium starch glycolate. The prepared tablets were evaluated for hardness, friability, thickness, drug content uniformity. According to the results of optimized batches the concentration of superdisintegrant were given rapid disintegration in 24 seconds which showed 99.78 % drug release within 45 minutes. Crospovidone superdisintegrant, gives a rapid disintegration and when used in formulation of ODT.

Key Words: *Perindopril, Croscarmellose Sodium, Crospovidone, Sodium starch glycolate and Oral Disintegrating Tablets.*

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INTRODUCTION:

The oral route of administration is considered as the most widely accepted route because of its convenience of self-administration, compactness and easy manufacturing. But the most evident drawback of the commonly used oral dosage forms like tablets and capsules is difficulty in swallowing, leading to patients incompliance particularly in case of pediatric and geriatric patients, but it also applies to people who are ill in bed and to those active working patients who are busy or traveling, especially those who have no access to water [1]. Most of the pharmaceutical dosage forms are formulated for oral administration where, direct ingestion is intended. In such cases like those with conventional dosage forms, chewing imposes issue in pediatric and the geriatric patients form in. Further psychiatric patients, hospitalized or bedridden patients with chronic diseases finds difficult to swallow solid oral dosage. It is expected that Orally disintegrating tablets (ODTs) can address such critical issues. ODTs are solid dosage form that provides the rapid disintegration or dissolution of solid to present as solution or suspension form even when placed in the mouth under limited bio-fluid. These Orally disintegrating tablets have various synonyms such as or dispersible tablets, quick disintegrating tablets, and mouth dissolving tablets, fast disintegrating tablets, fast dissolving tablets, rapid dissolving tablets, porous tablets, and rapimelts. The excipients which are used in ODT technology are usually hydrophilic in nature that could be selected on the basis of drug's physicochemical properties, especially, hydrophillicity or hydrophobicity. If the drug is hydrophobic then dosage form is termed disintegrating tablets whereas, if the drug is hydrophilic then it is called fast dissolving tablets [2-3].

IDEAL CHARACTERISTICS OF ODTS

ODTs should depict some ideal characteristics to distinguish them from traditional conventional dosage forms. Important desirable characteristics of these dosage forms include

1. It should dissolve or disintegrate in the mouth usually within fraction of seconds. There is no requirement of water for swallowing purpose.
2. It should provide pleasant feeling in the mouth.
3. It should be compatible with taste masking agents.
4. It should be portable without fragility concern.
5. ODTs leave negligible or no residue in the mouth after oral administration.
6. ODTs exhibit low sensitivity to altered environmental conditions such as humidity and temperature.
7. ODTs allow high drug loading.
8. Adaptable and amenable to conventional processing and packaging equipment at nominal expense.

ADVANTAGES OF ODTs

1. ODT can be administer to the patients who cannot swallow tablets/cap., such as the elderly, stroke victims, bedridden patients, patients with esophageal problems & patients who refuse to swallow such as pediatric, geriatric & psychiatric patients and thus improves patient compliance. 2. It contain the certain studies which concluded increased bioavailability and proved rapid absorption of drugs through pregastric
3. Absorption of drugs from mouth, pharynx & esophagus as saliva passes down.
4. ODT is most convenient for disabled, bedridden patients, travelers and busy people, who do not always have access to water.
5. Good mouth feel property of ODT helps to change the perception of medication.
6. As bitter pill particularly in pediatric patients.
7. The risk of chocking or suffocation during oral administration of conventional formulations due to physical obstruction is avoided, thus providing improved safety.
8. ODT opened new business opportunity like product differentiation, product promotion, patent extension and life cycle management.
9. Suitable during traveling where water may not be available.
10. No specific packaging required can be packaged in push through blisters.
11. Allow high drug loading.
12. No chewing needed.
13. Provides rapid drug delivery from dosage forms.

DISADVANTAGES OF ODTs

1. ODT is hygroscopic in nature so must be keep in dry place.
2. It is also shows the fragile, effervescence granules property.
3. ODT requires special packaging for properly stabilization & safety of stable product
4. The tablets usually have insufficient mechanical strength. Hence, careful handling is required. 5. The tablets may leave unpleasant taste and/or grittiness in mouth if not formulated properly [4,5]

SUITABILITY OF DRUGS FOR ODTs

For developing ODT of a specific drug several factors should be kept forth while selecting drug, excipients and formulation method. These are as follows:

1. Drugs to be used for sustained action are not suitable candidate for ODT.
2. Drugs having very disagreeable taste are not suitable like clopidogrel.

3. Patients suffering from Sjogren's syndrome and those with less saliva secretion and not suitable for FDT dosage form.
4. Drugs of very short half life and requiring frequent dosing are not appropriate candidate. Patients on anticholinergic therapy are not suitable for ODT.
5. Drugs showing altered pharmacokinetic behavior if formulated in such dosage form with respect to their conventional dosage form are not suitable, like selegiline, swallowing bulky conventional dosage forms.

Requirements of fast dissolving tablets

Patient factors

- ✓ Fast dissolving dosage forms are suitable for those patients are not able to swallow tablets and capsules like pediatric and geriatric patients.
- ✓ Patients who have difficulty in swallowing or chewing solid dosage forms.
- ✓ Patients in compliance due to fear of choking.
- ✓ Very old patients of depression who may not be able to swallow the solid dosage forms.
- ✓ An eight-year-old patient with allergies desires a more convenient dosage form than antihistamine syrup.
- ✓ A middle-aged patient undergoing radiation therapy for breast cancer may be too nauseous to swallow her H2-blocker.
- ✓ A schizophrenic patient who may try to hide a conventional tablet under his or her tongue to avoid their daily dose of an atypical antipsychotic.
- ✓ A patient with persistent nausea, who may be a journey, or has little or no access to water.

Effectiveness factor

Increased bioavailability and faster onset of action are a major claim of these formulations. Dispersion in saliva in oral cavity causes pregastric absorption from some formulate ions in those cases where drug dissolves quickly. Buccal, pharyngeal and gastric regions are all areas of absorption for many drugs. Any pre-gastric absorption avoids first-pass metabolism and can be a big advantage in drugs that undergo hepatic metabolism. Furthermore, safety profiles may be improved for drugs that produce significant amounts of toxic metabolites mediated by first-pass liver metabolism and gastric metabolism, and for drugs that have a substantial faction of absorption in the oral cavity and pre-gastric segments of GIT.

Excipients used for the preparation of FDT

FDT contain one superdisintegrant, a diluent, a lubricant. Contain optionally a swelling agent, a permeabilizing agent, sweeteners and flavouring agents.

Super disintegrants

As day's passes, demand for the faster disintegrating formulation is increased. For, that pharmacist needs to formulate disintegrants i.e. Super disintegrants which are effective at less concentration and have greater disintegrating efficiency. The superdisintegrant must quickly wick saliva into that tablet to generate the hydrostatic pressure and volume expansion necessary to provide rapid disintegration in the mouth.

Examples

- Croscarmellose Sodium
- Crospovidone
- Cross-linked alginic acid
- Gellan gum
- Sodium starch glycolate
- Soy polysaccharide meant for diabetics.
- Xanthan gum

Bulking materials

Bulking materials are very important in the development of fast dissolving tablets. They contribute the functions of a diluent, filler and cost reducer. Bulking agents improve the texture of the tablets that consequently enhances the disintegration in the mouth, besides adding volume and reducing the concentration of the active in the formulation. The bulking agents for this formulation should be sugar-based such as mannitol, polydextrose, lactose derivatives such as directly compressible lactose (DCL) and starch hydrolysate for higher aqueous solubility and good sensory perception. Mannitol especially has high aqueous solubility and good sensory perception, as it provides a cooling effect due to its negative heat of solution. Bulking agents are added in the range of 10% to about 90% by weight of the final composition. Sugar based excipients are two types they classify on the basis of moulding and dissolution rate:

Type 1 saccharides: (lactose and mannitol) which exhibit low moldability but high dissolution rate.

Type 2 saccharides: (maltose and maltitol) which exhibit high moldability but low dissolution rate.

Emulsifying agents

Emulsifying agents are more significant for formulation of fast dissolving tablets they help in quick disintegration and drug release without the need for chewing, swallowing or drinking water. Also, emulsifying agents stabilize the immiscible blends and increase bioavailability. A variety of emulsifying agents for fast dissolving tablet formulations include

alkyl sulfates, propylene glycol esters, lecithin, sucrose esters and others. These can be added in the range of 0.05% to about 15% by weight of the final formulation.

Lubricants

Though not essential excipients, these can aid in making the tablets more palatable after they disintegrate in the mouth. Lubricants reduce grittiness and help in the drug transit process from the oral to the stomach.

Flavours (taste masking agents) and sweeteners

Flavours and taste masking agents are useful for the formulation they make the products more palatable and pleasing for patients. The incorporation of these ingredients assists in overcoming bitterness and undesirable tastes of some actives. Natural as well as synthetic flavours can be used to enhance the organoleptic characteristic of fast dissolving tablets. A wide range of sweeteners including sugar, dextrose and fructose, as well as non-nutritive sweeteners such as aspartame, sodium saccharin, sugar alcohols and sucralose are available. The addition of sweeteners imparts a pleasant taste as well as bulk to the formulation.

Important criteria for excipients used in formulation of ODTs

1. Should disintegrate rapidly.
2. Do not interact with the drugs in formulation.
3. Should be chemically inert.
4. Should not affect the efficacy of formulation.
5. Should not alter the organoleptic characteristics of the product.
6. Should be stable.
7. Should melt in the range of 30-35°C

MATERIALS

Perindopril-Procured From Glenmark Pvt. Ltd., Mumbai. Provided by SURA LABS, Dilsukhnagar, Hyderabad, Croscarmellose Sodium-Oxford Laboratories Pvt. Ltd, Mumbai, India, Crospovidone Rubicon-Research Pvt. Ltd., Mumbai, India,Sodium starch Glycolate-S.D. Fine chemicals, Mumbai, India, Talc-S J Chemicals, Mg.Stearate-Nikita Chemicals, India, Mannitol-Merck Specialities Pvt Ltd, Mumbai, India, Lactose-Oxford Laboratories Pvt. Ltd, Mumbai, India

METHODOLOGY:

Buffer preparation:

Preparation of 0.2 M Potassium dihydrogen orthophosphate solution: Accurately weighed 27.128 gm of monobasic potassium dihydrogen orthophosphate was dissolved in 1000 ml of distilled water and mixed.

Preparation of 0.2 M sodium hydroxide solution : Accurately weighed 8 gm of sodium hydroxide pellets were dissolved in 1000 mL of distilled water and mixed.

Preparation of pH 6.8 phosphate buffer : Accurately measured 250 mL of v0.2 M potassium dihydrogen orthophosphate and 112.5 mL of 0.2 M NaOH was taken into the 1000 mL volumetric flask. Volume was made up to 1000 mL with distilled water.

Analytical method development for Perindopril:

a) Determination of absorption maxima

A spectrum of the working standards was obtained by scanning from 200-400 nm against the reagent blank to fix absorption maxima. The λmax was found to be 387.2 nm. Hence all further investigations were carried out at the same wavelength.

b) Construction of standard graph

100 mg of Perindopril was dissolved in 100 mL of pH 6.8 phosphate buffer to give a concentration in 1mg/mL (1000µg/mL) 1 ml was taken and diluted to 100 ml with pH 6.8 phosphate buffer to give a concentration of 0.01 mg/ml (10µg/ml). From this stock solution aliquots of 1.0 ml, 2.0ml, 3.0 ml, 4.0 ml, 5 ml, were pipette out in 10 ml volumetric flask and volume was made up to the mark with pH 6.8 phosphate buffer to produce concentration of 10, 20, 30, 40 and 50µg/ml respectively. The absorbance of each concentration was measured at respective (λmax) i.e., 387.2 nm.

Formulation development:

Drug and different concentrations of super disintegrants (Sodium starch glycolate, Cross caramellose Sodium, Cross povidone) and required ingredients were accurately weighed and passed through a 40-mesh screen to get uniform size particles and mixed in a glass motor for 15 min.

- The obtained blend was lubricated with magnesium stearate and glidant (Talc) was added and mixing was continued for further 5 min.
- The resultant mixture was directly compressed into tablets by using punch of rotary tablet compression machine. Compression force was kept constant for all formulations.

Table : Formulation table showing various compositions

INGREDIANTS	FORMULATIONS								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Perindopril	8	8	8	8	8	8	8	8	8
Croscarmellose Sodium	4	8	12	-	-	-	-	-	-
Crospovidone	-	-	-	4	8	12	-	-	-
Sodium starchglycolate	-	-	-	-	-	-	4	8	12
Talc	5	5	5	5	5	5	5	5	5
Mg.Stearate	5	5	5	5	5	5	5	5	5
Mannitol	10	10	10	10	10	10	10	10	10
Lactose	68	64	60	68	64	60	68	64	60
Total weight	100	100	100	100	100	100	100	100	100

The tablets were prepared by using tablet compression machine . The hardness of the tablet was maintained as (2.25-2.48) kg/cm^2

RESULTS AND DISCUSSION:

Preparation of calibration curve of Perindopril:

The regression coefficient was found to be 0.999 which indicates a linearity with an equation of y=0.018 x-0.005. Hence Beer-Lmbert's law was obeyed.

Table : Calibration curve data of Perindopril in pH 6.8 phosphate buffer

Concentration	Absorbance
0	0
10	0.178
20	0.353
30	0.537
40	0.724
50	0.913

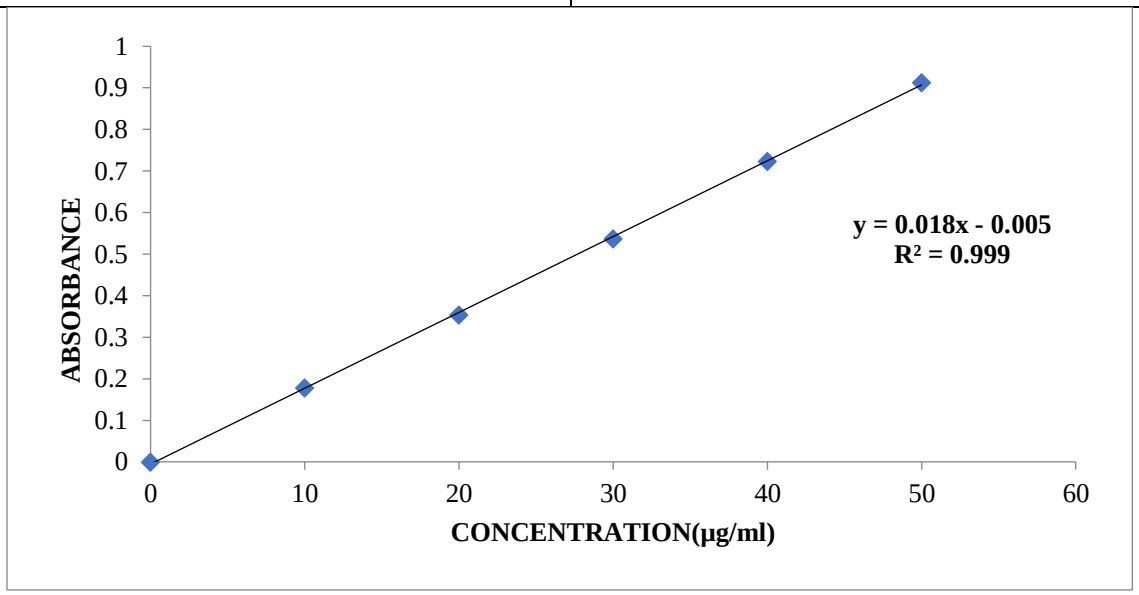

FIG : Calibration curve data of Perindopril in pH 6.8 phosphate buffer

EVALUATION OF PRE-COMPRESION PARAMETERS OF POWDER BLEND

Table : Evaluation of pre-compression parameters of powder blend

Formulation code	Angle of repose	Bulk density(gm/mL)	Tapped density (gm/mL)	Carr's index(%)	Hausner's ratio
F1	23.04 ±0.3	0.54 ±0.01	0.57 ±0.01	5.26 ±2.0	1.06 ±0.02
F2	23.77 ±0.4	0.55 ±0.01	0.59 ±0.02	6.78 ±2.0	1.07 ±0.03
F3	23.53 ±0.5	0.55 ±0.02	0.61 ±0.03	9.84 ±2.0	1.11 ±0.03
F4	23.37 ±0.4	0.53 ±0.03	0.58 ±0.04	8.62 ±2.2	1.09 ±0.03
F5	22.16 ±0.2	0.48 ±0.02	0.55 ±0.01	12.14 ±4.9	0.65 ±0.23
F6	23.44 ±0.4	0.50 ±0.01	0.58 ±0.01	14.96±2.2	1.17±0.03
F7	23.31±0.3	0.47 ±0.02	0.55±0.03	14.23±2.0	1.16±0.23
F8	22.83±0.4	0.43 ±0.03	0.50±0.02	13.2±2.0	1.15±0.02
F9	22.44±0.2	0.58 ±0.01	0.66±0.01	11.81±2.2	1.13±0.02

- For each formulation blend of drug and excipients were prepared and evaluated for various pre compression parameters described earlier in methodology chapter.
- The bulk density of all formulations was found in the range of 0.43 ±0.03 - 0.58 ±0.01 and tapped density was in the range of 0.50±0.02 - 0.66±0.01
- The Carr's index and Hausner's ratio was calculated from tapped density and bulk density.

EVALUATIONS OF POST COMPRESSION PARAMETERS OF PERINDOPRIL ODTs

Table : Evaluation of post compression parameters of Perindopril Fast dissolving tablets

Formulation codes	Average weight(mg)	Hardness (kg/cm^2	Friability (%loss)	Thickness (mm)	Drug content (%)	*In vitro* disintegration Time(sec)
F1	98.25	2.28	0.48	1.67	99.96	51
F2	99.68	2.45	0.39	1.61	97.21	46
F3	98.41	2.32	0.58	1.75	96.20	58
F4	100.02	2.25	0.35	1.58	99.35	24
F5	96.69	2.37	0.44	1.64	97.18	62
F6	97.47	2.48	0.51	1.89	98.65	55
F7	99.59	2.38	0.49	1.65	99.86	65
F8	98.23	2.46	0.47	1.77	98.41	57
F9	99.72	2.35	0.51	1.82	98.62	51

Weight variation and Thickness : All the formulations were evaluated for uniformity of weight using electronic weighing balance and the results are shown above. The average tablet weights of all the formulations were noted down.

Hardness and friability: All the ODT formulations were evaluated for their hardness using Monsanto hardness tester and the results are shown above. The average hardness for all formulations was found to be between (2.25- 2.48) kg/cm^2 which was found to be acceptable. Friability was determined to evaluate the ability of the tablets to with stand the abrasion during packing, handling and transpoting. All the ODT formulations were evaluated for their percentage friability using Roche friabilator and the results are shown above. The average percentage friability for all the formulations was between 0.35 - 0.58 which was found to be within the limit.

Drug content : All formulations were evaluated for drug content according to the procedure described in methodology section and the results were shown above . The assay values for all formulations were found to be in the range of (96.20 -99.96). According to IP standards the tablets must contain not less than 95% and not more than 105% of the stated amount of the drug. Thus, all the ODT formulation comply with the standards given in IP.

***In vitro* disintegration time :** *In vitro* disintegration studies showed from 24-65 sec. The F4 formulation showed very less *in vitro* disintegration time i.e.44 sec.

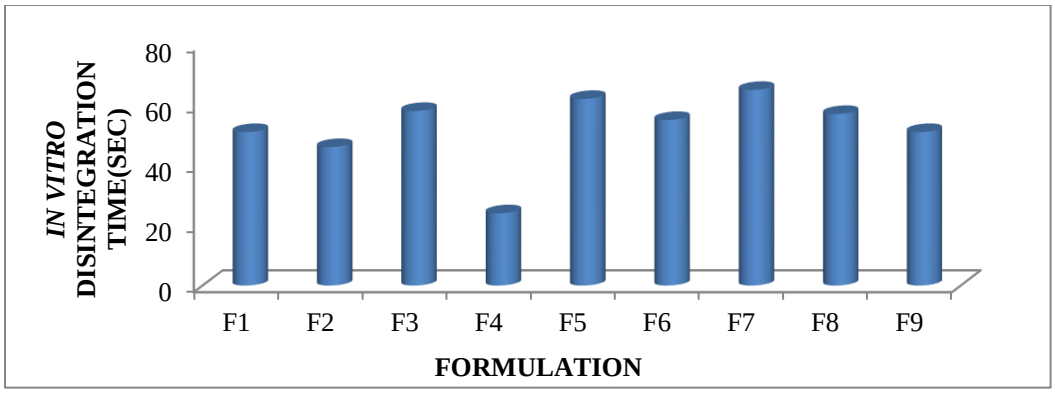

Figure : *In vitro* disintegration time

***IN VITRO* DRUG RELEASE SYUDIES OF PERINDOPRIL**

Table : *In vitro* Dissolution data of Perindopril

Time (mints)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	12.25	15.08	21.18	27.32	25.47	30.55	29.19	31.95	26.47
10	28.88	33.62	42.38	49.34	44.92	38.71	35.62	46.35	41.76
15	35.49	46.71	55.67	64.04	58.75	45.68	51.37	54.09	49.52
20	58.22	63.35	72.85	75.91	67.29	59.18	68.88	62.76	55.68
30	76.19	79.48	81.57	86.31	82.17	77.32	73.49	68.19	74.32
45	88.37	92.82	95.22	99.78	95.36	91.48	88.67	85.22	81.61

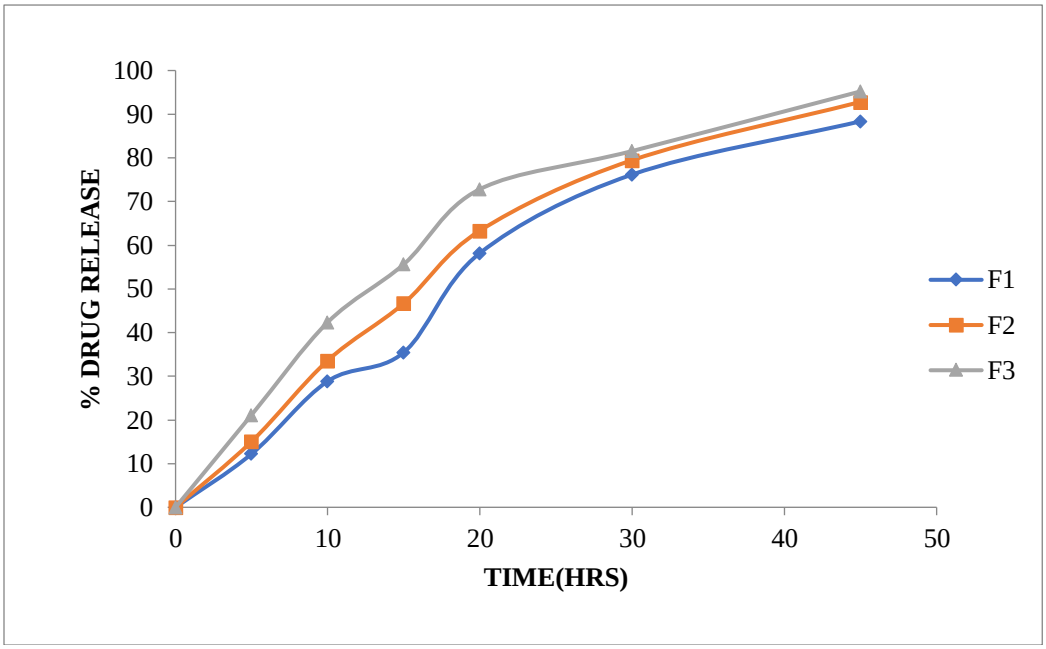

Fig : Dissolution profile of formulations F1, F2, F3

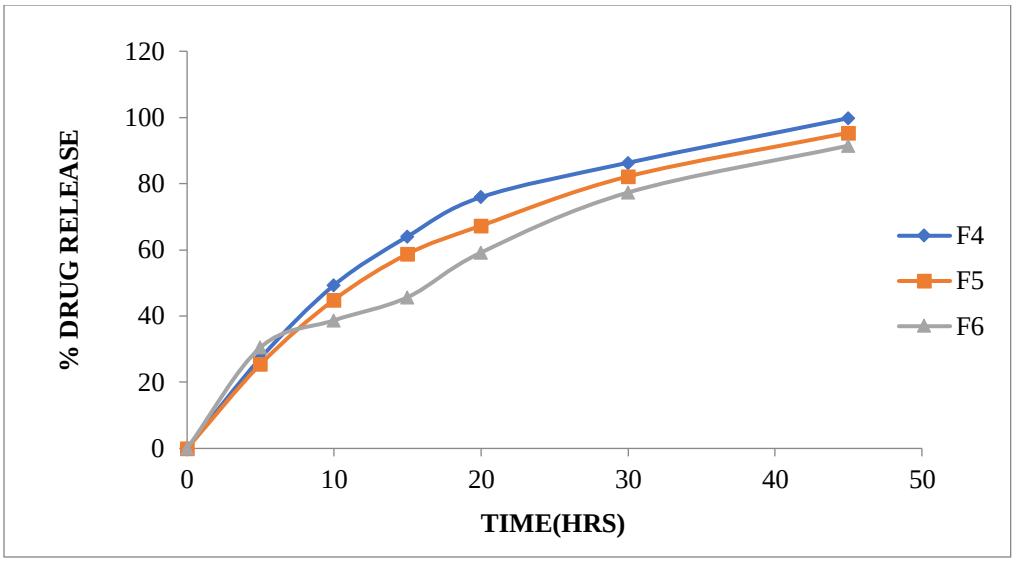

Fig : Dissolution profile of formulations F4, F5, F6

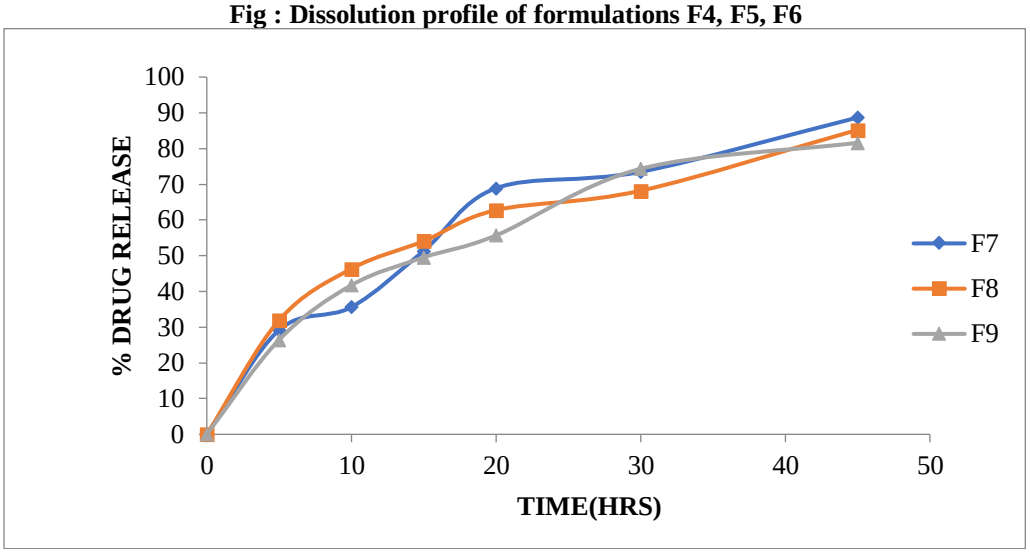

Fig : Dissolution profile of formulations F7, F8, F9

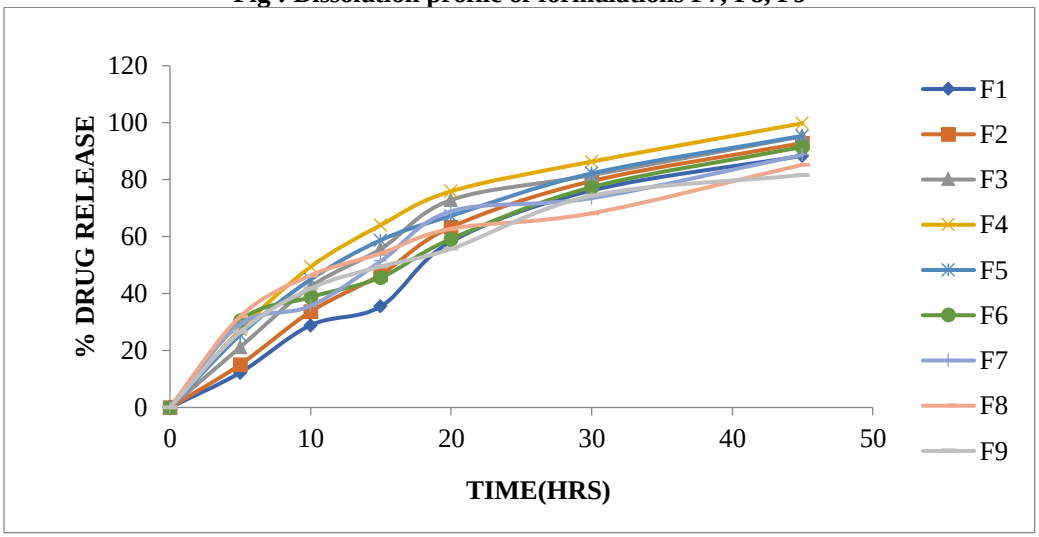

Fig : Dissolution profile of all formulations F1-F9

From the Table it was evident that the formulations prepared with Croscarmellose Sodium powder were showed good drug release i.e.,95.22 % (F3 Formulation) in higher concentration of blend i.e. 12 mg. Formulations prepared with Crospovidone showed good drug release i.e., 99.78 % (F4 Formulation) in 4 mg concentration when increase in the concentration of Crospovidone drug release unable to retarded. Formulations prepared with Sodium starch glycolate showed maximum drug release i.e., 88.67 % (F7 Formulation) at 45 min in 4 mg of blend.

Among all formulations F4 formulation considered as optimised formulation which showed maximum drug release at 45 min. i.e. 99.78 %. Croscarmellose Sodium were showed good release when compared to Sodium starch glycolate. Finally concluded that f4 formulation (contains Crospovidone) was optimised better formulation.

FTIR RESULTS :

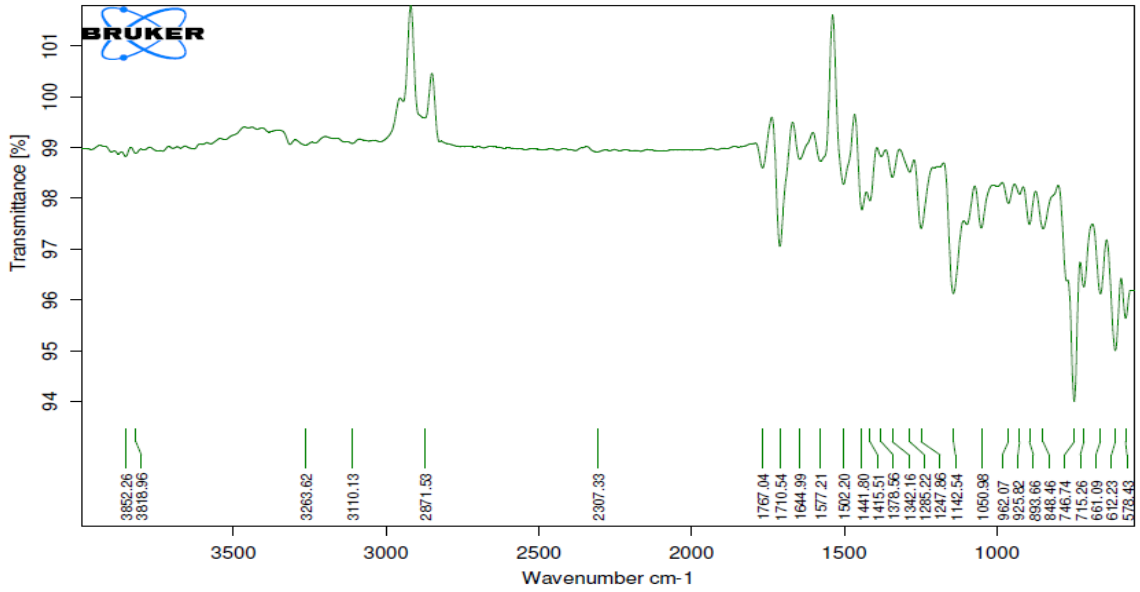

Fig : FTIR of Perindopril Pure Drug

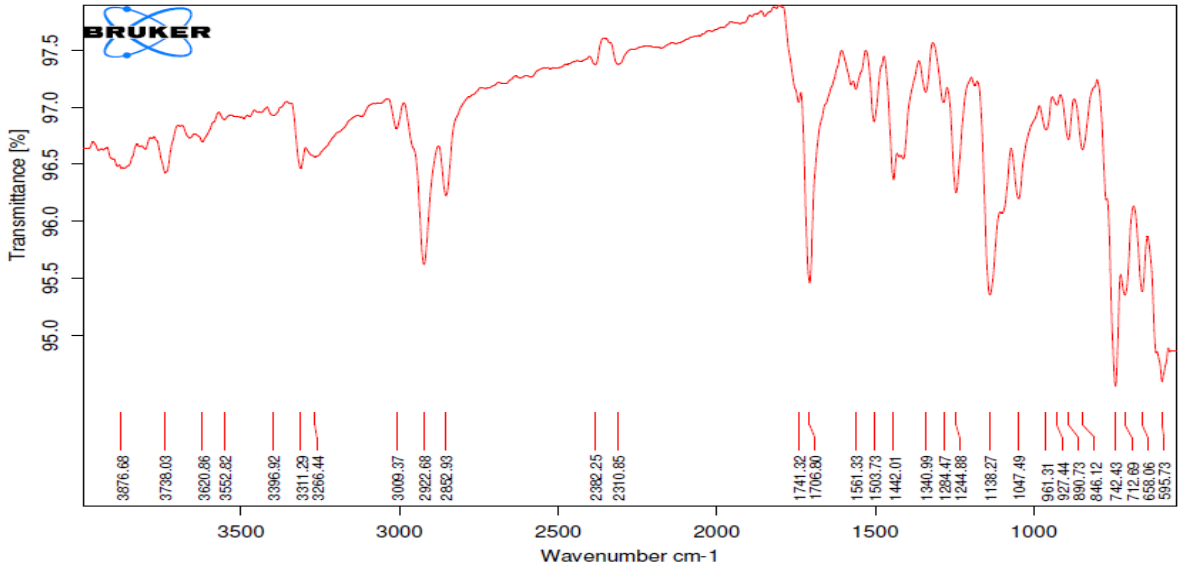

Fig : FTIR of Perindopril optimized formulation

Perindopril was mixed with proportions of excipients showed no colour change providing no drug-excipient interactions

CONCLUSION:

The Oral disintegrating tablets of Perindopril were formulated by using super disintegrants like Sodium Starch Glycolate, Cross Caramellose Sodium And Crosspovidone. FTIR study reveals that there is no drug-excipients interaction between Perindopril and excipients. The prepared tablets were shown good post compression parameters and they passed all the quality control evaluation parameters as per I.P limits. The use of super disintegrant Crospovidone at the concentration of 4 mg given better release of drug when compared to other superdisintegrants. The Optimised Formulation (F4) was showed Highest Drug Release (99.78%) in 45 minutes. The proposed ideal and reproducible characteristics of disintegration time and drug release profile.

By employing commonly available pharmaceutical Glycolate, Cross Caramellose Sodium And Crosspovidone and Lactose a fast disintegrating tablet of Perindopril can be developed which can be commercialized. The developed formulation of Perindopril ODT showed good efficacy, rapid onset of action, better patient compliance.

REFERENCES:

1. Ankaj kaundal, tarun k. Sharma, archana choudhary, dev raj sharma, upasana thakur. International Journal Of Pharmaceutical Research And Bio-Science. IJPRBS, 2018; Volume 7(4): 37-49.
2. Velmurugan S and Sundar Vinushitha, Oral Disintegrating Tablets: An Overview. International Journal of Chemical and Pharmaceutical Science, 2016, 1(2), 1-12.
3. Asthana A, Aggarwal S, Asthana G, Oral Dispersible Tablets: Novel Technology and Development. Int. J. Pharm. Sci. Rev. Res., 2013, 20(1), 193-199.
4. Nagar P, Singh K, Chauhan I, et. al., Orally disintegrating tablets: formulation, preparation techniques and evaluation. Journal of Applied Pharmaceutical Science, 2011, 01 (04), 35-45.
5. Lavakumar V, Divya L, Sowmya C, et. al., Oral Dispersible Tablets - An Overview International Journal of Research in Pharmaceutical and Nano Sciences. 2013, 2(3), 394 - 401.
6. Hannan PA, Khan JA, Khan A, Safiullah S, Oral Dispersible System: A New Approach in Drug Delivery System. Indian Journal of Pharmaceutical Sciences. 2016, 78(1), 2-7.
7. Aher smita s, saudagar r. B, shinde mayuri s. Review: Fast Dissolving Tablet. Vol 10, Issue 2, 2018.
8. Hannan PA, Khan JA, Khan A, Safiullah S. Oral dispersible systems: a new approach in drug delivery system. Indian J Pharm Sci 2016;78:2-7.
9. Sharma S. New generation of the tablet: fast dissolving tablet. Latest Rev Pharma Info Net 2008. p. 6.
10. Kumari S, Visht S, Sharma PK, Yadav RK. Fast dissolving drug delivery system: a review article. J Pharm Res 2010;3:1444-9.
11. Kumaresan C. Orally disintegrating tablet-mouth dissolving sweet taste and target release profile. Pharm Rev 2008;6:1.
12. Patel TS, Sengupta M. Fast dissolving tablet technology. World J Pharm Sci 2013;2:485-508.
13. Khan AB, Tripuraneni A. Fast dissolving tablets–a novel approach in drug delivery. Rguhs J Pharm Sci 2014;2:17-6.
14. Nagasamy Venkatesh Da,, Kiran H.Ca , Shashikumar. Sa , Jenisha Karmacharyaa ,Veeramachaneni Krishna Priyaa , Kosaraju Bhavithaa and Ayush Shresthab. orally disintegrating tablets (odts)- a comprehensive review. Vol 4, Issue 08, 2015.
15. Dobetti L. Fast-Melting Tablets: Developments and technologies. Pharm Tech Drug Deliv (Suppl.). 2001; 44-50.