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Formulation and Optimization of *In-Situ* Buffered Formulation Containing Indomethacin In Combination With Pantoprazole

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ABSTRACT

In-Situ buffer formulation contain agents which immediately buffer the internal environment of the body and increases the stability of acid labile drugs inside the body. Here this approach is used for making combination capsule formulation to reduce the side effects of Nonsteroidal anti-inflammatory drugs. Pantoprazole is an acid labile drug which is very useful for the prevention and treatment of NSAID related gastric ulcer, so to reduce its side effects this approach was used. In this Indomethacin is used as Nonsteroidal anti-inflammatory drugs. For this purpose, macroenvironment buffering method was used. Based on their acid neutralizing capacity the best buffering combination was selected. In this method immediate release excipients or superdisintegarnts (SSG & CCS) whereas rate retarding polymers (Guar gum, Xanthan gum & ethyl cellulose) were added in the formula. *Ex vivo* permeation study was performed by using Non-everted intestinal sac method in selected optimized batch. For optimization full factorial 2^3 design was used along with mathematical models. Prepared optimized formulation was compared with marketed formulation. Final pH from optimized formulation was found to be 5 to 6. The prepared optimized capsule is having 98.86% immediate release of pantoprazole sodium sesquihydrate upto 30 min and 99.84% sustain release of indomethacin upto 12 hrs. The prepared formulation showed immediate release of stable pantoprazole sodium sesquihydrate (due to the presence of in-situ buffering agents inside the capsule) along with the sustained release Indomethacin with very less adverse effects.

Keywords: Stable pantoprazole sodium sesquihydrate, sustained release, macroenvironment, Ex vivo permeation.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs are commonly used for the treatment of acute or chronic conditions where pain and inflammation are present [1]. Indomethacin is an anti-inflammatory drug under NSAID category. In many cases long term therapy of indomethacin is required. But along with the advantages it has one major disadvantage is it causes gastrointestinal adverse effects like ulcer in many cases [2]. Many approaches have been used to overcome these gastrointestinal side effects associated with Nonsteroidal anti-inflammatory drugs. One of the approach is to modify the NSAID structure so that the prevention of such side-effects can be done, but this approach is very less successful. The other approach is use of acid inhibitor along with NSAID. This approach is less complex and more effective than the previous approach [3]. There are many antiulcer drugs which can be used along with Indomethacin, such as H_2 receptor blockers, proton pump inhibitors and mucosal protective agent [4]. The comparison between these antiulcer agents on the basic of potency, acid inhibition and many other properties are given in the table 1 [5]**.** As reported by American Gastroenterological Association recent guidelines PPIs are more effective than H_2 receptor blocker [6].

Table 1: Comparison between acid suppressants for prophylaxis and treatment of ulcer due to NSAID.

Properties	**PPI (pantoprazole)**	**H2 receptor antagonist (Ranitidine)**	**Prostaglandin analogue (misoprostol)**
Potency	80-95%	**70 %**	85-95%
Gastric acid inhibition	upto 48 hrs	**upto 24 hrs**	**12 hrs**
Continuous long term therapy of Nonsteroidal anti-inflammatory drugs	Efficacy remains same	**Less effective**	**Less effective**
Adverse effects	No serious adverse effects	No serious adverse effects	**Diarrhoea and other GI side effects**
Onset of action	**Delay**	Rapid	Rapid
Stability in gastric pH	**Unstable**	stable	stable
Per day dose	40 mg	**300 mg**	800 mcg

For making an effective therapy of Indomethacin it is necessary to administer it along with an antiulcer drug. Concurrent administration of antiulcer drug with indomethacin reduces the chances of gastrointestinal side effects associated with it, but the main fact is that the release of antiulcer drug in the formulation has to be optimized so that the ulcerogenic effect of the indomethacin can be reduced [7].

As discussed earlier PPIs are one of the best agent for NSAID related GI ulceration, but they are unstable in acidic environment i.e. acid labile. Proton pump inhibitors are used to suppress the acid of the stomach. Long term use Nonsteroidal anti-inflammatory drugs causes serious ulcer [8]. Pantoprazole is an acid labile drug so it cannot be given as uncoated form while making immediate release formulation. For the prevention of acid labile drug from degradation or making the immediate release formulation of acid labile drug buffer can be used [9]. So for this *in situ* buffer is an effective approach. It increases the pH of the environment in the stomach by buffering action. Two approaches may be used either the micro environment or the macro-environment. In macro-environment whole stomach will buffer by buffering agents [10].

One of the most common approach is enteric coating approach and the second is novel *in situ* buffered approach. Enteric coating is most common approach but in spite of advantages there are many disadvantages such as [11]:

- Disruption of enteric coating by chewing
- Late onset of action by these type of formulation.
- Onset may vary with gastric emptying.

Second approach is in situ buffer formulation which is novel technique and it has many advantages over enteric coating such as [12]:

- Maintenance of pH
- Enhancement of stability
- Decrease in gastric irritation
- Increase in onset of action
- Patient compliance

MATERIALS AND METHOD

Materials:

Pantoprazole sodium sesquihydrate [API], Indomethacin [API], Sodium Starch Glycolate [superdisintegrant], Croscarmellose Sodium [superdisintegrant], Xanthan gum [rate retarding polymer], Guar gum [rate retarding polymer], Ethyl cellulose [rate retarding polymer], Lactose [Diluent], Mannitol [Diluent], PVPK-30 [Binder], Magnesium Stearate and Talc were used during the formulation development.

Methods:

Preparation of Calibration Curve:

Different aliquots [5,10,15,20,25,30 µg/ml] of drug were accurately prepared by stock solution containing 100 µg/ml drug [by dissolving 10 mg drug in 100 ml solvent] and transferred into a series of 10 mL volumetric flasks and volume made up to the mark with the given solvent. Then all dilutions were scanned between 200-400 nm against blank, which was resulted with a specific λ max. The solvent used for this study was distilled water, chloroform, simulated gastric fluid [pH 5] and 0.1 N HCl for pantoprazole sodium and distilled water, chloroform, 6.8 pH mixed phosphate buffer and 0.1 N HCl for Indomethacin. The same λ max was used for further measurement of drug. A calibration curve for absorbance vs. concentration was plotted[9].

Compatibility Studies:

Binary mixture compatibility testing is one of the commonly used method. In the present investigation binary mixture compatibility testing was used. In this the drug and all the excipients were mixed [1:1 ratio] separately and kept in a previously washed, clean and dry glass vial in stability chamber [humidity chamber] at 40^0C and 73% RH for 6 months. For determining compatibility samples were tested at three time points i.e. 0 months,3 months and 6 months as per ICH guidelines [13]. The selected samples were analysed on the basis of general appearance and IR spectra.

Buffer Selection:

The term buffer means any compound or combination of compounds that increase the pH of the environment in which they are dissolved or dispersed. In this research there is a need to create macro environment having a pH of 5 to 6 in the stomach for immediate release of acid labile PPI pantoprazole stable at a pH between 5 to 10, the maximum stability at pH 5.5 and to provide the concept of a macro-environment pH instead of a micro- environment pH. Number of buffers, both soluble and insoluble, were tested for their acid neutralizing capacity. Total 16 formulations [BF1-BF16, Table 2 & Table 3] of buffer were prepared and tested for acid neutralizing property. Based on their acid neutralizing capacity, buffers both individual and combination, were evaluated.

Table 2: Acid neutralizing property of single buffering agent.

Serial No.	Selected Ingredients	Quantity Used (mg)	Observed pH
BF-1	Sodium Bicarbonate	100	9.2
BF-2	Sodium Carbonate	200	8.5
BF-3	Magnesium Hydroxide	50	5.3
BF-4	Calcium Carbonate	300	7.7
BF-5	Magnesium Oxide	100	7.4
BF-6	Calcium lactate	150	5.8

Table 3: Acid neutralizing property of single buffering agent.

Serial No.	Selected Ingredients	Quantity Used (mg)	Observed pH	pH after 15 min*	pH after 30 min*	pH after 45 min*	pH after 60 min*
BF-7	Sodium Bicarbonate: Magnesium Hydroxide	50:50	5.8	1.9	NA	NA	NA
BF-8	Sodium Bicarbonate: Magnesium Hydroxide	100:50	6.7	3.2	NA	NA	NA
BF-9	Sodium Bicarbonate: Magnesium Hydroxide	100 :100	7	3.8	2.6	NA	NA
BF-10	Sodium Bicarbonate: Magnesium Hydroxide	250 : 250	8.7	NA	NA	NA	NA
BF-11	Sodium Bicarbonate: Calcium Carbonate	50:50	6.3	2.7	1.6	NA	NA
BF-12	Sodium Bicarbonate: Calcium Carbonate	100 : 100	8.1	6.6	5.9	4	2.1
BF-13	Calcium Carbonate: Magnesium Hydroxide	100 : 100	6	5.8	5.1	4.3	3.8
BF-14	Calcium Carbonate: Magnesium Hydroxide	120 : 120	6.8	6	5.5	5	4.2
BF-15	Calcium lactate: Magnesium Hydroxide	100 : 100	6.5	4.7	3.6	NA	NA
BF-16	Calcium lactate: Magnesium Hydroxide	120 : 120	6.5	5.6	5.4	5.2	5

pH stability analysis of PPI:

pH stability profile shows the rate of degradation of drug in different pH. As per the literature Pantoprazole which is a PPI, is unstable at acidic pH whereas as the pH increases the stability of the PPI increases. So for the determination of stability of PPI in different pH this experiment was design.

In this method the solution of drug [20 mg] was first prepared in different pH [pH1 to pH 7] solutions [50 ml]. Then lamda max of all the prepared solutions was determined in UV spectroscopy. The absorbance was also noted down in respective lamda max, then the amount of PSS was determined with the help of calibration curve after eight hours in different pH solutions. As well as the change in colour also observed as appearance parameter. On the bases of amount of drug present in the solution and appearance the conclusion has been made.

Acid neutralizing capacity [ANC] of different buffers: -

In this technique excess acid was provided according to the need of stomach. According to Lentner [1981] and Yamada [1999]. The basal stomach fluid contains 9.6 ml of 0.1 N HCI and releases 0.5 ml of 0.1 N HCI per minute. So for making simulated gastric conditions 9.6 ml of

0.1 N HCI + 210 ml of water and titrated with excess acid [0.1 HCI] at the rate of 0.5 ml /minute for a period of 1 hour [total volume= 250 ml] [14].

Selection of buffer on the basis of ANC: -

Based on acid neutralizing capacity of different buffers the suitable combination of buffer was selected. The requirement was the buffer combination which raise the pH of environment around 6 and should maintained the pH 5 to 6 for about 30 min or more preferably for 60 min.

Pantoprazole Sodium Sesquihydrate IR Granulation

Wet granulation method was used for making pantoprazole immediate release granules. In this method immediate release excipients or superdisintegarnts were added in the formula. All the ingredients were weighed and sieved according to formula [table 4] through sieve # 40. Mix all the sieved ingredients with the help of plastic polybag except PVPK-30, talc and magnesium stearate. Binding solution of PVPK-30 was then added into the mixed ingredients. And dough was formed. Then the dough was passed with the sieve # 10 to form desired size of granules. The prepared granules were then dried in oven for approximately 1 hrs at 60°C. then the dried granules were passed through sieve # 20 and mixed with talc and magnesium stearate. Finally, prepared granules were evaluated and stored in the air tight polybag, and evaluated by different parameters.

Table 4: Formulation of IR pantoprazole sodium sesquihydrate trial batches.

Ingredients	Formulation Code: Quantity per capsule (mg)							
	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8
Pantoprazole	20	20	20	20	20	20	20	20
Sodium Starch Glycolate	6.6	10	6	4	8	8	-	8
Croscarmellose Sodium	4	3	5	10	5	4	8	-
Mannitol	40.4	38	39.2	34.2	36	34	36	36
PVPK-30	4	4	2.8	2.8	2	2	4	4
Water	qs.	qs.	qs.	qs.	qs.	qs.	qs.	qs.
Talc	1	-	2	4	4	4	2	2
Magnesium Stearate	4	5	5	5	5	5	10	10
Total	80	80	80	80	80	80	80	80

Optimization of Pantoprazole Sodium Sesquihydrate:

Full factorial 2^3 design was used on selected formulation [P-8]. Amount of sodium starch glycolate [10% & 12%] [X_1], amount of PVPK-30 [3.5% & 5%] [X_2] and amount of mannitol [45% & 50%] were used as independent variable. Coded value for 2 levels were -1, +1. Total 8 possible outcomes were prepared. The percentage drug release was selected as dependent variable. The optimization batches were also prepared by same procedure as trial batches [table 5].

Table 5: - Formulations of IR Pantoprazole sodium sesquihydrate optimized batch.

Ingredients	Formulation Code: Quantity per capsule (mg)							
	OP-1	OP-2	OP-3	OP-4	OP-5	OP-6	OP-7	OP-8
Pantoprazole	20	20	20	20	20	20	20	20
Sodium Starch Glycolate*	8	8	8	8	9.6	9.6	9.6	9.6
Mannitol*	36	36	40	40	36	36	40	40
PVPK-30*	4	2.8	4	2.8	4	2.8	4	2.8
Water	qs.	qs.	qs.	qs.	qs.	qs.	qs.	qs.
Talc	2	3.2	2	2	2	2	2	2
Magnesium Stearate	10	10	6	7.2	8.4	9.6	4.4	5.6
Total	80	80	80	80	80	80	80	80

Indomethacin SR Granulation

For indomethacin sustained release granules also the same wet granulation method was used. In this case for sustain release of indomethacin polymers or sustain release excipients were added in the formula.

Table 6: - Formulation of SR Indomethacin trial batches.

Ingredients	Formulation Code: Quantity per capsule (mg)							
	IN-1	IN -2	IN -3	IN -4	IN -5	IN -6	IN -7	IN -8
Indomethacin	75	75	75	75	75	75	75	75
Xanthan Gum	6	15	10	7	10	27	-	-
Guar Gum	6	11	6	8	10	-	-	9
Ethyl cellulose	25	11	11	7	-	-	27	27
PVPK-30	9	9	9	4.5	4.5	9	9	9
Water	qs.	qs.	qs.	qs.	qs.	qs.	qs.	qs.
Lactose	41	41	51	60.5	62.5	51	51	44
Magnesium stearate	10	10	10	10	10	10	10	10
Talc	8	8	8	8	8	8	8	8
Total	180	180	180	180	180	180	180	180

All the ingredients were weighed and sieved according to formula [Table 6] through sieve # 40. Mix all the sieved ingredients with the help of plastic polybag except PVPK-30, talc and magnesium stearate. Binding solution of PVPK-30 was then added into the mixed ingredients. And dough was formed. Then the dough was passed with the sieve # 10 to form desired size of granules. The prepared granules were then dried in oven for approximately 1 hrs at 60°C. then the dried granules were passed through sieve # 20 and mixed with talc and magnesium stearate. Finally, prepared granules were evaluated and stored in the air tight polybag.

Optimization of Indomethacin:

Full factorial 2^3 design was used on selected formulation [IN-8]. Amount of ethyl cellulose [15% & 20%] [X_1], amount of PVPK-30 [3.5% &5%] [X_2], and amount of guar gum [5% & 10%] [X_3] were used as independent variable. Coded value for 2 levels were -1, +1. Total 8 possible

outcomes were prepared [Table 7]. The percentage drug release was selected as dependent variable. The optimization batches were also prepared by same procedure as trial batches.

Table 7: - Formulations of SR Indomethacin optimized batch.

Ingredients	Formulation Code: Quantity per capsule (mg)							
	OIN-1	OIN-2	OIN-3	OIN-4	OIN-5	OIN-6	OIN-7	OIN-8
Indomethacin	75	75	75	75	75	75	75	75
Ethyl cellulose*	27	27	27	27	36	36	36	36
PVPK-30*	9	9	6.3	6.3	9	9	6.3	6.3
Guar Gum*	9	18	9	18	9	18	9	18
Water	qs	qs	qs	qs	Qs	qs	qs	qs
Lactose	44	33	44.7	35.7	33	24	35.7	26.7
Magnesium stearate	10	10	10	10	10	10	10	10
Talc	8	8	8	8	8	8	8	8
Total	180	180	180	180	180	180	180	180

Evaluation of prepared granules:

Drug Release [Dissolution Test]:

Pantoprazole Sodium Sesquihydrate:

Apparatus: USP type 1 [basket type], RPM: 100, Temp: 37°C, Time: 30 min, Volume of dissolution medium: 500ml [80ml 0.1 N HCl mixed with 420 ml water then [120+120 mg buffering agent], Final pH- 5.5, Number of samples: 04

Indomethacin SR:

Apparatus: USP type 1 [basket type], RPM: 100, Temp: 37°C, Time: 12 hrs, Volume of dissolution medium: 750ml of 0.1N HCl for 2 hrs then 250 ml of 0.2M tricalcium phosphate was added], Final pH- 6.8, number of samples: 09.

***Ex vivo* permeation study:**

Non-everted intestinal sac method was used for this study [15]. firstly, the Krebs ringer bicarbonate buffer solution was prepared according to the formula. Chicken intestine was procured from a slaughter house and small intestine was taken for the study. The lumen was carefully cleared from mucus by rinsing with pH 6.8 buffer solution [Krebs–Ringer solution]. An intestinal segment of the first 6-cm length was removed and transferred to oxygenated Krebs – Ringer solution. It was washed thoroughly with Krebs–Ringer solution.

The intestinal segment of about 6 cm in length were tied at one end and the sacs were filled with 5ml of kerbs-Ringer Bicarbonate buffer containing suspension of optimized formulation, then the other end was ligated carefully. The non-everted sac was submerged in a conical flask containing 100ml of kerbs-Ringer Bicarbonate buffer with maintained temp 37°C by heating mental.

The samples were then collected in different time interval; the equivalent fresh KR buffer was added to solution at the time of sampling. Each experiment was performed six times and proper dilutions of sample were done with KR buffer solutions and absorbance was measured using UV Spectrophotometry. Same experiment was repeated with marketed formulation.

Evaluation of prepared formulation:

Dissolution test on prepared capsule:

Dissolution procedure was same as follow in the dissolution of granules. In this case first 500 ml dissolution medium [as in case of PSS IR Formulation] was used after 30 min the dissolution medium was made upto 750 ml with 0.1 N HCl. Then it was again made upto 900 after 2 hrs with250 ml of 0.2M tricalcium phosphate. Sampling time was upto 12 hrs.

Statistical Analysis & Mathematical model:

In optimized batches Analysis of variance [ANOVA] and Response surface model was used as statistical analysis and by this the significance of the work was justified. Zero-order model, first order model, Higuchi model and Korsmeyer-Peppas model was used as mathematical model and the value of different rate constant was calculated to determine the type of release.

RESULTS AND DISCUSSION:

Preformulation studies were performed on pantoprazole sodium sesquihydrate in which the angle of repose was found to be 33.66° and the average compressibility index was found to be 15.9%, indicates fair to good flow properties [Table 8].

Table 8: Flow properties of pantoprazole sodium sesquihydrate.

S. No.	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Angle of Repose (Degree)	Compressibility Index (%)
1	0.370 ± 0.004	0.454± 0.007	35.02± 0.005	18.5± 0.002
2	0.370± 0.012	0.434± 0.003	33.22± 0.004	14.7± 0.010
3	0.370± 0.007	0.434± 0.005	32.75± 0.011	14.7± 0.007

n=3, data presented as mean ± SD.

The solubility study showed that pantoprazole sodium sesquihydrate was water soluble drug. Other than that the preformulation studies on Indomethacin were also performed the result showed average angle of repose was found to be 27.27° and the average compressibility index was 38.86%. it was also indicated the fair flow properties [Table 9].

Table 9: Results of flow properties of Indomethacin

S.No.	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Angle of Repose (Degree)	Compressibility Index (%)
1	0.462± 0.002	0.769± 0.004	30.45± 0.010	39.92± 0.002
2	0.476± 0.006	0.793± 0.012	25.22± 0.002	39.97± 0.006
3	0.526± 0.007	0.831± 0.003	26.15± 0.004	36.70± 0.005

n=3, data presented as mean±SD

The solubility was found to be water insoluble drug. After preformulation studies compatibility studies were performed according to ICH guidelines and the results were evaluated on the basis of IR spectra and assay. The results showed that there was no incompatibility between the drugs and selected excipients with both PSS and indomethacin [Table 10 & Table 11].

Table 10: Observed IR peak of pantoprazole after compatibility study.

Observed peaks of pantoprazole sodium sesquihydrate			
Interpretation	**Peak (zero months)**	**Peak (Three months)**	**Peak (Six months)**
N-H	3490	3490	3490
O-H	3382.91	3382.91	3382.91
CH2	3078.18	3078.18	3078.18
CH3	2981.74	2981.74	2981.74
C-O	1589.23	1589.23	1589.23
C-F	1382.87	1382.87	1382.87
S=O	1039.56	1039.56	1039.56

Table 11: Observed IR peak of indomethacin after compatibility study.

Observed peaks of Indomethacin			
Interpretation	**Peak (zero months)**	**Peak (Three months)**	**Peak (Six months)**
O-H	3371.34	3371.34	3371.34
C=O	1714.6,1691.46	1714.6,1691.46	1714.6,1691.46
C=C	1614.31,1479.30	1614.31,1479.30	1614.31,1479.30
C-H	1427.23	1427.23	1427.23
C-N	1371.29	1371.29	1371.29
C-O	1307.65	1307.65	1307.65
C-CO-O	1234.36	1234.36	1234.36
Aromatic ring	1188.07,1147.57	1188.07,1147.57	1188.07,1147.57

After that the buffer was selected on the bases of acid neutralizing capacity of different buffers. The combination of buffering agents [calcium lactate and magnesium hydroxide] was selected to achieve the required pH buffer [pH 5-6]. Other than that the pH stability profile was done on PSS and the result showed [Table 12] that selected PPI pantoprazole sodium sesquihydrate was unstable in lower pH [acidic environment]. The results also showed that as the pH of the solution increases the stability of the PPI increases. The selected PPI was best stable on pH 6.

Table 12: Results of pH stability of pantoprazole.

Solution	0.1 N HCl	HCL Buffer	HCL Buffer	Buffered SGF	Phosphate Buffer	Phosphate Buffer
pH	pH 1.3	pH 2	pH 3	pH 5	pH 6	pH 7.2
Change in colour	Brown colour	Dark yellow	Light yellow	Clear white	Light precipitated solution	Precipitated white
PSS (mg/ml) after eight hours	0.291	2.98	4.23	12.27	14.09	13.52

Than On the basis of preformulation studies on drugs, first the immediate release granules of PSS were prepared by using 8 different formulae [P1- P8] with different combination of ingredients. After evaluation of prepared granules, it was seen that SSG was better superdisintegrant then CCS. The amount of PVPK 30 responsible for the initial release of drug from the formulation [Table 13].

Table 13: Percentage Cumulative drug release of pantoprazole sodium sesquihydrate IR granules (Trial batch).

Formulation	DR after five min	DR after fifteen min	DR after thirty min
P1	0	61.95± 1.48	76.42± 0.96
P2	0	58.64± 1.53	88.36± 1.65
P3	0	69.82± 1.41	82.67± 0.82
P4	0	70.84± 0.44	92.39± 0.41
P5	0	78.33± 0.56	91.85± 0.86
P6	0	76.74± 1.47	89.71± 0.72
P7	0	80.95± 1.50	95.48± 1.43
P8	0	82.49±0.72	97.64±0.95

n=6, data presented as mean±SD

On the basis of results one of the best combination [P8] formulation was selected for optimization. After that the optimization was performed and for this again 8 formulations [OP1-OP8] were prepared.

The granules were then again evaluated all the parameters of flow properties showed good flow of granules. The cumulative percentage drug release showed the best release rate with max release in 30 min from the formulation [Table14]. The marketed formulation selected was enteric coated tablet of pantoprazole. The tablet was first crushed to form suspension with Krebs ringer bicarbonate buffer. After crushing its enteric coating was damaged so the marketed formulation showed release immediately.

Table 14: Percentage Cumulative drug release of pantoprazole sodium sesquihydrate IR granules (Optimization batch).

Formulation	DR after five min	DR after ten min	DR after fifteen min	DR after thirty min
OP1	0	70.12±0.31	82.49±0.72	97.64±0.95
OP2	0	78.45±0.94	82.04±0.81	96.16±0.45
OP3	0	70.50±0.10	79.56±0.22	95.55±1.24
OP4	0	75.61±0.83	80.43±0.38	96.96±0.78
OP5	0	70.59±0.74	83.63±0.75	98.26±0.92
OP6	0	74.40±0.41	85.00±0.19	98.35±0.34
OP7	0	71.16±1.64	84.48±1.48	99.61±0.70
OP8	0	73.96±1.20	86.54±0.66	99.72±0.42

n=6, data presented as mean±SD

After evaluation the statistical analysis of optimized formulations were performed on the bases of full factorial design by using response surface plot, ANOVA and order of reaction [Table 15 & Table 16].

Table 15: Analysis of variance (ANOVA) of immediate release pantoprazole sodium sesquihydrate for dependent variables from factorial design

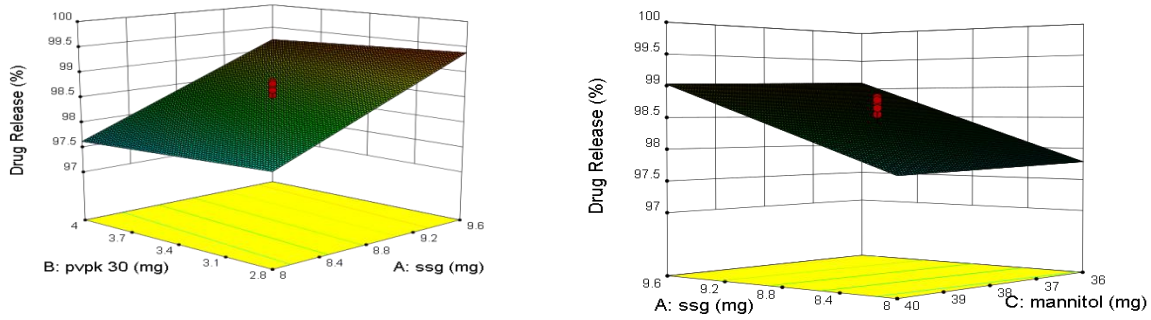
Factors	Sum Square	Degree of Freedom	Mean Square	F Value	p Value
Ssg	9.844	1	9.844	119.239	0.0002
Mannitol	0.501	1	0.501	6.063	0.8092
Pvpk-30	0.363	1	0.363	4.397	0.0396
Polynomial	$y = 0.0822x^2 + 4.571x + 36.47$				

Table 16: Mathematical model of drug release profile immediate release pantoprazole sodium sesquihydrate

Formulation	Regression values				
	Zero order	First order	Higuchi	Korsmeyer peppas	Release exponent (n)
OP1	0.9512	0.9212	0.9741	0.9917	0.676
OP2	0.8809	0.8986	0.9176	0.9823	0.732
OP3	0.9696	0.9532	0.9871	0.9904	0.686
OP4	0.9318	0.9681	0.9446	0.9782	0.681
OP5	0.9369	0.9432	0.9633	0.9908	0.713
OP6	0.9429	0.9633	0.9675	0.9860	0.725
OP7	0.9292	0.9583	0.9573	0.9912	0.698
OP8	0.9301	0.9780	0.9571	0.9918	0.710

The drug release profile of pantoprazole sodium sesquihydrate immediate release formulation followed korsmeyer peppas model, as the R^2 was found to be highest [0.9782 to 0.9918]. The value of release exponent [n] was 0.676 to 0.732 which indicates non fickian diffusion mechanism of the optimized formulation. On the bases of statistical results, it can be concluded that there was significant difference between concentration of SSG and PVPK-30 on drug

release. Whereas there was no significant difference between the concentration of Mannitol, as the p value was [0.8092] greater than 0.05 [Fig 1 (A), Fig 1 (B)].



(A) **(B)**

Figure1: Response surface model showing the effect of PVPK 30 and SSG (A) and mannitol and SSG (B) on drug release of PSS.

Than sustain release granules of indomethacin were also formulated and optimized in the same way. In sustain release granules also 8 different formulae [IN1-IN8] with different combination of ingredients [Table 17]. Then the granules were evaluated and results concluded that ethyl cellulose in combination with guar gum can be used to retard drug release. As the amount of PVPK 30 decreases the release of the drug increases. So one of the best combination [IN8] of formulation was selected for optimization. After that the optimization was performed and for this again 8 formulations [OIN1-OIN8] were prepared.

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Table 17: Percentage Cumulative release of indomethacin SR granules (Trial batch).

Formulation	DR 5 min	DR 30 min	DR 1 Hr	DR 2Hr	DR 4Hr	DR 6Hr	DR 8Hr	DR 10Hr	DR 12Hr
IN1	0	10.20± 0.033	25.24± 0.048	50.14± 1.34	63.11± 0.050	75.41± 0.038	85.72± 0.047	98.26± 1.27	-
IN2	0	11.54± 0.074	26.48± 0.029	50.84± 0.037	65.78± 0.024	74.26± 0.033	86.38± 0.058	98.12± 0.084	-
IN3	0	10.00± 0.090	20.17± 0.070	46.22± 0.027	60.76± 0.020	71.83± 0.038	79.34± 1.28	83.65± 1.57	-
IN4	0	18.85± 0.038	35.80± 0.064	63.06± 0.058	71.15± 0.078	80.35± 1.75	97.25± 0.093	-	-
IN5	0	15.17± 0.032	28.76± 0.092	42.74± 0.058	69.00± 0.069	80.54± 1.47	95.23± 0.067	-	-
IN6	0	9.95± 0.049	20.35± 0.070	26.13± 0.058	40.72± 0.034	49.69± 0.063	68.10± 0.084	79.36± 0.058	96.81± 1.24
IN7	0	6.64± 0.043	26.27± 0.045	30.99± 0.058	49.91± 0.060	55.27± 0.072	69.78± 0.057	88.82± 0.076	95.79± 0.030
IN8	0	11.47±0.12	15.77±0.35	27.83±0.71	50.85±0.74	64.48±0.92	70.22±0.15	81.78±0.97	97.89±0.70

n=6, data presented as mean±SD The evaluation was done which concluded the good flow property of prepared formulations. The drug release was found to be highest in OIN8 with better release rate [Table 18]

Table 18: Percentage Cumulative drug release of Indomethacin SR granules (Optimization batch).

Formulation	DR 5 min	DR 30 min	DR 1 Hr	DR 2Hr	DR 4Hr	DR 6Hr	DR 8Hr	DR 10Hr	DR 12Hr
OIN1	0	11.47±0.12	15.77±0.35	27.83±0.71	50.85±0.74	64.48±0.92	70.22±0.15	81.78±0.97	97.89±0.70
OIN2	0	11.29±0.40	16.12±0.57	27.28±0.78	52.14±0.43	63.72±0.19	72.93±0.64	80.77±0.58	97.26±0.32
OIN3	0	10.34±0.22	15.26±0.52	24.76±0.80	50.57±0.14	68.22±0.37	74.15±0.82	83.73±0.81	98.53±0.64
OIN4	0	12.85±0.59	15.91±0.16	27.98±0.74	55.13±0.72	65.71±0.68	75.74±1.27	85.84±0.46	98.72±0.72
OIN5	0	12.25±0.56	15.73±0.77	28.49±0.74	50.87±0.75	66.49±0.54	71.99±0.61	82.91±0.58	97.05±0.81
OIN6	0	11.48±1.25	15.96±0.78	27.28±0.59	50.41±0.72	64.07±0.74	71.82±0.54	81.94±0.23	98.84±0.94
OIN7	0	11.76±0.82	26.78±0.39	30.16±0.42	59.95±0.73	68.00±0.61	76.36±0.94	85.58±0.22	99.26±0.54
OIN8	0	11.83±1.85	15.71±0.46	28.00±0.50	51.32±0.26	68.55±0.75	75.44±0.30	83.47±0.93	99.15±0.79

n=6, data presented as Mean±SD

Then the ex vivo study was performed in OIN8 and the results were concluded that the optimized formulation [OIN8] showed better release profile then marketed formulation. The marketed formulation selected was sustained release tablet. It was first crushed to form suspension with Krebs ringer solution [Table 19].

Table 19: Percentage drug permeated from Indomethacin SR granules (Optimized batch).

Time (hrs)	OIN 8 (% Cumulative drug permeated)	Marketed (% Cumulative drug permeated)
0.5	20.24±0.12	18.25±0.03
1	25.87±0.06	26.69±0.48
2	44.84±0.05	35.48±0.50
4	62.11±0.17	52.78±0.08
6	70.58±0.21	65.71±0.17
8	80.42±0.42	73.28±0.33
10	87.06±0.14	79.11±0.06
12	99.15±0.02	97.52±0.15

n=3, data presented as mean±SD

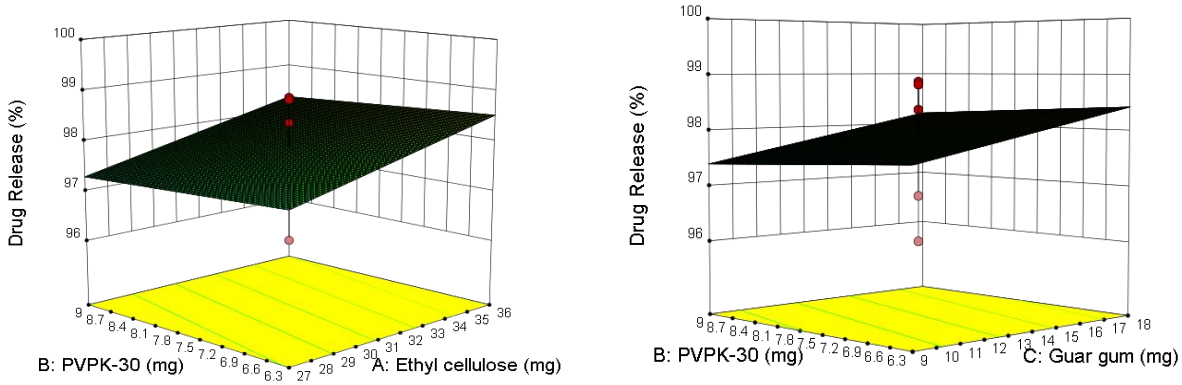
After that statistical analysis of optimized formulations were performed on the bases of full factorial design by using response surface plot, ANOVA and order of reaction [Table 20 & Table 21, Fig 2 (A) & Fig 2 (B)].

Table 20: Analysis of variance (ANOVA) of Sustain Release Indomethacin for dependent variables from factorial design using SPC

Factors	Sum Square	Degree of Freedom	Mean Square	F Value	p Value
Ethyl Cellulose	0.253	1	0.253	0.907	0.0037
Guar Gum	0.012	1	0.012	0.008	0.0214
PVPK-30	3.735	1	3.735	13.413	0.0061
Polynomial	$y = 0.0179x^4 - 0.3911x^3 + 2.0977x^2 + 7.7858x + 6.9117$				

Table 21: Mathematical model of drug release profile on Sustain Release

Formulation	Regression values				
	Zero order	First order	Higuchi	Korsmeyer peppas	Release exponent (n)
OIN1	0.9610	0.9929	0.9824	0.9099	0.793
OIN2	0.9568	0.9728	0.9732	0.9038	0.771
OIN3	0.9611	0.9703	0.9856	0.8952	0.827
OIN4	0.9556	0.9845	0.9836	0.9036	0.785
OIN5	0.9658	0.9937	0.9728	0.9093	0.774
OIN6	0.9628	0.9846	0.9813	0.9432	0.782
OIN7	0.9254	0.9907	0.9854	0.8940	0.781
OIN8	0.9627	0.9929	0.9873	0.9109	0.722



(A) (B)

Figure 2: Response surface model showing the effect of PVPK 30 and ethyl cellulose (A) PVPK 30 and guar gum and (B) on drug release of Indomethacin.

The results concluded that there was significant difference between concentration of ethyl cellulose, guar gum and PVPK-30 on drug release, as the p value was less than 0.05. The mathematical model concluded that the drug release profile of sustain release indomethacin formulation followed first order release model, as the R^2 was found to be highest [0.9703 to 0.9929]. The value of release exponent [n] was 0.771 to 0.827 which indicates non fickian diffusion mechanism of the optimized formulation.

After preparation of all three phases i.e. buffer phase, Immediate release phase and sustain release phase the capsule formulation [CP1] was made by hand operating capsule filling machine. And the prepared capsules were yellow in colour with avg weight 604 mg having 98.86% immediate release of pantoprazole sodium sesquihydrate and 99.84% sustain release of indomethacin upto 12 hrs [Table 22].

Table 22: Evaluation of optimized *In-situ* capsule formulation.

General appearance	Weight variation (mg)	Avg. Wt. (mg)	D.T. (min)	DR 30 min (PN)	DR 30 min (IN)	DR 2 Hr (IN)	DR 6 Hr (IN)	DR 10 Hr (IN)	DR 12 Hr (IN)
				%age cumulative drug release					
Yellow Cap and Body	Max+ 4.15 Min -0.15	604.15	12±2	98.86± 0.12	10.52± 0.09	28.00 ±0.04	67.55±0.17	82.17 ±0.06	99.84± 0.05

n=6, data presented as mean±SD

Comparison of Optimized Formulation with Existing Marketed Formulation:

A comparison was made between marketed formulation and the optimized formulation on the basis of drug release pattern [Table]. As per the search there was no such formulation available

in the market so for pantoprazole IR formulation the comparison was done with marketed enteric coated pantoprazole [**Pantocid 20mg tablet: Sun Pharmaceutical Industries Ltd.]**.

On the basis of observation, it can be concluded that in prepared optimized formulation the release of pantoprazole was fast and immediate as compared to the selected marketed formulation [Table 23].

Table 23: Comparison of Optimized Formulation with Existing Marketed Formulation.

Formulation	DR 15 min (PN)	DR 30 min (PN)	DR 30 min (IN)	DR 1 Hr (IN)	DR 2 Hr (IN)	DR 4 Hr (IN)	DR 6Hr (IN)	DR 8Hr (IN)	DR 10 Hr (IN)	DR 12 Hr (IN)
Marketed	0	0	13.44 ±0.11	18.25± 0.04	22.23 ±0.07	39.16 ±0.03	58.88 ±0.45	67.63 ±0.52	77.24± 0.18	96.08 ±0.09
CP1	82.04 ±0.05	98.8± 0.06	10.52 ±0.43	15.12± 0.07	28.00 ±0.32	51.32 ±0.02	67.55 ±0.25	73.44 ±0.09	82.17± 0.15	99.84 ±0.46

n=3, data presented as mean±SD

Accelerated Stability Studies [ICH: 40 ± 2°C Temp. & 75 ± 5% Rh] of Optimized Formulation:

As per the ICH guidelines optimized formulation was subjected to accelerated stability testing. For this the prepared capsules were placed in a humidity chamber at 40 ± 2°C Temp. & 75 ± 5% Rh for 6 months. The samples were tested for stability at 0,3 and 6 months on the bases of general appearance and infrared spectroscopy. The results concluded that the optimized formulation [CP1] was the stable because there was no change was observed in general appearance as well as in drug release when exposed to accelerated environmental conditions [Table 24].

Table 24: Accelerated Stability Studies of Optimized Formulation (CP1).

Time	General appearance	DR 15 min (PN)	DR 30 min (PN)	DR 30 min (IN)	DR 1 Hr (IN)	DR 2 Hr (IN)	DR 4 Hr (IN)	DR 6Hr (IN)	DR 8Hr (IN)	DR 10 Hr (IN)	DR 12 Hr (IN)
Zero Month	Yellow Cap and Body	82.04 ±0.50	98.86 ±0.41	10.52 ±0.07	15.12 ±0.40	28.00 ±0.08	51.32 ±0.47	67.55 ±0.71	73.44 ±0.63	82.17 ±0.09	99.84± 0.19
Three Month	Yellow Cap and Body	81.13 ±0.02	98.32 ±0.32	9.75± 0.43	14.86 ±0.33	27.44 ±0.06	49.71 ±0.80	66.42 ±0.36	74.72 ±0.51	82.33 ±0.23	99.43± 0.15
Six Month	Yellow Cap and Body	82.54 ±0.64	98.41 ±0.02	11.02 ±0.41	15.34 ±0.47	28.92 ±0.31	49.47 ±0.08	67.83 ±0.37	73.26 ±0.21	82.97 ±0.18	99.05± 0.05

n=3, data presented as mean±SD

CONCLUSION

On the bases of above experimentation, it can be concluded that the designed capsule containing novel sustain release formulation of indomethacin [Nonsteroidal anti-inflammatory drugs] may be used as anti-inflammatory for the inflammatory diseases such as gout arthritis etc. in long term without any gastrointestinal adverse effects due to the presence of pantoprazole [PPI] in combination. It can also be concluded that the formulation showed immediate release of stable pantoprazole sodium sesquihydrate due to the presence of in-situ buffering agents inside the capsule. Further work can be done to reduce the size of the formulation and to improve the release pattern by using novel technologies such as microencapsulation, nanotechnology ect.

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