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Formulation & *In-Vitro, Ex-Vivo* Evaluation of Suppositories of Mebeverine Hydrochloride by Using Cocoa Butter Base

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ABSTRACT

The Rectal route of drug delivery have been recognized as an alternative to the oral route in situations such as when the patient is comatose, unable to swallow or when the drug produces nausea or vomiting. Suppositories are solid bodies of various weights and shapes, adapted for introduction into the rectal, vaginal, or urethral orifice of the human body. They usually melt, soften, or dissolve at body temperature. IBS or spastic colon is a symptom-based diagnosis characterized by chronic absdominal pain, discomfort, bloating, and alteration of bowel habits. Mebeverine is used in the treatment of IBS as musculotropic antispasmodic drug without anticholinergic side-effects. the present work has been done to formulate and evaluate Mebeverine HCl suppositories for the treatment of IBS by using different surfactants and osmotic modifiers in different ratios.21 formulations of Mebeverine Hydrochloride suppositories were prepared by fusion method with different ratios of surfactants (Polysorbate20, Polysorbate80, SLS) and osmotic modifier (Urea) for the maximum drug release through the rectal route of administration. All the formulations were executed for in vitro and in vivo evaluation parameters & compatibility studies and all the values were within the limits. In vivo studies were performed using male albino rabbits. F2 (2% Urea) & F10 (2% Polysorbate20) were determined as optimized formulations by considering their drug release profiles.

Keywords: Cocoa Butter, Urea, Surfactants, IBS, Bioavailability, USP Type I (Rotating basket)

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INTRODUCTION

The rectal route of drug delivery have been recognized as an alternative to the oral route in situations such as when the patient is comatose, unable to swallow or when the drug produces nausea or vomiting. There are several therapeutic reasons why a drug should be administered rectally than orally. One of these is that, it is possible to avoid partly hepatic first pass elimination following rectal administration [3].

Suppositories are medicated, solid bodies of various sizes and shapes suitable for introduction into body cavities. The medicament is incorporated into a base such as cocoa butter which melts at body temperature, which slowly dissolves in the mucous secretions[2, 13].

IBS or spastic colon is a symptom-based diagnosis characterized by chronic abdominal pain, discomfort, bloating, and alteration of bowel habits. The primary symptoms of IBS are abdominal pain or discomfort in association with frequent diarrhea or constipation and a change in bowel habits. There may also be urgency for bowel movements, a feeling of incomplete evacuation (tenesmus), bloating, or abdominal distension [9, 10].

In some cases, the symptoms are relieved by bowel movements. People with IBS, more commonly than others, have gastro-esophageal reflux, symptoms relating to the genito-urinary system, chronic fatigue syndrome, fibromyalgia, headache, backache, and psychiatric symptoms such as depression and anxiety[11,12].

Mebeverine HCl is an anti spasmodic agent which exerts direct action on the GI smooth muscle. It is mostly used for the IBS as musculotropic antispasmodic drug without anti-cholinergic side-effects. Mebeverine is also an inhibitor of calcium-depot replenishment. Therefore, it has dual mode of action which normalizes the small bowel motility [6].

It acts directly on the gut muscles at the cellular level to relax them. This relieves painful muscle spasms of the gut, without affecting its normal motility. Mebeverine is used to relieve symptoms of irritable bowel syndrome and related intestinal disorders that are the result of spasms in the intestinal muscles. These include colicky abdominal pain and cramps, diarrhoea alternating with constipation and flatulence (wind).

The side effects associated with mebeverine include indigestion, heartburn, constipation, anorexia, insomnia, itchy skin or rashes [14].

MATERIALS AND METHOD

Mebeverine HCl (Nihal traders, Hyd), HPMC (Neha chemicals Hyd), Cocoa butter (Neha chemicals, Hyd), Urea (Neha chemicals, Hyd), Polysorbate 20 (Neha chemicals, Hyd), Polysorbate 80 (Neha chemicals, Hyd), Sodium Lauryl Sulphate (Neha chemicals, Hyd), cemtrifuge, male albino rabbits.

METHODOLOGY

Pre-formulation Studies:

Pre-formulation testing is the first step in the rationale development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced.

Identification of drug*:*

The IR spectrum of pure drug was found to be similar to the reference standard IR spectrum of mebeverine hydrochloride.

Solubility:

Solubility of Mebeverine Hydrochloride was determined in distilled water, ethanol, 0.1M HCl.

Melting point determination:

Melting point of Mebeverine Hydrochloride was determined by open capillary method at 130˚C.

Compatibility studies:

The vials containing samples were observed 2^{nd} and 4^{th} week and compared with vials kept at 4^0C as control. They were compared for incompatibility like lump formation and color change. From the results it was observed that there is no change as shown in table 1.

Table: 1 Drug and Excipient Compatibility Studies

S.No	Ingredients	Ratio	Physical Description		
			Initial	55°C (2 weeks)	40±2°C /70±5 % RH(4 weeks)
1.	API (Mebeverine HCl)	--	White	No change	No change
2.	API+ cocoa butter	1:1	Off white	No change	No change
3.	API+HPMC	1:1	Off white	No change	No change
4.	API+ urea	1:1	Off white	No change	No change
5.	API+ poly sorbate 20	1:1	Off white	No change	No change
6.	API+ poly sorbate 80	1:1	Off white	No change	No change
7.	API+ SLS	1:1	Off white	No change	No change

DSC curve of Mebeverine Hydrochloride:

The resultant Thermo grams generated for the analysis of each of the materials under investigation are presented in figure 1. The drug exhibited a sharp melting endotherm at 130°C. No significant thermal shifts were observed for Mebeverine hydrochloride, when it was assessed in combination with the other excipients intended for use in suppository formulation.

Therefore, based on thermal analysis data, it was concluded that Mebeverine could be formulated with combinations of the excipients tested, as potential major incompatibilities were not evident. However, more rigorous, long-term stability testing of manufactured dosage forms should also be conducted to rule out real-time long term dosage form instabilities.

FORMULATION STUDIES:

Preparation of suppositories with cocoa butter:

A brief overview is provided for the common method of suppository preparation with cocoa butter. Drug(s) and other ingredients are weighed accurately and are finely powdered. The appropriate quantity of cocoa butter is grated, and a small amount of grated cocoa butter is melted at about 34°C. The finely powdered drug mixture is then mixed with molten cocoa butter, and the remaining cocoa butter is added by keeping the entire contents at 34°C or less. The contents are stirred until a creamy liquid is formed, which is poured in lubricated molds and is allowed to congeal. Because the suppository mass generally shrinks upon cooling, it is a good practice to slightly overfill and then to trim after congealing. If the ingredients lower the melting point of the cocoa butter base, stiffening agents should be added in order to obtain suppositories that are solid at room temperature and melt when inserted in the body. The lowering of the melting point is common with soluble ingredients [1, 2, 14]

Preparation of control suppositories (F1):

Cocoa butter was first melted and then Mebeverine HCl, HPMC were added and poured into the moulds which were lubricated with liquid paraffin. Then the mould is kept for solidification in refrigerator. The excess base was trimmed and the solidified suppositories were packed and stored in cool and dry place to prevent the melting of the suppositories.

Using different concentrations of osmotic modifier

Urea (F2-F6):

The common method of suppository preparation with cocoa butter was followed. Then Mebeverine HCl, HPMC [5] were added along with Urea (osmotic modifier), in 2%, 4%, 6%, 8% and 10% concentrations to the liquefied base. The mixture was poured into the moulds which were lubricated with liquid paraffin. Then the mould is kept for solidification in refrigeration. The excess base was trimmed and the solidified suppositories were packed and stored in cool and dry place to prevent the melting of the suppositories.

Using different concentrations of variable surfactants*:*

Polysorbate 20 (F7-F11):

The common method of suppository preparation with cocoa butter was followed. Then Mebeverine HCl, HPMC were added along with polysorbate 20, in 0.5%, 1%, 1.5%, 2% and

2.5%concentrations to the liquefied base. The mixture was poured into the moulds which were lubricated with liquid paraffin. Then the mould is kept for solidification in refrigerator. The excess base was trimmed and the solidified suppositories were removed and packed and stored in cool and dry place to prevent the melting of the suppositories.

Polysorbate 80 (F12-F16):

The common method of suppository preparation with cocoa butter was followed. Then Mebeverine HCl, HPMC were added along with polysorbate 80, in 0.5%, 1%, 1.5%, 2% and 2.5%concentrations to the liquefied base. The mixture was poured into the moulds which were lubricated with liquid paraffin. Then the mould is kept for solidification in refrigerator. The excess base was trimmed and the solidified suppositories were packed and stored in cool and dry place to prevent the melting of the suppositories.

Sodium Lauryl Sulphate (F17-F21):

The common method of suppository preparation with cocoa butter was followed. Then Mebeverine HCl, HPMC were added along with SLS, in 0.5%, 1%, 1.5%, 2% and 2.5% concentrations to the liquefied base. The mixture was poured into the moulds which were lubricated with liquid paraffin. Then the mould is kept for solidification in refrigerator. The excess base was trimmed and the solidified suppositories were packed and stored in cool and dry place to prevent the melting of the suppositories. The ingredients and their concentrations for all the formulations were mentioned in the tables 2-5.

Table: 2 Formulation of suppositories using osmotic modifier (Urea, F2-F6

Ingredients	Control (F1)	Using osmotic modifier (urea)				
		F2	F3	F4	F5	F6
Drug (mg)	200	200	200	200	200	200
HPMC	5%	5%	5%	5%	5%	5%
Urea	0%	2%	4%	6%	8%	10%
Cocoa Butter	q.s	q.s	q.s	q.s	q.s	q.s

Table: 3 Formulation of suppositories using surfactants (Polysorbate 20, F7-F11)

Ingredients	Using surfactants (polysorbate 20)				
	F7	F8	F9	F10	F11
Drug (mg)	200	200	200	200	200
HPMC	5%	5%	5%	5%	5%
Polysorbate- 20	0.5%	1%	1.5%	2%	2.5%
Cocoa Butter	q.s	q.s	q.s	q.s	q.s

Table: 4 Formulation of suppositories using surfactants (polysorbate 80, F12-F16)

Ingredients	Using surfactants (polysorbate 80)				
	F12	F13	F14	F15	F16
Drug (mg)	200	200	200	200	200
HPMC	5%	5%	5%	5%	5%
Polysorbate- 80	0.5%	1%	1.5%	2%	2.5%
Cocoa Butter	q.s	q.s	q.s	q.s	q.s

Table: 5 Formulation of suppositories using surfactants (SLS, F17-F21)

Ingredients	Using surfactants (SLS)				
	F17	F18	F19	F20	F21
Drug (mg)	200	200	200	200	200
HPMC	5%	5%	5%	5%	5%
SLS	0.5%	1%	1.5%	2%	2.5%
Cocoa Butter	q.s	q.s	q.s	q.s	q.s

EVALUATIONS OF SUPPOSITORIES[14]

Appearance:

The suppository was examined with the naked eye for the uniform appearance of internal and external surfaces to access absence of fissuring, fat blooming, exudation and absence of migration of active ingredients. Longitudinal section of suppository was checked for homogeneity of active ingredient within the mass. The appearance of all the suppositories was found to be good.

Uniformity of weight:

20 suppositories were taken and weighed individually by using digital weighing balance and the average weight was calculated. The average weight of the suppository was found to be 2gm. The uniformity of weight values of all the formulations were mentioned in the table 6.

Disintegration[4]:

All the formulations were subjected for the disintegration test in 6.8 pH phosphate buffer which was found to be more than 30 min. The results were shown in the table 6.

Drug content Evaluation:

5 suppositories were cut into small pieces and an appropriate mass was placed into a 250 ml volumetric flask. pH 6.8 phosphate buffer was added upto the mark and it was heated slightly to melt the suppository. Then it was allowed to cool. The solution was filtered through whattman filter paper. The absorbance of filtrate was measured at 263nms using UV spectrophotometer and the amount of the drug present was calculated using calibration curve.

Hardness:

It was measured by using Erweka suppository hardness tester to determine breaking point of a suppository or brittle, elastic nature of the suppository. The results were shown in table 6.

Table: 6 Evaluation Parameters Of All Formulations

For.code		Appearance	Hardness (kg/m^3)	Dis. time (min)	Wt. variation (gm)	Content uniformity
F1 (control)		Good	2.2	33	2.10	98.08
Urea	F2(2%)	Good	2.3	35	2.11	98.04
	F3(4%)	Good	2.1	37	2.01	98.2
	F4(6%)	Good	2.5	31	1.95	100.01
	F5(8%)	Good	2.7	32	1.99	100.42

Polysorbate 20	F6(10%)	Good	2.1	38	2.04	101.9
	F7(0.5%)	Good	2.9	36	2.03	100.03
	F8(1%)	Good	2.8	39	2.06	99.6
	F9(1.5%)	Good	2.4	34	1.97	97.98
	F10(2%)	Good	2.3	35	1.98	98.5
	F11(2.5%)	Good	2.7	30	1.96	97.3
Polysorbate 80	F12(0.5%)	Good	3	31	2.01	99.94
	F13(1%)	Good	3.1	35	2.03	99.65
	F14(1.5%)	Good	2.7	37	2.07	100.03
	F15(2%)	Good	3.2	39	2.05	101.05
	F16(2.5%)	Good	2.5	38	1.98	98.65
SLS	F17(0.5%)	Good	2.7	36	1.99	101.73
	F18(1%)	Good	2.4	35	2.01	100.97
	F19(1.5%)	Good	2.3	31	2.04	100.67
	F20(2%)	Good	2.6	35	2.06	99.01
	F21(2.5%)	Good	2.8	31	2.03	99.97

Table 7: %DR Of Control Suppositories

Time (min)	%DR of control
5	18
10	28
20	59
30	72
40	78
50	95
60	97

Table 8: %DR of urea (F2-F6)

Time (min)	%DR of Urea (F2-F6)				
	F2 (2%)	F3 (4%)	F4 (6%)	F5 (8%)	F6 (10%)
5	27	32	36	38	40
10	45	52	54	58	60
20	74	80	80.5	81	82
30	82	84	86	88	90
40	92	93	94	95	95
50	96	96	97	97	97
60	97.5	97	97	97	97

Table 9: %DR of Polysorbate 20 and 80 (F7-F16)

Formulation code	%DR of surfactants (F2-F6)	
	Polysorbate 20	Polysorbate 80
F7 (0.5%)	29	-
F8 (1%)	34	-
F9 (1.5%)	37	-
F10 (2%)	48	-
F11 (2.5%)	50	-
F12 (0.5%)	-	29.5
F13 (1%)	-	32
F14 (1.5%)	-	38
F15 (2%)	-	45
F16 (2.5%)	-	47

Table 10: %DR of SLS (F17-F21)

Formulation code	%DR of SLS (F17-F21)
F17 (0.5%)	29
F18 (1%)	31
F19 (1.5%)	32
F20 (2%)	33
F21 (2.5%)	35

***In-Vitro* Dissolution Studies[13]:**

The developed formulations of Mebeverine HCl out in dissolution apparatus (USP I), filled with 400ml buffer.The temperature of the system was maintained at 37°C ± 0.5°C. 5ml of buffer was withdrawn periodically at 5, 10, 20, 30, 40, 50, 60min of and sink condition was maintained by replacing equal volume of fresh buffer. The drug concentration samples was measured by using UV Visible Spectroscopy at 263 nm.The *in-vitro* release profiles of formulation F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15, F16, F17, F18, F19, F20 and F21 containing different ratios of surfactants and osmotic modifiers were shown in Figure: 2, 3, 4, 5 & 6.

***In-Vivo* Study Design:**

All experiments were conducted according to the protocol approved by the Animal Ethics Committee (AEC). The experiment was deported according to the guidelines of Committee for the purpose of control and supervision of the experiment on animal.[15] The test was carried out on animal study protocol was reviewed and approved by the institutional animal ethical committee, St. Peter's Institute of Pharmaceutical Sciences, (SPIPS/AEC-29), Hanamkonda, India using Healthy male Albino Rabbits.

Procedure:

Male Albino rabbits of weight 3.5-4.5 kg were used as the experimental animal. Prior to the experiment four albino rabbits were fasted for 24hrs allowing water intake. The formulated suppositories (F2 and F10) were cut longitudinally and the rabbits were treated with one half containing 100mg of drug. The suppositories were inserted into rectum with glass injector at about 3cm depth from the anus. To prevent the leakage of the drug the anus was closed with

the adhesive tape. At least one week of washout period was allowed between successive dosing.

Following drug administration, blood samples were withdrawn using an implanted cannula from marginal ear vein with a sample size of 2ml at regular intervals of time. The samples were immediately centrifuged at 3000rpm for 5mins. The topmost plasma was then extracted for determination of availability of the drug using HPLC.

RESULTS AND DISCUSSION

F1 formulation is taken as control (Drug + HPMC + Cocoa Butter)

Activity of urea:

By using urea (osmotic modifier), (F2-F6) formulations were formulated.

Urea is having the pore forming ability and it was reported to increase the number of "sub micron voids" resulting in a more porous permeable structure from which the drug was released. Since urea is highly soluble substance, thus the internal pressure produced by the entry of water could force the drug solution out of the suppositories. The enhancing effect was observed with the addition of 2% urea, besides being compatible with the body fluids.

Activity of surfactants:

By using SLS (F17-F21), formulations were formulated.

SLS, sodium lauryl sulphate is an anionic surfactant, where the hydrophilic group carries a negative charge. By using polysorbate20 (F7-F11) & polysorbate80 (F12-F16) formulations were formulated.

Polysorbate 20 (mono laurate) and polysorbate 80 (mono oleate), are the ether ester type of non-ionic surfactants where the hydrophile carries no charge but derives its water solubility from highly polar groups[7,8]. The nature of the fatty alcohol or fatty acid chain that is present in the surfactant molecule affects drug release. Polysorbate 20 and 80 contain the same hydrophilic chain and the difference between their enhancing activities on the release of Mebeverine HCl was due to their lipophillic chain. The enhancement of the release rate produced by polysorbate 20 was more than that produced by polysorbate 80. This could be explained on the basis of the structure and HLB values as well as the size and number of micellar aggregates of the surfactant which increase when the length of its lipophillic chain becomes longer. Polysorbate 20 and 80 contain the same hydrophilic chain and the difference between their enhancing activities on the release of Mebeverine HCl was due to their lipophillic chain. The HLB values of polysorbate 20 and 80 are 16.7 and 15.0 respectively

In vivo studies:

From the in vitro studies the formulations F2 (2% urea) and F10 (2% polysorbate 20), gave maximum drug release profiles. Hence in vivo studies were conducted for F2 and F10

formulations. The sample containing 2% urea (F2), produced bioavailabililty of 71.15%. The increased systemic bioavailability of the drug after rectal administration could be due to partial avoidance of hepatic first pass metabolism. By addition of urea, the drug plasma concentration increases due to the effect of urea as a penetration enhancer, keratolytic agent and as a protein denaturant. The sample with 2% polysorbate 20, produced bioavailability of 67.3%. The enhancing effect of polysorbate 20 could be attributed to the ability of this non-ionic surfactant to lower the surface tension between the base and the surrounding rectal fluids, thus improving the wetting and contact with the epithelium, as well as distribution of the drug. It would interact with the lipid portions of the membrane thus increase the permeability of rectal membrane.

The mean plasma levels of mebeverine HCL in rabbits following rectal administration with urea (F2) and polysorbate 20 (F10) was shown in the figure 7.

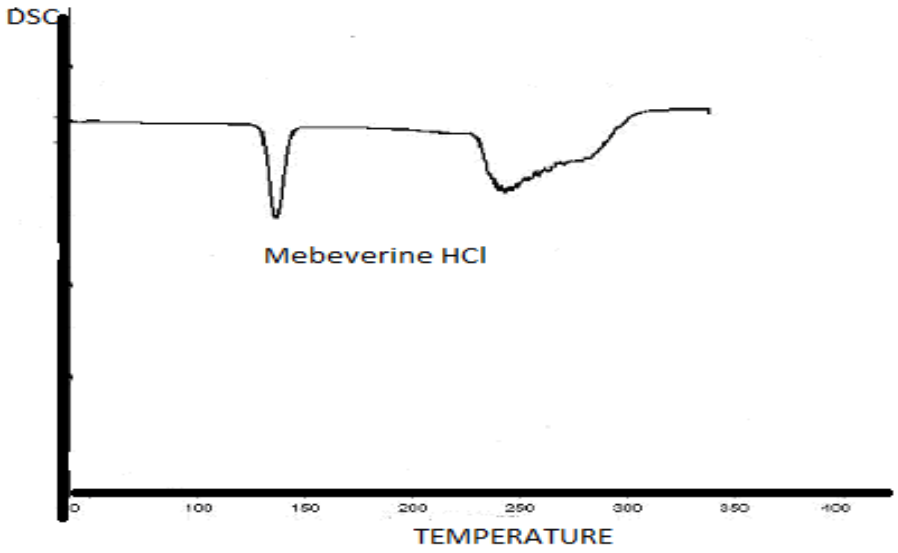

Figure: 1 DSC Curve of Mebeverine HCl

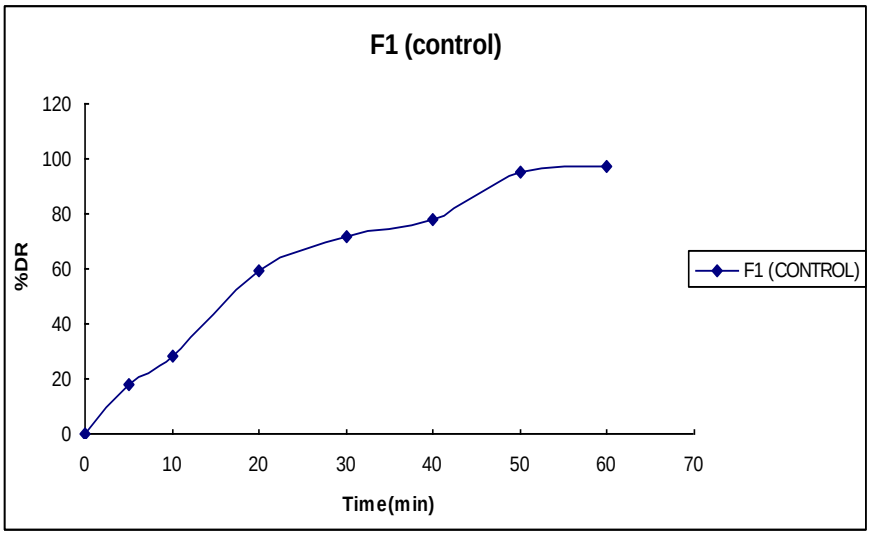

Figure: 2 %DR of Control Suppositories

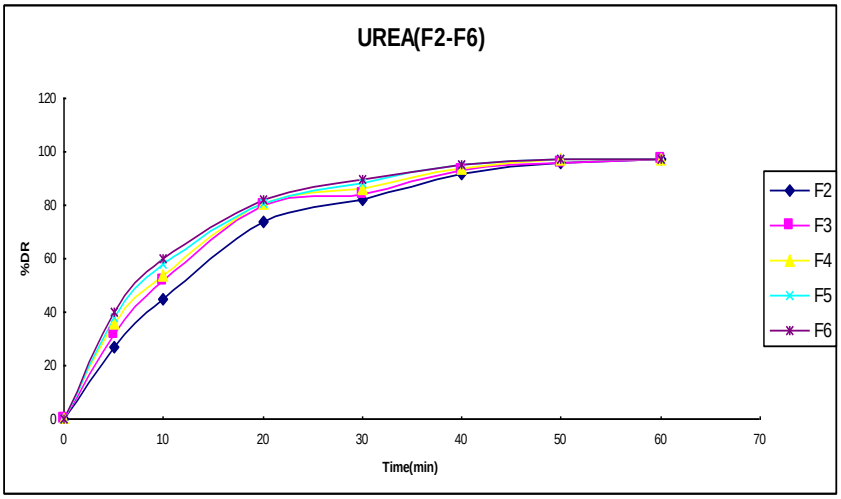

Figure: 3 %DR of urea (F2-F6)

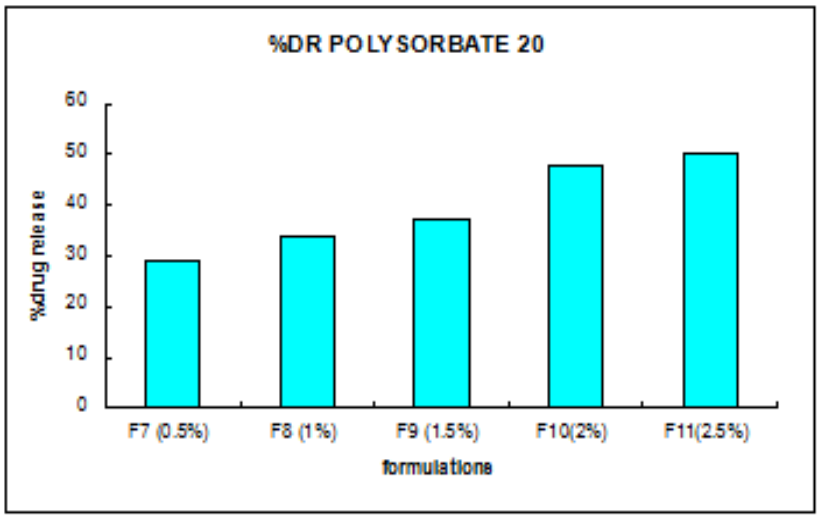

Figure: 4 %DR of polysorbate 20 (F7-F11)

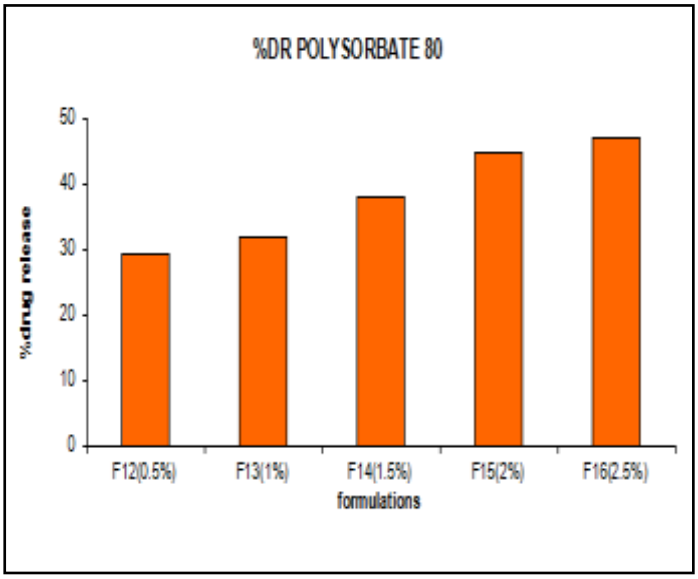

Figure: 5 %DR of polysorbate 80 (F12-F16)

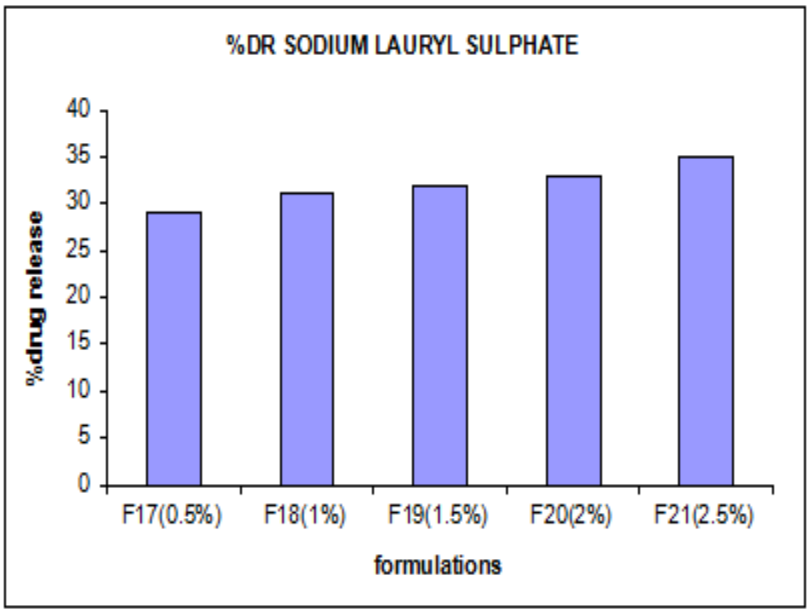

Figure: 6 %DR of SLS (F17-F21)

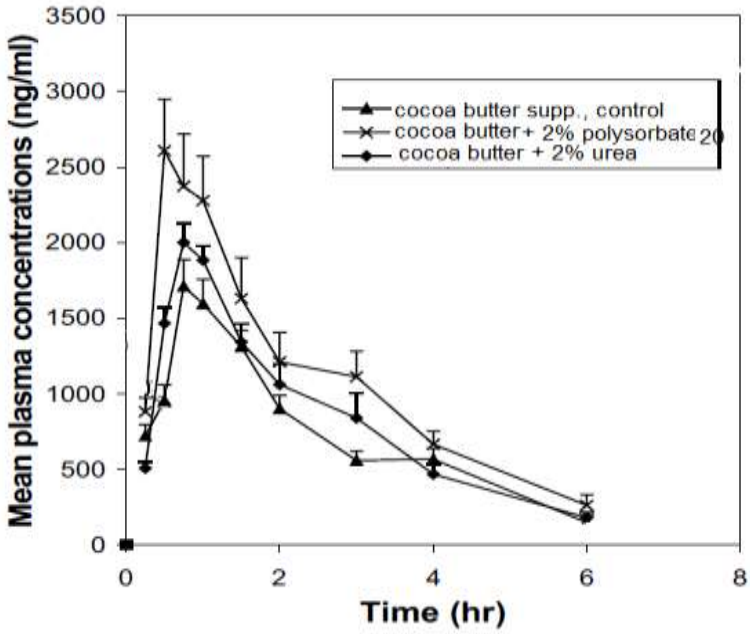

Figure: 7 The mean plasma levels of mebeverine HCL in rabbits following rectal administration with urea (F2) and polysorbate 20 (F10)

CONCLUSION:

From the experimental findings formulation & *in-vitro* evaluation of Mebeverine hydrochloride suppositories, it can be concluded that the analytical techniques indicate that the drug sample obtained was pure & does not show any incompatibilities with the excipients. In the present investigation, Mebeverine HCl drug has been attempted to deliver from rectal route of administration using albino rabbits. Suppositories were prepared by using urea as osmotic modifier (F2-F6) and SLS (F17-F21), polysorbate 20 (F7-F11) and 80 (F12-F16) as surfactants in different concentrations by fusion method. F1 is taken as control

prepared with cocoa butter. The in vivo bioavailability values of the formulation F2 with 2% urea 71.15 % and from formulation F10 with 2% polysorbate 20 was found to be 67.3%. Based on the above furnished details, considering *in vitro* drug release and bioavailability values, I conclude that F2 formulation with 2% urea & F10 with 2% Polysorbate20 are the best formulations with maximum release profiles.

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