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## Development, optimization and evaluation of solid dosage form of Thiocolchicoside by using absorption enhancers

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### ABSTRACT

Thiocolchicoside has a selective affinity for  $\gamma$ -amino-butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA-nergic inhibitory pathways thereby acting as a potent muscle relaxant. Thiocolchicoside (Muscoril, Myoril, Neoflax) is a muscle relaxant with anti-inflammatory and analgesic effects. It is used for the treatment of orthopedic, traumatic and rheumatologic disorders. This drug can not be absorbed orally owing to its very less permeation through GI epithelia. The physico-chemical properties of thiocolchicoside are not favorable for its permeation across the skin. Thiocolchicoside has a relatively high molecular weight (563), relatively high water solubility (16.1mg/ml) and low octanol/water partition coefficient ( $\log P = -0.34$ ). The present study attempts to increase the intestinal permeability of BCS class III drug Thiocolchicoside by using certain intestinal absorption enhancers. To enhance the permeation, intestinal permeation enhancers were used in various molar ratios with the drug. The lipophilicity of Thiocolchicoside was determined by means of the *n*-octanol/water system to check the effect of absorption enhancers. The changes in partition coefficient by the octanol/water system were confirmed using an *in vitro* transport model with the excised animal tissue membrane. The Data Expert software is applied for the  $3^2$  factorial design and the ANOVA study of the linear regression model, response surface plot as well as contour plot confirms the predicted batch with the desirability of 0.982. Result of  $R^2$  and *T*-test confirmed that predicted and observed responses showed no significant difference. The result indicated that there is significant improvement in the permeability of the drug and the extent of enhancement was highly dependent on the type of absorption enhancers used. Thus the study indicated that Thiocolchicoside could be successfully delivered orally when formulated with permeation enhancers.

**Keywords:** Thiocolchicoside, optimization, permeation enhancers, Franz diffusion cell, intestinal permeability.

### INTRODUCTION

The traditional drug administration routes used were oral administration for systemic effects and topical for local effects. Drugs could also be self-administered by inhalation, suppository and sometimes injections. The other routes of delivery usually required the intervention of a healthcare provider and the pain, fear and the possibility of infections associated with injections often resulted in low patient compliance and therefore have aided the development of suitable non-parenteral routes of administration. Oral administration still dominates drug therapy and more than 60 % of marketed drugs are oral products. This type of drug administration is preferred due to its convenience, high patient compliance, less stringent production conditions and lower costs. Delivering a drug by oral route is also preferred for its convenience. Tablets and capsules can be prepared in large quantity at low price.

Therefore in lead optimization step of drug discovery, oral bioavailability of a drug is important. It depends on various factors the most common being intestinal permeability, solubility during gastrointestinal transit, liberation from dosage form, liability to efflux and metabolism. Development in the field of combinatorial chemistry and high throughput screening has made it possible to generate a large number of drug candidates but it has also resulted in a number of poorly soluble and or poorly absorbable drugs. A new trend of drug development based on pharmacogenomics or development of molecular targeted drugs is also encouraging the tendency, and it does not necessarily lead to good output in terms of new drug development. Therefore it is necessary to improve the membrane permeability as well. The pharmacokinetic profile of a drug is dependent on the drugs ability to cross biological membranes. The permeability characteristics of compounds therefore affect its absorption, distribution and elimination<sup>[1-6]</sup>. It is estimated that approximately 40 % of the failures in drug development programs during clinical phases are due to problems in pharmacokinetics and drug delivery . The absorption of an orally administered compound depends on many different parameters, such as the chemical structure of the drug, permeability of the intestine brush border membrane (BBM), intestinal motility, gastrointestinal transit times, fluid volume, bile salt composition, enzyme systems, food and the physiological state of the human intestinal tissue. It is therefore clear that the absorption of a drug by the intestine is a complex process<sup>[7-9]</sup>. All drugs are now classified according to the biopharmaceutical classification BCS into four categories on the basis of solubility and permeability to rationalize science of drug delivery and simplify complications in the drug registration of newly evolving diverse compounds for regulatory authorities. Among the different classes of BCS the per oral delivery of class 3 and 4 drugs is partially or completely decreased due to their poor intestinal permeability. A great number of currently available drugs fall under the class III of the biopharmaceutical classification system, possess high therapeutic potential but cannot be delivered by oral route because of its poor permeation across the GIT epithelia.<sup>[10-15]</sup> It is important that the limiting factor to intestinal absorption can be modelled when using *in vitro* models to predict *in vivo* performance. Release of the active compound and absorption must occur within the available transit time. The drug must be released before reaching the absorptive site of the GIT and must be stable in the luminal fluids. The physicochemical properties of an orally administered drug are generally the major determinants of intestinal permeability. This includes molecular size and shape, pKa, lipophilicity (log P/log D), charge/ionisation and hydrogen bonding properties. Solubility of the compound in the intestinal tract is an extremely important factor that dictates the dissolution characteristics of the drug and eventually influences the bioavailability of the compound. Even though the underlying driver for solubility in the gastrointestinal fluids is the aqueous solubility of the drug, the solubility of the drug in the GIT may be influenced by pH profile, solubilisation *via* naturally occurring surfactants and food components, as well as complex formation with food and native components of the gastrointestinal contents<sup>[16-20]</sup>.

## MATERIALS AND METHODS

### Materials

Thiocolchicoside Drug (Saico Healthcare (P) Ltd, New Delhi) Sodium Glycolate and Sodium Caprylate (Himedia Laboratories (P) Ltd, Mumbai) EDTA(Qualikems Laboratories Reagent, New Delhi ) Lactose Monohydrate (CDH Analytical Reagent, Central Drug House (P) Ltd, New Delhi) were obtained as gift samples.

### Permeability enhancement of the drug<sup>[21-22]</sup>

Following steps were carried out:

#### Determination of o/w partition profile of the drug with selected permeation enhancers:

The partition coefficients of Thiocolchicoside drug with or without absorption enhancers in different molar ratio were determined between pH-7.4 phosphate buffer and n-octanol. These two phases were saturated with each other. The compounds were dissolved in aqueous phase (5 mg/ml). The buffer/octanol solutions were shaken for 8 hr at room temperature. After separation of the samples, into two phases, the drug content was analyzed spectrophotometrically at  $\lambda_{\max}$  259.0 nm.

The partitioning coefficient was calculated using the following equation:

$$P_{o/w} = a_o / a_b$$

Where  $a_o$  and  $a_b$  are the concentrations of the drug in n-octanol and buffer respectively.

**Table1. Partition coefficient of the drug with Permeation enhancers**

S. No	Permeation enhancer	Drug : Permeation enhancer ratio (mM)	P <sub>o/buffer</sub>	Batch code
1	Sodium glycolate	1:0.2	0.450	B1
		1:0.3	0.450	B2
		1:0.4	0.453	B3
		1:0.5	0.453	B4
2	Sodium caprylate	1:0.1	0.466	B5
		1:0.2	0.470	B6
		1:0.3	0.482	B7
		1:0.4	0.478	B8
		1:0.5	0.472	B9
		1:0.6	0.464	B10

**Table2. Partition coefficient of the drug with Ethylene diamine tetra acetate**

S. No	Permeation enhancer	Drug : Permeation enhancer ratio (mM)	P <sub>o/buffer</sub>	Batch code
1	Ethylene Di amine Tetra Acetic Acid	1:3	0.448	B11
		1:4	0.455	B12
		1:5	0.459	B13
		1:6	0.462	B14
		1:7	0.498	B15
		1:8	0.501	B16
		1:9	0.472	B17
		1:10	0.460	B18
		1:11	0.452	B19
		1:12	0.450	B20

**Table3. Partition coefficient of drug with Sodium Glycolate and Sodium Caprylate**

S.No	Batch code	Drug: SG: SC (mM ratio)	P <sub>o/buffer</sub>
1	B21	1 : 0.4 : 0.3	0.463

*S.G = sodium glycolate , S.C = sodium caprylate .*

**Table 4. Partition coefficient of drug with Sodium Caprylate & EDTA.**

S.No	Batch code	Drug: SC: EDTA	P <sub>o/buffer</sub>
1	B22	1:0.3:7	0.652
2	B23	1:0.3:8	0.715
3	B24	1:0.3:9	0.630
4	B25	1:0.3:10	0.602
5	B26	1:0.1:8	0.612
6	B27	1:0.2:8	0.624
7	B28	1:0.4:8	0.682
8	B29	1:0.5:8	0.673

*ST-Sodium Caprylate (Mm), EDTA- Ethylene Diamine Tetra Acetic Acid(Mm)*

Thiocolchicoside, a hydrophilic drug containing many polar groups, exhibited very small P<sub>o/buffer</sub> pH 7.4 value (0.450). However, the combination with absorption enhancers led to improvement in the P<sub>o/buffer</sub> pH 7.4 values. It was observed that the partition coefficient value did not increased significantly with all above used absorption enhancers. Hence I selected Batch P22, P23, P24, P27, P28 and P29 for further studies.

#### **In vitro permeation studies using excised animal intestinal tissue<sup>[23-26]</sup>**

The permeability studies were conducted using the static Franz cell system. The Franz cell is a diffusion chamber made of glass comprising an upper donor and lower acceptor compartment between which the tissue is clamped, with the mucosal side oriented upwards. The effective permeation area of the intestinal epithelium was 1.54 cm<sup>2</sup>. Transport medium was Hank's Balanced Salt Solution (HBSS) buffer (pH-7.4). 2.5 ml of sample solution (2mg/ml) was placed in donor compartment and 18.5 ml of the buffer were filled into the acceptor compartment. The acceptor medium was continuously stirred and the experiment was performed at 37°C. Samples were periodically removed from the acceptor compartment over 4 h. The volume of the acceptor compartment kept constant by adding fresh

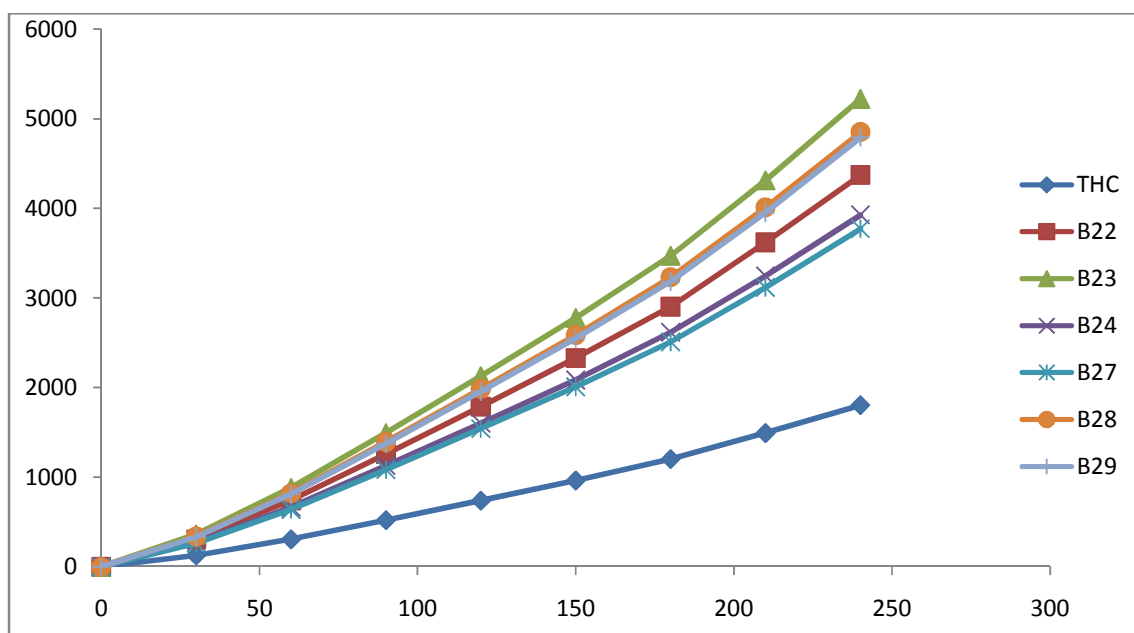
HBSS after each withdrawal. The samples were appropriately diluted and their absorbance determined at a wavelength of 259.0 nm.

**HBSS Buffer pH-7.4 :** Calcium chloride(anhydrous) 0.14g/L, Magnesium sulphate (anhydrous)0.0977 g/L, Potassium chloride 0.40g/L , Potassium di hydrogen phosphate 0.06g/L, Sodium bicarbonate 0.35g/L , Sodium chloride 8.00g/L, Di sodium hydrogen phosphate (anhydrous) 0.0477, D-Glucose 1g/L . Adjust the pH by NaOH.

#### Permeation profile:

**Table 5.**Permeability study of Batches having good  $P_{o\text{buffer}}$  value

Time(min)	Cum amount permeated ( $\mu\text{g}/\text{cm}^2$ )						
	THC	Batch B22	Batch B23	Batch B24	Batch B27	Batch B28	Batch B29
0	0	0	0	0	0	0	0
30	125.59	304.56	360.85	272.43	260.48	337.83	331.81
60	305.78	740.51	881.51	664.98	639.08	820.34	810.31
90	518.65	1256.72	1490.89	1127.61	1082.91	1391.16	1373.42
120	737.06	1786.12	2125.10	1601.31	1540.23	1982.69	1955.20
150	960.93	2327.00	2777.08	2082.90	2007.34	2584.45	2546.46
180	1201.18	2900.86	3470.21	2616.17	2509.43	3231.17	3182.54
210	1490.58	3618.65	4310.72	3245.48	3115.31	4009.66	3951.03
240	1801.82	4370.12	5220.29	3925.00	3770.30	4850.23	4790.21



**Figure1.** Permeation rate of thiocolchicoside ( $\mu\text{g}/\text{cm}^2$ ) using biological membrane

#### Calculation of permeability coefficient / apparent permeability coefficient:<sup>[27]</sup>

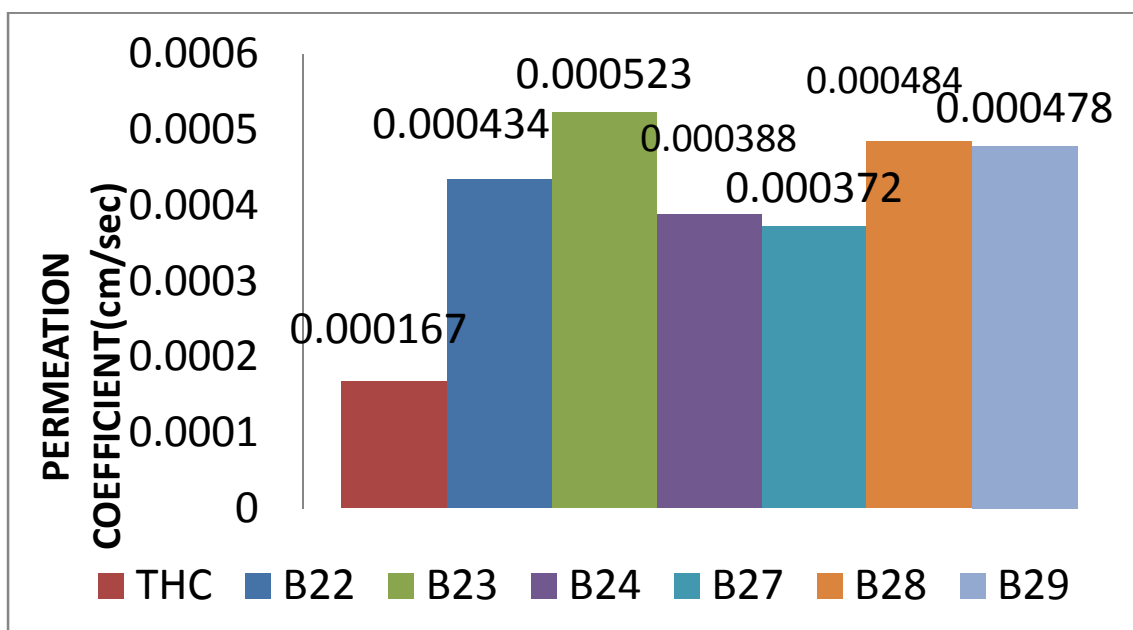
The apparent permeability ( $P_{app}$ ), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

$$P_{app} = \left( \frac{V_A}{\text{Area} \times \text{time}} \right) \times \left( \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{initial,donor}}} \right)$$

Where  $V_A$  is the volume (in mL) in the acceptor well (18.5ml), Area is the surface area of the intestinal membrane ( $1.54 \text{ cm}^2$ ), and time is the total transport time in seconds (14400 sec).

**Table 6. Permeability coefficient of selected batches**

S No.	Batch code	$P_{app}(\text{cm/sec})$
1	THC	$1.67 \times 10^{-4}$
2	B 22	$4.55 \times 10^{-4}$
3	B 23	$5.44 \times 10^{-4}$
4	B 24	$4.09 \times 10^{-4}$
5	B27	$3.93 \times 10^{-4}$
6	B 28	$5.05 \times 10^{-4}$
7	B 29	$4.99 \times 10^{-4}$



**Figure 2. Permeation coefficient of Thiocolchicoside**

#### Factorial design:<sup>[28-32]</sup>

Factorial design are used in experiments where the effects of different factors or conditions on experimental results to be elucidated. These are the design of choice for simultaneous determination of the effect of several factors and their interactions. The simplest one is the two-factorial design where two factors are considered, each at two levels, leading to four experiments, which are situated in 2-dimensional factor space at the corner of a rectangle. If there are three factors, each at two levels, eight experiments are necessary which are situated at the corners of the orthogonal cube in a 3-dimensional space. The number of experiments is given by  $2^n$  where 'n' is the number of factors.

If the number of factors and levels are large, then the number of experiments needed to complete a factorial design is large. To reduce the number of experiments, fractional factorial design can be used (i.e.,  $\frac{1}{2}$  or  $\frac{1}{4}$  of the original numbers of experiments with full factorial design). The fitting of an empirical polynomial equation to the experimental results facilitates the optimization procedure.

The general polynomial equation is as follows:

$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + \dots + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \dots + B_{123}X_1X_2X_3$ . Where Y is the response,  $X_1, X_2, X_3$  are the levels (concentration) of the 1,2,3 factors.  $B_0, B_1, B_2, B_3, B_{12}, B_{13}, B_{23}, B_{123}$ , are the polynomial coefficients.  $B_0$  is the intercept (which represents the response when the level of all factors is low).

**Full Factorial Design**<sup>[33]</sup>

A 3<sup>2</sup> randomized full factorial design was used to optimize the variables in the present study. In the design 2 factors were evaluated, each at 3 levels, and experimental trials were performed for all 9 possible combinations. The amount (20, 40, 60 mg) of starch (X<sub>1</sub>), and (2,2.5,3mg) of gelatin(X<sub>2</sub>), were selected as independent variables. The disintegration time and the % drug release (t<sub>45</sub>) were selected as dependent variables.

**Table7. Predicted Solution**

Factor A	Factor B	Disintegration time	Drug release	Desirability	Remarks
46.53	2.52	11	74.7007	0.982	Selected

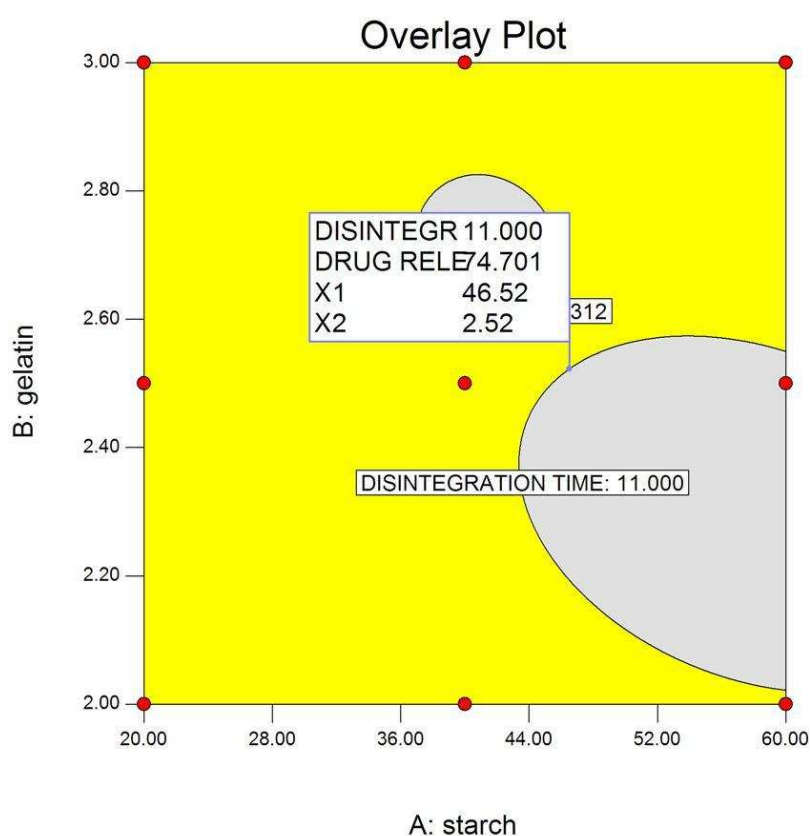
**Table8. Optimized batch B<sub>2</sub>**

STARCH (mg)	BINDER (%)	DISINTEGRATION TIME(min)	DRUG RELEASE (%)
40	2.5	11	75.312

Design-Expert® Software  
Factor Coding: Actual  
Overlay Plot

DISINTEGRATION TIME  
DRUG RELEASE  
● Design Points

X1 = A: starch  
X2 = B: gelatin

**Figure 3: Desirability graphical optimization of batch B<sub>2</sub>****Evaluation:**<sup>[34]</sup>**4.2. Post-compression Parameters****4.2.1. Tablet Hardness**

The strength of tablet is expressed as tensile strength (Kg/cm<sup>2</sup>). The tablet crushing load, which is the force required to break a tablet into pieces by compression. It was measured using a tablet hardness tester (Monsanto hardness tester).

The tablet hardness is found to be **4.0 kg/cm<sup>2</sup>**

**4.2.2. Tablet diameter**

The diameter of the tablet was found with the help of screw guaze.

**Table9. Tablet Average diameter readings:**

S.no	Diameter (mm)	Average
1	229.26	229.3025
2	229.31	
3	229.34	
4	229.30	

**Table10. Tablet average thickness readings:**

S.no	Thickness (mm)	Average
1	225.09	225.1925
2	225.25	
3	225.11	
4	225.32	

Randomly selected 20 tablets were weighed individually and together in a single pan balance. The average weight was noted and standard deviation was calculated. IP limit for weight variation in case of tablets weighting up to 120 mg is  $\pm 10\%$ , 120 mg to 300 mg is  $\pm 7.5\%$  and more than 300 mg is  $\pm 5\%$ .

**Table11. Weight Variation Test**

S.No	Weight (g)
1	0.222
2	0.221
3	0.223
4	0.221
5	0.220
6	0.224
7	0.224
8	0.221
9	0.223
10	0.224
11	0.221
12	0.222
13	0.225
14	0.221
15	0.223
16	0.220
17	0.224
18	0.223
19	0.222
20	0.224

$$PD = \frac{(W_{\text{avg}}) - (W_{\text{initial}})}{(W_{\text{avg}})} \times 100$$

Where, PD= Percentage deviation,

$W_{\text{avg}}$  = Average weight of tablet,

$W_{\text{initial}}$  = Individual weight of tablet.

Percentage deviation in weight of tablets is in limit i.e.0.450.

**4.2.3. Friability**

Roche friabilator was used for the purpose. This device subjects a number of tablets to the combined effect of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm dropping the tablets at a distance of 6

inches with each revolution. Pre-weighed 20 tablets were placed in the friabilator, which was then operated for 100 revolutions. Tablets were dusted and reweighed.

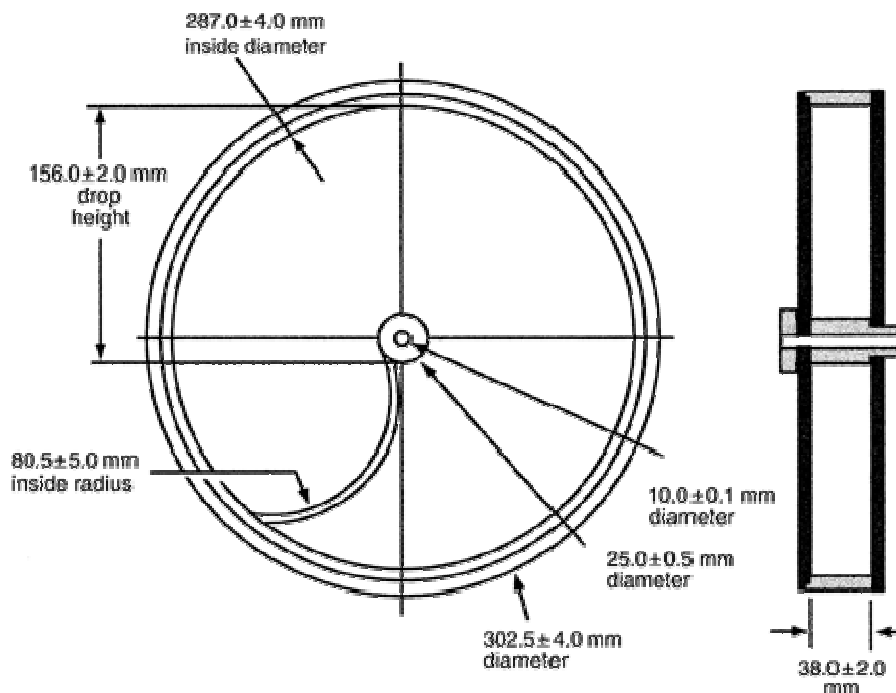


Fig4. Roche friability apparatus

#### CRITERIA:

For tablets with a unit weight equal to or less than 650 mg, take a sample of whole tablets corresponding as near as possible to 6.5 g. For tablets with a unit weight of more than 650 mg, take a sample of 10 whole tablets

$$\text{Percentage Friability} = \frac{W_1 - W_2}{W_1} \times 100$$

The maximum mean weight loss from the three samples is not more than **1.0 %** and is considered acceptable.

## RESULTS AND DISCUSSION

#### Permeability study:

Table11-permeability study of selected batches

Time (min)	Thiocolchicoside	Batch B23	Batch B28
0	0	0	0
30	125.59	360.85	337.83
60	305.78	881.51	820.34
90	518.65	1490.89	1391.16
120	737.06	2125.10	1982.69
150	960.93	2777.08	2584.45
180	1201.18	3470.21	3231.17
210	1490.58	4310.72	4009.66
240	1801.82	5220.29	4850.23

Thiocolchicoside alone shows a small Po/w of 0.450 with the permeation enhancers it exceeds to 0.715. Thiocolchicoside alone exhibited limited absorption via the lipid membranes. The combination with permeation



enhancers leads to a permeation rate of Thiocolchicoside from  $1801.82\mu\text{g}/\text{cm}^2$  to  $5220.29\mu\text{g}/\text{cm}^2$  and a permeation coefficient ranging from  $1.67\times 10^{-4}\text{ cm}/\text{sec}$  to  $5.44\times 10^{-4}\text{ cm}/\text{sec}$  (tables 5 and 6)

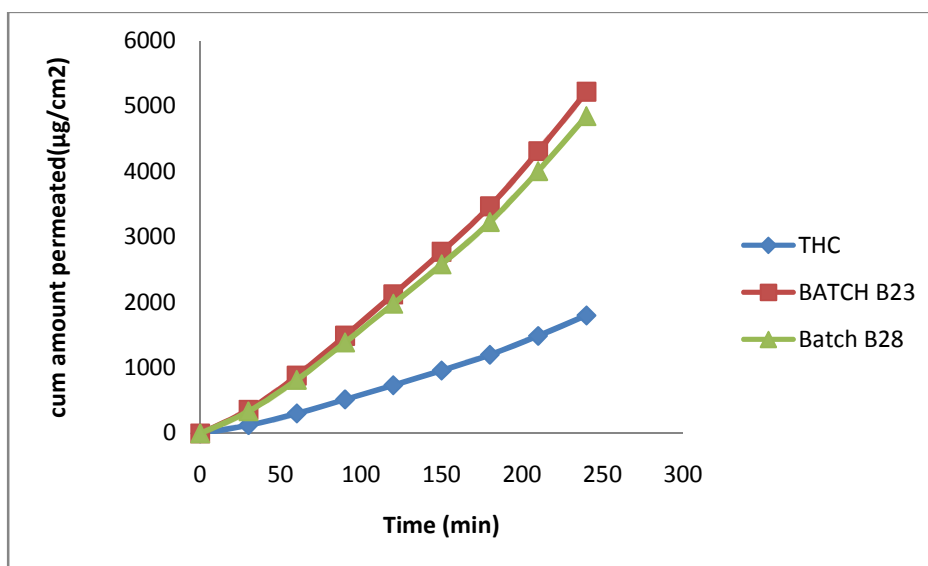


Figure5. Permeation rate( $\mu\text{g}/\text{cm}^2$ ) of Thiocolchicoside, B 23& B28 using biological membrane

The influence of enhancers on the absorption of Thiocolchicoside was studied. It was observed that the partition coefficient was increased significantly. The experiment using in vitro model with biological membranes shows enhanced penetration of Thiocolchicoside in the membrane through the combination of Sodium Caprylate and Edta. The result show that the optimal effect was obtained through the combination rather than using enhancers alone.

#### Optimization:

A  $3^2$  randomized full factorial design was used to optimize the variables in the present study. In the design 2 factors were evaluated, each at 3 levels, and experimental trials were performed for all 9 possible combinations. The amount (20, 40, 60 mg) of starch ( $X_1$ ), and (2,2.5,3%) of gelatin( $X_2$ ), were selected as independent variables. The disintegration time and the % drug release ( $t_{60}$ ) were selected as dependent variables.

Table12: optimized batch B<sub>2</sub>.

STARCH (mg)	BINDER (%)	DISINTEGRATION TIME(min)	DRUG RELEASE (%)
40	2.5	11	75.312

Thus the ANOVA study of the linear regression model, response surface plot as well as contour plot confirms the predicted batch with the desirability of 0.982. By the application of the Data Expert software it is confirmed that out of the 9 formulation prepared the batch B<sub>2</sub> is showing the best with the drug release and the disintegration time as the dependent variable, starch and gelatin as the independent variable. Result of  $R^2$  and T-test confirmed that predicted and observed responses showed no significant difference.

#### Evaluation:

##### Post compression Parameters:

The hardness of the tablet was found between  $4.0\text{ kg}/\text{cm}^2$  which have good mechanical strength. The tablet thickness was found to be **225.1925 mm**. The tablet diameter was found to be **229.3025 mm** weight variation the average percentage deviation of 20 tablets of each formula was **less than  $\pm 7.5\%$**  Friability of tablet was found **below 1%** indicating good mechanical resistance. In vitro disintegration time is found to be **11 minutes**, %CDR **58.131-75.312**.

### CONCLUSION

It can be concluded that the use of combination of permeation enhancers is more effective for enhancing the intestinal permeation of Thiocolchicoside, the extent of enhancement was found to be highly dependent on the absorption enhancers species used. Thiocolchicoside as a class III drug, shows that the use of absorption enhancers to enhance bioavailability of Thiocolchicoside offers viable method to deliver them by oral route.

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