PREPARATION AND IN-VITRO EVALUATION OF LEVAMISOLE HYDROCHLORIDE AS A CANDY BASED ANTHELMINTIC MEDICATED LOLLIPOPS FOR PEDIATRICS

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ABSTRACT:

Helminthiasis is prevalent globally, but is more common in developing countries with poorer personal and environmental hygiene, which is the major cause of undernourishment, anaemia, eosinophilia and pneumonia. The development of taste masking for bitter-tasting drugs administered orally for children has always been a formidable challenge for formulation scientists and this study reflects one of the contemporary advancements in the pediatric dosage forms. This study involves the preparation of candy based medicated lollipops of drug Levamisole, a synthetic imidathiazole derivative which acts by targeting the nematode nicotinergic acetylcholine receptor for pediatrics, by heating and congealing technique, using polymers like Sodium carboxy methyl cellulose, Methyl cellulose, Hydroxy propyl methyl cellulose and comparing with lollipops with no hydrocolloids. It was found that the formulation containing methyl cellulose showed better drug release and was more stable, unlike the other formulations.

KEY WORDS: Levamisole Hydrochloride, Medicated lollipops, Heating and congealing technique, NaCMC, Microcrystalline Cellulose, HPMC

INTRODUCTION:

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas, with poorer personal and environmental hygiene. [1]

Multiple infestations in the same individual are not infrequent. In the human body, GIT is the abode of many helminths, but some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of ill health.[2] Male fern and chenopodium had been used for worm infestations for centuries. Many drugs were discovered in the early part of the present century. However, over the past 4 decades many new, highly efficacious and well tolerated anthelmintics have been developed. These have largely replaced the older drugs. The choice of drug for each worm infestation is based not only on efficacy, but also on lack of side effects/toxicity, ease of administration and low cost.[2]

Levamisole Hydrochloride is the *S*-enantiomer of tetramisole- a synthetic imidazo-thiazole derivative acting as an anthelmintic[1]. The single enantiomer was introduced in 1969 since the other enantiomer (dexamisole) showed more adverse effects[1, 2]. Levamisole proved to be also effective in combination with 5-fluorouracil as adjuvant therapy in patients with colon carcinoma and current investigations of Levamisole HCl are focused on its immunomodulatory effects.

MATERIALS AND METHODS:

The following materials that were either AR/LR grade or the best grade available is used as supplied by the manufacturer without further purification or investigation.

Drug: - Levamisole HCl purchased from

- Yarrow Chem Products, Wadala, Mumbai

Polymers:

Table No.1 List of Polymers

Sl.	Name	Grade	Supplier
1.	Methyl Cellulose	Pharma Grade	Yarrow Chem Products
2.	Sodium carboxy methyl cellulose	Pharma Grade	Himedia
3.	Hydroxy propyl methyl cellulose	Pharma Grade	Yarrow Chem Products

Other Materials:

Table No.2 List of Excipients

Sl. No.	Name	Suppliers
1.	Sucrose	Himedia
2.	Dextrose	Yarrow Chem Products
3.	Citric acid	Yarrow Chem Products

EXPERIMENTAL:

FORMULATION OF MEDICATED LOLLIPOPS

Preparation of Medicated lollipops by Heating and congealing method[44]

Medicated lollipops 5gms in weight oval in shape were prepared. The method followed for the preparation was heating and congealing technique. Syrup base was prepared in a copper vessel dissolving the required amounts of sugar in water on heating and stirring continued for 2 hours by raising the temperature to 160^0C.The temperature was brought down 90^0C till a plastic mass was obtained. Drug, polymer, color, flavor were added and mixed the material for 30min. The mixture was poured into the molds. Air dried for 2 hours. The prepared lollipops were seal wrapped in polythene wrappings. An altogether three batches of formulations were prepared i.e., without added hydrocolloid, methylcellulose (MC) added, HPMC (Hydroxy propyl methyl cellulose, Sodium Carboxy methyl cellulose added medicated lollipops.

EVALUATION OF MEDICATED LOLLIPOPS

Evaluation:

1) Drug Content:

The drug content was calculated for all the six formulations of Medicated lollipops. Table No.15 and Fig No.12 shows the result of drug content of each formulation. Three replication of each test were analyzed for mean and standard deviation. The drug content found in the formulations containing methyl cellulose i.e., F1 and F2 contains 100.24±0.05 and 101.56±0.12% of the drug respectively, which indicates drug content in F1< F2. The drug content in the formulations F3 and F4 containing polymer Sodium carboxy methyl cellulose contains 100.24±0.10 and 99.36±0.08% respectively which implies drug content in F3> F4. Among the formulations the highest percentage of drug was in F2 and the lowest being F4. The formulation without Hydrocolloids i.e., F0 contain 99.68±0.06% of drug. Hence, all the formulations were found to be within the standard limits, which states that the drug content should be in the range of 95%- 105% for Levamisole.

2) Weight variation:

20 individual lollipops of F0 to F4 formulations were weighed; mean and standard deviation were calculated for

each Table No.15 shows the mean value of uniformity of weight. The formulation F1 and F2 containing Methyl cellulose weighed 5.13±0.04gm and 5.18±0.09gms respectively, hence weight variation in F1< F2. The formulation containing Sodium carboxy methyl cellulose F3 and F4 weighs 5.16±0.06gms and 5.22±0.08gms respectively, hence weight variation in F3< F4. The highest weighing formulation is F2 and lowest F1. The formulation containing no hydrocolloids weighs 5.07±0.02gms.

3) Thickness:

The thickness of the lollipops (F0 to F4) were measured by using Vernier calipers by selecting five lollipops in random. The mean values are shown in Table No.15.The values were almost uniform in four formulations i.e., F0, F1, F2, F3, and F4 and it was found to vary between 10.01±0.08mm to 10.61±0.005mm which was found in F0 and F4 respectively.

4) Hardness:

The hardness of the lollipops (F0 to F4) were measured by Monsanto Hardness Tester by selecting six lollipops of each formulation in random. The highest hardness was found in formulation F4 i.e., 10.11±0.006kg/cm^2 and least hardness was determined in F5 i.e., 9.21±0.005kg/cm^2. Since there is no specified standard limits for the deviation in the hardness of lollipops, comparing each formulation with one another it could be concluded that, because the difference between the standard deviations are not too large, the formulations had good uniformity in the hardness.

5) *In-vitro* release studies:

All the six formulation prepared were subjected to *in-vitro* release study. The *in-vitro* method for studying the release rate should be so that it must simulate the mouth condition. In the present work *in-vitro* release study was carried out using dissolution apparatus. For different time interval, sample was withdrawn and cumulative drug release was calculated. The dissolution apparatus USP II paddle type was used. The temperature was maintained at 37± 0.5 ^{0}C and stirred at 100rpm. The dissolution medium being phosphate buffer of pH 6.8. The samples were withdrawn at 5mins interval for 30mins.

Since the drug release in the formulations F0, F1, F2 were faster the samples were withdrawn at 2mins interval. And for F3, and F4 the samples were withdrawn at 5mins interval. Cumulative percentage drug release and percentage drug retained unreleased were calculated on the basis of mean amount of Levamisole Hydrochloride present in the respective lollipops. The results are given in Table No: 16 to 22 and Fig. No: 12 and 13.

The cumulative percentage drug release of F1 at the end of 12 minutes was found to be 99.08036±0.78% and F2 98.764±0.88 at the end of 14 minutes. The release was faster in F1 than F2. The cumulative percentage drug release of F3 was 99.687±0.23% by the end of 20mins and in F4 98.748±0.11 at 25mins.

Hence by the determination of the in-vitro release data, it can be concluded that the drug release was faster in case of F1 and F2 which was within 15mins. The formulations containing Sodium carboxy methyl cellulose and Hydroxy propyl methyl cellulose showed slower release rates when compared to Methyl cellulose. The use of polymers showed extended release of the drug.

This shows that F1 and F2 have comparatively moderate time of release, than the other formulations which shows longer time of release in comparison to F1 and F2, which can be considered as a desirable characteristic for the preparation of medicated lollipops. Hence it is considered as preferred formulations.

RESULTS AND DISCUSSION:

Several methods were described in the previous chapter for the formulation and evaluation of candy based medicated lollipops. These lollipops were intended for the treatment of anthelmintic. The lollipops were prepared as per the procedures and formulated according to the formulas given in Table No.13. The prepared lollipops were then subjected to evaluation like in-vitro dissolution studies, hardness, thickness, weight variation, stability studies and compatibility studies. The procedure adopted to perform the above mentioned parameters were discussed in methodology section and the obtained data were shown in results section.

Identification of max

The drug was identified by the light absorption in the UV range of 400-200nm at max 215nm. This was in accordance with reported values. The result were shown in Fig.No.3

Standard curve for Levamisole:

Table No:6 shows the absorbance of Levamisole standard solution containing 2,4,6,8 and 10 mg/ml of drug in pH 6.8 phosphate buffer solution. Fig No:4 shows the standard calibration curve with the correlation coefficient of 0.996 and regression value of Y=+0.028 the calculation of drug content uniformity in-vitro dilution studies are based on this calibration curve.

Drug-Excipient Compatibility studies:

The IR spectra of the formulation was compared with the standard spectrum of pure drug Levamisole

Hydrochloride and the characteristic absorption peaks associated with specific functional groups and bonds of the molecule and their presence of absence in the excipients were noted. The prominent peaks associated with Aromatic stretching, N-H amine stretching and C-N vibrations of Levamisole Hydrochloride were obtained (alone and with different polymer) were analyzed.

The principle IR absorption peak of Levamisole Hydrochloride and all excipients are as follows:

- The peak at 2791.46 cm^{-1}, 2883.06 cm^{-1} , 2955.38 cm^{-1} , 2882.09 cm^{-1} and 2955.38cm^{-1}in drug, drug + methylcellulose, drug + NaCMC, drug , drug+ excipients respectively is due to aromatic stretching.
- The peak at 1516.74 cm^{-1}, 1566.88 cm^{-1}, 1567.84 cm^{-1}, 1568.88 $cm^{-1}$and 1566.88 cm^{-1} drug, drug + methylcellulose, drug + NaCMC, drug, drug+ excipients respectively is due to N-H amine stretching.
- The peak at 1088.62 cm^{-1}, 1040.41 cm^{-1}, 1078.99 cm^{-1}, 1028.84 $cm^{-1}$and 1078.98 cm^{-1} drug, drug + methylcellulose, drug +NaCMC, drug, drug+ excipients respectively is due to C-N vibrations.

The range of peak values were found to be the same indicating that there were no interaction of Levamisole Hydrochloride with different polymer confirming the stability of drug in the formulation. The spectra for all the three polymer along with the drug are shown from Fig No: 6, Fig.No:7, Fig No:8 and Fig No.9. The spectra for drug Levamisole Hydrochloride, is shown in Fig No: 5 and the peak pickings in the spectra are shown in Table.No:7, 8, 9, 10 and 11.

CONCLUSION:

In the present study, an attempt was made to formulate and evaluate candy based medicated lollipops of Levamisole Hydrochloride for the treatment of Helminths. The main interest in such a dosage form was for the development of new dosage form and the effect of different polymers on the in-vitro release of the drug and also their stability. At the outset, estimation of drug by UV visible spectrophotometer was carried out. The possible interaction between the drug and excipient was studied by FT-IR spectroscopy which showed that there was no interaction between the selected drug and polymers under study.

Candy based medicated lollipops of Levamisole Hydrochloride was prepared by Heating and congealing method. In this study, various formulations were developed using Methyl cellulose, Sodium carboxy methyl cellulose. Evaluation parameters like thickness, weight variation, hardness show that they were within the limits. Drug content uniformity was also found to be within the limit. In vitro release rate studies showed that the drug release was maximum in formulation F0 and minimum in F4. The rate of release of F1 and F2 was found to be 99.08% in 12 minutes and 98.76% in 14mins respectively. The rate of release of F3 and F4 was 99.68% at 20 minutes and F4 at 25 minutes respectively. This shows that F1 and F2 have comparatively moderate time of release, than the other formulations which shows longer time of release in comparison to F1 and F2, which is considered as a desirable characteristic for the preparation of medicated lollipops. Hence it is considered as preferred formulations. All the formulations were found to be stable over the storage period and at different conditions tested.

TABLES AND FIGURES

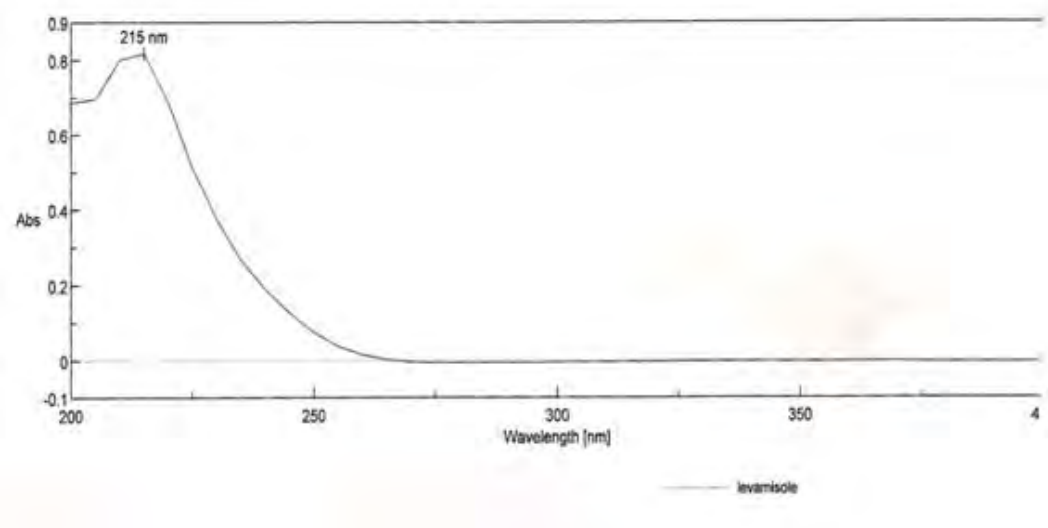

Fig. No.1 UV visible spectrum for drug Levamisole

Table No.3 Standard Graph for Levamisole

Concentration (µg/ml)	Absorbance (nm)	Standard deviation (±)
0	0	0
2	0.058	0.006
4	0.116	0.013
6	0.165	0.034
8	0.219	0.007
10	0.295	0.011

*Each value is an average of three determinations

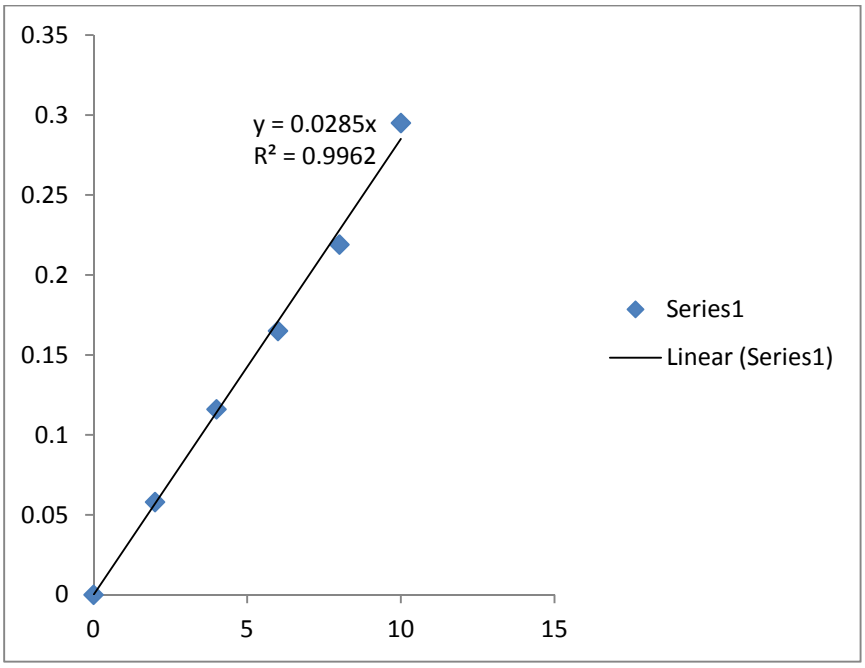

Fig No: 2 Standard Graph For Levamisole

DRUG EXCIPIENT COMPATIBILITY STUDIES

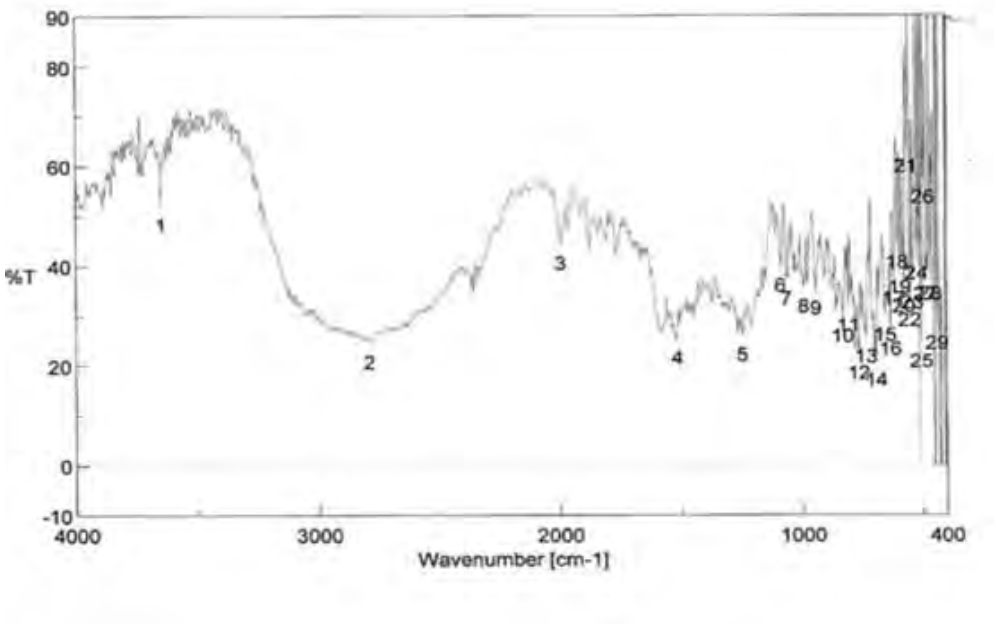

Fig. No: 3 FTIR for Pure Drug Levamisole

Table No:4 Peak picking for pure drug Levamisole

Sr no	Position	Intensity	Sr no	Position	Intensity
1	3647.7	52.3174	8	994.125	35.8179
2	2791.46	24.6863	9	943.985	35.4752
3	1996.93	44.4922	10	834.062	29.8879
4	1516.74	25.5432	11	809.956	32.0644
5	1248.68	26.1364	12	766.566	22.4368
6	1088.62	40.0357	13	736.674	25.7771
7	1065.48	37.4268	14	694.248	21.1318

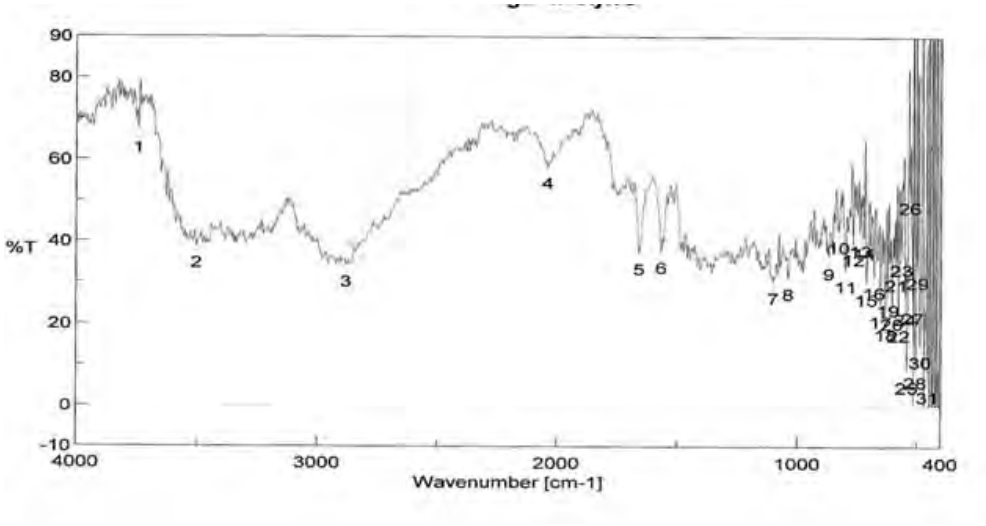

Fig.No:4 FTIR for pure drug+ Methyl cellulose

Table No:5 Peak picking for pure drug and methylcellulose

Sr no	Position	Intensity	Sr no	Position	Intensity
1	3743.15	66.6756	8	1040.41	31.1129
2	3501.13	38.7003	9	870.703	36.1239
3	2883.06	34.1172	10	830.205	42.5441
4	2040.32	58.1972	11	800.314	32.9754
5	1659.45	37.173	12	767.53	39.5872
6	1566.88	37.5378	13	738.603	41.5979
7	1102.12	30.0877	14	723.175	41.1332

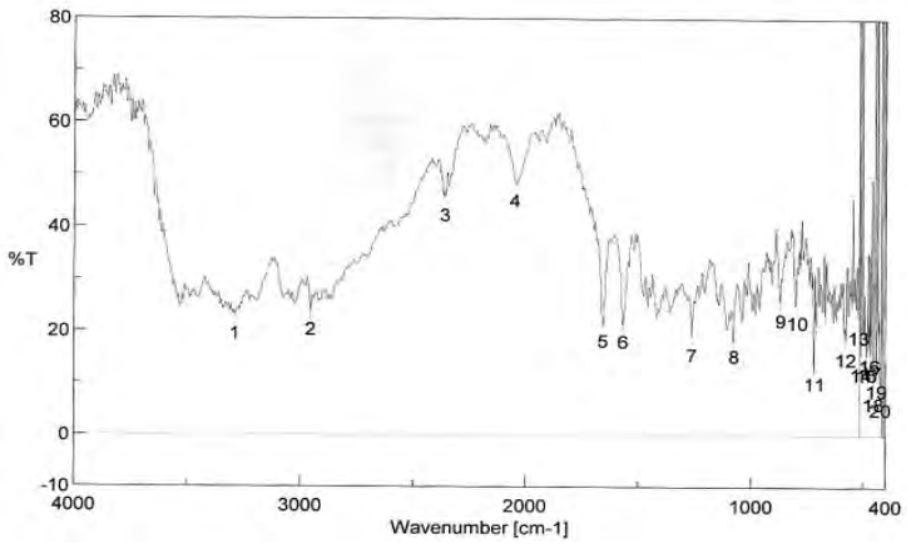

Fig.No:5 FTIR for pure drug + Sodium Carboxy methyl cellulose

Table No:6 Peak picking for pure drug and Sodium carboxy methyl cellulose

Sr no	Position	Intensity	Sr no	Position	Intensity
1	3290.93	22.9625	8	870.703	25.5148
2	2955.38	23.781	9	801.278	24.1066
3	2041.28	48.0189	10	720.282	12.0014
4	1654.62	20.9484	11	668.214	23.0761
5	1567.84	20.9902	12	577.576	18.1545
6	1262.18	20.9485	13	519.722	22.3933
7	1078.98	15.953	14	510.08	15.2858

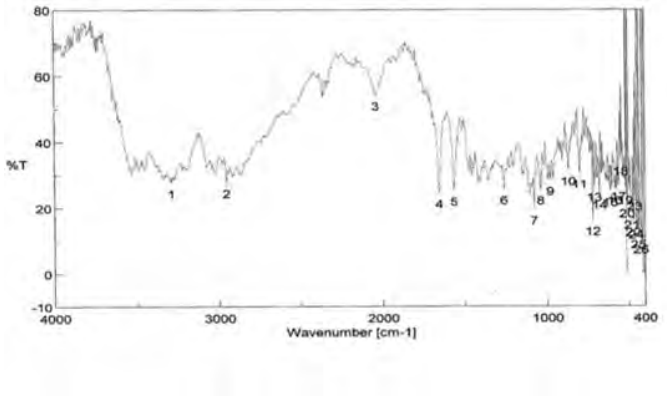

Fig. No: 6 FTIR for pure drug +All Excipients

Table No:7 Peak picking for pure drug and all the excipients

Sr no	Position	Intensity	Sr no	Position	Intensity
1	3290.93	27.7841	8	1039.44	25.5118
2	2955.38	27.8301	9	980.625	28.3007
3	2042.25	54.2802	10	870.703	31.2782
4	1655.59	24.5373	11	801.278	30.3867
5	1566.88	25.2557	12	720.282	16.0848

Table No:8 Interpretation of FT-IR spectra of Pure drug and Polymer

Ingredients	Peaks of functional groups (cm^{-1})		
	Aromatic stretching	N-H Amine stretching	C-N vibrations
Levamisole	2791.46	1516.74	1088.62
Levamisole+MC	2883.06	1566.88	1040.41
Levamisole+ NaCMC	2955.38	1567.84	1078.98
Levamisole+Excipents	2955.38	1566.88	1078.98

PREPARATION OF CANDY BASED MEDICATED LOLLIPOPS

Table No:9 Formulation Chart for Candy based Medicated Lollipops

BATCH	F0	F1	F2	F3	F4
DRUG LEVAMISOLE	50mg	50mg	50mg	50mg	50mg
SUCROSE	3.52365 gms	3.4476 gms	3.37155 gms	3.4476 Gms	3.37155gms
DEXTROSE	1.45002 gms	1.45002 gms	1.45002 gms	1.45002 Gms	1.45002gms
METHYL CELLULOSE		0.0507 gms	0.07605 gms	-	-
SODIUM CARBOXY METHYL CELLULOSE	-	-	-	0.0507 gms	0.07605 gms
CITRIC ACID	0.0507 gms	0.0507 gms	0.0507 gms	0.0507 gms	0.0507 gms
COLOURING AGENT	q.s	q.s	q.s	q.s	q.s
FLAVOURING AGENT	0.0344 gms	0.0344 gms	0.0344 gms	0.0344 gms	0.0344 gms

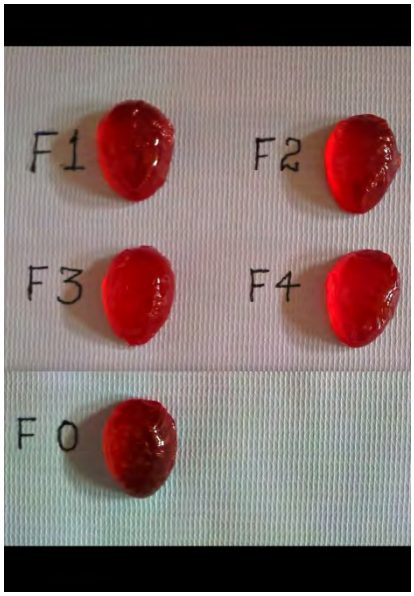

Fig.No:7 Models of Prepared Medicated Lollipops II

CHARACTERIZATION OF PREPARED MEDICATED LOLLIPOPS

Table No: 10 Physical Appearance of prepared Medicated lollipops

Formulation	Appearance
F0	Orange, smooth, little sticky, easily removed from the mold
F1	Orange, smooth, little less sticky, easily removed from the mold
F2	Orange, smooth, little less sticky, easily removed from the mold
F3	Orange, smooth, sticky
F4	Orange, smooth, little sticky

Table No: 11 Physicochemical Characterization of Medicated Lollipops

Batch no.	Weight Variation(gm)±SD	Hardness(kg/cm^2) ±SD	Thickness (mm)±SD	Drug content (%) ±SD
F0	5.07±0.02	9.30±0.06	10.01±0.08	99.68±0.06
F1	5.13±0.04	9.56±0.07	10.11±0.08	100.24±0.05
F2	5.18±0.09	9.84±0.09	10.21±0.05	101.56±0.12
F3	5.16±0.06	10.05±0.03	10.56±0.09	100.24±0.10
F4	5.22±0.08	10.11±0.06	10.50±0.02	99.36±0.08

***** Each value is an average of three determinations

***IN-VITRO* DISSOLUTION STUDY**

Table No:12 *In-vitro* dissolution of F0 in Phosphate buffer of pH 6.8

Time	%Cumulative drug released*	% Drug remain unreleased	Log % drug released
0	00	100	00
2	29.08929±0.26	70.91071	1.46373
4	55.35757±0.78	44.64243	1.74317
6	99.22143±0.56	11.77857	1.99660

***** Each value is an average of three determinations

Table No:13 *In-vitro* dissolution of F1 in Phosphate buffer of pH 6.8

Time	%Cumulative drug released*	% Drug remain unreleased	Log % drug released
0	00	100	00
2	25.857±0.78	74.14286	1.41258
4	53.473±0.89	46.52679	1.72813
6	70.755±0.53	29.24464	1.84975
8	82.485±0.45	17.51429	1.91637
10	90.953±0.76	9.04643	1.95881
12	99.080±0.34	0.91964	1.9959

***** Each value is an average of three determinations

Table No: 14 *In-vitro* dissolution of F2 in Phosphate buffer of pH 6.8

Time (Min)	%Cumulative drug released*	% drug remain unreleased	Log % drug released
0	00	100	00
2	24.241±0.89	75.75893	1.38455
4	51.848±0.56	48.15179	1.71473
6	66.544±67	33.45536	1.82311
8	78.260±0.43	21.73929	1.89354
10	86.082±0.53	13.91786	1.93491
12	92.266±0.87	7.73393	1.96504
14	98.764±0.88	1.23571	1.99459

***** Each value is an average of three determinations

Table No: 15 *In-vitro* dissolution of F3 in Phosphate buffer of pH 6.8

Time (Min)	%Cumulative drug released*	% drug remain unreleased	Log % drug released
0	0	100	0
5	41.371±0.81	58.6283	1.616758
10	71.658±0.65	28.34107	1.855270
15	83.783±0.56	16.21607	83.78393
20	99.687±0.23	0.3125	0.3125

***** Each value is an average of three determinations

Table No: 16 *In-vitro* dissolution of F4 in Phosphate buffer of pH 6.8

Time (Min)	%Cumulative drug released*	% Drug remain unreleased	Log % drug released
0	00	100	00
5	37.169±0.89	62.83036	1.5701
10	67.433±0.74	32.56607	1.82887
15	76.326±0.45	23.67321	1.88267
20	89.303±0.67	10.69643	1.95086
25	98.748±0.11	1.25179	1.99452

***** Each value is an average of three determinations

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