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Formulation of topical oral gel for the treatment of oral sub mucous fibrosis (OSMF)

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ABSTRACT

In the present work oral mucoadhesive semi solid jels were prepared for the treatment of oral sub mucous fibrosis, which provide effect for extended periods of time. Stress was given for improvised local action of the drug with the addition of mucoadhesive polymer in the formulation. Curcumin was taken as a model drug as it exhibits profound antitumeric & antimutogenic activity. The semisolid preparations were comprising of stabilizer like sodium metabisulphite, muco retention / mucoadhesive polymer HEC and were subjected for various pysicochemical parameters like pH, spreadability, drug content uniformity, extrudability, viscosity & I.R. studies. In-vitro drug release studies were carried out in phosphate buffer (6.4 pH). Stability studies were also done at room temperature for a period of eight weeks. The formulation containing HEC as base showed good in-vitro release and good adhesion to oral mucosae. IR studies showed that there was no drug-excipient interaction. The in-vivo studies were carried out in two phases using 18 mice with the permission of ethical committee under the supervision and help of staff, Department of Pathology, M.R. Medical College, Gulbarga. In first phase oral sub mucous fibrosis was induced in mice using marketed Gutkha preparation and formulating into a mucoadhesive gel form and applying to mice oral mucosa with the help of cotton bud for a period of 6 months. In second phase, treatment was carried out following the above method using curcumin formulation. The tissue samples collected for 1, 3 & 6 months induction period & 1, 3 & 6 months of treatment period on 6 months oral sub mucous fibrosis induced mice. Histopathological observations reported that there was considerable induction of oral sub mucous fibrosis and excellent treatment results on curcumin usage. The results of the present study of mucoadhesive semi-solid drug design for the treatment of oral sub mucous fibrosis will be useful for drug industry for the benefit of patients suffering from oral sub mucous fibrosis.

Key Words: Mucoadhesive, semi solid preparations, curcumin, oral sub mucous fibrosis (OSMF).

INTRODUCTION

Oral submucous fibrosis is a condition reported mainly from India and is seen in 33% to 40% of patients with oral cancer. In early stages vesicles or fibrous bands are present on the labial mucosa associated with pigment changes. In later stages, the mucosa become stiff, causing difficulty in opening the mouth. Histologically, the mucosa varies from atrophic to normal. A characteristic feature is a prominent sub epithelial eosinophilic band. The juxtaepithelial connective tissue is amorphous and non bundular as against the normal undulated bundular collagen [1,2,3]. The main cause for Oral submucous fibrosis are chewables like gutkha, tobacco, pan masalas, areca nut [4]. A thorough literature survey has been carried out on the proposed topic and most prominent references found are Hastak K, Lubri N, Jakhi SD, More C, John A, Bhaisa SD, Bhide SV, (1997), studied the effect of turmeric oil and turmeric oleoresin on cytogenetic damage in patients suffering from OSMF. In vitro studies on the effect of alcoholic extracts of turmeric, turmeric oil and turmeric oleoresin, on the incidence of micronuclei in lymphocytes from normal healthy subjects showed that the test compounds did not cause any increase in the number of micronuclei as compared with those found in untreated controls [5]. Katharia S K. Singh S P. K Kulshretra V K studied the effect of placenta extract in management of Oral submucous fibrosis and stated that there was significant improvement in mouth opening, colour of oral mucosa and reduction of fibrous bands [6]. Krishna Prasad N S. Sarasija Suresh developed a simple and easy method of estimation of curcumin based on the solubility of curcumin in methanol [7]. Sarasija Suresh. Shobha Rani R Hiremath. Praveen S. Aney Thomas prepared and characterized curcumin gels using a bioadhesive polymer like pluronic F-127 for local application as topical therapeutic system [8]. Dr.Paranjothy K L K, Dr. Than1pi formulated zinc sulphate gel using a Sodium Carboxy Methyl Guar as oral gel for mouth ulcers [9]. Pandey S. Pai M. Singh U V, Udupa N prepared buccal mucoadhesive films and mucoadhesive gels of captopril using Hydroxy propyl methyl cellulose, ethyl cellulose and carbopol. The drug release pattern was higher with formulations containing carbopol [10], Uma Devis. Gancsh M, Mohanta G P & Manavalan R designed and evaluated tetracycline hydro chloride gels. The tetracycline gels formulated with hydroxy propyl methyl cellulose and carbopol showed increase in drug release with increase in polymer concentration [11].

There is no effective treatment for OSMF and there is a need for drug research for its treatment in any type of dosage form. In the present study an attempt has been made to develop mucoadhesive semisolid preparations of Curcumin for oral application directly on to the inflamed site to produce local action, using mucoadhesive Hydrophilic polymer like Sodium carboxy methyl cellulose (HEC) [12].

MATERIAL AND METHODS

Curcumin was procured from Sami Labs, Bangalore, Glycerin from Ranbaxi Lab Ltd. Chandigarh, Hydroxy ethyl cellulose was purchased from S. D. Fine Chem. Ltd., Mumbai, Sodium meta bisulphite and methanol from Qualigens Mumbai.

Preparation of formulation

The semisolid formulation was prepared using Hydroxy ethyl cellulose, as base. Prehydrated polymer samples for 12 hours were dissolved in 85 ml of distill water on constant stirring for about one hour. Then added 15 ml of ethanolic curcumin solution. On continued stirring, glycerine, sodium meta bisulphite were dissolved in the above Polymer-Drug solution.

Table-1: Formulae used to prepare mucoadhesive gels

Sl. No.	Ingredients	Qty
1.	Curucumin	1.0 gm
2.	HEC	4.0 gms
3.	Glycerine	2.0 gms
4.	Sodium meta bisulphate	0.5 gms
5.	Ethanol	15 ml.
6.	Distilled water q.s. (ml)	100 gms

Evaluation of physiochemical parameters [13, 14]

The prepared formulation were subjected for various physicochemical parameters such as spreadability, extrudability, pH, viscosity, Mucoadhesive, drug content estimation.

Spreadability:

Spreadability was determined by an apparatus suggested by Muttimer et al., which was suitability modified in the laboratory and used for the study. It consisted of a wooden block which was provided by a pulley at one end. A rectangular ground glass plate was fixed on this block. An excess of ointment (about 3 gm) under study was placed on this ground plate. The ointment was then sandwiched between this plate and another glass plate having the dimensions of the fixed ground plate and provided with the hook. A 1 Kg. weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the ointment between the plates. Excess of the ointment was scrapped off from the edges. The top plate was then subjected to a pull of 50 gms, with the help of a string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10 cm is noted. A shorter interval indicates better spreadability. (Table-2)

The spreadability can be calculated using the formula:

$$S = \frac{m \times l}{t}$$

Where S = Spreadability
m = weight tied to the upper slid
l = length of the glass slid
t = time.

Extrudability:

The formulation under study was filled in a clean, lacquered aluminum collapsible one-ounce tube with a nasal tip of 5 mm opening, extrudability was then determined by measuring the amount of ointment, cream and gels extruded through the tip when a constant load of 1 Kg. was placed on the pan were collected and weighed. The percentage of ointment, cream and gel extruded was calculated: recorded and grades were allotted (+++ Good; ++ Fair; + Poor). (Table-2)

Determination of Viscosity:

All the products formulated in the semi-solid form were subjected to viscosity studies. Instrument used to measure viscosity is Brookfield digital viscometer. (Table-2)

Determination of pH:
Weigh accurately 5±0.1 gm. of the cream in a 100 ml beaker, add 45 ml. of water and disperse the cream in it. Determine the pH of the suspension at 27°C using the pH meter (Table-2).

Determination of Drug Content Uniformity:
Drug content uniformity was carried out by taking 5 gm sample of prepared formulation and subjected for analytical assay to calculate the drug present in the sample using UV spectrophotometer at λ_{max} 430 nm (Table-2). The drug content was uniform in all formulations.

Mucoadhesive Studies:
The glass plates are coated with the polymer and suspended from a microbalance. The glass plate is immersed in a temperature controlled mucous solution. The force required to pull the plate out of solution is determined under constant experimental conditions. A number of methods use liquid adhesive mass for evaluation. Duration of mucosal adhesion i.e., the time span required until the adhesive patch completely looses its adhesive contact with the mucosa was measured, HEC and hydroxy propyl cellulose possess superior mucosal adhesion in human subjects and time span value are 30 and 15 minutes respectively. The results are given in the (Table-2).

Drug polymer interaction studies:
The studies were carried out using IR method with the help of Perkin-elmer 1615 spectrophotometer to check the possible drug polymer interaction.

Evaluation of Drug Release:
Release of the curcumin from various semisolid preparation was studied by using the permeation apparatus as directed by Fitter et al. A glass cylinder with both the ends open, 10 cm in height, 3.7 cm. in outer diameter and 3.1 cm. in inner diameter, was used as a permeation cell. A cellophane membrane soaked in distilled water (24 hr. before use) was fixed to the end of the cylinder with aid of an adhesive to result the permeation cell. Five gram of medicament was kept in permeation cell. A beaker of (150 ml.) containing 100ml. of 6.4 pH buffer solution as receptor compartment. The sample was immersed to a depth of below the surface of medium in the receptor compartment. The medium in the compartment was agitated using a magnetic stirrer at the temperature 37 °C ± 1 °C. Samples were withdrawn (10 ml) at the interval of 10 min and assayed at 430 nm. The volume withdrawn each time was replaced by equal amount of medium each time.

The data obtained from Tables- 3, 4, 5 & 6 was graphed as follows:

1. Percent cumulative drug release versus time. (Figure-3)
2. Log percent cumulative drug released versus time. (Figure-4)
3. Percent cumulative drug release versus log time. (Figure-5)
4. Log percent cumulative drug released versus time. (Figure-6)

In-vivo Studies:
The in-vivo studies were carried out in mice with the permission of ethical committee under the supervision and help of staff, Department of Pathology, M. R. Medical College, Gulbarga.

The in-vivo studies were carried out in two phases using 18 mice.

- ❖ Induction of OSMF in animals for a period of six months.
- ❖ Treatment of OSMF on the induced animals.

Induction of OSMF:

18 (eighteen) Swiss male albino mice weighing 25 - 30 gms were selected for the experimental design. In the present work OSMF was induced with the causative ingredients of marketed brands of gutkhas. The gutkha powder was pulverized with the help of mortar and pessel and passed through Seive No. 200. Mucoadhesive gel formulations containing 1% gutkha powder prepared in the laboratory, were applied with the help of cotton bud on to the buccal mucosa of the animals for a period of 6 months. During the induction period the animals were without water and food for a period of 6 hours and other times with regular food and water. To study the effect of induction, a punch biopsy technique was used, by sacrificing the animals using skin punch biopsy forceps (No. 5). The biopsy samples of buccal mucosa of 3 animals, collected in normal saline vials were subjected for histopathological slide preparation and study of observation. The similar procedure was followed to check the induction after 3 months and 6 months. A biopsy sample of buccal mucosa of 3 healthy animals, were collected and set aside for comparative purpose.

Ingredients of Gutkha:

Betelnuts, Catechu, Lime, Cardamom, Menthol, Tobacco, Natural perfumes, Sandal oil, species & flavours.

Formula used to prepare muco adhesive semi solid preparation gutkha of marketed product

Ingredients	Quantity
Gutkha (Seive No. 200)	1.0 gms.
Polymer (HEC)	4 gms
Glycerine	2.0 gms.
Sodium Meta bisulphite	0.5 gms.
Distilled water q.s. (ml.)	100 gms.

Procedure details

85 ml of distilled water was taken in 250 ml glass beaker. Then add the polymer, glycerine and the preservative (Sodium metabisulphite) and mix with a glass rod. Cover the beaker with a glass plate and keep aside for 24 hrs. for hydration of the polymer. Then add gutkha powder to 15 ml of water. This solution was added slowly to the hydrated base and mixed using a Rem stirrer at 100 rpm.

Treatment:

After six (6) months of induction study. The remaining nine animals were tested for the purpose of treatment of OSMF, 1% curcumin muco adhesive gel prepared in the laboratory was used. The curcumin gel was applied on to the buccal mucosa of mice with the help of cotton bud and the procedure followed for application is same as used in induction method. For histopathological observations of treatment, the biopsy samples were collected on 3 animals each after 1 month, 3 months & 6 months. Unlike in induction process, the slides of smears of the biopsy sample were processed for comparative evaluation of treatment.

Stability studies:

The formulations were then packed in the collapsible tube and stored at room temperature for 8 weeks and studied for viz., spreadability, extrudability, pH, drug content, viscosity (Table-7).

RESULTS AND DISCUSSION

The gels were subjected to physical evaluations such as viscosity, extrudability, spreadability, pH, drug content uniformly and results are shown in Table-2.

Table-2: Characterization of prepared formulations

Spreadability (Sec.)	14.11
Extrudability	+++
Viscosity (CPS)	2.6×10^5
Ph	6.7
Drug Content (%)	99.63
Duration of Mucosal Adhesion (min.)	31

During our physico-chemical evaluation studies all the formations were within pH range. Drug content estimation, drug present in formulation was found to be 99.27. The formulation showed good mucoadhesion for 33 minutes. Mucoadhesive curcumin gels were evaluated for drug polymer interaction by infrared spectral studies. On going through the infra red spectra of curcumin we observe broad peak at 3422.05 cm^{-1} is due to O-H structure and peak at 1599.25 cm^{-1} is due to the C=O confirms the drug structure (Figure-1). In the formulation (Curcumin + Hydroxy ethyl cellulose), broad peak at 3428.95 cm^{-1} is due to O-H structure and peak at 1639.40 cm^{-1} is due to C=O structure confirm the undisturbed drug in formulation (Figure-2).

Figure-1: Infrared Spectra of Pure Drug

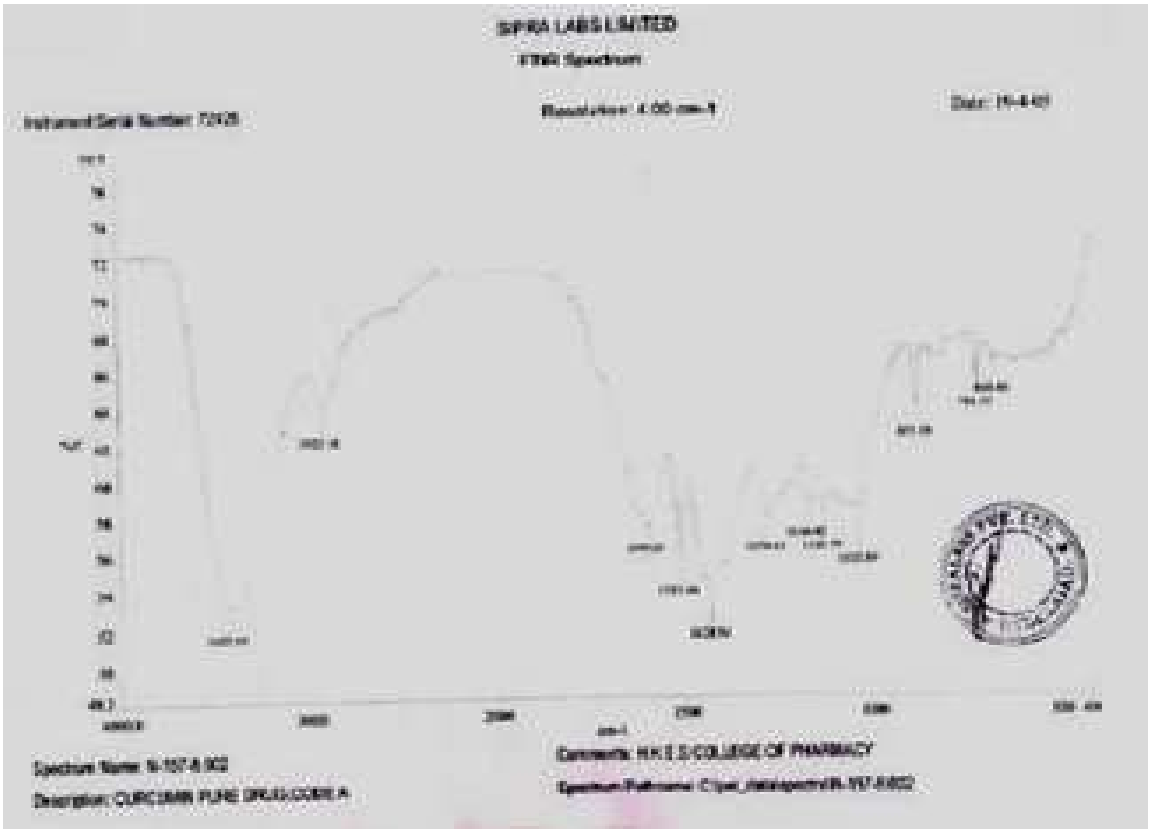
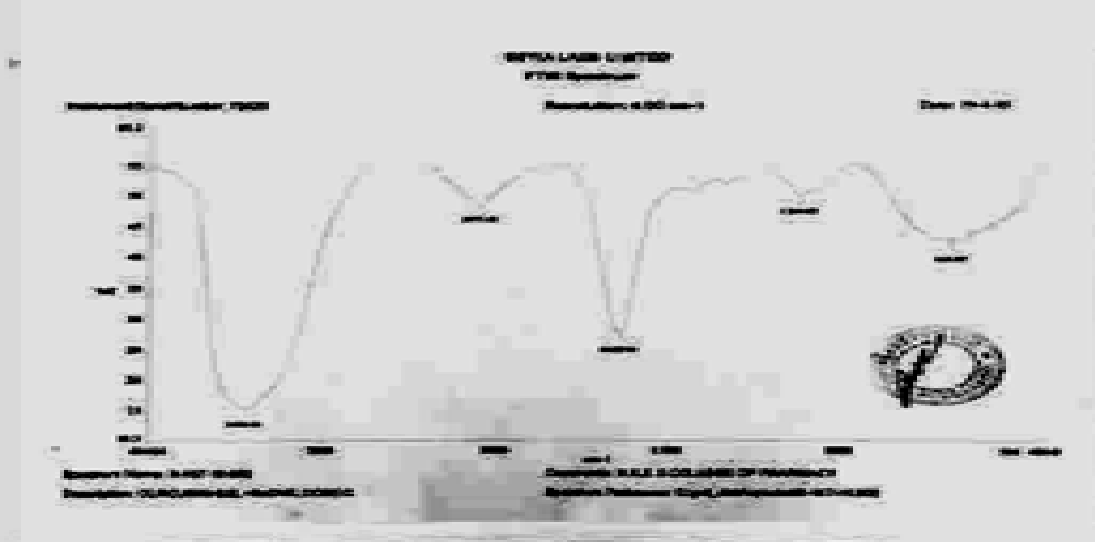


Figure-2: Infrared Spectra of Mucoadhesive Curcumin Anti-Tobacco Semisolid Formulations



After comparing the spectra i.e., absorption bands of pure drug with the spectra of formulations, the absorption bands of the pure drug were retaining in the formulations without undergoing any interaction with the polymers.

In-vitro drug release from curcumin gels was studied and in-vitro data obtained has been shown in graphical presentation in Figure- 3, 4, 5 & 6 using Tables- 3, 4, 5 & 6 respectively. In the formulation at the end of 120 min, percent cumulative drug release was 22.56 %. To know precisely, the rate of drug release, the basic in-vitro data was plotted according to first order release (Figure-3). The result shows that the graph fairly linear. The regression coefficient was found to 0.9616

Table-3: In-vitro release of Curcumin (1%) from Mucoadhesive Semisolid preparations Containing HEC

Sl. No.	Time	Absorbance	Concentration	Cumulative Drug Released	Percentage Cumulative Drug Released
1.	0	0	0	0	0
2.	10	0.228	2.26	0.226±0.030	2.26
3.	20	0.551	5.75	0.587±0.061	5.87
4.	30	0.839	8.95	0.923±0.240	9.23
5.	40	1.105	11.75	1.219±0.041	12.19
6.	50	1.340	14.20	1.478±0.070	14.78
7.	60	1.565	16.60	1.731±0.060	17.31
8.	70	1.672	18.00	1.883±0.065	18.83
9.	80	1.796	19.23	2.013±0.068	20.13
10.	90	1.848	19.73	2.069±0.030	20.69
11.	100	1.884	20.16	2.114±0.020	21.14
12.	110	1.980	21.28	2.228±0.030	22.28
13.	120	2.037	21.50	2.256±0.057	22.56

** Each reading is a replicate of three determinations.; * 5 gm. of semi-solid preparation contains 50mg of curcumin.*

Table-4: First Order Release Plots of Curcumin (1%) from Mucoadhesive Semisolid preparations Containing HEC

Sl. No.	Time	Absorbance	Concentration	Cumulative Drug Released	Percentage Cumulative Drug Released	Percentage Cumulative Drug Remaining	Log Percentage Cumulative Drug Remaining
1.	0	0	0	0	0	0	0
2.	10	0.228	2.26	0.226±0.030	2.26	97.74	1.990
3.	20	0.551	5.75	0.587±0.061	5.87	94.13	1.973
4.	30	0.839	8.95	0.923±0.240	9.23	90.77	1.957
5.	40	1.105	11.75	1.219±0.041	12.19	87.81	1.943
6.	50	1.340	14.20	1.478±0.070	14.78	85.22	1.930
7.	60	1.565	16.60	1.731±0.060	17.31	82.69	1.917
8.	70	1.672	18.00	1.883±0.065	18.83	81.17	1.909
9.	80	1.796	19.23	2.013±0.068	20.13	79.87	1.902
10.	90	1.848	19.73	2.069±0.030	20.69	79.31	1.899
11.	100	1.884	20.16	2.114±0.020	21.14	78.86	1.896
12.	110	1.980	21.28	2.228±0.030	22.28	77.72	1.890
13.	120	2.037	21.50	2.256±0.057	22.56	77.44	1.888

** Each reading is a replicate of three determinations.; * 5 gm. of semi-solid preparation contains 50mg of curcumin.*

Table-5: Diffusion controlled release plots of Curcumin (1%) from Mucoadhesive Semisolid preparation Containing HEC

Sl. No.	Time	Square root of time	Absorbance	Concentration	Cumulative Drug Released	Percentage Cumulative Drug Released
1.	0	0	0	0	0	0
2.	10	3.162	0.228	2.26	0.226±0.030	2.26
3.	20	4.472	0.551	5.75	0.587±0.061	5.87
4.	30	5.477	0.839	8.95	0.923±0.240	9.23
5.	40	6.324	1.105	11.75	1.219±0.041	12.19
6.	50	7.071	1.340	14.20	1.478±0.070	14.78
7.	60	7.745	1.565	16.60	1.731±0.060	17.31
8.	70	8.366	1.672	18.00	1.883±0.065	18.83
9.	80	8.944	1.796	19.23	2.013±0.068	20.13
10.	90	9.486	1.848	19.73	2.069±0.030	20.69
11.	100	10.000	1.884	20.16	2.114±0.020	21.14
12.	110	10.480	1.980	21.28	2.228±0.030	22.28
13.	120	10.954	2.037	21.50	2.256±0.057	22.56

** Each reading is a replicate of three determinations.; * 5 gm. of semi-solid preparation contains 50mg of curcumin.*

Table-6: Plot of Log Cumulative Percent Drug Release versus Log time of Curcumin (1%) from Mucoadhesive Semisolid preparation Containing HEC

Sl. No.	Time	Log time (min)	Absorbance	Concentration	Cumulative Drug Released	Percentage Cumulative Drug Released	Log Percentage Drug Released
1.	0	0	0	0	0	0	0
2.	10	1.000	0.228	2.26	0.226±0.030	2.26	0.354
3.	20	1.301	0.551	5.75	0.587±0.061	5.87	0.768
4.	30	1.477	0.839	8.95	0.923±0.240	9.23	0.965
5.	40	1.602	1.105	11.75	1.219±0.041	12.19	1.086
6.	50	1.698	1.340	14.20	1.478±0.070	14.78	1.169
7.	60	1.778	1.565	16.60	1.731±0.060	17.31	1.238
8.	70	1.845	1.672	18.00	1.883±0.065	18.83	1.274
9.	80	1.903	1.796	19.23	2.013±0.068	20.13	1.303
10.	90	1.954	1.848	19.73	2.069±0.030	20.69	1.315
11.	100	2.000	1.884	20.16	2.114±0.020	21.14	1.325
12.	110	2.041	1.980	21.28	2.228±0.030	22.28	1.347
13.	120	2.074	2.037	21.50	2.256±0.057	22.56	1.353

** Each reading is a replicate of three determinations.* 5 gm. of semi-solid preparation contains 50mg of curcumin.*

Figure-3: Comparative In-vitro drug release of Curcumin semi-solid preparations containing HEC as base

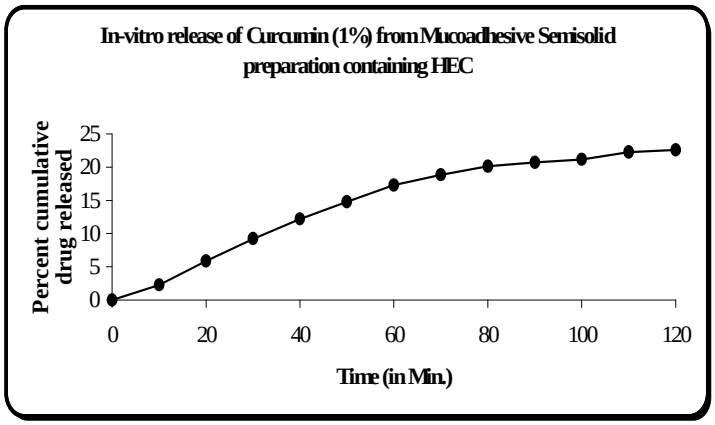


Figure-4: Comparative First order release of Curcumin semi-solid preparations containing HEC as base

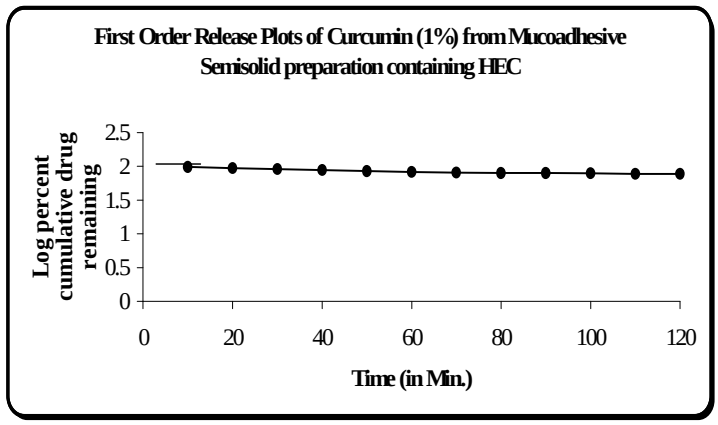


Figure-5: Comparative Diffusion controlled release of Curcumin semi-solid preparations containing HEC as base

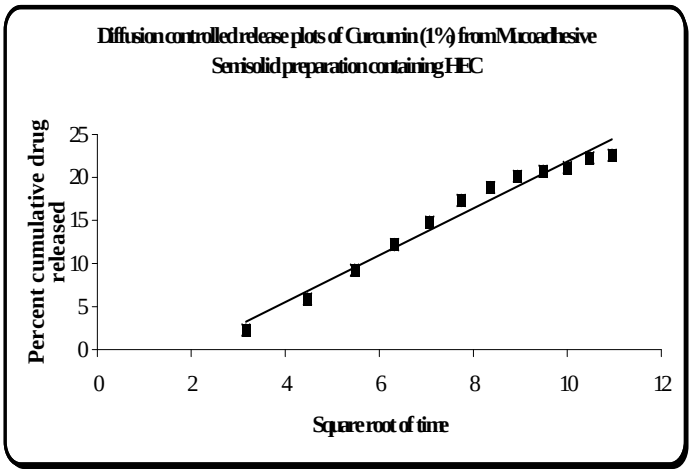
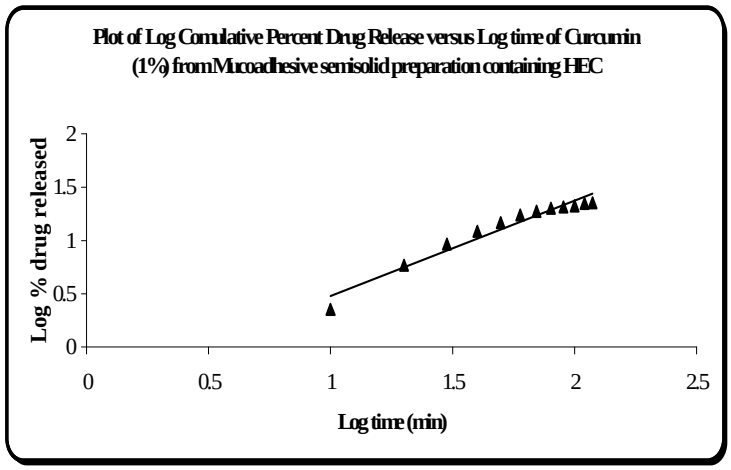


Figure-6: Comparative Plot of Log percent cumulative drug release of Curcumin semi-solid preparations containing HEC as base



In our present investigation of stability studies, all formulations did not segregate, ferment of physically deteriorated during normal condition of storage and use. When stored at room temperature for a period of 8 weeks all the formulation did not undergo phase separation of gassing formulation or otherwise deterioration aesthetically (Table-7)

Table-7: Stability studies data

Storage Temp.	Time of Analysis	Spreadability (Sec.)	Extrudability	pH	Viscosity (CPS)	Drug Content (%)
Room Temperature	1st Week	14.13	+++	6.7	2.6×10^5	99.27
	2nd Week	14.13	+++	6.7	2.6×10^5	99.27
	3rd Week	14.12	+++	6.6	2.5×10^5	99.26
	4th Week	14.12	+++	6.5	2.5×10^5	99.27
	5th Week	14.11	+++	6.4	2.4×10^5	99.26
	6th Week	14.13	+++	6.4	2.4×10^5	99.26
	7th Week	14.11	++	6.4	2.5×10^5	99.27
	8th Week	14.11	++	6.4	2.5×10^5	99.26

The formulation was then planned for in-vivo studies using mice as model animal. The present study tries to focus the array of histomorphological changes in oral mucosa of albino mice after oral application of gutkha and histomorphological changes in already OSMF induced albino mice after oral application of curcumin and to see whether curcumin can heal OSMF [15]. In first phase of histopathological studies of OSMF induction in mice there was gross change of mucosa is observed and increased significance seen the use of gutkha gel from 1 month application to 6 months applications. In second phase of treatment part of OSMF using prepared curcumin semi-solid preparation and encouraging results were observed. There is a marked reduction (more than 50%) of OSMF seen from the histopathological studies on the specimen samples taken after 1 month, 3 month and 6 months.

CONCLUSION

The results of the present study of mucoadhesive semi-solid drug design for the treatment of OSMF will be useful for drug industry for the benefit of patients suffering from OSMF

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