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FORMULATION AND EVALUATION OF SUSTAINED RELEASE AMBROXOL HYDROCHLORIDE MICROSPHERES

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ABSTRACT

Polymethyl mehtacrylates polymers such as Eudragit RL100 have been used in preparation of matrix type oral sustained release, coating and in microencapsulation of drug. Embedding of drug within an insoluble matrix is convenient way of controlling the drug release.The prepared microspheres by emulsion solvent evaporation technique was simple and reproducible, possessed good sphericity, smooth surface morphology, uniform and narrow size distribution (170-200 µm), when analyzed by scanning electron microscopy, and optical microscopy. Method of preparation has influenced the particle size and drug loading efficiency. The carrier ethyl cellulose and Eudragit RL100 are easily available and compatible. Drug-polymer compatibility was confirmed by Fourier transform infrared spectroscopy and DSC and X-ray diffraction studies revealed that the drug was dispersed inside the microspheres in the form of an insoluble matrix. The formation of microspheres was affected by glass transition temperature of the polymer, surfactant, type of plasticizers, volume of internal phase, stirrer speed etc. IR of Eudragit RL indicated high thermal stability of the polymer. Percentage loading increases with the increase in concentration of ethyl cellulose (F5). The resulting microspheres have smooth surface and good physical stability. Micromeritic studies of prepared microspheres showed good flowability (according to Carr's index) and uniformity in size and satisfactory results for compression (according to Hausner's ratio). In vitro studies showed formulation F5 well suited to be sustained release product. From the data obtained it is concluded that drug loaded microspheres appears to be a suitable delivery system for Ambroxol, which may reduce number of doses of drug and frequency of administration.

INTRODUCTION: Due to increase in awareness of medical and pharmaceutical community, about the importance of safe and effective use of drug, much attention has-been paid to develop the controlled drug delivery system. Microencapsulation is method used to obtain product with controlled release properties.

Ambroxol is an active metabolite of bromohexine a mucolytic. It is chemically described as trans-4-[(2-Amino-3, 5-dibromobenzyl) amino]-cyclohexanol. It is an expectoration improver and a mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucous.

It has been successfully used for decades in the form of its hydrochloride as a secretion-releasing expectorant in a variety of respiratory disorders. This drug has a shorter half life 4 hrs that requires frequent daily dosing, but chronic respiratory diseases necessitates its formulation into a sustained release dosage form.

Once or twice daily administration of controlled release preparations is recommended and improves patient compliance. In this study an attempt has been made to develop a sustained release dosage form by formulating ambroxol embedded Eudragit microspheres by solvent evaporation technique. It is more effective than its precursor bromohexine and is non-toxic and well endured by patients. It has fewer side effects.

Mucolytics are class of drug, which are thought to act by decreasing mucous production and decreasing the viscosity of mucous, which is made in the lungs, thereby facilitating its clearance. There are large number of examples carbocysteine, N-acetylcysteine (NAC), S-Carboxymethyl cysteine, Bromhexine, Ambroxol, Sobrerol, Cithiolone, Letosteine, Iodinated glycerol, Nisobutyrylcysteine (NIC) and Myrtol. Mucus consist of primarily water and is thought to have only 5-7% solid material consisting principally of mucins but also containing secreted antimicrobials proteins and peptides, phospholipids and particulate and cellular debris.

Mucin is large glycoproteins, that are expressed in two forms, the mucous gel layer, and membrane tethered mucins present on the epithelial surface that may act as surface receptors.

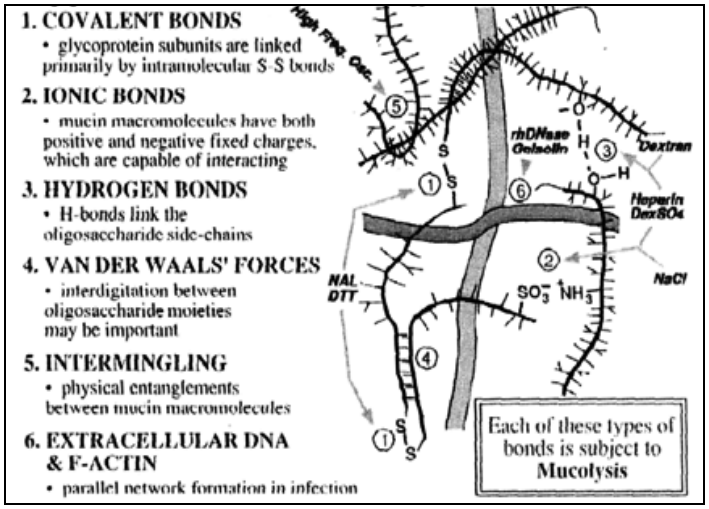


FIG. 1: TYPES OF BOND OCCURRING IN MUCUS GEL

As indicated in diagram structures that form mucins gel is dependent upon a number of forms of bonding (**fig. 1**). The main elements include following;

1) Disulfide bonds – these covalent links join glycoprotein's subunits into extended mucin oligomers.

2) Because of there extended size, these mucin polymers readily form entanglements with neighbouring macromolecules; these act as time dependent cross links, which are susceptible to mechanical degradation.

3) The sugar units that make up the oligosaccharide side chains form hydrogen bonds with complimentary units on neighbouring mucins.

4) Much stranger bonds due to vanderwaal's attractive forces can potentially occur between complementary saccharide moieties on neighbouring of chains. However the diversity of mucin oligosaccharides may make such strong interactions uncommon.

5) Mucin are also ionized, containing both positively charged amino acid residues as well as negatively charged sugar units, principally sailic acid and sulphated residues. These increase in airway disease in general; in CF the proportion of sulphated residues is particularly elevated.

6) There are extra networks of high molecular weight DNA and actin filaments released by dying leukocytes, exopolysaccharides secreted bacterial and glycosaminoglycans such as chrondroitin sulphate during infection and inflammation.

Eudragit is Polymethyl mehtacrylates are synthetic cationic or anionic polymer of dimethyl aminoethyl methacrylates, methacrylic acid and methacrylic acid ester. Eudragit RL, RS, E300. Eudragit RL is hydrophobic slightly cationic polymer. It has been reported that quaternary ammonium groups present in eudragit RL are more than Eudragit RS. These groups present as chlorides are completely dissociated in physiological pH range 1-8. These groups are cationic in nature and therefore are expected to interact with anionic species in solution. Films obtained by this polymer swell in water buffer solution and digestive juices and are

readily permeable to these liquids, compared to other water insoluble polymer.

The method of preparation of Ambroxol microspheres by emulsion solvent evaporation was found to be simple and reproducible. The carrier ethyl cellulose and Eudragit RL100 are easily available and compatible.

MATERIALS AND METHODS:

Reagents and Chemicals: Ambroxol (Jackson labs Pvt Ltd. Punjab), Eudragit RL100 (Rohm Pharma) Germany, Ethyl cellulose LR, (Loba Chemie Pvt Ltd) Mumbai, Dichloromethane, Polyvinyl alcohol, Methanol.

Preparation of Microspheres by o/w Solvent Evaporation Technique [10, 11, 12]: Microspheres were prepared by o/w emulsion solvent evaporation technique (**fig. 2**). Blend of two polymers (Eudragit RL100 and Ethyl cellulose) were weighed and dissolved in 10ml of Dichloromethane (oil phase). Drug was dispersed in it. The oil phase containing polymer and drug to be incorporated was dispersed into continuous aqueous phase (1% PVA solution). Stirring was continued at slow speed for three hours to evaporate the organic phase. Microspheres were filtered, washed with water collected and dried.

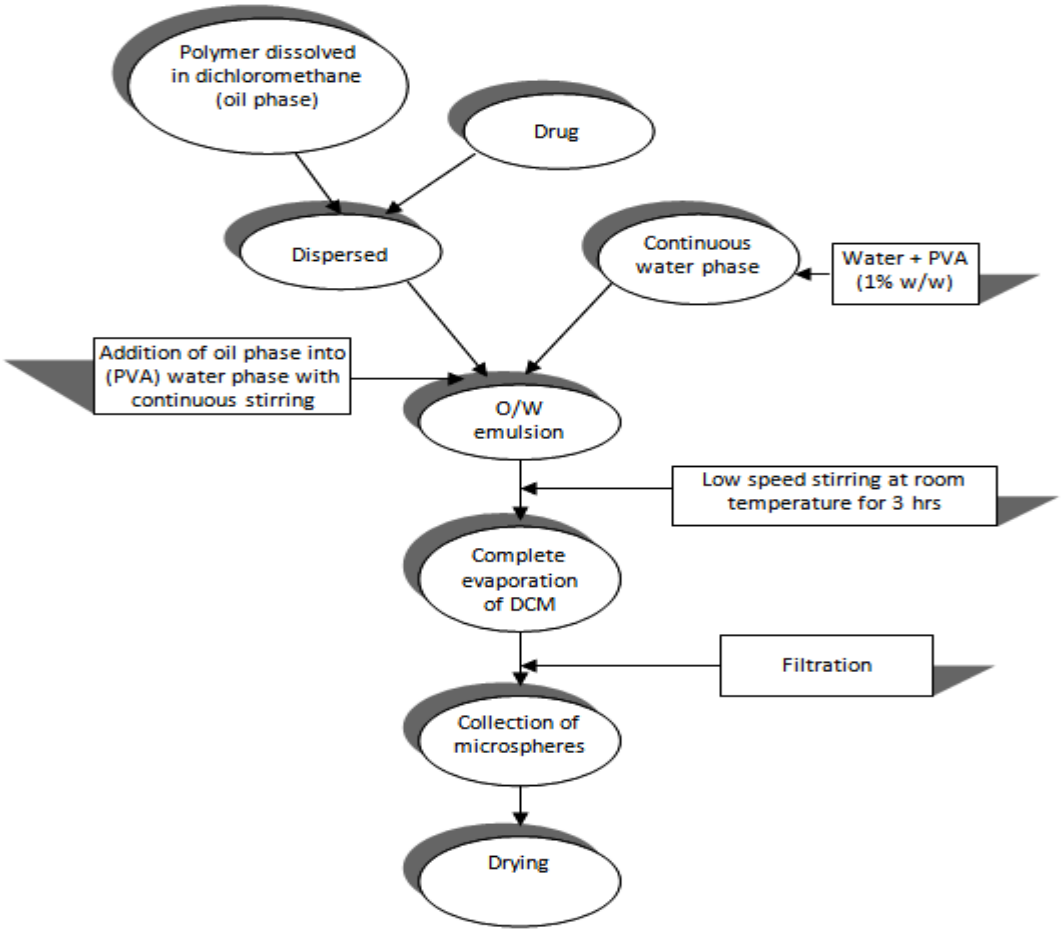


FIG. 2: FLOW CHART OF METHOD USED FOR THE PREPARATION OF MICROSPHERES

TABLE 1: FORMULATION CODE AND RATIOS OF DRUG AND POLYMER

Formulation code	Drug (mg)	Ethyl cellulose (gm)	Eudragit (gm)
F_1	500	1	0.5
F_2	500	1	1
F_3	500	1	2
F_4	500	1	3
F_5	500	2	1

FT-IR spectrophotometer [1, 2]**:** IR spectra of physical mixture drug and pure polymer were obtained with FT/ IR-4100 type a spectrophotometer, using the KBr disk technique.

Differential Scanning Calorimeter (DSC) [3, 4, 5]**:** Thermal analysis of drug, physical mixture and pure polymer were obtained with Perkin Elmer thermal analysis,

USA. A certain amount of ethanolic polymer, drug solution was placed into DSC aluminium pan and then the solvent was evaporated at room temperature. The sample was dried and examined by differential scanning calorimetry at heating rate 10°C / min with open pan system in stream of N_2 gas.

Characterization of Microspheres:

- **Determination of yield of Microspheres [6]:** Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formulae given below.

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

- **Determination of Percentage Drug loading [6]:** 50 mg sampled microspheres were accurately weighed and dissolved in 8ml of DMC, which dissolved polymer only. Then drug was extracted by adding 0.1 M HCl in separator funnel and vortexed for 15 to 20 min upper layer (HCl that contain drug) was separated out and suitable dilution was made and assayed spectrophotometrically at 247 nm, all determinations was done in triplicate.

$$\% \text{ loading} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} \times 100$$

TABLE 2: PERCENTAGE OF YIELD AND DRUG CONTENT

Formulation	% yield	Drug content
F_1	62	80.0
F_2	58.4	72.5
F_3	84.5	65.0
F_4	88.0	85.0.
F_5	75.0	95.0

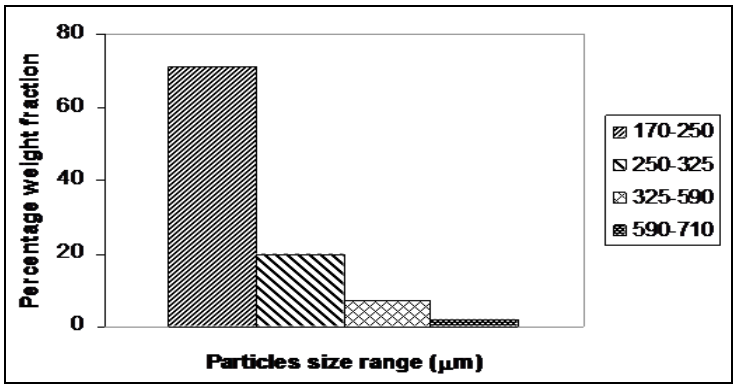
Morphological Characterization:

Particle size determination by Sieve Method: Particle size was expressed by sieve which describes diameter of sphere that passes through the sieve aperture as asymmetric particles.

Scanning Electron Microscope (SEM) [7]: The morphological characteristics of Ambroxol micro-spheres were studies using a scanning electron microscope. The free flowing dried microspheres were sprinkled onto stubs with double face adhesive tape on stubs, coated under a vacuum for 3 min with a thin layer of gold to depth of 50°A and examined under a scanning electron microscope.

TABLE 3: MICROSPHERES SIZE DISTRIBUTION DETERMINE BY SIEVE ANALYSIS FOR FORMULATION F5

Sieve no.	Particle size range (µm)	Amount of spheres retained (mg)	% weight fraction	Cumulative % retained
22/30	710-	30	1.5	1.5
30/44	590	145	7.25	8.75
44/60	590-	300	15.0	23.75
60/80	325	1525	76.25	100
	325-			
	250			
	250-			
	170			



GRAPH 1: PARTICLE SIZE DISTRIBUTION OF AMBROXOL MICROSPHERE BY SIEVE METHOD FOR FORMULATION F5

Micromeritics: Microspheres are characterized by their micromeritic properties like bulk density true density, porosity, Hausner's ratio, consolidation index, angle of repose of microspheres to determine whether sufficient flow ability is present or not.

- **Bulk density [8]:** When the particles are packed loosely, lots of gaps between particles are observed. Hence bulk volume increases making the powder light. Based on bulk volume powder are classified as light, heavy. Light powders have high

bulk volume or low bulk density. Apparent bulk density was determined by pouring a weighed quantity of blend into graduated cylinder and measuring the volume and weight "as it is" mathematically expressed as.

$$\text{Bulk density} = \frac{\text{Mass of microspheres (w)}}{\text{Bulk volume } (V_b)}$$

- **True Density:** True density is defined as ratio of weight of powder and the tapped volume of powder. It was determined by placing a graduated cylinder, containing a known mass of microspheres. The cylinder was allowed to fall under its own weight onto a hard surface from the height of 10cm at 2 second interval. The tapping was continued until no further change in volume was noted.

$$\text{True density } (\rho_p) = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping } (V_p)}$$

- **Porosity**: It is also called as voids, if the powder is non porous i.e., no internal pores or capillary spaces.

Void volume = Bulk Volume – True Volume

$$\text{Porosity or voids} = \frac{\text{Void volume}}{\text{Bulk volume}}$$

$$\text{Percentage porosity void (E)} = \frac{1 - \text{Bulk density}}{\text{True density}}$$

- **Flow Properties [8]:** The flow characteristics are measured by angle of repose, Hausner's ratio, consolidation index. Improper flow of powder due to frictional forces are quantified by angle of repose

- **Angle of Repose:** Angle of repose was determined by using funnel method; the accurately weighed spheres were taken in funnel. The height of funnel was adjusted in such as way that the tip of funnel just touches the apex of heap of blends. The blends were allowed to flow through funnel freely on to surface. The diameter of powder cone was measured; angle of repose was calculated by using following equation.

$$\tan \theta = h/r$$

- **Hausner's ratio [9]:** It is one of method for determining flow properties

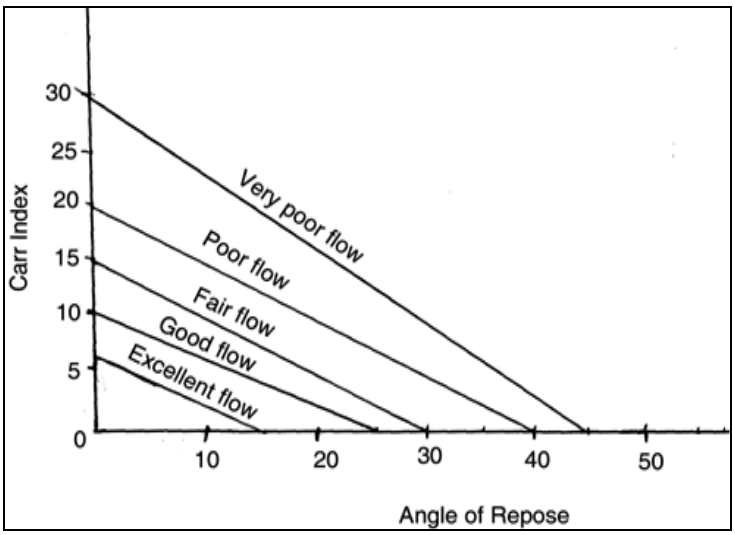
$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density / fluff density}}$$

< 1.25 Good flow, > 1.25 Poor flow

- **Consolidation index % [9]:** It is one of method for determining flow properties and also Carr's index of compressibility;

Percentage of Consolidated index =

$$\frac{\text{True density} - \text{bulk density}}{\text{Bulk density}} \times 100$$



GRAPH 2: ANGLE OF REPOSE VS CARR INDEX

***In-Vitro* Release Profiles:** *In-vitro* release profile of Ambroxol from microsphere were determined by USP dissolution apparatus I (basket type) which contain 900 ml of dissolution medium, the release studies were carried out by using two different bio fluids such as simulated gastric fluid (HCl buffer pH 1.2) and simulated intestinal fluid (phosphate buffer pH 6.8). Microspheres equivalent to 100 mg of drug were filled in hard gelatine capsule and this capsule was placed in dissolution medium maintained at 37±0.5°C.

All dissolution studies were carried out in triplicate for 24 hrs. at 100 rpm and samples were with drawn periodically, same volume of fresh medium was replaced after every sampling. The concentration of drug released at different time interval was then determined by measuring the absorbance using UV spectrophotometer at 247 nm and with help of standard graph.

RESULTS AND DISCUSSION: Microspheres containing Ambroxol as model drug was prepared by emulsion solvent evaporation method by using different polymer ratio (1:0.5, 1:1, 1:2, 1:3, 2:1) (500 mg.). The drug and polymer compatibility studies were determined by IR spectroscopy and differential thermal analysis. IR spectra of Eudragit show peaks in the range between 3100 and 2800 cm^{-1} related to C-H stretching bands, the peak in range between 1350 and 900 cm^{-1} corresponds to C-O stretching vibration mode.

Above discussion shows the absence of cyclic anhydride related IR peaks at 1801, 1759, 1006 cm^{-1} suggesting that no anhydride formation in Eudragit RL. IR spectra of Ambroxol show the peak in range of 3500 – 2500 cm^{-1} due to overlapping between –OH, $–NH_2$, and >NH vibrations. Peak at 3396.99 cm^{-1} is observed due to primary amine and band occurred due to N-H stretching vibration.

Methylene group is present due presence of peak at 2911.02 cm^{-1} occur due C-H stretching from methylene group. Secondary alcohol, alicyclic six membered ring, band occur due to –C–O stretching vibration at 1064.51 cm^{-1}. Peak at 1456 cm^{-1} is observed due to C=C stretching in aromatic molecular peak at 737 cm^{-1} show presence of ortho and Para substituted benzene. IR of ethyl cellulose peak at 1120.4 cm^{-1} and 3471 cm^{-1} peak is observed due to presence of ethoxy group.

Microsphere morphology: The microspheres prepared by o/w emulsification solvent evaporation method (before dissolution) was almost spherical with rough and porous surface, as shown below under different magnification (**fig. 3-6**).

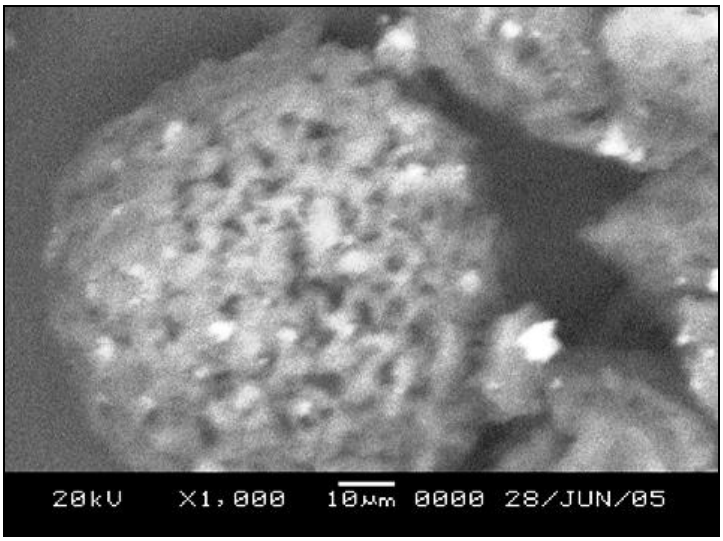
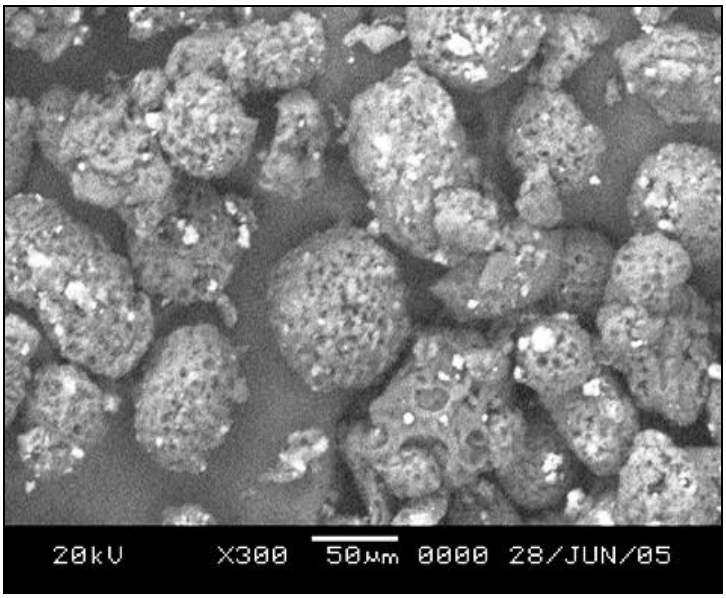


FIG. 3: SCANNING ELECTRON MICROGRAPHS OF AMBROXOL LOADED MICROSPHERES (BEFORE DISSOLUTION)



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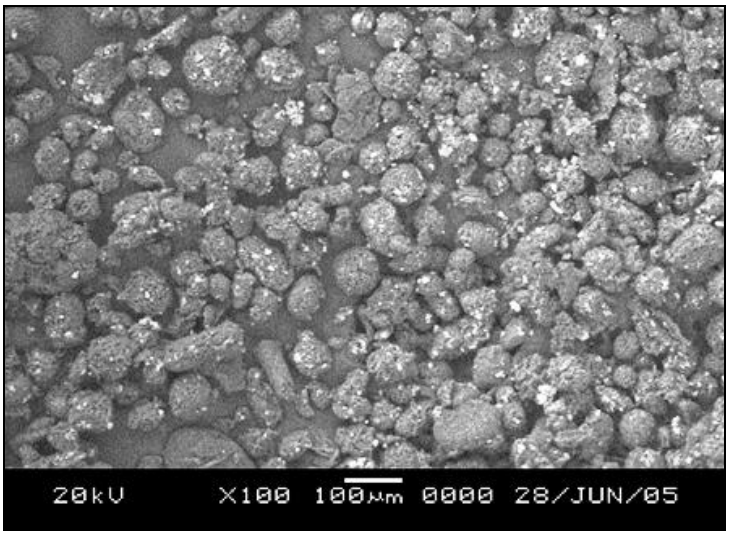


FIG. 5: SCANNING ELECTRON MICROGRAPHS OF AMBROXOL LOADED MICROSPHERES (BEFORE DISSOLUTION)

SEM of microspheres after dissolution showed very rough and eroded surface. Numerous big pores, pits and cracks were also observed.

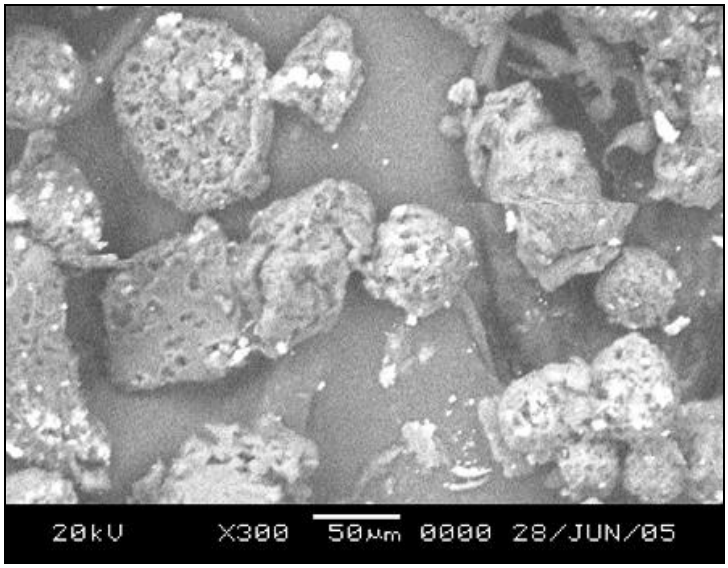


FIG. 6: SCANNING ELECTRON MICROGRAPHS OF AMBROXOL LOADED MICROSPHERES (AFTER DISSOLUTION)

Micromeritic: Micromeritic investigations such as Particle size, bulk density, true density, porosity, angle of repose, Hausner's ratio and consolidation index were carried out on Ambroxol, microspheres in order to standardize the product and to optimize the pilot production of dosage forms.

Particle size distribution of prepared microspheres was found by sieve analysis and resulting data are tabulated in **table 3**. Obtained values for angle of repose, Carr's index, Hausner's ratio, and percentage of compressibility index are shown in **Table 5**. The values for bulk density, true density, and porosity are shown in **Table 4**. The result showed that angle of repose is less than 25°; Hausner's ratio less than 1.25 and consolidation index also lie between 9.3, 12, 16.47, 19.75, and 16.66. Which indicate good flow properties. The flowability of microspheres was found to be excellent according to Carr's index of compressibility and good according to Hausner's ratio.

The results were confirmed by relationship between angle of repose (θ), flow and Carr's index as given in **graph 1 & 2**. Porosity indicates compressibility of prepared microcapsules. The above micromeritic studies show that the prepared spheres were spherical, non aggregated, and uniform in size and also have good compressibility and flowability properties.

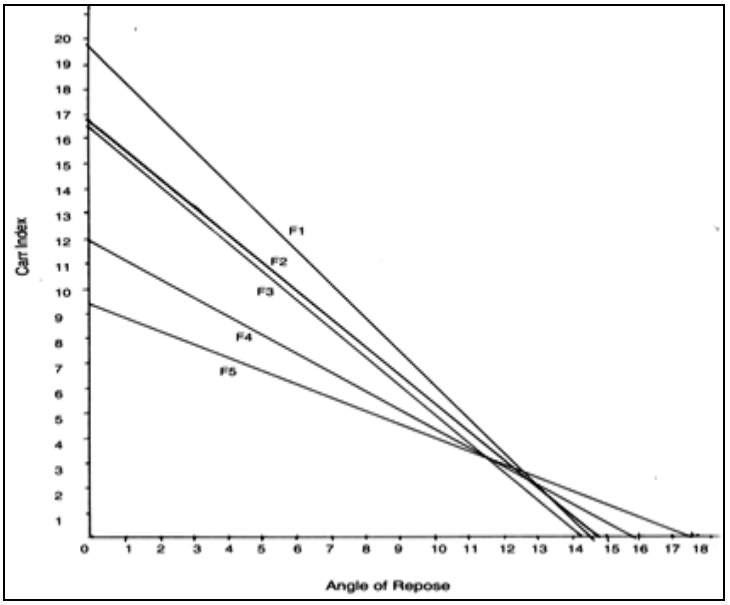
TABLE 4: TRUE DENSITY, BULK DENSITY, PERCENTAGE POROSITY VOID

Formulation	Bulk density	True density	% Void porosity
F_1	0.70	0.84	53.5
F_2	0.65	0.81	51.9
F_3	0.70	0.85	52.9
F_4	0.47	0.50	12.0
F_5	0.78	0.86	45.3

Flow Properties:

TABLE 5: FLOW PROPERTIES

Formulation	Angle of repose (θ°)	Carr's index	Hausner's ratio	% Consolidation index
F_1	14.3	16.6	1.2	16.66
F_2	14.4	19.75	1.24	19.75
F_3	14.4	16.47	1.19	16.47
F_4	15.48	12.0	1.13	12.0
F_5	17.5	9.30	1.10	9.30



GRAPH 2: ANGLE OF REPOSE VS CARR'S INDEX FOR FORMULATION F1, F2, F3, F4 & F5

***In Vitro* Release Profiles (Dissolution Studies):** The *in vitro* studies of Ethyl cellulose and Eudragit microspheres was determined by USP dissolution apparatus containing dissolution medium as biofluids such as simulated gastric fluid (pH 1.2) and simulated intestinal fluid (phosphate buffer 6.8 pH). Among five formulations, the formulation number F1, F2, F3, F4 show decrease in drug content as the amount of loaded drug decreases, with polymer ratio kept constant as shown in Table 2. In F5 as the concentration of ethyl cellulose increased retaining of drug load also increased.

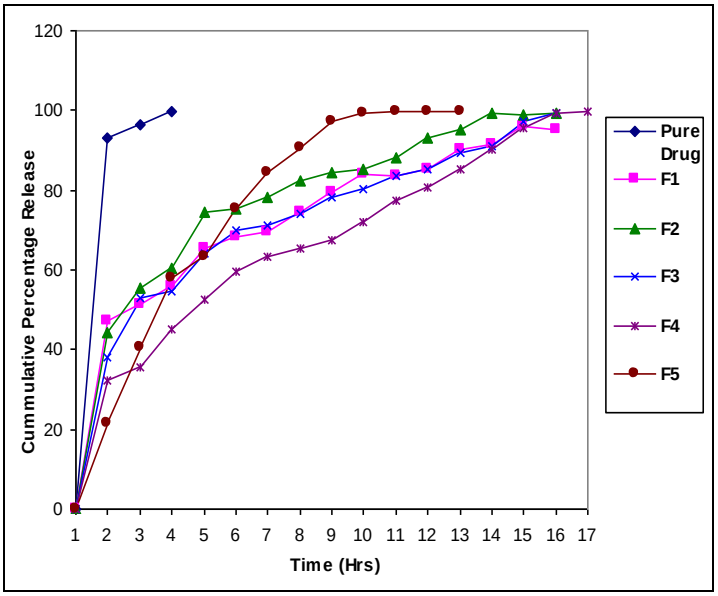
In formulation F1, F2, F3, F4 and F5 release rates are shown in graph .3 .As it can be seen that initial drug release in F1, F2, F3 and F4 are ranging from 47%, 44%, 37% and 30% respectively but in formulation F5 was found to have retained the drug from initial burst release followed by controlled release of about 99% of drug for a period of 16 hours.

Though good correlation was obtained with 16 hours drug release patter with all formulation F1, F2, F3, F4 & F5, the initial burst release was found to be a choosy factor among them. The initial burst release associated with formulation F1, F2, F3 & F4 may be due to surface located drug, which was left over after intense solvent elimination and due to porous nature of microspheres which was formed by more concentration of Eudragit RL100.

As in formulation F5 the concentration of ethyl cellulose was increased, the initial burst effect was decreased. The order of decrease in initial burst release from all formulation was found to be as below

F5 < F4 < F3 < F2 < F1

Alteration of pH of the medium resulted in swelling or de swelling of polymer. At low pH the pores are created as result of penetration of dissolution medium to insoluble membrane of ethyl cellulose. At higher pH due to swelling properties of Eudragit the drug diffuses, through the pores created, into medium. As ratio of ethyl cellulose increased (F5) the initial burst effect was found to be decreased and sustained release (of 99%) was observed for 16 hrs.



GRAPH 3: COMPARISON BETWEEN IN VITRO RELEASE OF AMBROXOL, MICROSPHERES FOR FORMULATION F1, F2, F3, F4 & F5

Order of release ratio was calculated from *in vitro* release profile by plotting log (percentage undissolved drug) Vs time. From the graph 5, slope was calculated between points and rate constant K was calculated.

TABLE 6: RELEASE PATTERN FOLLOWED FIRST ORDER RATE

Formulation	Correlation coefficient	Intercept	Slope	K= - Slope X 2.303	$T_{50\%}$
F1	– 0.982	2.563	– 0.066	0.158 (hr^{-1})	4.40 hrs
F2	– 0.993	2.324	– 0.097	0.224 (hr^{-1})	3.09 hrs
F3	– 0.984	1.903	– 0.071	0.165 (hr^{-1})	4.20 hrs
F4	– 0.995	1.951	– 0.062	0.144 (hr^{-1})	4.80 hrs
F5	– 0.998	2.033	– 0.131	0.303 (hr^{-1})	2.30 hrs
Pure drug	– 0.936	1.912	– 0.012	0.027 (min^{-1})	25.67 mins

GRAPH 4: GRAPH BETWEEN LOG OF PERCENTAGE REMAINING VS TIME (HRS) OF DIFFERENT FORMULATIONS

SUMMARY AND CONCLUSION: The method of preparation of Ambroxol microspheres by emulsion solvent evaporation was found to be simple and reproducible. The carrier ethyl cellulose and Eudragit RL100 are easily available and compatible. Drug and polymer compatibility studies were carried out by IR spectroscopy, the peak in IR spectroscopy and DSC showed no interaction between polymer and drug. IR of Eudragit RL indicated high thermal stability of the polymer. Percentage loading increases with the increase in concentration of ethyl cellulose (Formulation F5).

Micromeritic studies of prepared microspheres showed good flowability (according to cars index) and uniformity in size and satisfactory results for compression (according to Hausner's ratio). Above conclusion was confirmed by the relationship between angle of repose flow and cars index that flowability of microspheres was excellent according to cars index of

compressibility and good according to Hauser ratio as shown in graph 2 & 3. *In vitro* studies showed formulation F5 well suited to be sustained release product. From the data obtained it is concluded that drug loaded microspheres appears to be a suitable delivery system for Ambroxol, which may reduce number of doses of drug and frequency of administration. The work can be extended to in vivo studies.

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