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Research Article

FORMULATION AND EVALUATION OF LEVOFLOXACIN HEMIHYDRATE AND AMBROXOL HCl BILAYER TABLETS

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ABSTRACT

The present work has been done to formulate Levofloxacin hemihydrate immediate release and Ambroxol HCl sustain release bilayer tablets in order to improve patient compliance. All the formulations were prepared by wet granulation method because of poor flow property exhibited by pure drugs. HPMC K4M and HPMC K100M were used for sustaining the release rate of Ambroxol from Ambroxol layer and superdisintegrant like sodium starch glycolate was used for immediate release of levofloxacin from immediate release layer. All the prepared formulations were evaluated for weight variation, hardness, thickness, friability, disintegration and dissolution tests. The release pattern of Ambroxol was fitted to different kinetic models and among all the formulations, F11 of Ambroxol has shown a release rate up to 12 hours. All the formulations could be good expressed by Higuchi equation as the plots shows good linearity, and the correlation coefficient (r^2) for the best formulation F11 was 0.9991 with slope n=0.530, which appears to show a coupling of diffusion and erosion mechanism-so called anomalous diffusion . F2 of levofloxacin disintegrated less than 12min. So, F11 of Ambroxol and F2 of levofloxacin were selected as best formulations. Stability studies were conducted at 25^0 c for long term studies and 45^0 c for accelerated studies for the best formulations.

Keywords: Levofloxacin hemihydrates, Ambroxol HCl, Sustain release, Immediate release, HPMC K4M and K100M, Sodium starch glycolate.

INTRODUCTION

People taking antibiotic alone experience repeated exacerbations and also it takes time to cure. But the patient taking antibiotic along with a mucolytic drug, there is reduction of acute exacerbation and days of illness. But it is quite difficult to take both drugs at a time in two dosage forms by the elderly patients and children, since they feel uneasy to take in two dosages and it is high in terms of cost also. So, both the drugs (antibiotic and mucolytic) are taken in unit dosage form. In that, solid dosage form (especially layered tablet) is the better choice for successful delivery of both drugs in combined form. Also it is cost effective and gives well patient compliance; there is also an advantage in formulating the two drugs as layered tablets as it prevents the formation of incompatibilities between the two drugs. In addition, fixed dose combination of two drugs treat and prevent the symptoms of upper and lower respiratory tract infection. Furthermore, Ambroxol HCl also helps in the penetration power of antibiotic [1].

Levofloxacin is a broad spectrum antibiotic, active against both gram positive and gram negative bacteria. It function by inhibiting DNA gyrase, a type II topoisomerase which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. It has 99% bioavailability and metabolized in renal and excreated through urine [2].

Ambroxol is a muco active drug with secretolytic and secretomotoric action that restore the physiological clearance mechanisms of the respiratory tract. It induces synthesis and release of surfactant by type II pneumocytes. Surfactant acts as an anti glue agent by reducing the adhesion of mucus to the bronchial wall, by which improving the transport and providing protection against infection and irritating agents [3].

MATERIALS AND METHOD

Materials

Levofloxacin and Ambroxol are gift samples obtained from the Madras Pharmaceuticals Ltd(Chennai), HPMC K4M and HPMC K100M (Pioma Chemicals ,Mumbai), Microcrystalline cellulose and Sodium starch glycolate (Vikash Chemicals, Chennai), PVP K30 (Kawarlal & Co., Chennai), Lactose and Dicalcium Phosphate (Cabot Sunmar Pvt Ltd, Naddoor). All the other ingredients were of Analytical grade.

Preparation of Immediate and Sustain release layer

Bilayered tablet of sustained release Ambroxol and immediate release Levofloxacin were prepared through wet granulation method.

Table 1: Formulation of Ambroxol SR layer with HPMC K4M and HPMC K100M

Sr no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
1	Ambroxol Hcl	75	75	75	75	75	75	75	75	75	75	75	75
2	HPMC K4M	22.5	30	37.5	45	-	-	-	-	30	30	32.5	36
3	HPMCK100M	-	-	-	-	30	37.5	45	52.5	30	30	37.5	45
4	MCC 1 17	175	168	160	153	168	160.5	153	145.5	138	136	128	117
5	PVP K30	9	9	9	9	9	9	9	9	9	11	9	9
6	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
7	Talc	6	6	6	6	6	6	6	6	6	6	6	6
8	Aerosil	6	6	6	6	6	6	6	6	6	6	6	6
9	Magnesium Stearate	6	6	6	6	6	6	6	6	6	6	6	6

All units in milligrams

Granulation of Ambroxol:

The sustain release granules were prepared by wet granulation technique. All the ingredients like Ambroxol, HPMC K100M, HPMC K4M, and MCC were passed through sieve#40 and transferred into poly bag and mixed for 5minutes to ensure uniform mixing of the ingredients with the drug. Binder solution was prepared by accurately weighing PVP K30 in Iso propyl alcohol and stirred vigorously to get a clear solution. The Granules were prepared by adding the binder solution to the dry mixed ingredients with constant mixing till to get solid mass to form uniform and optimum granules. Then the obtained granules were dispersed on trays and air is passed for drying. Samples were removed randomly at

different time intervals from the totl bulk of t he granules and then checked out for moisture content. Finally the dried granules were passed through the sieve#20.

Table 2 Formulation of immediate release layer with Levofloxacin

Sr no	Ingredients	F1	F2	F3	F4
1	Levofloxacin hemihydrates	500	500	500	500
2	MCC	89	79	76	83.5
3	Sodium starch glycolate	19.5	32.5	32.5	25
4	PVP K30	22	19	22	22
5	Purified Talc	6.5	6.5	6.5	6.5
6	Aerosil	6.5	6.5	6.5	6.5
7	Magnesium Stearate	6.5	6.5	6.5	6.5
8	IPA	q.s.	q.s.	q.s.	q.s.

All units in milligrams

Granulation of Levofloxacin

The immediate release granules were also prepared by wet granulation technique. Levofloxacin, Sodium starch glycolate were mixed properly and binder solution was prepared as per the formula given in table 2. The immediate release granules were also subjected to similar processing steps as that of sustain releasing granules.

Evaluation of granules flow property

The prepared granules were evaluated for parameters like Bulk density, Tapped density[4, 5], Hausner's ratio, Carrs index, Angle of repose[6]. The results are shown in table 3 and 4.

Lubrication and compression of bilayered tablets:

Magnesium stearate, Aerosil and talc were weighed and they were passed through sieve#20.Then mixed with dried granules of Ambroxol and Levofloxacin separately in a polybag for 5minutes to get a uniform blend. Then the lubricated granules of Ambroxol and Levofloxacin were added in the separate hopper in double rotary punching machine and compressed into bilayered tablets using 18.6 X 9mm caplet shape punches, at weight of 950mg each.

Evaluation of Tablets [7, 8]

Hardness test

Hardness of the prepared tablet was measured using Monsantro hardness tester.

Disintegration Test:

The disintegration for levofloxacin immediate release layer was determined using the disintegration apparatus. One tablet was placed in each of 6 tubes laid in a beaker containing 1000 ml of purified water maintained at 37 ± 2^0 C and the apparatus was operated. The time taken for the tablets to disintegrate and pass through the mesh was noted.

Drug content determination by UV method:

Standard preparation of Ambroxol and levofloxacin:

Accurately weighed 50mg of Ambroxol and 50 mg of levofloxacin were dissolved in 250ml of methanol separately. Further dilutions were made with methanol to prepared standard Ambroxol and levofloxacin preparation. These standard preparations were then analyzed spectrophotometrically.

Drug content uniformity:

Twenty tablets were selected randomly. Then the bilayer was separated. Average weight of Ambroxol layer and average weight of levofloxacin were calculated. Separated layers were crushed in mortar separately. Then accurately weighed amount of tablet triturate of Ambroxol layer equivalent to 75mg and levofloxacin layer equivalent to 500mg were taken and added into the 250 ml volumetric flask separately, add the methanol to dissolve and make up the volume.

Further dilutions were made with again methanol. Then these dilutions were analyzed spectrophotometrically at the λmax of 244nm [9, 10,] for Ambroxol layer and at the λmax of 288 nm [11] for levofloxacin layer.

In vitro Dissolution Studies

The drug release studies were carried out using USP type II dissolution apparatus, with 900ml of dissolution medium maintained at 37±1°C for 12 h, at 100 rpm. 0.1 N HCl (pH 1.2) was used as a dissolution medium for first 2h continued by pH 6.8 phosphate buffer for further 10 h. 5ml of the sample was withdrawn at preset time intervals, substituting with an equal quantity of drug free dissolution fluid. The samples withdrawn were filtered through 0.45µ membrane filter, and the drug content in each sample was analyzed after suitable dilution by UV/Vis Spectrophotometer applying simultaneous estimation method for Ambroxol and levofloxacin, and cumulative percent drug release was calculated. The study was performed in triplicate.

Analysis of drug release kinetics

The drug release mechanism and kinetics were determined by using the fallowing equations. The model that best fits the release data is selected based on the correlation coefficient (r) values in various model. The model that gives the high value of 'r' is selected as the best fit of the release data.

$$Q_t = Q_0 + k_0 t$$

$$\text{Log } Q_t = \text{Log } Q_0 + Kt/2.303$$

Where Q_0 is initial amount of drug, Q_t is Cumulative amount of drug release at time t, t is time in hours, K is First order release constant and K_0 is Zero order release constant.

$$3\sqrt{Q_0} - 3\sqrt{Q_t} = K$$

Where K_{HC} is Hixson crowell release constant

$$Q = K_H t^{1/2}$$

Where K_H is Higuchi constant

$$F = (M_t / M) = K_m t^n$$

Where F is fraction of drug release, M_t is amount of drug release at time t, M is total amount of drug in dosage form, n is Diffusion or release exponent and K_m is Kinetic constant.

Stability studies of the tablets

Stability of a formulation can be defined as the time from date on manufacture of the formulation until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

Stability studies for the present work were carried out at 25^0 C for long term study and in the temperature of 45^0 C for accelerated studies. Samples were withdrawn after 3month and evaluated for their physical parameters and *in-vitro* dissolution behavior. The results were tabulated.

RESULTS AND DISCUSSION

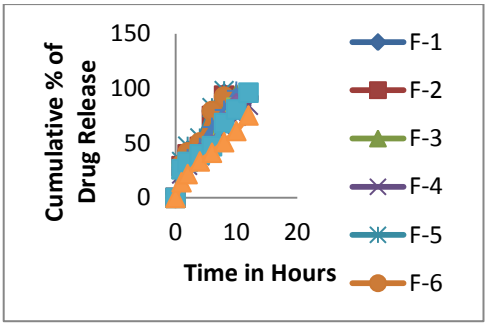

Figure1: Cumulative % Drug Release of Ambroxol SR layer of Formulations F1-F12 Formulated with HPMC K4M & HPMC K100M of different concentration

Table 3: Physico chemical properties of Ambroxol granules

Formulation	Angle of Repose(⁰)	Tapped bulk density	Carr's index(%)	Hausners	Loose bulk density
F1	21.31±0.04	1.154	0.351	13.41	0.304
F2	21.21±0.09	1.157	0.367	13.63	0.317
F3	21.16±0.46	1.161	0.360	13.89	0.310
F4	24.12±0.88	1.188	0.378	15.87	0.318
F5	24.32±0.23	1.176	0.346	15.02	0.294
F6	24.26±0.09	1.172	0.360	14.72	0.307
F7	24.17±0.78	1.183	0.368	15.48	0.311
F8	25.32±0.56	1.177	0.312	15.06	0.265
F9	23.14±0.98	1.177	0.391	15.08	0.332
F10	21.11±0.14	1.157	0.346	13.58	0.299
F11	22.20±0.53	1.174	0.317	14.82	0.270
F12	24.21±0.96	1.191	0.336	16.07	0.282

Density in g/ml

Table 4: Physicochemical properties of levofloxacin granules

Formulation	Angle of Repose(⁰)	Loose bulk density	Tapped bulk density	Carr's index(%)	Hausners
F1	21.34±0.52	0.272	0.3161	3.92	1.161
F2	21.21±0.09	0.289	0.3341	3.47	1.155
F3	21.16±0.46	0.324	0.3761	3.82	1.160
F4	24.12±0.88	0.294	0.3441	4.53	1.170

Table 5: Invitro release kinetics of ambroxol

Formulation	Zero order	First order	Higuchi	Hixogon	Peppas	n
F1	0.94	0.9854	0.9982	0.9541	0.9960	0542
F2	0.949	0.9865	0.9945	0.9786	0.9912	0.651
F3	0.964	0.9831	0.9978	0.9809	0.9960	0.546
F4	0.966	0.9734	0.9965	0.9845	0.9890	0.532
F5	0.93	0.9866	0.9987	0.9612	0.9730	0.543
F6	0.943	0.9712	0.9923	0.9723	0.9890	0.678
F7	0.964	0.9820	0.9998	0.9943	0.9956	0.512
F8	0.965	0.981	0.9968	0.9816	0.9813	0.590
F9	0.931	0.972	0.9976	0.9740	0.9900	0.656
F10	0.935	0.9890	0.9959	0.9963	0.9900	0.675
F11	0.958	0.9887	0.9991	0.9987	0.980	0.530
F12	0.98	0.9769	0.9989	0.9620	0.996	0.510

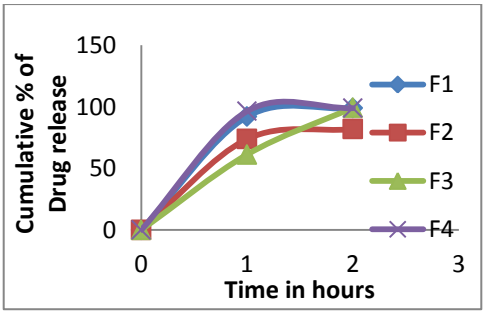

Figure 2: Cumulative % Drug Release of Levofloxacin IR layer of F1-F4 formulated with sodium starch glycolate

Table 6: Stability Data of Optimized batch of Formulation F11 Bilayered tablets of Ambroxol SR and Levofloxacin IR

Sr No	Tests	Initial 0 month		After 3 Months	
1	Description	Complies		Complies	
2	Average Weight	951.2mg		951.2 mg	
3	Average thickness	5.6mm		5.6mm	
4	Average Hardness	6.4 kg/cm^2		6.4kg/cm^2	
5	Friability	0.17%		0.17%	
6	Dissolution Profile	**AMB**	**LEV**	**AMB**	**LEV**
		1hr-26.4	70.2	1hr-26.3	70.8
		2hr-32.9		2hr-33.2	
		4hr-39.2		4hr-41.1	
		6hr-48.1		6hr-49.8	
		8hr-65.3		8hr-65.7	
		10hr-81.8		10hr-83.5	
		12hr-96.1		12hr-96.7	
7		99.4%		98.8%	

Storage Conditions: 24^0±2^{0}C at 60%±5%RH

Table 7: Stability Data of Optimized batch of Formulation F11Bilayered tablets of Ambroxol SR and Levofloxacin IR

Sr No	Tests	Initial 0 month		After 3 Months	
1	Description	Complies		Complies	
2	Average Weight	951.2mg		951.2 mg	
3	Average thickness	5.6mm		5.6mm	
4	Average Hardness	6.4 kg/cm^2		6.4kg/cm^2	
5	Friability	0.17%		0.29%	
6	Dissolution Profile	**AMB**	**LEV**	**AMB**	**LEV**
		1hr-26.4	70.4	1hr-26.3	71.9
		2hr-32.9		2hr-33.2	
		4hr-39.2		4hr-41.1	
		6hr-48.1		6hr-49.8	
		8hr-65.3		8hr-65.7	
		10hr-81.8		10hr-83.5	
		12hr-96.1		12hr-96.7	
7		99.7%		97.6%	

Storage Conditions: 40^0±2^{0}C at 75%±5%RH

DISCUSSION

The results of micromeritic properties are demonstrated in the table 3. Plain Ambroxol and levofloxacin exhibited angle of repose value of 47.15^0 and 46.01^0 respectively indicated that the both drugs contain extremely poor flow property. It was further supported by high Carr's index value. Hence suitable MCC was added for increasing the flow of power. The incorporation of these fillers into plain drugs improved the flow properties as indicated by a reduction in the values of the angle of repose and Carr's index. But still the expected good flow property was not achieved by all the three vehicles selected, even though the MCC properties showed the possible flow property. So, the granulation method (Wet) was tried to achieve the expected flow property by using the filler MCC which gave the possible flow property. From the above tables, it was confirmed that both the drugs were exhibited excellent flow property (AOR= 20 to 24), when the drug was granulated with excipients. It was also supported by the results of Carr's index value.

The results showed that all trial tablets have their weight within 949 to 952 mg/ tablet and except the formulation 1 and 4, all trials have the sufficient hardness i.e., within the limit. All the tablets of different trials were uniform in thickness (5 – 6 mm). According to Friability parameter, the tablets of trials F1 and F4 were beyond the limit, but remaining trials were within the prescribed limits i.e., (<1). Good and Uniform drug content (>98%) was observed within the batches of different tablet formulations.

The availability of literature on solubility profile of levofloxacin and Ambroxol indicates that the drugs were freely soluble in methanol and water. And also it tells that both drugs solubility increases by increasing the pH nearly alkaline. This was confirmed by observing the solubility studies of Ambroxol hydrochloride and Levofloxacin hemi hydrate practically. However, pH 6.8 phosphate buffer may be suitable for dissolution studies as sufficient solubility was attained at this pH for both the drugs. But on the other hand, the present formulation contains sustained release layer and immediate release layer too; it would be more meaningful to use both acidic and alkaline media for dissolution studies.

From the table, it was confirmed that the Trials of F1- F8 of SR layer does not fulfill the sustained release theory, in that the HPMC grades K4M and K100M were used separately in the formulations, but increasing the polymer concentration, it was clearly identified that the drug release was retarded, and also from the figure 1, it was also confirmed that the formulation made with HPMC K4M (F1 to F4) releases the drug in less amount compared to the formulation made with HPMC K100M (F5 to F8), since its viscosity is somewhat higher than HPMC K100M.

In order to produce an optimized formulation, both the grades were used together in formulations of remaining trials. Yet the Trail F9 of Ambroxol SR layer made of both HPMC K4M and HPMC K100M was not attained the sustained release. The trial F10 was made with the same concentration as that of F9 with a little altering in binder concentration. But there was no major change in drug release and also the hardness of the tablet goes beyond the limit.

The next trial F11 of Ambroxol SR layer made of HPMC K4M (11%) and HPMC K100M (12.5%) sustained the drug release upto 12th hour. When the concentration of HPMC K4M and HPMC K100M was again increased for the trial F12, the drug from the tablet was very hard to release. So trial F11 made of 32.5mg of HPMC K4m and 37.5mg of HPMC K100m was considered as optimized formulation for SR layer.

All the formulations were fitted into different kinetic models to find out the mechanism of drug release. All formulations followed Higuchi model, which followed korsmeyer-Peppas model. Then value for all the formulations was fallen between 0.45-0.89, which indicates that the drug is diffused from the polymer matrix and the

release rate is independent of drug concentration. The mechanism behind the release of drug from the polymer is basically because of hydration, followed by dissolving of drug and swelling of the polymer which induces to diffuse drug out of the polymer.

Then for the tablet of IR layer, 4 trials were made. In those trials, the formulation (Trial F4 of Levofloxacin layer) made with sodium starch glycolate of 25mg released the drug within one hour and also has the sufficient hardness and friability than other formulations. So the formula made for Trial F4 of Levofloxacin layer was considered as optimized formulation and that formula was used for the remaining trials (F8 to F12) of Ambroxol SR layer to produce the Bilayered tablet.

Table 6,7 shows the stability of F11 tablet in two different storage conditions. Storing the tablet at $24^0 \pm 2^0$c at 60%±5 %RH did not change the physicochemical characterietics much upto 3 months (Table 6). But storing the tablet at $40^0 \pm 2^0$c at 75%±5%RH, there was some changes found in drug release characteristics and drug content (Table 7). Moreover the color of the tablet was change from pink to pale pink color at 3 months storage period.

CONCLUSION

The Bilayered tablets containing Ambroxol hydrochloride SR and Levofloxacin IR were successfully prepared by wet granulation method. The prepared granules were also maintained the physiochemical properties of tablets such as thickness, hardness, weight variation, friability and drug content. The optimized formulation contains the average thickness of 5.4±0.042, average hardness of 6.4±0.14, average weight of 950±0.56, friability of 0.021 and 99.72% of drug content. The stability studies confirmed that there was no significant difference over a stability testing period.

Hence it may be summarized that the trial F11 tablets prepared by wet granulation method might be a perfect and effective formulation to treat the respiratory tract diseases especially for sinusitis and bronchitis.

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