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Behavior of uptake of moisture by drugs and excipients under accelerated conditions of temperature and humidity in the absence and the presence of light. 1. Pure anti-tuberculosis drugs and their combinations

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Abstract

An investigation was carried out to determine the behavior of moisture gain by four anti-tuberculosis drugs, viz. rifampicin, isoniazid, pyrazinamide and ethambutol, when exposed in pure form and in combinations to accelerated conditions of 40 °C and 75% RH, in the absence and the presence of light. Weight gain was seen only in those samples that contained ethambutol, and this behavior was observed both in dark and lighted chambers. There was a decrease in moisture uptake with an increase in the number of drugs in the mixture. Another observation was a higher weight gain by the mixture of ethambutol and isoniazid in a dark chamber, than either pure ethambutol or drug combinations containing ethambutol. The most interesting finding was an overall acceleration of weight gain in the presence of light as compared with dark conditions, which is a hitherto unknown phenomenon.

Keywords: Moisture gain; Storage; Accelerated conditions; Dark; Light; Rifampicin; Isoniazid; Pyrazinamide; Ethambutol; Pure drugs; Combinations

1. Introduction

The problem of reduced bioavailability of rifampicin from fixed dose combination (FDC) products of anti-tuberculosis drugs is a matter of global concern. The deficiency in delivery of proper dose of rifampicin has serious implications as it is known that doses of the drug less than 9 mg/kg body weight can result in therapeutic failure (Long et al., 1979) and hence can be the cause of development of drug resistance.

Looking into this seriousness, an efficient research program is being carried out in our laboratories to look out for the reasons for the

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loss of bioavailability of rifampicin from anti-tuberculosis FDC products containing the drug in combination with isoniazid, pyrazinamide or ethambutol. In our initial studies, we reported that a significant fall of rifampicin could occur in the presence of isoniazid under in situ fasting stomach acidic conditions (Singh et al., 2000a). A mechanism for the same was outlined (Singh et al., 2000b). We also suggested solutions to overcome the problem (Singh et al., 2001). In subsequent studies, our focus was on to explain as to why the problem of reduced bioavailability of rifampicin was particularly shown when studies were conducted on multiple products collected from different parts of the globe (Acocella, 1989; Pillai et al., 1999). For the purpose, FDC tablets procured from market were stored in unpacked condition in humidity and light chambers set at 40 °C ± 1 °C/75% RH ± 3%. A much stronger physical change was shown in photostability chamber in comparison to the same products stored in a dark humidity chamber. The products stored in photostability chamber virtually had a red liquid bleeding out, whereas the tablets in the dark humidity chamber only had red surface spots in the same period (Mohan, 2001).

It indicated that light was perhaps responsible for the accelerated physical change, in all probability, the uptake of moisture. As the influence of light on moisture uptake was a previously unknown phenomenon, it prompted us to confirm the same. Therefore, moisture uptake studies in the absence and the presence of light were carried out on all four anti-tuberculosis drugs, in pure form and combinations that were prepared in a manner that they contained drugs and their ratios similar to those contained in the marketed FDC products. The results are given in this communication.

2. Materials and methods

Rifampicin, isoniazid, pyrazinamide and ethambutol were gift samples from Lupin Laboratories Ltd., Aurangabad, India. Weighing was done on a precision analytical balance (AG 135, Mettler Toledo, Switzerland). The following were the weights of the four drugs, rifampicin: 150 mg, isoniazid: 100 mg, pyrazinamide: 250 mg, and ethambutol: 267 mg. The same proportion was employed when preparing mixtures.

The weighed samples were transferred to 10 ml beakers, which were stored separately in a humidity chamber (KBF720, Binder, Germany) and a photostability chamber (KBF240, Binder, Germany). The latter was equipped with an illumination bank on inside top consisting of a combination of two black-light UV lamps (OSRAM L73) and four white fluorescent lamps (OSRAM L20) in accordance with option 2 of International Conference on Harmonization (ICH) guideline Q1B (ICH, 1996). Both UV and visible lamps were put on simultaneously. The samples were placed at a distance of 9 in. from the light bank. The overall illumination at the point of placement was 5500 l × fluorescent light and 0.5 W/m^2 UV light. Both chambers were set at 40 °C ± 1 °C/75% RH ± 3%. The samples were withdrawn at various intervals and weighed.

3. Results and discussion

The data on the mean percent weight gain by pure rifampicin, isoniazid, pyrazinamide and ethambutol in the absence and the presence of light are given in Tables 1 and 2, respectively. The corresponding values for various drug combinations are given in Tables 3 and 4. In all cases, the data are mean of three observations.

The data in Tables 1 and 2 clearly show that among the four anti-tuberculosis drugs, it is only ethambutol, which shows moisture gain with time. This is further confirmed from the values contained in Tables 3 and 4, which show that moisture uptake again is significant only in those drug combinations that contain ethambutol. This observation was on the expected lines, as ethambutol alone of the four anti-tuberculosis drugs is known to be hygroscopic. The samples containing ethambutol and combination of isoniazid and ethambutol became liquid over the time due to dissolution of the drugs in the gained moisture. All those containing pyrazinamide and/or rifampicin along with ethambutol and isoniazid resulted in a wet

Table 1
Percent weight gain at different time periods for the four pure anti-tuberculosis drugs when stored at 40 °C/75%RH in the absence of light

Drug	Percent weight gain ± S.D. at various time periods (h)											
	1	2	4	6	8	10	12	16	20	24	28	32
R	0.013 ± 0.005	−0.026 ± 0.005	0.010 ± 0.017	0.173 ± 0.015	−0.023 ± 0.03	−0.033 ± 0.015	−0.106 ± 0.02	0.1465 ± 0.015	0.143 ± 0.041	0.153 ± 0.030	0.130 ± 0.017	0.233 ± 0.049
H	−0.019 ± 0.001	0.127 ± 0.001	0.129 ± 0.001	0.167 ± 0.001	0.483 ± 0.002	0.286 ± 0.001	0.116 ± 0.002	0.166 ± 0.001	0.267 ± 0.001	0.265 ± 0.002	0.279 ± 0.001	1.306 ± 0.020
Z	0.006 ± 0.002	−0.131 ± 0.007	−0.015 ± 0.004	0.150 ± 0.003	0.162 ± 0.004	0.050 ± 0.005	0.025 ± 0.003	0.056 ± 0.004	0.088 ± 0.002	0.107 ± 0.003	−3.72 ± 0.172	−3.05 ± 0.066
E	5.89 ± 0.86	7.89 ± 1.36	10.40 ± 1.23	21.30 ± 1.62	27.53 ± 2.43	32.75 ± 3.16	39.70 ± 3.62	49.98 ± 2.78	61.73 ± 2.61	64.92 ± 0.79	69.74 ± 0.21	73.93 ± 0.65

Mean of three readings. R, rifampicin; H, isoniazid; Z, pyrazinamide and E, ethambutol.

Table 2
Percent weight gain at different time periods for the four pure anti-tuberculosis drugs when stored at 40 °C/75% RH in the presence of light

Drug	Percent weight gain ± S.D. at various time periods (h)											
	1	2	4	6	8	10	12	16	20	24	28	32
R	0.036 ± 0.015	−0.006 ± 0.047	−0.035 ± 0.015	0.246 ± 0.023	0.250 ± 0.034	0.236 ± 0.346	0.386 ± 0.335	0.436 ± 0.025	0.363 ± 0.025	0.426 ± 0.02	0.466 ± 0.011	0.566 ± 0.040
H	0.058 ± 0.001	0.048 ± 0.001	0.080 ± 0.001	−0.008 ± 0.001	−0.002 ± 0.010	0.109 ± 0.001	0.724 ± 0.001	0.704 ± 0.009	0.222 ± 0.165	0.049 ± 0.002	0.117 ± 0.002	0.247 ± 0.001
Z	0.129 ± 0.003	−0.22 ± 0.001	−0.234 ± 0.001	0.092 ± 0.003	0.09 ± 0.005	0.067 ± 0.005	0.049 ± 0.007	0.005 ± 0.004	0.074 ± 0.003	0.074 ± 0.004	−0.031 ± 0.014	0.071 ± 0.004
E	6.63 ± 1.58	13.13 ± 1.36	19.90 ± 2.20	40.94 ± 1.32	53.83 ± 0.74	59.75 ± 0.84	63.34 ± 1.97	65.82 ± 1.10	69.29 ± 1.21	72.93 ± 0.57	74.09 ± 0.99	73.30 ± 1.27

Mean of three readings. R, rifampicin; H, isoniazid; Z, pyrazinamide and E, ethambutol.

Table 3
Percent weight gain at different time periods for different combinations of anti-tuberculosis drugs when stored at 40 °C/75% RH in the absence of light

Drug	Percent weight gain ± S.D. at various time periods (h)											
	1	2	4	6	8	10	12	16	20	24	28	32
R+H	−0.090 ± 0.001	0.002 ± 0.001	0.019 ± 0.001	0.037 ± 0.001	0.108 ± 0.001	0.103 ± 0.001	0.183 ± 0.001	0.046 ± 0.001	0.207 ± 0.002	0.247 ± 0.001	0.211 ± 0.001	0.257 ± 0.001
H+E	5.69 ± 0.85	8.58 ± 1.19	13.00 ± 1.33	27.36 ± 1.03	33.81 ± 1.3	39.05 ± 1.36	44.68 ± 1.51	48.86 ± 0.93	55.29 ± 0.33	56.38 ± 0.52	58.64 ± 0.45	59.56 ± 1.41
R+H+Z	0.083 ± 0.001	−0.064 ± 0.001	−0.053 ± 0.001	−0.004 ± 0.001	−0.012 ± 0.001	0.006 ± 0.001	−0.072 ± 0.001	−0.096 ± 0.001	−0.060 ± 0.001	−0.040 ± 0.001	−0.045 ± 0.001	−0.035 ± 0.002
R+H+E	3.10 ± 0.83	5.42 ± 1.33	7.46 ± 1.16	16.16 ± 1.37	20.92 ± 1.12	24.27 ± 1.00	27.94 ± 1.08	30.49 ± 1.02	35.66 ± 0.95	38.45 ± 0.85	40.34 ± 0.70	40.62 ± 0.82
R+H+Z+E	2.16 ± 0.69	4.00 ± 0.92	5.60 ± 0.83	12.10 ± 1.21	15.22 ± 1.46	17.9 ± 1.66	21.71 ± 0.10	23.78 ± 0.58	26.00 ± 1.10	27.64 ± 0.26	29.26 ± 0.28	29.72 ± 0.52

Mean of three readings. R, rifampicin; H, isoniazid; Z, pyrazinamide and E, ethambutol.

Table 4
Percent weight gain at different time periods for different combinations of anti-tuberculosis drugs when stored at 40 °C/75% RH in the presence of light

Drug	Percent weight gain ± S.D. at various time periods (h)											
	1	2	4	6	8	10	12	16	20	24	28	32
R+H	0.098 ± 0.001	−0.07 ± 0.001	0.012 ± 0.001	0.174 ± 0.001	0.146 ± 0.001	0.204 ± 0.001	0.056 ± 0.002	0.193 ± 0.002	0.232 ± 0.001	0.321 ± 0.001	0.344 ± 0.001	0.399 ± 0.001
H+E	3.92 ± 0.32	9.10 ± 1.39	19.40 ± 0.81	41.88 ± 1.30	47.77 ± 0.11	50.46 ± 0.80	53.16 ± 0.38	54.62 ± 0.41	56.19 ± 0.22	59.10 ± 0.85	59.44 ± 1.49	59.75 ± 0.90
R+H+Z	−0.043 ± 0.005	−0.123 ± 0.001	−0.134 ± 0.001	−0.161 ± 0.001	−0.134 ± 0.001	−0.134 ± 0.001	−0.067 ± 0.001	−0.147 ± 0.001	−0.156 ± 0.001	−0.200 ± 0.009	−0.172 ± 0.001	−0.166 ± 0.001
R+H+E	6.48 ± 1.12	9.50 ± 0.25	14.04 ± 1.81	24.99 ± 1.45	28.75 ± 1.16	31.89 ± 0.87	34.29 ± 0.82	36.45 ± 0.88	39.09 ± 0.91	40.24 ± 0.18	40.85 ± 0.14	41.29 ± 0.50
R+H+Z+E	3.23 ± 0.49	5.07 ± 0.50	7.30 ± 0.71	14.88 ± 1.14	19.55 ± 1.78	22.23 ± 1.88	24.70 ± 2.06	26.15 ± 1.62	27.64 ± 0.57	28.54 ± 0.75	29.83 ± 0.49	29.88 ± 0.28

Mean of three readings. R, rifampicin; H, isoniazid; Z, pyrazinamide and E, ethambutol.

mass. The samples devoid of ethambutol did not show any physical change.

An interesting and new behavior was observed on construction of a plot of percent weight gain by pure ethambutol with time in the absence and the presence of light (Fig. 1). The plot shows that ethambutol gains moisture much faster in the presence of light than in dark conditions, till the saturation is reached. The behavior of weight gain, for the data at 8 h, the time when maximum difference occurs in the moisture uptake in the absence and the presence of light (Fig. 1), is shown in Fig. 2a. It is clearly evident that moisture gain occurs more profoundly under light conditions, than samples stored in the dark in a humidity chamber. Another key observation is that while total moisture uptake in the presence of light decreases with an increase in number of drugs in the combination, however, the pattern is evidently not the same in the humidity chamber in the dark conditions. The combination of isoniazid and ethambutol shows more moisture gain at 8 h than even pure ethambutol or combinations containing three or four drugs. The rapid change in this combination was also observed physically with the powder becoming watery more rapidly than other combinations.

The behavior of weight gains at 32 h, the time when saturation was reached, is depicted in Fig. 2b for all samples containing ethambutol. It clearly shows that saturation weight gain is similar in light and dark conditions for all the combinations. However, overall moisture uptake gets decreased as the number of drugs increase in the combination. While ethambutol alone showed a total weight gain of 73%, the percent weight gain decreased to 59.5% when the drug was mixed with isoniazid. The weight gain further reduced to ~41% when ethambutol was present along with rifampicin and isoniazid, and the value fell still lower to ~30%, when the same drug was mixed with rifampicin, isoniazid and pyrazinamide. Scatter or dilution of ethambutol particles in increasing bulk best explains this incremental reduction of hygroscopicity on addition of drugs, one after another.

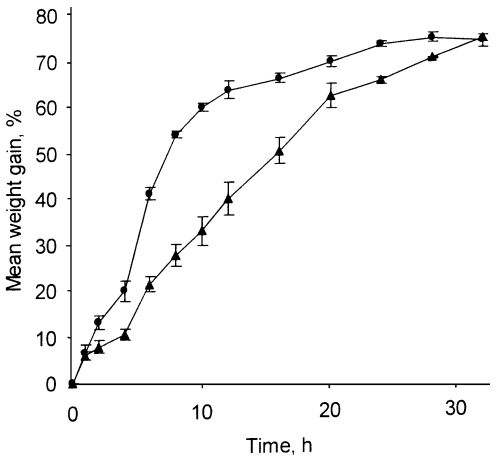

Fig. 1. Behavior of weight gain by ethambutol with time on exposure to stability chambers without (▲) and with light (●).

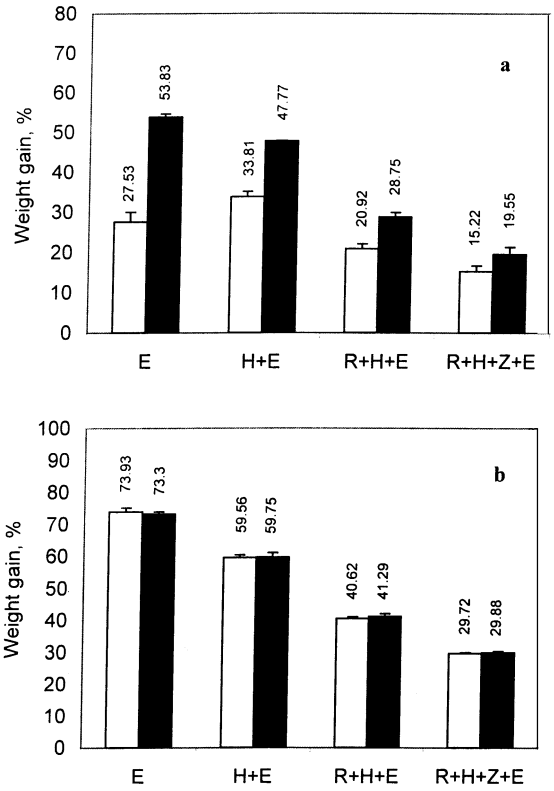

Fig. 2. Weight gain by ethambutol (E) and combinations of this drug with isoniazid (E+H), rifampicin and isoniazid (R+H+E), and rifampicin, isoniazid and pyrazinamide (R+H+Z+E) on exposure to stability chambers without (□) and with light (■) after 8 h (a) and 32 h (b). The values indicated on the bars are means of three observations.

4. Conclusions

The study prominently shows that:

1) The moisture gain by ethambutol is accelerated in the presence of light as compared with dark conditions. This is a new finding. Literature search reveals no previous report on this phenomenon for any other drug.
2) There is a significant overall moisture uptake in all drug combinations containing ethambutol, but the gain of weight decreases as the number of drugs in the combination increases.
3) The combination of isoniazid and ethambutol shows higher rate of moisture gain in dark conditions than ethambutol alone or combinations containing ethambutol.

Based on the results of this pilot study, multipronged investigations have been initiated in our laboratories to (a) determine the extent of moisture uptake in the absence and the presence of light by unpacked and packed marketed anti-tuberculosis FDC formulations, (b) find out the reason for the typical behavior shown by the combination of isoniazid and ethambutol, and (c) test whether the accelerated moisture uptake in the presence of light is a general phenomenon, applicable to other drugs and even to excipients. Results of these studies will be reported subsequently in series publications.

References

Acocella, G., 1989. Human bioavailability studies. Bull. Int. Union Tuberc. Lung Dis. 64, 38–40.

ICH, 1996. Photostability testing of new drug substances and products. International Conference on Harmonization, Geneva.

Long, M.W., Snider, D.R., Farer, L.S., 1979. US Public Health service cooperative trial of three RIF–INH regimens in the treatment of pulmonary tuberculosis. Am. Rev. Respir. Dis. 119, 879–894.

Mohan, B., 2001. Development of a stability indicating assay method for rifampicin, isoniazid, and pyrazinamide in combination, M.Pharm. thesis, NIPER. S.A.S. Nagar, India.

Pillai, G., Fourie, P.B., Padayatchi, N., Onyebujoh, P.C., McIlleron, H., Smith, P.J., Gabriels, G., 1999. Recent bioequivalence studies on fixed dose combination antituberculosis drug formulations available on the global market. Int. J. Tuberc. Lung Dis. 3, S317–S321.

Singh, S., Mariappan, T.T., Sharda, N., Singh, B., 2000a. Degradation of rifampicin, isoniazid and pyrazinamide from prepared mixtures and marketed single and combination products under acid conditions. Pharm. Pharmacol. Commun. 6, 491–494.

Singh, S., Mariappan, T.T., Sharda, N., Kumar, S., Chakraborti, A.K., 2000b. The reason for an increase in decomposition of rifampicin in the presence of isoniazid under acid conditions. Pharm. Pharmacol. Commun. 6, 405–410.

Singh, S., Mariappan, T.T., Sankar, R., Sharda, N., Singh, B., 2001. A critical review of the probable reasons for the variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.