STANDARDIZATION AND BIOAVAILABILILTY OF AYURVEDIC DRUG LAUHA BHASMA PART-1 PHYSICAL AND CHEMICAL EVALUATION

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ABSTRACT: Lauha Bhasma, an ayurvedic drug, is widely used in iron deficiency anaemia, this ancient drug is claimed to be better absorbed gastrointestinally, and is also claimed to be devoid of the usual side effects associated with administration of the allopathic iron preparations. Physical and chemical methods of standardization required for any quality preparation, is not found in the ayurvedic and the modern literatures for Lauha Bhasma. Thus an approach has been made to standardize Lauha Bhasma. For the purpose of analysis, samples of all the three commonly available variants of Lauha Bhasma were considered. Qualitative analysis indicates the presence of iron both in the ferric and the ferrous forms. A simple spectrophotometric method has been used for simultaneous determination of ferric ferrous and the total iron content in a single aliquot.

INTRODUCTION

The iron deficiency anaemia is the most common type of anaemia. The body is normally in a state of positive iron balance. When a negative iron balance occurs either due to blood loss, increased requirement, or impaired absorption; the deficit is made good by the iron mobilized from the tissue stores, thus an adequate supply of iron for haemoglobin formation is maintained. However, when the tissue stores are exhausted, the supply of iron to the bone marrow for haemoglobin synthesis becomes inadequate and hypochromic anaemia develops (Penington, 1984). Regarding the comparative efficacy of oral and parenteral iron preparation, McCurdy found the parenteral preparations except in case of defective iron absorption as in gastrectomy and in cases where iron salts may be irritating as in peptic ulcer, ulcerative colitis (Block, 1974).

In the ayurvedic system of medication, it is claimed that the oral iron preparation Lauha Bhasma is better absorbed from gastrointestinal tract and is devoid of the side effects, generally associated with oral/parenteral iron preparations or the allopathic system of medication. Due to this claim, it would be interesting to compare the marketed allopathic and ayurvedic iron preparations.

For the present study, three variants of the marketed Lauha Bhasma, the calcined iron preparation of ancient ayurvedic medicine (Ayurvedic Formulary of India 1978), namely sahashastraputti (Calcined thousand times) satputti (calcined hundred times) and ordinary (calcined seven times), were analysed.

Methodology
I Physical evaluation
Particle size distribution

The size and the surface area of a particle can be related in a significant way significant way
to the physical, chemical and pharmacological properties of a drug. As Bhasma is a polydisperse system, estimation of the size range together with the particle number and the weight fraction of each particle size is required (Martin, 1983). The size of the particles of a solid substance is of considerable importance, as it affects its dissolution rate and the rate of absorption (Lackman and Lieberman, 1976). For the estimation Coulter counter method has been used (Bean, 1967).

The data obtained is presented in Table 1 in the form of cumulative weight percentage vs diameter, and the same is depicted graphically in the form of histobor (Graph 1). Further, the number of particles per unit weight and the specific surface area per unit weight are calculated, and their average values are depicted in Table 2.

**Loss on ignition**

Lauha Bhasma samples were first dried under an infrared lamp to do away with moisture, A weighed amount of sample was taken in a silica crucible and was incinerated for several hours so as to get a constant weight of the ample (Pharmacopoeia of India, 1978). An average of three readings expressed in terms of the percentage loss in weight, are tabulated in Table 2.

**II. Chemical evaluation**

**Qualitative analysis**

Number of tests were performed to find out whether Lauha Bhasma contained the ferrous, the ferric or both the forms of iron. Various tests were performed using potassium ferricyanide, potassium or ammonium thiocyanate, potassium ferricyanide, thioglycolic acid, 1,10 phenanthrolin, cupferron, α, α\(^1\) dipridyl, 8-hydroxy 7-iodoquinoline 5- sulfuric acid (Shapiro, 1975; Altherden, 1979).

It is evident from the tests performed that the samples of Lauha Bhasma contain both the forms of iron i.e the ferric and the ferrous forms. The intensity of the colour obtained in the various tests made it amply clear that the proportion of ferric iron is more than ferrous iron in all the variants taken.

**TABLE 1**

| Diameter range of samples (µm) | Cumulative Weight Percentage of Samples (Lauha Bhasma) |
|-------------------------------|------------------------------------------------------|
|                               | Sahastraputti | Satputti | Ordinary |
| 2-10                          | 24.77         | 21.95    | 4.34     |
| 10-20                         | 21.69         | 27.95    | 3.89     |
| 20-30                         | 31.85         | 12.91    | 10.91    |
| 30-40                         | 9.29          | 27.01    | 4.82     |
| 40-50                         | 12.4          | 7.49     | 6.03     |
| 50-60                         | -             | 2.69     | 21.71    |
| <60                           | -             | -        | 48.25    |
| Lauha Bhasma | Particle Number per unit Wt. ‘N’ | Surface area per unit wt. ‘S_w’ | Loss on Ignition |
|-------------|---------------------------------|---------------------------------|-----------------|
| Sahastrputti | 1.130 x10^9                     | 1.901 x 10^3 cm^2/G            | 1.56%           |
| Satputti    | 3.714x10^8                      | 1.009 x 10^3 cm^2/G            | 3.44%           |
| Ordinary    | 2.265x10^8                      | 0.460 x 10^3 cm^2/G            | 3.56%           |

| S.No | Batch No | Amount of Iron taken from stock solution (100 µg/ ml) | Dilution | Conc. of Iron/ml in dilute sample (µg/ ml) | Amount recovered after analysis |
|------|----------|------------------------------------------------------|----------|------------------------------------------|--------------------------------|
| 1    | I        | 25                                                   | Ferric   | 1.0                                      | 1.0049                         | 100.49                         |
| 2    | 25       | 50                                                   | Ferrous  | 1.5                                      | 1.5                            | 100.00                         |
| 3    | 100      | 00                                                   | Total (µg) | 2.0                                      | 2.0049                         | 100.24                         |
| 4    | 00       | 25                                                   |           | 0.50                                     | 0.4975                         | 99.51                          |
| 5    | II       | 100                                                  | Ferric   | 2.1                                      | 2.0985                         | 99.93                          |
| 6    | 100      | 10                                                   | Ferrous  | 2.2                                      | 2.2069                         | 100.31                         |
| 7    | 100      | 20                                                   | Total (µg) | 2.4                                      | 2.3941                         | 99.76                          |
| 8    | 100      | 30                                                   |           | 2.6                                      | 2.6108                         | 100.41                         |
| 9    | 100      | 40                                                   |           | 2.8                                      | 2.773399                       | 99.05                          |
| 10   | III      | 70                                                   | Ferric   | 1.9                                      | 1.8867                         | 99.30                          |
| 11   | 75       | 25                                                   | Ferrous  | 2.0                                      | 1.99                           | 99.50                          |
| 12   | 100      | 25                                                   | Total (µg) | 2.5                                      | 2.4877                         | 99.51                          |
## Table 4
ASSAY OF LAUHA BHASMA

| Sample No | Varient of Lauha Bhasma | Amount of Lauha Bhasma | Amount of total iron present in the sample (mg) A | Amount of ferrous present in the sample (mg) B | Amount of ferric present in the sample (mg) (A-B) | % of ferric present in the sample | % of ferrous present in the sample | % of total Iron present in the sample |
|-----------|-------------------------|------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------|---------------------------------|-----------------------------------|
| 1         | Ordinary                | 30.1                   | 25.824                                        | 5.276                                         | 20.458                                        | 68.266                           | 17.528                          | 85.794                           |
| 2         | Ordinary                | 33.7                   | 29.829                                        | 5.795                                         | 23.034                                        | 68.349                           | 17.198                          | 85.547                           |
| 3         | Ordinary                | 39.2                   | 33.467                                        | 6.995                                         | 26.472                                        | 67.531                           | 17.844                          | 85.375                           |
| 4         | Satputti                | 29.9                   | 24.391                                        | 0.745                                         | 23.646                                        | 79.085                           | 2.490                           | 81.575                           |
| 5         | Satputti                | 32.5                   | 26.508                                        | 0.713                                         | 25.795                                        | 79.369                           | 2.194                           | 81.563                           |
| 6         | Satputti                | 32.3                   | 26.164                                        | 0.634                                         | 25.530                                        | 79.041                           | 1.962                           | 81.003                           |
| 7         | Sahastraputti           | 34.7                   | 29.668                                        | 0.792                                         | 28.876                                        | 83.215                           | 2.283                           | 85.498                           |
| 8         | Sahastraputti           | 30.4                   | 25.989                                        | 0.634                                         | 25.355                                        | 83.403                           | 2.085                           | 85.488                           |
| 9         | Sahastraputti           | 30.1                   | 25.000                                        | 0.745                                         | 25.055                                        | 83.239                           | 2.474                           | 85.713                           |
Quantitative analysis

In order to analyse quantitatively ferrous, ferric and total iron simultaneously in a single aliquot, spectrophotometric method was selected for accuracy and reproducibility.

The early calorimetric methods, using the thiocyanate or the ferricyanide reagents for determination of iron, are relatively insensitive and have been suitable replaced by organic reagents. The reagent 1,10 phenanthrolin (C12H8N2) was selected because: (i) its molar absorptivity is 1.11 x 107, hence it can be used to determine iron in very small amounts (ii) its detection limit (A= 0.01) is about 1x 106 M and the usual range for analysis is 0.4 to 8 ppm of iron in 1 cm cell. (iii) Its iron complex has absorption maxima at 510-515 mm (iv) it reacts only with the ferrous and not with the ferric iron (Varley, 1980; Fritz, 1969).

Principle of analysis

To a sample containing both the ferrous and the ferric iron in aqueous solution, having a pH range of 3-6, 4 cm3 of 1,10 phenanthrolin was added. The reddish orange color developed, was due to the ferrous iron and had its absorption maxima at 515nm (Beckman UV spectrophotometer model 34 with recorder, USA). In a second set of sample the ferric iron present was reduced to the ferrous form by reacting with hydroxyl ammonium chloride and the color was developed. The color now was due to the total iron in the sample. The amount of the ferrous iron obtained in the first set, deducted from the amount of the total iron obtained in the second set, gave the amount of the ferric iron present in the sample.

Verification of the contemplated method

A known amount of sample in different ratios of ferrous to ferric iron, form the stock solution of ferrous ammonium sulphate G.R and ferric ammonium sulphate G.R were taken in volumetric flasks. The color was developed by adding 4 Cm3 of 1,10 phenanthrolin and its optical density was measured at 515nm. This gave the amount of ferrous iron present in the sample. In a second aliquot 5 cm3 of hydroxyl ammonium chloride was added, and the pH was adjusted to 3-6 with sodium acetate solution followed by 4 cm3 of 1,10 phenanthrolin. Its optical density was measured similarly. This gave the total iron present in the sample.

Various known concentrations of the ferrous and the ferric mixtures were analysed. The results in Table 3 demonstrate the validity of the method the degree of accuracy.

Assay of Lauha Bhasma

Weighed amount of Lauha Bhasma was dissolved by heating with dilute hydrochloric acid. The temperature was not allowed to exceed 50°C. During heating, carbondioxide gas was bubbled so as to avoid the oxidation of ferrous iron to ferric iron. The aliquot from this solution was analysed and the results of analysis depicted in Table 4.

RESULTS

The Coulter Counter analysis data revealed a wide range of difference in the particle size distribution (PSD) between the samples of Sahastraputti Lauha Bhasma, Satputti Lauha Bhasma, an Ordinary Lauha Bhasma (Table1). The particle number and the specific surface area were also different in the three variants taken, as observed (Table 2).
Determination of loss on ignition showed a meager loss in weight for each of the three variants of Lauha Bhasma ((Table 2).

On assay, the samples of Lauha Bhasma were found to contain about 81-85% of the total iron content. Ferric oxide was found in a greater proportion than ferrous oxide in all the samples (Table 4).

DISCUSSION AND CONCLUSION

From the PSD data, the particle size range of 2.55 to 40.9 µm was found in Sahastraputti Lauha Bhasma, 5.1 to 51.6 µm in Satputti Lauha Bhasma.

From the histobar data and the data depicted in Table 1 it has been observed that, in the range of 2-10 µm the Sahastraputti Lauha Bhasma accounts for 24.77%, the Ordinary Lauha Bhasma accounts for only 4.34% and the Satputti Lauha Bhasma finds its place between them i.e 21.95% of the cumulative weights, In the range of 10-20 µm, the percentage of sahastraputti Bhasma is intermediate, where as the percentage fo satputti Bhasma is maximum and for ordinary Bhasma accounts for the least percentage, In the range of 20 -30 µm the percentage of Sahastraputti Bhasma accounts for the maximum cumulative weight (31.85%), where as satputti Bhasma and ordinary Bhasma contain 12.91% and 10.91% respectively. The range of 30-40 µm also shows Sahastraputti Bhasma to be in between satputti Bhasma and ordinary Bhasma in their respective cumulative weight percentage. The range of 40-50 µm shows the cumulative weight percentage of Sahastraputti, Satputti and ordinary Bhasma in a decreasing order. In the range of 50-60 µm, there is no particle of Sahastraputti Lauha Bhasma, where as Satputti accounts only for 2.69% and ordinary Lauha Bhasma accounts for 21.71% of the cumulative weights. Particles of more than 60 µm are found only in the case of ordinary Lauha Bhasma i.e 48.25%.

Sahastraputti Lauha Bhasma consisting of very fine particles, represents the specific surface area (1.901 x 103 cm2/g) which is about our times more than the of ordinary lauha bhasma whereas satputti Bhasma accounts for twice the specific surface area that ordinary Bhasma (Table 1). Similarly, with respect to ordinary Lauha Bhasma the particle numbers of sahastraputti and satputti Bhasma account for 5 times and 1.6 times respectively.

Bhasma are oxides of their metal. On qualitative analysis it was found to contain both the ferric and ferrous iron (Table 4). The minor loss on ignition (Table 2) indicated that Bhasma do not contain a considerable portion of the organic matter and also it was not in the form of salts. Thus it was not in the form of salts, thus it contained both the ferric oxide and the ferrous oxide. On assay it was found to contain about 85% of total iron oxide. Remaining portion (about 15%) in the Lauha Bhasma is expected to consist of mainly the silicious matter because it is prepared in earthen pots with repeated ignition, Samples of Lauha Bhasma are found to contain a greater proportion of ferric oxide, 79.16 % and 83.29% for satputti and sahastraputti respectively than the ferrous oxide (about 2.28%) However, the proportion of ferrous oxide (17.5%) in the case of ordinary Lauha Bhasma is more than the satputti and sahastraputti samples, the total amount of iron remaining same. Still the proportion of ferric oxide remains larger than ferrous oxide. The difference in the amount of ferrous oxide maybe attributed to the fact that satputti and sahastraputti Lauha bhasma are prepared after 100 and 1000 times ignition respectively, where as ordinary
Lauha Bhasma is exposed to 7 times burning only. Increase in the number of times burning exposes the material to atmospheric oxygen, which may be converting the ferrous form of iron to the ferric form.

The above data obtained through physical and chemical evaluation of Lauha Bhasma may conveniently be used to standardize the Ayurvedic iron preparations of Lauha Bhasma.

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