CHAPTER V

SUMMARY AND CONCLUSIONS

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An attempt was made to evolve a new model for predicting the iron availability from cereal meals, consumed in India. The model which is presently available in the literature for predicting iron bioavailability is based only on enhancers of iron bioavailability namely ascorbic acid, meat, fish and poultry (MFP) (Monsen and Balintfy, 1982). The present study tested the hypothesis that an equation derived on the basis of the interaction effect of different enhancers and inhibitors, rather than the equation evolved on the basis of enhancers only, would predict the avaiability of iron from cereal based Indian meals better.

The in vitro availability of iron, measured by the method of Narasinga Rao and Prabhavati (1978) was used as the measure of potentialy available iron, since this method has been shown to have a high correlation with the in vivo bioavaiability of iron in humans.

The high correlation demonstrated by Narasinga Rao and Prabhavati (1978) of the in vitro avaiable iron with the in vivo bioavailable iron, was based on studies in 47 human subjects who had absorption of iron within a limited range of 1.6 to 3.8%. Therefore, before using this measure of the in vitro available iron for developing the prediction equation, studies were undertaken to further validate this method by carrying out in vitro analysis of twelve meals, selected from various in vivo human studies in the literature and correlating the in vitro

available iron with the in vivo iron absorption values reported in the respective studies. These experiments formed the first phase of the present study.

In the next two phases, experiments were carried out using a pure system containing 3 mg elemental iron in the form of $FeCl_3$ and different levles of enhancers and inhibitors, to evolve a regression equation for prediciting iron availability. Five dose levels of six food constituents, namely ascorbic acid, citric acid, tannic acid, phytic acid, oxalic acid and calcium phosphate were selected for the study, based on the dietary data from various sources. These constituents were added in a pure chemical form, to study the dose effect on iron availability from the pure system in Phase II. On the basis of the dose relationship curves obtained in this phase, two levels of each of the six variables were selected and their interaction effect was studied in 64 combinations in the next phase (III). A regression equation was evolved from the pure system in this phase, termed as equation no.1

Parallel set of experiments were carried out in the next two phases, using a standard cereal meal as the source of iron. For selection of the standard meal, a diet survey was carried out in urban Baroda, on 18 Gujarati women, belonging to high, medium and low socio-economic group families. The most commonly consumed, main meal of the day (lunch), in amounts equivalent to those consumed by the women, was selected as the standard meal. This provided 60 % of the day's iron intake. This meal

consisted of Rotla[1], Khichdi[2], Kadhi[3], and Vegetable[4]. The standard meal was simulated in the laboratory using standardised recipe each time for analysis. The dose effect of different enhancers and inhibitors on iron availability was studied in Phase IV, from the standard meal homogenate, providing 3 mg nonheme iron.

As the study progressed, it became evident that of the six variables studied, phytate and oxalate, in pure chemical form did not show the expected trends with iron availability, as those reported in the in vivo human studies in the literature. Hence, a sub study was carried out in Phase IV, where the effect of phytate rich wheat bran and rice bran were evaluated on the in vitro availability of iron from pure $FeCl_3$ solution. Both were inhibitory in nature, larger the dose, greater the inhibition. Further, dephytinising the bran reversed the inhibitory effect partially. It was concluded that at the levels used in the present study these two constituents added in the pure chemical form did not represent the in vivo effect. Hence it was decided to exclude these two variables from the experiments of the next phase (V) where interaction effect of rest of the four variables, namely ascorbic acid, citric acid, tannate and calcium phosphate was studied in the standard meal. Based on the data, a second regression equation was evolved, termed as equation No. 2.

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1. Rotla is a thick wheat chapati.
2. Khichdi - rice and tuver dal (Cajanus cajan) cooked together for 30 minutes till soft.
3. Kadhi - curry prepared from buttermilk and bengalgram (Cicer arictinum) flour, brought to boil (1 minute).
4. Vegetable - potatoes (Solanum turerosum) and onions (Allium cepa) chopped and shallow fried till tender (7 to 8 minutes).

In the final Phase (VI), the two equations, evolved from the pure system and the standard meal respectively were evaluated for their predictive powers, as applied on a set of ten, typical Indian meals of varying compostion. These meals were selected on the basis of the diet surveys carried out by Narasinga Rao et al (1983) and Billa (1990). The selected meals were prepared in the laboratory, using the same recipes as those suggested by the above investigators. All the ten meals were analysed for in vitro iron availability, enhancers and inhibitors. The content of enhancers and inhibitors chemically analysed or calculated from food tables was incorported in the two predictive models evolved in the present study, and their predictive powers were evaluated as applied to these meals. Attempts were also made to compare the two models with the only other model available in the literature, evolved by Monsen and Balintfy (1982).

The results of the present study are summarised below.

1. The observations that reconfirmed the documented findings were :

   a) The in vitro iron availability of the 12 meals tested in Phase I had high correlation (r= 0.97) with the in vivo iron absorption values reported by respective authors, which agreed with the findings of the authors who proposed the in vitro method. Thus it was confirmed that this in vitro method can be used as a measure of bioavailable iron.

b) Of the six variables studied, ascorbic acid, tannic acid and calcium phosphate exhibited, both in the pure system and in the standard meal, trends, that were consistent with the in vitro and in vivo iron absorption studies reported in the lietrature.

c) When compared to the in vivo system the net effect of various enhancers and inhibitors as observed in the in vitro system in the present study was of a smaller magnitude.

d) Barring the first dose level of ascorbic acid, the magnitude of increase in iron availability, on addition of the two enhancers was greater in the STD meal as compared to the pure system. In case of inhibitors however, there was more pronounced inhibition in the pure system vis-a-vis the STD meal (for the dose levels that showed inhibition).

2. The new findings that emerged from the study were :

a) Citrate, which failed to show any effect when added alone to a pure system, however, turned out to be a strong enhancer of iron availability in combination with other enhancers and inhibitors. In the STD meal System, citrate when added alone produced a modest increase in available iron, which effect became even stronger when citrate was added in combination with ascorbic acid and other inhibitors. These findings indicated that citrate may serve as a promising enhancer, as good as ascorbic acid, if not better, in improving the availability of iron from cereal based vegetarian meals.

b) Phytate and oxalate, which have been reported to be inhibitory in nature did not show such an effect in the in vitro situation, when tested in pure chemical form. An interesting observation consistent for both these constituents was that at lower dose levels there was a reduction while at higher levels there was an increase in iron availability.

c) The predictive model, evolved from the cereal meal system was better (r=0.76) than that evolved from the pure system (r=0.59), in predicting iron availability from cereal meals. Also it provided a more precise quantitative estimate of iron availability in terms of absolute values.

d) In the absence of analysed values for enhancers and inhibitors present in a given meal, calculated values from food tables can also be used to predict iron availability from the present model, eventhough such a prediction would not be as accurate as that obtained with the actual estimation of the enhancers and inhibitors.

e) Finally, incorporation of both, enhancers and inhibitors into the predictive model, rather than only enhancers, results in a more practical and precise estimation of iron availability from typical Indian meals.