

## ABSTRACT

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The major goal of the study was to explore the possibility of developing an updated model that integrates the effect of various enhancers and inhibitors, for predicting the potential availability of iron from typical Indian vegetarian meals. The in vitro availability of iron, measured by the method of Narasinga Rao and Prabhavati (1978) was used as the measure of potential availability of iron. The study was carried out in six phases.

In the first phase (Phase I) in order to further validate the in vitro method, twelve meals were selected from different in vivo studies and these were analysed for nutrient composition and in vitro iron availability. The analysed values for iron availability were correlated with the in vivo iron absorption values, as reported by the respective authors. Results revealed a high correlation ( $r=0.97$ ) between the two sets of values, indicating that the above method could be used to provide a fairly good indication of iron bioavailability. For the experiments of the next two phases (Phase II and III) a pure ferric chloride solution was used as the source of iron, providing 3 mg elemental iron/250 ml, designated as the pure system. When the dose effect and interaction effect of various enhancers (ascorbic acid and citric acid) and inhibitors (tannate, phytate, oxalate and calcium phosphate), was studied in the pure system, it was found that ascorbic acid significantly enhanced iron availability. Citrate failed to show any effect when added alone. Tannate and calcium phosphate, both, exhibited

marked inhibitory effect on iron availability. The effects of ascorbic acid, tannate and calcium phosphate were found to be clearly dose related. Phytate and oxalate showed an unexpected increase in iron availability. The interaction effects of these six constituents were studied in Phase III, which revealed ascorbic acid to be a strong enhancer. Citrate which failed to show an effect when added alone, turned out to be as strong an enhancer of iron availability as ascorbic acid in the presence of other constituents. Tannate and calcium phosphate exerted a strong negative effect, reducing iron availability. Results with phytate and oxalate, contrary to other reported findings were positively correlated with iron availability indicating an enhancement rather than an inhibition. Based on the data of Phase III, a regression equation was evolved from the pure system, termed as equation 1.

Parallel experiments on dose effect and interaction effect were carried out using a complex cereal meal, designated as the standard meal (STD meal), providing 3 mg nonheme iron/250 ml homogenate. Experiments on the dose effects of various enhancers and inhibitors on iron availability from the STD meal (Phase IV) yielded essentially similar trends as those observed in the pure system (Phase II). Ascorbic acid turned out to be a strong enhancer; citrate showed a moderate enhancing effect when added alone to the STD meal. Among the inhibitors, both tannate and calcium phosphate significantly reduced iron availability. These effects were all dose related. Phytate and oxalate again exhibited the unexpected trend of increasing iron availability.

In view of the above observations, a sub-study was carried out in Phase IV, where the effect of phytate rich bran (wheat and rice) was evaluated on the in vitro availability of iron from pure  $\text{FeCl}_3$  solution. Both turned out to be inhibitory, 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 in the pure chemical form did not represent the in vivo effect. And so they were excluded from the experiments of the next phase.

In Phase V, interaction effect of rest of the four variables was studied. Results revealed that both ascorbate and citrate emerged as equally strong enhancers, while tannate and calcium phosphate demonstrated strong inhibitory effect on iron availability in the STD meal. The observation that citrate could enhance iron availability to an almost equal extent as ascorbate, when added in combination with the other variables was encouraging. Based on the STD meal data, another regression equation was evolved, termed as equation 2.

The two equations (1 and 2) were evaluated for their predictive powers in the last phase of the study (Phase VI). For this, 10 typical Indian, vegetarian meals were analysed for iron availability and the content of various enhancers and inhibitors. The analysed values for % iron availability were correlated with those computed using the two equations, by incorporating the content of enhancers and inhibitors. Comparisons revealed that equation 2 (STD meal) was far better in predicting iron

availability from the meals ( $r=0.76$ ) than equation 1 (pure system,  $r=0.59$ ). When food table values of the enhancers and inhibitors were used in equation 2, the correlation coefficient obtained was 0.60, which indicated the present model could be used to provide an estimate of iron availability even in the absence of analysed values of enhancers and inhibitors present in the meals.

Further, when an attempt was made to compare the present equation with the model of Monsen and Balintfy (1982), it was found that the computed values using the latter model had a poor correlation ( $r=0.19$ ) with the analysed iron availability values for the 10 typical Indian vegetarian meals. The findings of the present study substantiated the hypothesis that a regression model, evolved from a complex meal by integrating the effect of enhancers as well as inhibitors, rather than only enhancers, provides a more precise estimate of iron availability from typical Indian meals. The limitations and practical implications of the present model are discussed in the text. The hypothesis that the regression equation developed from a pure system can be used to predict the availability of iron from a complex meal was not accepted, the reasons for which are indicated in the thesis.