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Dynamic continuous-flow dialysis method to simulate intestinal digestion for in vitro estimation of mineral bioavailability of food

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Abstract

A system for dynamic continuous-flow dialysis during intestinal digestion for an in vitro simulation of gastrointestinal digestion is presented as an alternative to human and animal in vivo methods for estimation of the bioavailability of minerals. The method is based on the in vitro batch dialysis method described by Miller, which was developed into a continuous-flow system of a simple design to perform dynamic dialysis in the intestinal digestion stage. A flow dialysis system has the advantages of simulation being close to in vivo physiological conditions because pH change during dialysis is gradual and dialyzed components are continuously removed. The proposed new design performed dialysis during a continuous flow of dialyzing solution ($NaHCO_3$) around a dialysis bag containing peptic digest, which is placed inside a glass dialysis chamber. Gradual change of dialysis pH, similar to that occurring in the gastrointestinal tract, was obtained by optimization of flow rate and concentration of $NaHCO_3$. The dialysate collected in fractions was analyzed to determine dialyzed minerals and pH change in the course of dialysis. The method was tested by determination of calcium bioavailability of powder milk and calcium carbonate tablets.

Keywords: In vitro method; Bioavailability; Continuous-flow

1. Introduction

The total concentration of a mineral micronutrient in food does not provide information about its bioavailability. Speciation of a micronutrient or the determination of its chemical forms in food and in the gastrointestinal tract is essential to the understanding and the prediction of its availability for absorption [1]. This is often difficult to perform. Nutrient bioavailability has usually been estimated by in vivo human study. In vivo experiments, however, are time consuming and very expensive and often give variable results caused by uncontrollable physiological factors. Laboratory in vivo experiments in animals are sometime used as a model for human. Experiments with animals are less expensive but are limited by uncertainties with regard to differences in metabolism between animals and human. As an alternative to in vivo human and animal studies, nutrient bioavailability has also been estimated through in vitro methods [2–9]. These methods have gained popularity because of their simplicity, precision, speed of analysis and relatively low cost.

Interest in development of in vitro methods for estimating bioavailability of essential mineral elements dates back to at least the early 1930s [2]. These methods provide insights on minerals and trace element nutrition that are not achievable by human or animal experiments. The earliest trial [2] assumed ionizable minerals as potentially available and determined ionizable iron in food by extracting with complexing agents such as α,α'-dipyridyl and bathophenanthroline. Another approach attempted to simulate gastrointestinal digestion conditions and determined soluble or dialyzable minerals [3–9]. Particularly, the in vitro method developed in 1981 by Miller et al. [5] has been reported to provide availability measurements that correlate well with in vivo studies for iron. This method has been the basis for several in vitro methods for estimation of the bioavailability of iron and other minerals such as calcium and zinc [10,11]. The in vitro method

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involves a simulated gastrointestinal digestion with pepsin at pH 2 for 2 h during the gastric stage and with a mixture of pancreatin and bile salts along with a gradual pH change from 2 to 7 during the intestinal stage. The proportion of the compounds diffusing across a semipermeable membrane during the intestinal stage is used as a prediction of the elemental bioavailability.

In Miller's method, equilibrium dialysis is performed to obtain dialyzable compounds during intestinal digestion. The drawback is that dialyzed components are not removed during dialysis, as occurred in the real situation of the digestive tract. This may cause lower dialyzability. Therefore, a modified continuous dialysis in vitro method was developed by Minihane et al. [12] in which dialyzed components were removed continuously. The model developed by Minihane et al. used an Amicon stirred cell for continuous dialysis. The pH was adjusted gradually over a 30 min period from 2.0 to 7.0 before dialysis was started. Minihane's method was further modified by Shen et al. [13] to obtain a gradual pH change during the dialysis instead of adjusting the pH before dialysis. Shen et al. performed continuous dialysis by introducing a gradual pH adjustment using a small dialysis bag filled with an amount of $NaHCO_3$ equivalent to the predetermined titratable acidity of the peptic digest. The dialysis was carried out in a vessel under a pressure of 50 psi.

Wolters et al. [9] developed an in vitro method for continuous dialysis of minerals and trace elements based on a hollow-fiber system. The hollow-fiber system for continuous dialysis consists of a reaction vessel placing in a water bath at 41 °C. The food suspension in this vessel is pumped via a peristaltic pump through a suction tube into the hollow-fiber membrane. A fine filter cloth stretched across the inlet of the hollow-fiber and a magnetic stirrer was used to prevent clogging of the hollow-fiber by large particles. Components in the suspension that could pass through the hollow-fiber membrane were dialyzed and collected in a plastic bottle for subsequent analysis. That part of the suspension that could not pass through the hollow-fiber membrane was pumped back into the reaction vessel where these components could be digested further and recirculated into the hollow-fiber for complete dialysis.

A multicompartmental computer controlled simulated gastrointestinal digestion system has been developed [14] and applied [15,16] for evaluation of bioavailability. The system consists of several successive compartments to simulate the digestion in the stomach, duodenum, jejunum and ileum. Compartments are connected by peristaltic valve pumps to regulate the transfer of digestive enzymes. The system was also equipped with rotary pumps, syringe pumps for water pressure and secretion controls. Because the model aimed to mimic the whole GI-tract from stomach to ileum, it was rather complicated and not easy to perform. A simple method to access maximum bioaccessibility based on flow injection leaching of food sample by artificial saliva, gastric juice and intestinal juice was recently developed [17]. The method has the advantages of rapidity and simplicity. However, because leaching was accomplished in only a few minutes, the food sample may only be partially digested and leached.

In the present study, a simple setup for continuous-flow dialysis to perform an in vitro simulated intestinal digestion was developed. Considering that mineral absorption takes place mainly at the intestinal digestion stage [5], this setup was designed for dialysis in the intestinal digestion stage to occur by a continuous flow of dialyzing solution (dilute $NaHCO_3$ solution) around the dialysis tubing containing the gastric digestate. The gastric digestion was performed in a batch manner to effect high sample throughput because a large number of samples could be digested at the same time. In the simulated intestinal digestion stage, gradual change of pH, similar to that occurring in the intestinal tract, was obtained by optimization of flow rate and concentration of $NaHCO_3$. The dialysate collected in fractions was analyzed to determine the amount of dialyzed minerals. The graphical plot of dialyzed minerals with time of dialysis provides kinetic information of the dialysis process. The feasibility of the developed system was tested by applying it to evaluate dialyzability of calcium in calcium carbonate tablets and powder milk.

2. Experimental

2.1. Design and setup of continuous-flow dialysis system

A continuous-flow dialysis system was designed to serve three objectives: a gradual pH change at the early stage of dialysis, a convenient means of addition of enzymes at will and continuous removal of dialyzable components during dialysis.

The proposed dialysis system is presented schematically in Fig. 1. A dialysis chamber was designed to allow containment of a dialysis tubing, around which dialyzing solution could flow during dialysis. The chamber (ca. 20 cm in length and 0.8 cm inner diameter) and its cover were constructed in-house from borosilicate glass. Dialysis tubing MMCO 12,000–14,000 Da (Spectra/Por, Thomas Scientific, USA) was used. To prepare the dialysis chamber, a dialysis tubing

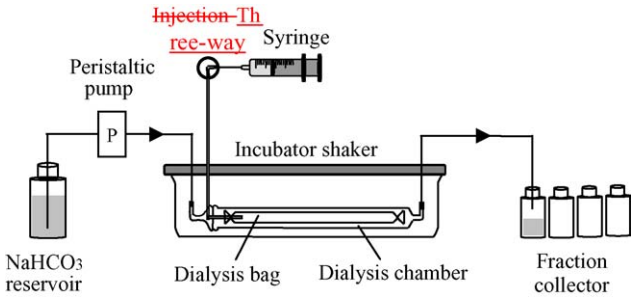

Fig. 1. Schematic diagram of the proposed continuous flow in vitro dialysis setup.