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Empirical Modeling of *in-vitro* Kinetics Study on Tamarind Fruit Tablet and its Microbial Stability

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Key words: *In-vitro*, tamarind fruit tablet, zero-order, first-order, Higuchi, microbial stability

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Abstract: The aim of this study is to determine the best fit model of the tamarind fruit tablet dissolution process and the effect of storage time to the microbial stability on the tamarind fruit tablet. Different dissolutions models; zero-order kinetic, first-order kinetic and Higuchi models were applied to active ingredient released from the tamarind fruit tablet. In order to evaluate the release mechanisms and kinetics of tamarind fruit tablet, the most appropriate model was selected based on the linearity coefficient of correlation (R^2). It was found that the release of vitamin C fit well to the Higuchi model. The fruit tablet has the potential to be spoiled due the growth of microorganisms, yeast and mold after a long time of production. Therefore, the microbial stability test was done to the tamarind fruit tablet following the Association of Analytical Chemists method. The colony forming units (CFU/g) were calculated to determine the quality of this fruit tablets. From the microbial count of tamarind fruit tablets, it was found that shelf life has not been deteriorated even after one month of storage. The results showed 4.3×10^3 CFU g^{-1} for its highest values. It is considered safe since the microbial countbelow the standard limit of 10,000 CFU g^{-1}.

INTRODUCTION

Tamarind tree-type plant also known as Tamarindus indica L. is coming from Leguminose, Caesalpiniciacae family and originated from the tropical Africa. The tamarind tree has also been planted in South and North of America from Florida to Brazil as well as in subtropical India, Philippines, China, Pakistan, Indonesiaand Malaysia. Tamarind fruit has the nutrient for laxative, digestive, expectorant, blood tonic and carminative (Komutarin *et al*., 2004). Tamarind was found to posse's hypolipemic activity (Martinello *et al*., 2006) and hypoglycemic activity (Maiti *et al*., 2005). Tamarind fruitpulp is famous and safe to consume as an important ingredient in sauces and spice in both Asian and Latin American cuisines. Tamarind fruit which has ripened is sweet and suitable for taking as a snack, dessert and can be used in drinks. Tamarind is well known as a candies

among the Mexican in Mexico such as Sino-Peruvian ood uses tamarind-based juice for its distinctive sweet flavor. Usually, in tamarind juice that consumed by the humans, 5% of tamarind pulp is present (Martinello *et al*., 2006).

Fresh fruits of tamarind usually are hard to handle and prepared as a healthy drink. Therefore, tamarind fruit is preserved in a powder form and tabletting process to counter the problems of handling and storage. Dissolution testing also is new for food tablet manufacturing and development. The new formulations of fruit tablets are seldom guided based on in-vitro dissolution rates using United States Pharmaceutical (USP) apparatus method (Baxter *et al*., 2005). Besides, the dry tablets are capable of undergoing some forms of microbial spoilage. This study aim to determine the best fit model to the tamarind fruits tablet dissolution process and the effect of storage time to the microbial stability on the tamarind fruits tablet for a better fruit tablet product development in the future as it can act as a healthy supplement in human health.

MATERIALS AND METHODS

Tamarind fruit pulp preparation: Fresh tamarind fruits were purchased from the wet market at Serdang, Selangor, Malaysia (Selangor wholesale market). After purchase, the fruits were transported immediately to the laboratory. For maximum sanitizing effect prior to processing, the working area, cutting board, knife, plastic containers and other utensils used were washed and sanitized with sodium hypochlorite solution at pH 7. The tamarind pulp was picked and the seeds were removed from the tamarind pulp. After the removal of the crown and skin, the whole fruit was crushed into a pulp using a domestic blender. The pulp was then placed in airtight plastic containers and kept in the freezer (-20°C) until further analysis.

The tamarind was prepared in several containers and added with 10% (w/v) maltodextrin. A food grade maltodextrin DE 10 (R&M Chemicals, Essex, UK) was used. The samples were transferred to a spray dryer (Ben Hay, United Kingdom) operating at constant operational conditions of inlet and outlet air temperatures at 190°C and 90°C, respectively, with a blower velocity of 25,000 rpm and a feed rate of 0.18 kg m^{-1}. The spray dried tamarind powders were then compressed in a sealed plastic container and stored in a refrigerator (4°C±0.5) until further tests were carried out.

Microbial stability (storage test): Microbial stability of tablets was assessed by storing the tablets at room temperature for 1 month. Total Plate Count (TPC) method was used in this experiment to study the growth of bacteria, yeast or mold in the tablets. This method involved serial dilution of the tablet. The dilution was then spreaded and on the plate the number of colonies of bacteria, yeast or mold was counted after incubation.

An amount of 9 g of Plate Count Agar (PCA) powder was put into a conical flask in order to produce 20 plates of agar. Then, 400 mL of distilled water was added to the conical flask and the solution was stirred to form a homogenous mixture. The mouth of the flask was then covered with aluminium foil and the flask was placed into the autoclave at a temperature of 121°C for about 15 min. The autoclave was opened only after the pressure of the autoclave return to zero. The dilution was poured into petri dish until 1/3 of the petri dish. The petri dish was then shaken lightly to distribute the agar around the petri dish. Weight and the agar was cooled down to room temperature.

An amount of 10 g of tablets were placed in a sterile stomacher bag and mixed with 90 mL of 0.1% peptone using stomacher lab machine for 60 sec. Serial dilution was made and 0.1 mL of each dilution was pipette into agar plate. The dilution was swabbed on each agar plate. After that, the plates were incubated for 24 h at a temperature of 35°C. The number of colonies of bacteria, yeast or mold that grow in the plate was calculated using the colony counter. The apparent number of the colonyin both plates of a dilution in dishes that contain 30-300 colonies were calculated using the formula given below:

$$N = \frac{\sum C}{(N1 + 0.1N2)D}$$

Where is the sum of colonies counted on all the dishes retained, N1 is the no of dishes retained in the first dilution, N2 is the no of dishes retained in the second dilution and D is the dilution factor corresponding to first dilution (AOAC, Virginia USA).

In-vitro dissolution kinetic study: Dissolution of fruit tablets was carried out using a dissolution tester (PT-DT8, Germany). Five tablets were put in three media solutions: distill water, simulated gastric medium 0.1N HCL solution pH 1.2 and simulated intestinal medium 0.1M sodium chloride buffer pH 6.8 at temperature of 37± 0.5°C at 50 rpm. About 900 mL of liquid medium was prepared and then poured into the dissolution beaker. Simultaneously, five tablets of 20 mm diameter were placed put inside the same dissolution beaker. At every 2 h time of the interval time sample, 10 mL solution was withdrawn from the dissolution medium and immediately replaced in an equal amount of fresh dissolution medium. The liquid was strained through a filter paper (Whatman No. 1, 0.45 μm) (Tokyo Roshi Kaisha Ltd., Japan) before kept in the 50 mL centrifugal tubes for further analysis. The samples were analyzed for kinetic release of vitamin C using a UV-spectrophotometer (Shimadzu, Model UV-

160A, Kyoto, Japan) at the wavelength of 521 nm. The amount of active ingredients presented in the samples were calculated from calibration curves constructed from the standard solution of United States Pharmacopeia (USP) reference standard test of Vitamin C.

Mathematical modeling: Model dependent methods are based on different mathematical functions which describe the dissolution profile. The dissolution profiles were evaluated depending on the derived model parameters. The model dependent approaches in this study were comparable between three different models; Zero-order kinetic model, First-order kinetic model and Higuchi model (Kenneth, 1990).

For the zero-order model, drug dissolution from tablets that did not disaggregate and release the drug slowly could be represented by the Eq. 1 and 2:

$$(Q_o - Q_t = K_o t) \quad (1)$$

Rearranging the Eq. 1:

$$Q_t = Q_o + K_o t \quad (2)$$

Where:

Q_t = The cumulative amount of drug dissolved in time t

Q_0 = The initial amount of drug in the solution (most times, $Q_0 = 0$)

K_0 = The zero order release constant expressed in units of concentration/time

The data obtained from in-vitro drug release studies were plotted as the cumulative amount of drug released versus time (Hadjiioannou *et al*., 1993).

The release of the drug which followed first order kinetics was expressed by the following Eq. 3:

$$\log Q_t = \log Q_o - Kt/2.303 \quad (3)$$

Where:

Q_0 = The initial amount of drug

k = The first order rate constant and t is the time

Qt = The cumulative amount of drug release at the respective time

The data obtained were plotted as log cumulative percentage of drug remaining versus time yielding a straight line with a slope of -K/2.303. This relationship can also be used to describe the drug dissolution for tablets (Bourne, 2002; Kenneth, 1990).

In addition, Higuchi (1963) described the release of drug frominsoluble matrix as a square root of time dependent process based on Fickian diffusion as in Eq. 4:

$$Q = K_H t^{1/2} \quad (4)$$

where, K_H is the Higuchi dissolution constant. All the data obtained were plotted as cumulative percentage drug release versus the square root of time (Shoaib *et al*., 2016; Kenneth, 1990).

RESULTS AND DISCUSSION

Mathematics empirical relationship (Zero order kinetics, First order Kinetics and Higuchi model): Figures 1-3 show the zero-order, first-order and Higuchi models of the dissolution of tamarind tablets, respectively. The tamarind tablet was dissolved in three different media which were distill water, simulated gastric juice at pH1.2 and simulated intestinal juice at pH 6.8 for 12 h. The coefficient of correlation (R^2) values was determined and tabulated in Fig. 1-3.

The dissolution of the tablets, influenced by the bonding structure within the tablet particle, pore structure of the tablet and the solvent dissolution media. These three factors have effect on the tablet dissolution itself. Tablet dissolution fundamental step is the reaction of the tablet with the fluid and the components of the dissolution medium. This reaction occurs in the solid-liquid interface. There were three factors that gave effect on the dissolution kinetics which is the reaction rate at the

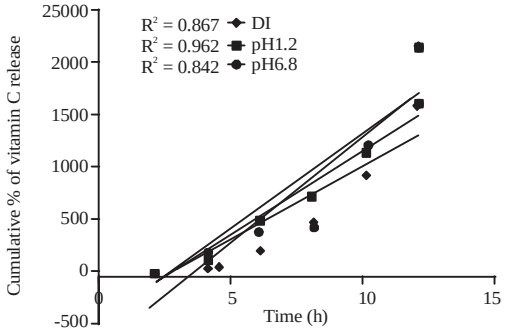

Fig. 1: Zero order kinetics for tamarind tablet

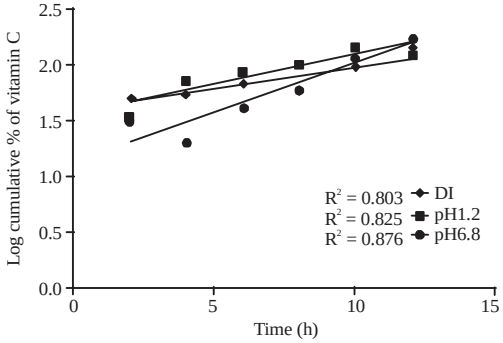

Fig. 2: First order kinetics for tamarind tablet

Table 1: Total plate count (TPC) for tamarind and pineapple for 1 month storage in room temperature

Weeks	Samples	10^{-4} g/mL	10^{-3} g/mL	10^{-2} g/mL	10^{-1} g/mL	Σ C	N1	N2	D	CFU	CFU g^{-1}
1	Tamarind tablet	0	7	8	10	25	2	2	0.01	1136	1.1×10^3
2	Tamarind tablet	3	9	12	23	47	2	2	0.01	2136	2.1×10^3
3	Tamarind tablet	8	13	20	37	78	2	2	0.01	3545	3.5×10^3
4	Tamarind tablet	9	17	24	45	95	2	2	0.01	4318	4.3×10^3

*Data represents CFU (Colony Forming Unit, CFU/g) for summation of all dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} g/mL)

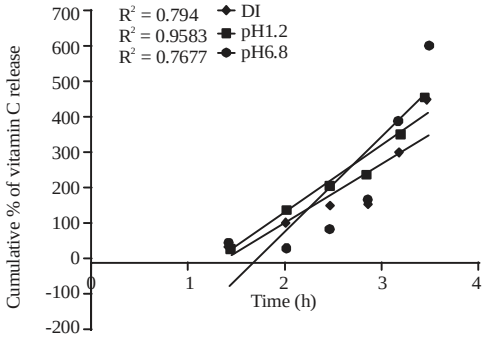

Fig. 3: Higuchi kinetics model for tamarind tablet

interface, the molecular diffusion of the dissolved tablet molecules from the interface toward the bulk solution and the flow rate of the dissolution medium toward the solid-liquid interface. The highest value of R^2 was obtained for the simulated gastric juice pH at 1.2. Among the entire three mathematical models, for simulated gastric juice, the values of R^2 were 0.9626 in zero-order model, 0.8935 in first-order model and 0.9683 in Higuchi model. It was also found that, low value of R^2 is obtained for dissolution in distill water and simulated intestinal juice at pH6.8. Therefore, it can be concluded that the active ingredient of Vitamin C from the tamarind fruit tablets is more efficient to be released in simulated gastric juice media at pH1.2 compared to the other media solution. The acidic solution weakened the strength of particle bonding of the tablets due to the ionic strength effect of the solution to the tablets particle bonding. Apart from that, low pH 1.2 solution has the ability to penetrate into pore structure of the tablets and therefore make the coherent bonding of the compaction tablets to fall apart from each other so that the tablets dissolve faster and the active ingredients in the tablets will release eventually.

In the concentration gradient of the tablets and solvents, the release of solute from the tablets also occurs from areas of high concentration to the area of low concentration. According to the three mathematical models, the best linearity was found to be from the Higuchi equation plot which exhibits the highest R^2 value of the cumulative percentage of vitamin C in all the three linear equations for all the media. This indicates that the vitamin C release rate is independent of concentration. For the other two models which were zero order and first-order kinetics, the coefficient correlation was in the average range as it is seen in the Fig. 1-3, respectively.

Apart from that, application of the empirical model such as the relationship of tablets dissolution of several types of modified release of active ingredients dosage forms,as in the case of some transdermal systems as well as matrix tablets with low soluble tablets in coated forms and osmotic systems can be described by zero-order models (Libo and Reza, 1996; Freitas and Marchetti, 2005). Besides, the tablet dissolution in fruit tablet's active ingredient dosage forms such as containing water-soluble solute in porous matrices was described by first-order model relationship (Narashimhan *et al*., 1999; Dash *et al*., 2010). Lastly, the tablet dissolution from several types of modified release active ingredients dosage forms as in the case of some transdermal systems and matrix tablets with water-soluble active ingredient can be described by Higuchi relationship (Higuchi, 1963; Grassi and Grassi, 2005; Shoaib *et al*., 2006).

Microbial stability test: Table 1 shows the total plate count for tamarind tablet after one month of storage and kept at the temperature of 24°C. The colony forming units (CFU g^{-1}) we recalculated for tamarind tablet. The results show that the highest CFU value was obtained after the week 4 of storage which was of 4.3×10^3 CFU g^{-1}. The counted microbial colonies were ranging from 1.1×10^3 CFU g^{-1} until 4.3510^3 CFU g^{-1} throughout week 1-4 of storage. This result indicatesthat the fruit tablets were safe from spoilage even after 1 month storage as the count of microbial colonies were not exceeded >10000 CFU g^{-1}. According to the South African regulations governing fruit juice in Section 15 (1) of the Foodstuffs Cosmetic and Disinfectants Act 1972, the count of microbialmust not exceed 10000 CFU g^{-1} (Maricel *et al*., 2008). Furthermore, tamarind fruit tablet has a very low surface area exposed for microbial contamination in tablet form, so it was found that there were hardly any bacteria and fungi growing on the tablet sample during 1 month of storage.These similar results were found by Adiba *et al*. (2011), for storage of food tablets made from dates and spirulina. The growth of microorganisms can spoil the fruit tablets. Common spoilage microorganisms associated with the fruits and food sample were yeasts and molds, Lactobacillus, Leuconostoc and thermophilic Bacillus, Escherichia coli, Saccharomyces cerevisiae and Listeria innocua are. In this study, all the microbes were counted in four different dilution concentration where the highest concentration was 10^{-1} and the lowest concentration of dilution was 10^{-4}. The counted colony

microbial was found to be higher in the high concentration of dilution compared to the low concentration of the sample. Moreover, increasing storage time increases the cohesiveness of the powder. The fruits powder cohesiveness can be increased and may lead to the caking of fruit powder it is likely due to the partial melting of the fruits fat resulting in the formation of liquid bridges between particles causing an increase in cohesion due to the capillary forces. According to Da Costa *et al*., (2013), the colony forming units value for passion fruit (*Paasiflora edullis f. flavicarpa*) powder was 0.6×10 CFU g^{-1} indicating that it was below 10^3 CFU g^{-1}.

CONCLUSION

Zero order kinetics model, first order kinetics model and Higuchi kinetic model have been established to describe the relationship of tablet dissolution and active ingredients of vitamin C release patterns mathematically. It is revealed that the active ingredients release kinetics in in-vitro dissolution of the tamarind fruit powder tablets correspond best to the Higuchi kinetics model as the R^2 value was high and consistent compared to zero-order and first-order release kinetics models. The tamarind tablets remained practically stable in the storage conditions tested and meets the microbiological standard, offering great potential for food beverage development.

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