

Devising Interactive Dissolution Experiment for Pharmacy Students (part I): Use of USP Type II Apparatus for Comparison of Immediate Release and Enteric Coated Tablets in Time Varying pH Conditions

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Received: 08.Aug.2016; Accepted: 15.Sep.2016; Published Online: 24.Sep.2016

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Abstract

Laboratory experiments are valuable tool for enhancing student's understanding of theory and practice of science. This manuscript is first part of series which reports interactive dissolution experiments to enhance student's perception about how conventional and advanced dosage forms are processed by the human body after oral administration. We used USP type II apparatus for this experiment at paddle speed at 50 rpm and temperature at 37°C. pH variations were simulated using hydrochloric acid (HCl) for acidic pH and sodium hydroxide (NaOH) to neutralize it. Commercially available immediate release (Disprin CV) and enteric coated tablets (Loprin) were used as model pH independent and pH sensitive drug delivery systems, respectively. Results showed that immediate release tablet released all contents at acidic pH in less than one minute. On the other hand, enteric coated tablets remained unaffected at acidic pH during 30 min exposure time. When pH of the medium reached to neutral, enteric coated tablet also started releasing drugs and whole drug was released in about 30 min. This study provided students with visual presentation of how enteric coated and immediate release tablets may differ in pattern of drug release in different regions of gastrointestinal tract. Students participated actively in the laboratory experiment and forecasting expected results.

Keywords: Laboratory experiments, dissolution studies, pH sensitive, drug release.

To cite this article: Madni, A., Kanwal, R., Tariq, M., Baloch, A., Shah, K., Jabbar, A., Khan, M.I., Rehman, M., 2016. Devising Interactive Dissolution Experiment for Pharmacy Students (part I): Use of USP Type II Apparatus for Comparison of Immediate Release and Enteric Coated Tablets in Time Varying pH Conditions. *PSM Biol. Res.*, 01(2): 83-87.

INTRODUCTION

Laboratory experiments are very interesting to students and increase their understanding of the scientific theories. They also allow students to get hand on practice of science. Research is a key component of the pharmaceutical science and laboratory experiments creates opportunities for pharmacy students to learn critical research skills and participate in ongoing faculty-led efforts to design new pharmaceutical agents, or pharmaceutical products (Levitt and List, 2007; Kiersma *et al.*, 2009). Devising interactive experiments will enhance involvement and active participation of students. In this way, they can acquire required practice and skill in order to produce results worthy of dissemination to scientific society (Berry *et al.*, 1999; Roche, 2007; Yu-Chihd *et al.*, 2015; Qiufen, 2015). One of the key challenges faced by faculty of pharmacy is to determine how to diminish time constraints for students interested in participating in research. In addition, students

lack interest in laboratory work if they are not aware of purpose and aim of the study (Hart *et al.*, 2000). To achieve this goal, students must be given comprehensive knowledge of the science behind the experiment and experimental design must be correlated with theory so that they can foresee the expected results. Dissolution experiments are part of Pharm D (5 years pharmacy graduation program) and M.Phil pharmaceutics (2 years postgraduate course) curriculum (HEC, 2016). Knowledge of operating variables for a dissolution apparatus is important to the pharmaceutical students interested in product development, quality assurance and research applications (Philip *et al.*, 2015).

Dissolution studies are *in vitro* indicators of *in vivo* performances. Dosage form is placed in excess of solvent, known as dissolution medium, which leads to release of drug from dosage form, into dissolution medium. Drug release from tablets occurs in three stages. First, tablet disintegrates into granules which is followed by dissolution of the drug in

gastric media. Thirdly, drug in solution form is absorbed through the biological membrane. USP type 2 apparatus used for dissolution studies consist of vessels containing dissolution medium which is stirred with the help of paddles (Singh, 2006). Along the GI tract, significant variations in pH have been observed which has been exploited for the purposes of delayed release therapies (Figure 1). pH of empty stomach lies between 1.2-2 and transient time is 3-4 hours. The acidic medium of stomach neutralizes to some extent (pH 6) as it passes to and reaches up to pH 7.4 in the terminal ileum. pH drops again in cecum to pH 6 or below and raises again as it reaches to the colon. The pH of rectum is around 6.7. However, pH ranges can exhibit variability between individuals, with factors such as water and food intake as well as microbial metabolism being major determinants. Residence in specific portion of GIT can alter delivery of the formulation and dissolution of the drug at the site of action, the intestinal fluid volume, and the propensity of the formulation or drug to be metabolized in the GI tract through enzymatic or microbial degradation. After oral administration, tablet will stay in stomach for 2-3 hours which is known as transit time. Transit time of small intestinal is around 4 hours although it dependence upon physical status and disease condition between 2 to 6 hours. Next, transit time of colon is 6 to 7 hours. However, these values are not absolute and can be influenced by gender and the time of dosing with respect to an individual's bowel movements. Intestinal fluid secretion also affects the viscosity of the mucous-gel layer, which may influence the ability of drugs to be taken up by cells at the site of action (Hua et al., 2015).

Part of GIT	Transit Time	pH
Mouth (Saliva)	— (Negligible)	6.8-7
Stomach	3-4 hours	1.2-2
Small Intestine	4 hrs (2-6 hrs)	6-7.4
Large Intestine	6-70 hrs	6-6.7

Fig. 1. Transit time and pH of different regions of GIT to which a dosage form is being exposed after oral administration.

Tablet is a pharmaceutical drug delivery system consisting of drug and inactive excipients, pressed or compacted into a solid unit dosage (Singh, 2006). Medicinal tablets are often called as pills. Conventional tablets are those in which the rate of release of drug from the formulation and/or the absorption of drug, is neither appreciably, nor intentionally retarded by galenic manipulations. Immediate release tablets are designed in such a way that drug is dissolved immediately when added into the dissolution medium. An enteric coating is a barrier applied to the tablet, so that it can target medication to specific portion of GI tract by responding to its pH and undergo dissolution. The term "enteric" means intestine i.e. enteric coated tablet will release drug in intestine. The enteric coating materials are usually polymers that remain unionized, an insoluble form, at acidic pH of stomach. They become ionize only at or near neutral pH of intestine and solubilize in aqueous medium. The advantages of enteric coating can be classified into four classes (Wen and Park, 2011; Shargel et al., 2007):

1. Protection of drug from degradation in acidic pH and enzymes of stomach.
2. Protection of stomach from unwanted effects of drugs such as gastric distress or nausea.
3. Drugs that show maximum absorption in the small intestine.
4. Delayed-release of loaded drug for repeat action tablets.

Although different polymers show different response to pH of stomach, thickness of polymer is another factor that can affect efficiency of enteric coated tablet. A thicker tablet physically retards drug release which may be undesirable. Generally, enteric coated tablets are made for NSAIDs that are prescribed for long term use such as aspirin, diclofenac and naproxen (Hussan et al., 2012). Dissolution experiments of conventional dosage forms are mostly included in pharmacy curriculum. However, dissolution experiments of enteric and immediate release tablets should also be included in curriculum to enhance student's knowledge in accordance with the advancement in the field. In this paper, expert from academia and postgraduate students designed a dissolution experiment for pharmacy students which will provide deeper insight into time course of dosage forms in GIT and effect of varying pH in different parts of GIT on tablets. Commercially available tablets of aspirin have been selected for this purpose.

MATERIALS AND METHODS

Materials

Disprin CV (immediate release tablet) and Loprin (enteric coated tablets) were purchased from retail pharmacy. Aspirin, sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck, Germany.

Preparation of Standard Curve

Standard curve is prepared from known concentration of drug and is used to identify concentration of drug in samples of dissolution studies (Sohail et al., 2015). Serial dilutions of aspirin from 0.0375 mg/ml to 0.6 mg/ml were analyzed spectrophotically at 254 nm. The absorbance of each dilution was measured three times and average of three absorbance values was recorded. Using MS excel, the concentration of dilutions was plotted against the absorbance to prepare the standard curve of aspirin (Figure 2). The trend line along with its equation and regression coefficient (R^2) was added using MS excel 2010.

In vitro dissolution studies

In vitro dissolution studies are carried out to surrogate behavior of tablets in the body (Shahzad et al., 2016). We used USP Apparatus 2, also known as paddle apparatus, at a speed of 50 rpm, dissolution medium volume of 500 mL, and temperature of $37 \pm 0.5^\circ\text{C}$. Samples were taken manually at different time intervals following pool sampling technique i.e. equal volume of dissolution medium was added after each sample. The samples were passed through 0.45 μm Whatman filter. Then, absorbance of each sample was measured at 265 nm by using a UV spectrophotometer (Irmeco U2020, Germany) and fitted in calibration curve to find drug concentration. Percentage of drug released data was plotted against time (Figure 3).

Time varying pH simulation

To simulate gastric pH of 1.2, we added 8 ml hydrochloric acid (HCl) in the distilled water in 6 vessels. We started the paddle apparatus at predefined stirring speed and added Dispirin CV in the first, second and third vessel and Loprin in fourth fifth and sixth vessel. Samples were taken for every 15 seconds for 1 minute and then after every 15 min to check which tablet remained undissolved or dissolved in acidic pH. After 30 min, pH was neutralized (as stomach pH convert to almost neutral pH of intestine that is the target site of the enteric coated tablet) by addition of 1M sodium hydroxide (NaOH) solution. Then, samples are taken every 15 min until whole drugs was dissolved in dissolution medium. Although gastric residence time is around three hours, we conducted dissolution in acidic pH for 30 min to reduce time of experiment and make it feasible for pharmacy curriculum, which allows around 2 hours for each experiment.

RESULTS AND DISCUSSION

The purpose of this study was to enhance student understanding of how different types of tablets i.e. immediate release and enteric coated, are processed in different parts of GIT as function of varying pH. An interactive dissolution experiment was designed to simulate time varying pH conditions of GIT. The tablets were exposed to acidic pH of stomach and, then, to the neutral pH which is encountered in initial portions of the intestine. For convenience, acidic pH was maintained for 30 min which was sufficient to complete

dissolution of tablets intended to dissolve in stomach. This will permit to complete the experiment in shorter time which is usually allocated for laboratory experiments (HEC, 2016).

Standard curve of aspirin was prepared for estimation of drug in dissolution samples (Figure 2).

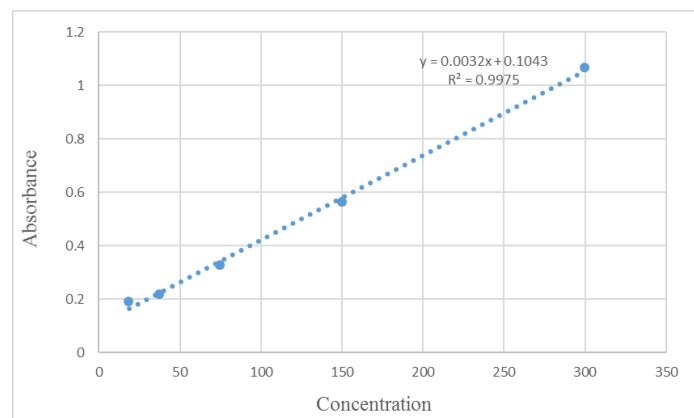


Fig. 2. Standard curve of aspirin for calculation of amount of drug in the samples.

An R^2 value of 1.0000 indicates perfect correlation (Hammarlund-Udenaes et al., 2013). The calibration curve showed R^2 of 0.9975 which is very close to 1. Thus, standard curve was considered reliable to predict amount of drug release from the tablets. Equation given in standard curve (eq. 1) was rearranged to predict the amount of drug from tablets from the absorbance of sample (eq. 2) as follows;

$$y = 0.0032x + 0.1043 \quad (\text{eq. 1})$$

$$x = \frac{y - 0.1043}{0.0032} \quad (\text{eq. 2})$$

Here, x indicates the amount of drug released and y indicates the absorbance of the sample obtained at different time intervals in dissolution studies.

In case of immediate release Dispirin CV tablets, for which stomach is favorable medium for drug release, dissolution of drug was faster and whole drug was released within first minute of dissolution experiment (Figure 3). Drug release in acidic conditions of stomach has been represented by first segment of figure 3 representing whole drug release from immediate release tablets and no drug release from enteric coated tablets. Immediate release profile of NSAIDs is desirable in emergency conditions to get instantaneous therapeutic level (Polski et al., 2013). Dispirin CV is manufactured for cardiovascular patients, hence named CV, and administered upon attack of angina pectoris (WWM, 2016). In case of enteric coated Loprin tablet, release profile appeared only after pH of dissolution medium was increased to neutral. As indicated in second segment of figure 3, whole drug release occurred at neutral pH in 45 min. This means that there may be no drug release from

Loprin in stomach due to acidic pH whereas drug may be released in intestine (PharmaGuide, 2016).

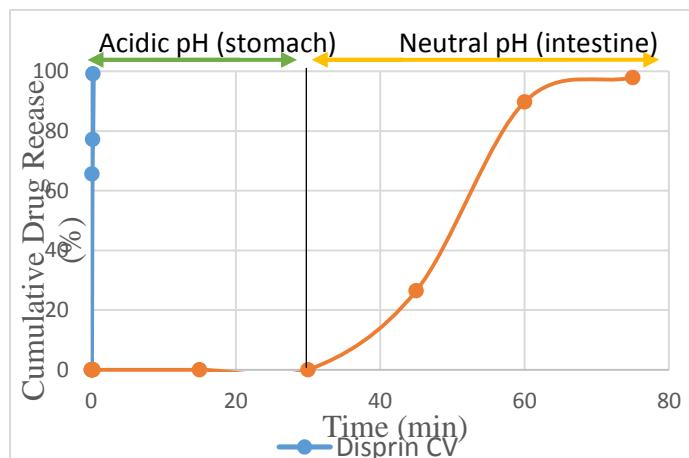


Fig. 3. Dissolution profile of immediate release Disprin CV (blue line) and enteric coated Loprin tablet in time varying pH conditions.

Loprin is enteric coated tablet containing aspirin which is indicated to the patients who need aspirin for longer period of time (Pharma Guide). In this way, stomach is not exposed to aspirin and is prevented from over secretion of acid, and hence, gastric ulceration (Hussan et al., 2012). Patients receiving NSAIDs in enteric coated tablets can take drug for longer period of time with improved efficacy and reduced side effects. This leads to improved quality of life and patients can actively participate in routine work (Ishikawa et al., 2014). Dissolution studies of commercially available brand NoClot EA was also conducted although not included in this manuscript. NoClot EA tablet contains clopidogril immediate release and aspirin enteric coated granules compressed together in single tablet (CCL, 2016). Initially in acidic pH, whole tablet was disintegrated into granules and clopidogril was released immediately whereas aspirin was released only after reaching neutral pH. However, authors consider these results out of the scope of this manuscript and considered it a subject of future directions.

This dissolution experiment performed in this manuscript will be interesting to pharmacy students because they can visualize time course of drug release from different dosage forms in one experiment (Figure 4). It also provides a platform for designing similar experiments to assess dissolution profiles of various controlled release formulations (Bhise and Deshpande, 2014). We observed that all students actively participated in the experimentation, data collection and analysis. Students were given comprehensive theoretical background of the study and they were able to forecast the expected results of the study. They were also able to state applications and future perspectives for devising new research techniques.

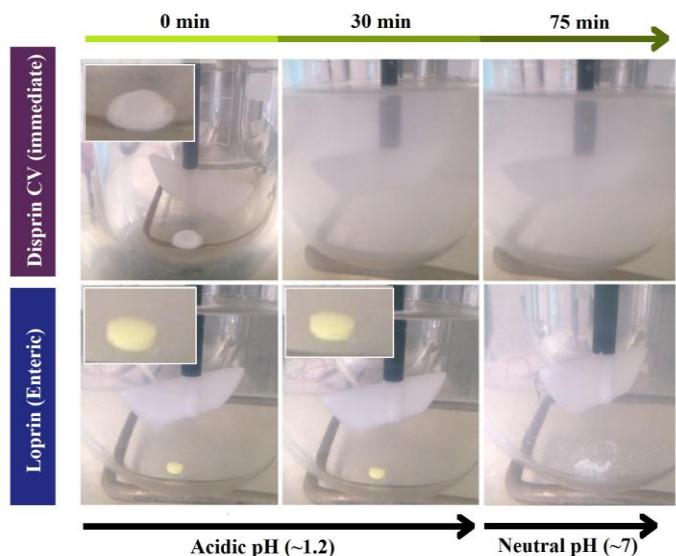


Fig. 4. Visual representation of drug release of immediate release tablet and enteric coated pH sensitive tablets in time varying pH dissolution conditions.

CONCLUSION

This interactive dissolution experiment provided visual representation to students of the drug release behavior of immediate release and enteric coated tablets in simulated time varying gastrointestinal pH. Regardless of the pH of dissolution medium, immediate release tablets released drug in less than one minute. Enteric coated tablets remained intact at acidic pH and released drug only when pH of the dissolution medium reached to neutral. As compared to immediate release tablets, enteric coated tablets safeguard stomach and release drug in intestine.

ACKNOWLEDGEMENT

Authors would like to thank the Dean Faculty of Pharmacy and Alternative medicine, and the Chairman, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan, for providing necessary facilities and materials.

CONFLICT OF INTEREST

Funding: none.
Conflict of interest: none.

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