

PREPARATION AND INVESTIGATION OF ACETYL SALICYLIC ACID-GLUTAMIC ACID COMPLEX: A NOVEL ORAL DELIVERY SYSTEM

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Acetyl salicylic acid and glutamic acid complex prepared by freeze-drying technique was proposed as a novel oral drug delivery system. The complex was analyzed by using differential scanning calorimetry (DSC), electron microscope, ¹H nuclear magnetic resonance (¹H NMR), mass spectrometry and fourier transform infrared (FTIR). The results revealed a formation of an ionic type complex between acetyl salicylic acid and glutamic acid in a molar ratio of 1:1. In-vitro dissolution testing of the formed complex was performed at pHs of 1.2 and 6.8. The results showed a complete dissolution of the complex within ten minutes. The effect of complex on the gastric mucosal tissues showed that the mucosal epithelium appeared normal (intact) without any change. The present results confirmed that acetyl salicylic acid glutamic acid complex is a promising and safe delivery system that can be utilized for oral administration.

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1. Introduction

Acetyl salicylic acid (ASA), Fig. 1A, has different therapeutic effects depending on the drug dose and administration pathway. Aspirin is the basic antiplatelet agent for all kinds of acute disease that may cause platelet-dependent thrombotic vessel occlusion [1]. In addition to its influence on platelet cyclo-oxygenase, aspirin inhibits the activity of platelet adenylate cyclase found in the platelet membrane. The effect is probably caused by an unspecific acetylation of platelet membrane proteins, an effect that has already been suggested following the observation of platelet shape changes during aspirin therapy. Thus, high doses of aspirin suppress not only prostaglandin plasma levels but also make the platelets less sensitive to the dis- and anti-aggregatory effects of this prostaglandin. Since platelet adenylate cyclase activity during low-dose aspirin treatment is not changed, but the anti-aggregating effect is well preserved, aspirin should be taken in relatively small doses if used as anti-platelet drug [2].

Acetylsalicylic acid inhibits both cyclo-oxygenases I and II, which may lead to a dramatic decrease of thromboxane A₂ synthesis, and that in turn leads to an inactivation of platelet aggregation. Because platelets are degenerated cells without any nucleus, the inhibitory effect induced by aspirin is irreversible [3]. Some nonsteroidal anti-inflammatory (NSAIDs) can damage the gastric mucosa by a direct effect. Acidic NSAIDs, including aspirin, become lipid soluble at low pH. They can cross the lipid barrier into gastric mucosal cells. At intracellular pH, they lose lipid solubility and become trapped, disrupting cell function, perhaps by inhibiting mitochondrial

oxidative phosphorylation [4, 5]. Damage to surface mucosal cells compromises the normal protective mechanisms, reducing resistance to acid damage. Mucosal erosions and hemorrhages can be seen within 90 minutes of ingestion of a single 75 mg tablet of aspirin [6].

Darling et al. [7] showed that aspirin can induce gastric injury in transgenic mice deficient in either COX-1 or COX-2. They found that the negative association between aspirin-induced gastric injury and mucosal surface hydrophobicity is consistent with the possibility that aspirin topically injures the mucosa by increasing the tissue's wettability to luminal acid. The role of surface phospholipids is the mechanism by which aspirin disrupts the gastric mucosal barrier was further supported by evidence that aspirin-induced gastric injury in COX-1 knockout mice could be prevented if the animals were administered phosphatidyl choline/aspirin. In view of the increasing use of low dose aspirin prophylaxis for cardiovascular disease, it was attractive for many researchers to formulate ASA as enteric coated dosage forms in order to prevent its gastric side effects [8-11].

Glutamic acid (Fig. 1B) or glutamate can be synthesized in the body from arginine, ornithine and proline and is found in high concentration in the brain. In the body it is converted to glutamine and is the only amino acid metabolized by the brain. Glutamic acid (Glu in the zwitterionic form) is a potential legend with the characteristic of having two carboxylate groups. It opens the possibility to obtain structures with different kinds of carboxylate bridges [12]. L-Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system that contributes not only to fast synaptic neurotransmission, but also to complex physiological processes like memory, learning, plasticity, and neuronal cell death [13, 14]. Glutamate is synthesized in the cytoplasm and stored in synaptic vesicles by an uptake system that depends on the proton electrochemical gradient, the vesicular glutamate transporters [15]. Glutamate is thought to be released synaptically and extrasynaptically by exocytosis [16], cystine-glutamate antiporter [17] and volume-regulated anion channels [18]. However, most of the glutamate is released synaptically and transits through the glutamate-glutamine cycle before being repackaged into synaptic vesicles [19, 20].

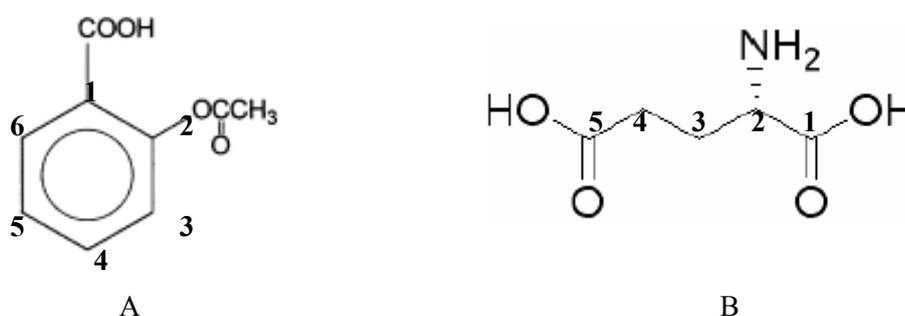


Fig. 1. Chemical structures of: (A)- Acetyl salicylic acid (ASA), and (B)- Glutamic acid

The present work was undertaken to: (1) prepare and investigate the formation of acetyl salicylic acid glutamic acid complex, (2) characterize the formed complex using nuclear magnetic resonance (¹H NMR) infrared spectrometry (FTIR), differential scanning calorimetry (DSC) and scanning electron microscope (SEM), and to investigate the effect of the formed complex on the gastric mucosal cells using Wister rats as an animal model.

2. Experimental

2.1 Materials

Aspirin was purchased from E. Merck Company (Darmstadt, Germany) and L-glutamic acid was obtained from Winlab Ltd. (Leicestershire, UK). All other chemicals were either of analytical or reagent-type grade.

2.2. Methods

2.2.1 Preparation of ASA glutamic acid complex by freeze-drying technique

Equimolar (0.01 mole) weights of ASA and L-glutamic acid were accurately weighed. L-glutamic acid was dissolved in 400 ml distilled water (HPLC grade). Then ASA was added and dissolved in the same vessel. The prepared solution was allowed to freeze overnight at (-20 °C) and then lyophilized over a period of 72 hrs using a freeze-drier (Alpha 1-4 LD-2, Martin Christ, Osterode, Germany) under the following conditions: (temperature= -59 °C, vacuum= 0.090 mbar). The dried powder were then collected and stored at room temperature in a dessicator until further investigations.

2.2.2 Evaluation of the ASA glutamic acid complex

2.2.2.1 Differential scanning calorimetry (DSC)

DSC studies were carried out using differential scanning calorimeter equipped with an intercooler (Shimadzu DSC-60, Shimadzu Corporation, Koyoto, Japan). Indium/zinc standard were used to calibrate the temperature and enthalpy scale. The samples were hermetically sealed in an aluminum pans and heated at a constant rate of 10 °C/min over a temperature range of 25- 400 °C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

2.2.2.2 Morphology of the prepared crystals

Samples morphology was examined under scanning electron microscope (Jeol, JSM-6360LV Scanning Microscope, Tokyo, Japan). Before microscopy, the dried microparticles were mounted at carbon tape and were sputter-coated using gold (Jeol, JFC-1100 fine coat ion sputter, Tokyo, Japan). The photomicrographies were taken at an acceleration voltage of 15 kV.

2.2.2.3 FTIR spectroscopy and mass spectroscopy

Fourier transform infrared (FTIR) spectrum was recorded on Perkin Elmer FTIR instrument (Perkin Elmer, Waltham, Massachusetts, USA). Samples were prepared as KBr pellet and scanned against a blank KBr pellet background at a wave number ranging from 4000 to 650 cm^{-1} with resolution of 1.0 cm^{-1} . The observed bands were given in cm^{-1} . For ASA: 2871, 2699, 2588, 2345, 1753, 1690, 1606, 1576, 1505, 1484, 1458, 1419, 1371, 1307; for glutamic acid: 3057, 2740, 1646, 1516, 1420, 1352.4, 1312 and for the complex: 3061, 2364, 2344, 1753, 1686, 1646, 1606, 1514, 1458, 1420, 1371, 1356, 1308. Studies of the mass spectra of the prepared complex, ASA and L-glutamic acid were conducted using a Perkin Elmer- Clarus MS 60T (Perkin Elmer Waltham, Massachusetts, USA).

2.2.2.4 Nuclear Magnetic Resonance (^1H NMR)

Nuclear Magnetic Resonance (^1H NMR) spectra were scanned on Bruker spectrometer instrument (Bruker Avance 500 MHz Ultrashield NMR, New Jersey, USA). Chemical shifts were

given in δ (ppm) relative to TMS as internal standard. The ^1H NMR spectra for ASA (DMSO- d_6): 2.25 (3H, d, $j=5$ Hz, $\text{CH}_3\text{C}=\text{O}$), 7.19 (1H, s, $\text{C}_3\text{-H}$), 7.36 (1H, d, $j=6$ Hz, $\text{C}_5\text{-H}$), 7.61 (1H, d, $j=6$ Hz, $\text{C}_4\text{-H}$), 7.96 (1H, d, $j=6$ Hz, $\text{C}_6\text{-H}$), 13.12 (1H, s, COOH), for ASA-glutamic acid complex (DMSO- d_6): 2.25 (3H, s, $\text{CH}_3\text{C}=\text{O}$), 7.2 (1H, d, $j=8$ Hz, $\text{C}_3\text{-H}$), 7.38 (1H, t, $j=15,7.5$ Hz, $\text{C}_5\text{-H}$), 7.64 (1H, m, $\text{C}_4\text{-H}$), 7.39 (1H, m, $\text{C}_6\text{-H}$), for L-Glutamic acid (D_2O): 2.09 (2H, m, $\text{C}_3\text{-H}_2$), 2.5 (2H, m, $\text{C}_4\text{-H}_2$), 3.76 (1H, m, $\text{C}_2\text{-H}$), and the ^1H NMR spectra for ASA-Glutamic acid complex (D_2O): 2.04 (3H, m, $\text{CH}_3\text{C}=\text{O}$), 2.11 (2H, m, $\text{C}_3\text{-H}_2$), 2.33 (2H, m, $\text{C}_4\text{-H}_2$), 3.8 (1H, m, $\text{C}_2\text{-H}$), 6.96 (1H, m, $\text{C}_3\text{-H}$), 7.17 (1H, m, $\text{C}_5\text{-H}$), 7.49 (1H, m, $\text{C}_4\text{-H}$), 7.88 (1H, m, $\text{C}_6\text{-H}$).

2.2.2.5 Dissolution testing of the complex

The dissolution profiles of ASA and the prepared complex was examined in triplicate using a DT 600 dissolution tester USP II type (Erweka, Heusenstamm, Germany). The used samples weight were 100 mg ASA powder passing from mesh size 50 μm or equivalent to 100 mg ASA in case of complex. Samples were placed in the dissolution vessel containing 500 ml of 0.1 N HCl, pH 1.2, maintained at 37 ± 0.5 $^\circ\text{C}$ and stirred at 75 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through Millipore filter 0.045 μm , concentrations ASA or of L-glutamic acid ASA complex were determined spectrophotometrically at 273 nm for ASA or 277 nm for complex. All the concentrations were calculated in molar bases.

2.2.3 Histopathological Study

Male Wister rats weighing 250 ± 20 g were fasted for 24 h prior to the experiments but were allowed free access to water. Solution of ASA or complex in 1% sodium carboxymethylcellulose (20 mg/ml) was administered orally into the stomach in a dose of 200 mg/kg (1 ml/100 gm) according to Hemmati et al. [21] was administered orally through the stomach sondle needle. At 4 h after administration, the stomach was isolated, opened along the greater curvature, rinsed with a saline solution, and fixed in 10% formalin phosphate buffer solution (pH 7.4) prior to embedding. Then, the specimens were embedded in paraffin using an embedding center and cut into slices. The slices were stained with hematoxylin-eosin and observed microscopically [22, 23] under a light microscope (Leitz Laborlux 12 Pols; Ernst Leitz GmbH, Wetzlar, Germany)

3. Results and discussion

3.1 Differential scanning calorimetry

Thermal and spectroscopic methods considered as the main tools in studying and identifying the physical and chemical interactions between drugs, excipients, polymers, or degradation [24-27]. Fig. 2 illustrates the thermal behavior for each of ASA, L-glutamic acid and their complex. According to Fig. 2 and Table 1, the thermogram of ASA showed a characteristic sharp endothermic peak at 138.02 $^\circ\text{C}$ corresponding to its melting point and ΔH 194.03 mj/g. However the thermogram of L-glutamic acid resulted in a sharp peak at 207.51 $^\circ\text{C}$ corresponding to its melting point and ΔH 496.72 mj/g. The freeze-dried powder showed an interaction conducted between the two compounds giving a new small endothermic peak having lower melting point, 113.3 $^\circ\text{C}$, and lower heat, ΔH 7.47 mj/g. At the same time, the peaks of ASA and L-glutamic acid were completely disappeared. The present result revealed the formation of complex between ASA and L-glutamic acid in water that can be collected by freeze-drying method.

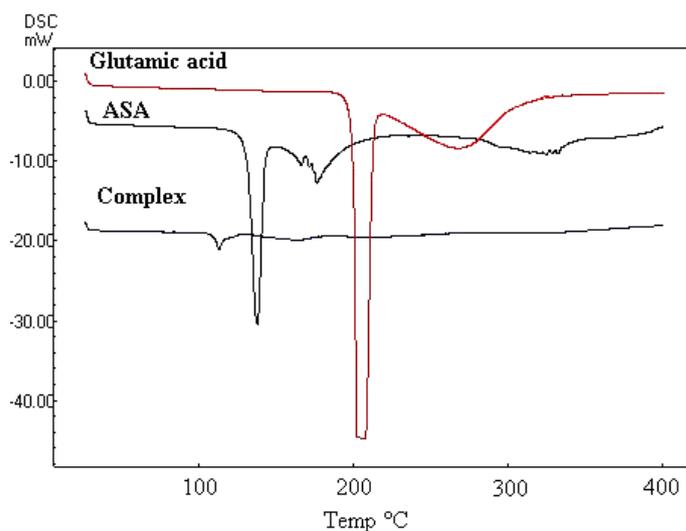


Fig. 2. DSC of ASA, L-glutamic acid and the formed complex

Table 1. Peak temperature and enthalpy (ΔH) obtained from DSC thermogram

Parameter	ASA	L-Glutamic acid	Complex
Peak °C	138.02	207.51	113.3
Heat (ΔH)	- 970.16 mj	- 2.48 j	- 37.35 mj
Heat mj/g	- 194.03	- 496.72	- 7.47

3.2 Scanning electron microscopy

Fig. 3 depicts the scanning electron microscope photographs of the used ASA, L-glutamic acid and the formed complex. The morphological shape of the formed complex crystals showed a clear and distinct difference in shape. These crystals of the formed complex appear as aggregation of flakes to form a snail like structure of a rough surface with holes. However, ASA crystals were needle-like shape and that of L-glutamic acid was a large of orthorhombic shape particles (Fig. 3).



Fig. 3. Scanning electron microscope micrographs and surface morphology of ASA, L-glutamic acid and complex crystals

3.3 Spectroscopic studies

3.3.1 Mass spectrometry

As the molecular weight of ASA is 180 and that of L-glutamic acid is 147, the expected molecular weight of the complex is the sum of the two molecular weights i.e 327. Mass spectrum showed a small peak at 327 representing the molecular ion peak which reflected the formation of a complex with a stoichiometric ratio 1:1.

3.3.2 FTIR spectroscopy

The FT-IR technique provides information concerning both the strength of the bonds between atoms and is a useful tool to study the interaction between the organic compounds. Fig. 4 showed the FTIR spectrum of ASA, glutamic acid and the formed complex, while Table 2 presented the bands frequencies of each of ASA and glutamic acid that were affected as a result of their interaction.

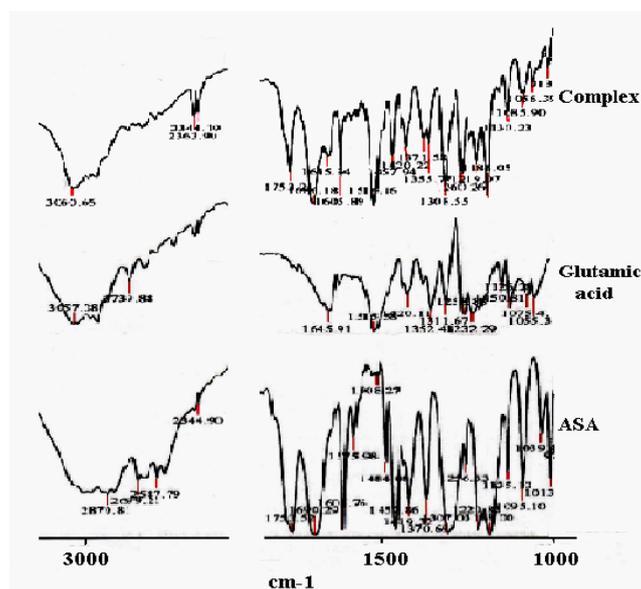


Fig. 4. FTIR chart for ASA, L-glutamic acid and the complex.

Table 2. The affected FTIR bands (cm^{-1}) of ASA and glutamic acid by complex formation

FTIR bands (cm^{-1})		
ASA	Glutamic acid	Complex
1690	-	1686
	3075	3061
	1516	1514
	1356	1356

As glutamic acid is an amino acid characterized by the presence of amino group and two carboxylic groups, it was noticed that the stretching and bending bands of the quaternary nitrogen bands wave numbers were decreased reflecting electron donation from the NH_3^+ . Consequently, there was an increase in the wave number of the stretching band of C-N from 1352 to 1356 cm^{-1}

reflecting an electron withdrawing effect. However, the bands of carboxylic groups were not affected. On the other hand, the effect of the interaction on the FTIR spectrum of ASA was noticed through the band of the C=O of the carboxylic group, where it was slightly shifted towards lower frequency value from 1690 to 1686 cm^{-1} . At the same time, the bands of C=O of the aryl ester of ASA did not show any changes in its wave numbers. This data may reflect an interaction between the carboxylate and the quaternary nitrogen.

3.3.3 ^1H NMR Spectroscopy

The ^1H NMR results of ASA and the effect of glutamic acid on its ^1H NMR, each of ASA and complex (dissolved in DMSO) showed the following characteristics: (1) the protons of the acetyl group did not show any change in their behavior, (2) the aromatic protons at carbons number 3, 4, and 5 showed a down-field shift, while the proton at carbon number 6 showed up-field shift. All these chemical shifts lies between 0.01 and 0.03 ppm which appear insignificant, (3) the carboxylic acid proton at 13.12 ppm disappeared in the chart of the complex and (4) the glutamic acid protons were detected but they were not integrated in an unexplained phenomenon. However, the data obtained from the ^1H NMR for the effect of ASA on the glutamic acid each of glutamic acid and the complex in D_2O , revealed: (1) the appearance of two protons around 2.09 ppm of C_3 which was insignificantly up-field shifted to 2.11 ppm in case of complex, (2) the two protons at C_4 and the one at C_2 showed up-field shifting by 0.17 and 0.04 ppm, respectively.

The ^1H NMR results indicated the complex formation; however, the contributed groups can not yet explained or estimated. Accordingly, the given spectral data indicated the formation of complex between ASA and glutamic acid in 1:1 molar ratio. This complex, most probably, can be described as an ionic type complex involved both the carboxylic and the amino function groups of ASA and glutamic acid respectively.

3.4 Histopathological evaluation

The effect of complex on the gastric mucosa was studied using Wister rats. As the aim of this work was to decrease the effect of ASA on stomach tissues, therefore, ASA glutamic acid complex was administered orally to male Wister rats and the histological changes were shown in Fig. 5. ASA was reported to irritate the gastric mucosa causing erosion and hemorrhage [6]. It was found that the administration of ASA glutamic acid complex had no dramatic effect on the gastric mucosa as it appeared normal without any noticeable changes.

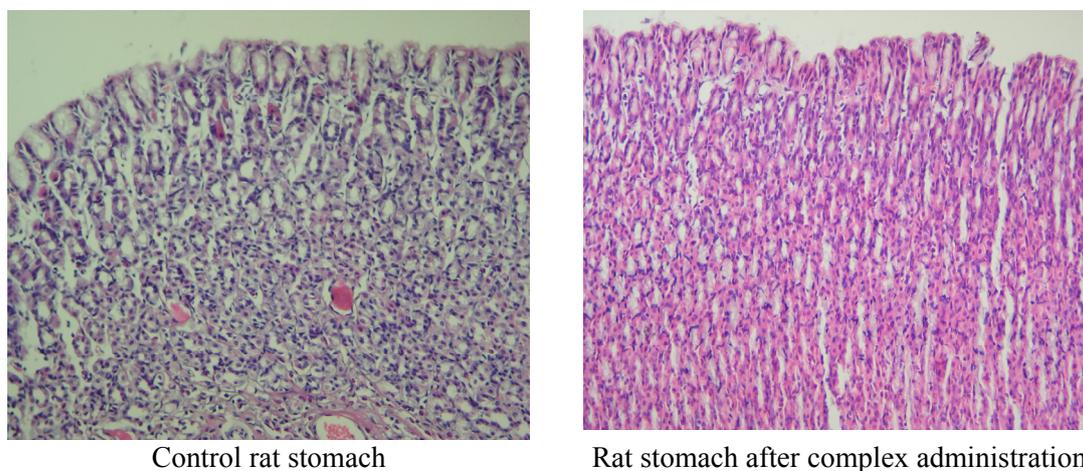


Fig. 5. Morphology of gastric mucosa of rats after oral administration of ASA glutamic acid complex solutions (x200)

3.5 In-vitro dissolution study

Dissolution is the process by which a solid solute enters a solution. In the pharmaceutical industry, it may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition. Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence. Dissolution behavior of drugs has a significant effect on their pharmacological activity. In fact, a direct relationship between in vitro dissolution rate of many drugs and their bioavailability has been demonstrated and is generally referred to as in-vitro/in-vivo correlation [28].

For the aim of studying the release of complex, it was important to establish the method of assessment of its concentration. Fig. 6 showed the maximum wave length of UV absorption of ASA and ASA glutamic acid complex. It was found that, 272 nm was the λ_{max} for ASA and 277 nm for complex (UV-1700, UV-visible spectrometer, Shimadzu, Kyoto, Japan). A standard calibration curves was constructed with r^2 of 0.999 for each of ASA and the complex. Consequently, ASA was determined at 272 nm, while complex was measured at 277 nm. As the complex is a physical complex in 1:1 ratio, each one mole at complete dissociation would give one mole of ASA. In this case, all the release calculations were performed in molar concentrations.

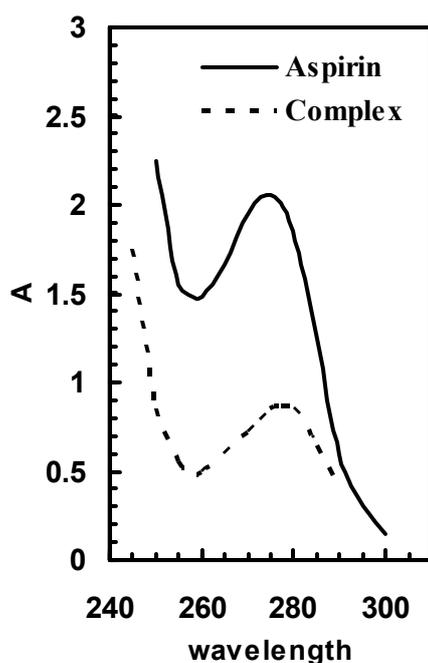


FIG. 6. UV absorbance for each ASA and ASA glutamic acid complex

Each of ASA and the complex was allowed to dissolve in HCl, pH 1.2, as presented in Fig. 7. ASA was found to completely dissolve between 40 and 45 min. However, the complex was completely soluble within ten minutes. Accordingly the relative dissolution rate RDR was calculated at times 5 and 10 min. as presented in Table 3. The results revealed an improvement in the dissolution of ASA by the formation of a complex with glutamic acid.

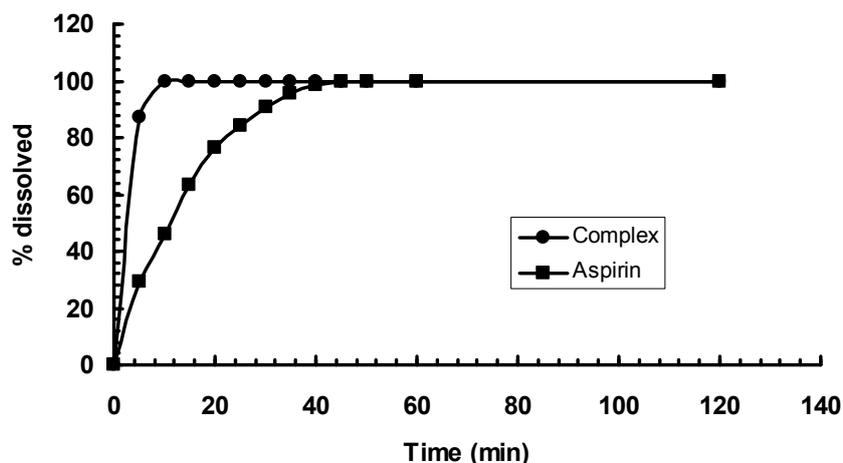


Fig. 7. Dissolution of ASA and ASA glutamic acid complex in HCl at pH 1.2

Table 3. Percent dissolution of ASA and its glutamic acid complex and the RDR

Time	Percent Dissolved		RDR*
	Complex	ASA	
5	87	29.469	2.952
10	100	46.154	2.167

* RDR= % ASA dissolved/%complex dissolved

4. Conclusions

A novel acetyl salicylic acid-glutamic acid complex has been successfully prepared using freeze-drying method. Histologically, the complex did not result in any change or damage on the rats' gastric mucosa after its oral administration. It is assumed that ASA glutamic acid complex will be a safe and a promising alternative to ASA for oral administration for long-term use of acetyl salicylic acid.

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