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FORMULATION AND *IN VITRO* EVALUATION OF ASPIRIN AND ISOSORBIDE 5-MONO-NITRATE SUSTAINED BILAYER TABLETS

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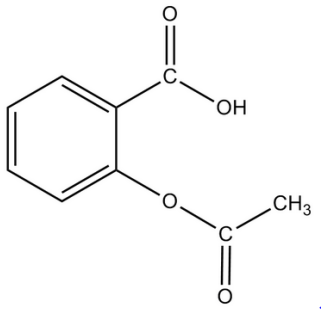
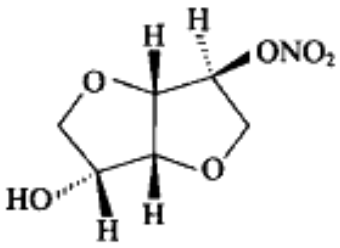
ABSTRACT: In this study, aspirin (AS) and isosorbide 5-mononitrate(ISM) sustained bilayer tablets were developed by wet granulation and compression technique, the influence of the contents of PVPP on the dissolution of AS from the tablets was investigated. An orthogonal experiment design was used to optimize ISM sustained release layer. The tablets were tested for their drug content, hardness, thickness, friability, and in vitro release characteristics. The optimized formulation of sustained bilayer tablets contains ISM 60 mg, HPMC K100M 45 mg, CMC-Na 12 mg, HPMC K15M 12 mg, lactose 50 mg and aerosol 3 mg for sustained release layer of ISM, PVP K30 Ethanol solution was used as adhesives. The optimized formulation of sustained bilayer tablets contains AS 75mg, PVPP 10mg, citrate acid 15mg, MCC 35mg, pregelatinized starch 15mg and 2mg talc for fast release layer. The drug release of AS was above 80% at 0.5h hour and ISM was above 70% at 7h in the optimized formulation.

INTRODUCTION: Aspirin chemically known as acetyl salicylic acid is used as a non-steroidal anti-inflammatory and analgesic drug. The structural formula of AS is as follows:



Isosorbide 5-mononitrate, chemically known as 1, 4:3, 6-dianhydro- D -glucitol 5-mononitrate, is used as antianginal drug. The structural formula of ISM is as follows:



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A combination of AS of 75 mg and ISM of 60 mg is commercially available tablet form. This medicine contains two active ingredients, namely AS and ISM. AS is a non-steroidal anti-inflammatory drug (NSAID) which is widely used for treatment of pain, fever and other inflammatory conditions [1].

In addition to the well-known anti-inflammatory, analgesic and antipyretic properties, AS also inhibits platelet aggregation, is useful in preventing myocardial infarction and stroke, has neuroprotective actions, and decreases the incidence of cancer [2-4]. Apart from these, AS shows a variety of pharmacological activities, including reducing ATP storage pools, increasing extracellular adenosine, lowering inducible nitric oxide synthase activity, modulating mitogen-activated protein kinases, and the expression of a plethora of genes induced under conditions of cell stress via the regulation of transcription factor NFkB activity.

AS is a commonly used non-steroidal anti-inflammatory drug capable of acetylating proteins in the course of a simple, non-enzymatic chemical reaction. AS usage is associated with decreased risk for colorectal, breast, esophageal, lung, stomach and ovarian cancer, and AS is both a chemopreventive and chemotherapeutic agent for breast and colon cancer [5–9]. ISM can be used to expand capacitance vessel, reduce blood flow of back to heart, thus reducing cardiac preload and protecting myocardium. Clinically, it is widely used to prevent angina, congestive heart failure and ischemic heart disease and so on [10].

Combination therapy has additive effect and can be used for prevention of angina and heart disease Ⅱ. At the same time, this can improve the patients' quality of life and medication compliance. In this preparation, ISM serves as the sustained release ingredient and AS the fast release ingredient. The two layers are pressed together and made into compound preparation. It is convenient and at the same time tolerability can be avoided.

Double-layer tablet refers to the tablet with the structure of two layers with two or more active pharmaceutical ingredients and each layer has different drug or excipients to avoid compatibility change between different drugs in compound preparation or to reach the effect of delayed release or controlled release. Double-layer tablets have the following advantages over conventional tablets: Firstly, it is convenient to regulate drug release rate or release order. Secondly, it can increase the capacity of drug loading, making it easier to use and improving patients' compliance and the preparation process is relatively simple.

The present study focused on development of sustained bilayer tablets with an incorporation of aspirin and isosorbide 5-mononitrate, achieving the fast release effect of aspirin and sustained release of isosorbide 5-mononitrate. The formulation of tablets was optimized and the physical characteristics such as their drug content, weight variation, hardness, thickness, friability, and in vitro release characteristics were evaluated.

MATERIALS AND METHODS:

Materials: Aspirin was obtained from Xinhua Pharmaceutical Limited Company (Shandong, China). Isosorbide 5-mononitrate was got from Keyuan Pharmaceutical Limited Company (Shandong, China). Hydroxypropyl methylcellulose(HPMC K100M,K15M) was supplied by Colorcon Company (Shanghai, China), crospovidone (PVPP), carboxymethylcellulose sodium (CMC-Na), microcrystalline cellulose (MCC), lactose, pregelatinized starch, talc and aerosil was purchased from Shanhe Pharmaceutical Company (Anhui, China), and citrate acid was obtained commercially from Fuchen Chemical Company (Tianjin, China).

Formulations of sustained release tablets: Drug (aspirin and Isosorbide 5-mononitrate), HPMC, PVPP, lactose, citrate acid, MCC, pregelatinized starch and CMC-Na were passed through 80 mesh sieve separately. Sustained release layer was prepared as follows: The drug ISM was mixed with other ingredients. Then adhesives were added to form damp mass, granulated with a 20 mesh sieve after dried in an oven at 60℃, and aerosil were added to the above granules and mixed for 20 min. Granules contained ISM were pressed with a single-punch tableting machine to form the first layer. Then aspirin mixed with other ingredients was added on this first layer and compressed at high pressure to obtain a bilayer tablet. The formulations are shown in **Table.1**.

Evaluations of Formulations:

1. **Determination of content:** Take out 20 tablets of this drug, weigh them precisely, grind them small and precisely weigh a certain amount

(that is, ISM 60mg and AS 75mg).Then set it into a flask of 50mL, add 1% acetic acid methanol solution of accurate amount, ultrasound for 30min to make ISM and AS dissolved and dilute it to the mark, shake, filtrate. Take out the above solution of 5mL into a flask of 50mL and dilute it to the mark using 1% acetic acid methanol solution, shake and take out 20uL precisely, inject into the liquid chromatograph and record the chromatograms. In addition, take reference substance of ISM and AS and weigh them precisely, then use acetic acid methanol solution of 1% to dilute quantitatively to a mixture of ISM 0.12mg/mL and AS 0.15mg/mL.Then use the same method to determine the concentration with the external standard method by peak area.

2. **HPLC conditions:** HPLC was carried out using a model LC-20AT system (Shimadzu, Japan) equipped with an SPD-20A UV-Visible detector. The column used was Thermo Quest C18 (250 *4.6 mm). A mixture of potassium dihydrogen phosphate buffer: methanol (took potassium dihydrogen phosphate 0.9g and added water of 1000mL, then shook to dissolve. The pH was adjusted to 3.2 using dilute orthophosphoric acid) (68:32 v/v) was used as mobile phase at a flow rate of 1 ml/min. Detection was done at 220 nm. The mobile phase was filtered through 0.45 μm membrane filter and degassed.

3. **Friability of the tablets:** Twenty tablets of the formulation were weighed and measured in a friabilator according to appendix XG in Chinese Pharmacopoeia of 2010 edition. The tablets were rotated at 25rpm for 4min, and the samples were reweighed. The friability was calculated using the equation: F% = W1 −W2 /W1 × 100%, in which F% represents the percentage of weight loss, W1 and W2 are the initial and final tablets weights respectively. The result was shown in **Table 1**.

4. **Hardness:** Hardness of ten randomly selected tablets was determined. The mean hardness and coefficient of hardness variation were calculated. And the hardness results met the requirements.

5. ***In-vitro* drug release:** Drug release studies were performed in 900ml of water using the basket method, at 100rpm and 37℃. The amount of drug release over time was determined by samples at various time intervals such as 0.5h, 1h, 3h and 7h. Then took the solution of 5mL, filtrated and immediately added release medium of the same temperature and the same volume. It was followed by precisely measuring continued filtrate of 20μL, injecting into the liquid chromatograph and recording the chromatograms; in addition, took ISM and AS reference substance and weighed them precisely then dissolved them using 1% acetic acid methanol solution and diluted quantitatively to make mixed solution of ISM 60μg/mL and AS 75ug/mL; took salicylic acid reference substance and weighed it precisely and used 1% acetic acid methanol solution to dilute quantitatively to 10μg/mL. Then, the same method was used to determine it. Calculated the concentration of AS and salicylic acid of per tablet at 0.5h and the release amount of ISM at 1h,3h,7h.

RESULTS AND DISCUSSION:

Effect of PVPP amount on dissolution of AS: The influence of the contents of PVPP on the dissolution of AS from the tablets was investigated. The result was shown in **Table 1**. From Table 1, we could see that the dissolution amount increased with increasing of the content of PVPP in tablets. As the concentration of PVPP was increased from 6mg (F1) to 10mg (F5), the dissolution was increased from 83.2 to 95.4% (**Fig. 1**). And there were no significant differences in the appearance of tablets among these formulations. Based on the results of dissolution, F5 was considered as the optimum formulation.

TABLE 1: EFFECT OF PVPP AMOUNT ON DISSOLUTION OF AS

INGREDIENTS (mg)	F1	F2	F3	F4	F5
AS	75	75	75	75	75
PVPP	6	7	8	9	10
Citrate acid	15	15	15	15	15
MCC	35	35	35	35	35
Pregelatinized starch	15	15	15	15	15
Talc	2	2	2	2	2
Dissolution (%)	83.2	86.4	87.5	91.7	95.4
Friability (%)	0.32	0.42	0.45	0.44	0.32

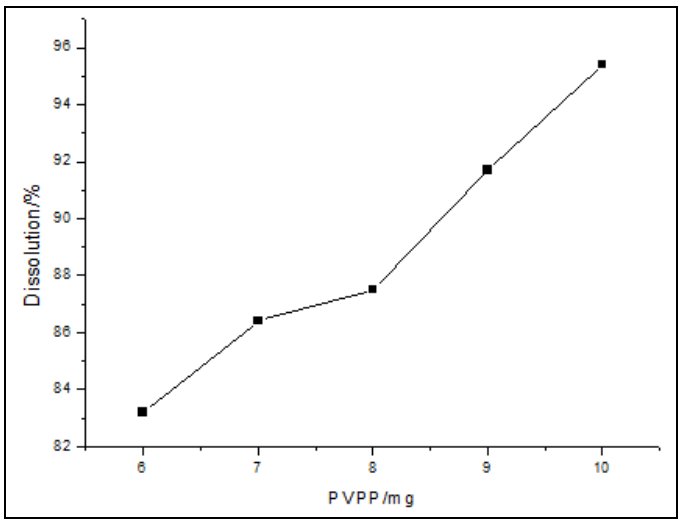

FIG. 1: EFFECT OF PVPP AMOUNT ON DISSOLUYION OF AS

Orthogonal experiment design for the selection of sustained release part: An orthogonal experiment design was used to optimize ISM sustained release layer. The concentration of HPMC K100M, CMC-Na, and HPMC K15M were chosen as the most influential factors (labeled as A, B, and C in **Table 2**). The three factors were investigated at three different levels taking the in vitro release as index. The L9 (34) orthogonal design was established as shown in Table 2 and **Table 3**.

In Table 3, Yi was an objective function value, the sum of difference between predicted value and the actual experiment data of drug release. It was calculated by Yi= | Y2-0.30 | + | Y4-0.55 | + | Y8-0.70 | ; Y2、Y4、Y8 respectively stood for actual accumulated release at 1、3、7 h and 0.30, 0.55, 0.70 were the predicted cumulative drug release at 1, 3, 7 h, respectively. K was the average of Yi for three factors under different levels. The difference between the highest and the lowest among K1, K2, and K3 was defined by the symbol "R". The higher the R, the greater the influence on the drug releases.

As seen from **Table.3**, we found that R of the three factors ranked as RA > RB> RC (0.20>0.17>0.13), which indicated the order of the three factors' effect on drug release is A>B>C, and concentration of HPMC K100M was found to be the most important determinant of the drug release. K was used to determine the optimal level in the three factors; level with minimum K value was the optimum level.

The individual levels within each factor were ranked as A: 3<2<1 (0.15<0.28<0.35), B: 3<2<1 (0.18<0.24<0.36), C: 3<2<1 (0.21<0.24<0.33), as shown in **Table.3**. Based on the optimized results of orthogonal design, the optimum formulation should be A3B3C3, which means the concentration of HPMC K100M, CMC-Na, and HPMC K15M were 45 mg/tablet, 12 mg/tablet and 12 mg/tablet respectively.

TABLE 2: THE ORTHOGONAL LEVELS AND FACTORS OF FORMULATION

Levels/ factors	A (mg/tablet)	B (mg/tablet)	C (mg/tablet)
1	25	8	8
2	35	10	10
3	45	12	12

TABLE 3: THE DESIGN AND RESULTS OF THE ORTHOGONAL EXPERIMENT

Formulation	Factors			Cumulative drug release			
	A	B	C	Y_2	Y_4	Y_8	Y_i
1	1	1	1	0.48	0.75	0.88	0.56
2	1	2	2	0.44	0.65	0.79	0.33
3	1	3	3	0.34	0.62	0.76	0.17
4	2	1	2	0.37	0.63	0.85	0.3
5	2	2	3	0.35	0.6	0.84	0.24
6	2	3	1	0.39	0.63	0.82	0.29
7	3	1	3	0.38	0.61	0.77	0.21
8	3	2	1	0.36	0.59	0.75	0.15
9	3	3	2	0.32	0.56	0.76	0.09
K_1	0.35	0.36	0.33				
K_2	0.28	0.24	0.24				
K_3	0.15	0.18	0.21				
R	0.20	0.17	0.13				

The evaluation of optimized formulation: In this paper, sustained release layer of ISM was prepared by wet granulation method using ISM 60 mg, HPMC K100M 45 mg, CMC-Na 12 mg, HPMC K15M 12 mg and lactose 50 mg. Ethanol solution of 80% of PVP K30 of 5% (w/v) was used as adhesives and aerosil of 3 mg as lubricant.

Granules contained ISM was pressed with a single-punch tableting machine to form a first layer. Then aspirin mixed with other ingredients (aspirin 75mg, PVPP 10mg, citrate acid 15mg, MCC 35mg, talc 2mg) was added on this first layer and compressed to obtain a bilayer tablet. Friability (0.32%-0.45%) showed good mechanical resistance of the tablets. And all the tablets had smooth surface without spots. The results of dissolution of this tablet were shown in A and B in **Fig. 2**. From the following figures we could learn that the dissolution results of both sustained release layer and fast release layer met the requirements.

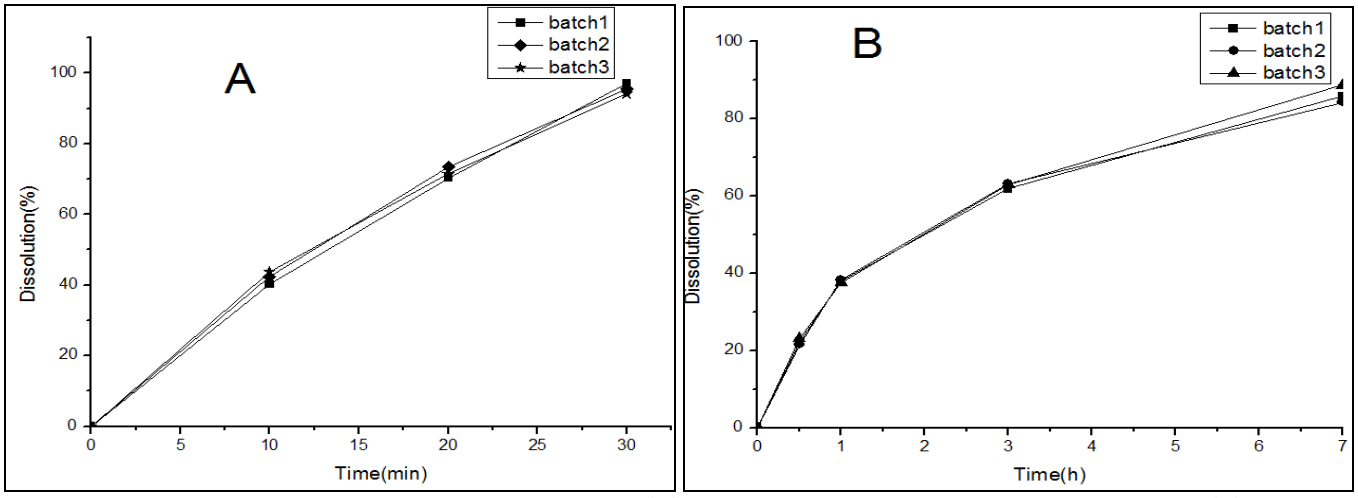

FIG. 2: THE RELEASE RESULTS OF ASPIRIN (A) AND ISOSORBIDE 5-MONONITRATE (B) IN THE SUSTAINED BILAYER TABLETS

CONCLUSION: Aspirin and isosorbide 5-mononitrate sustained bilayer tablets were developed and optimized in this study. The fast release layer of aspirin and sustained release layer of isosorbide 5-mononitrate were both investigated and optimized for the formulation of sustained bilayer tablets. The physical characteristics such as their drug content, hardness, thickness, friability, and in vitro release characteristics were evaluated. The tablets exhibited satisfactory drug release profiles for immediate drug release of aspirin and sustained drug release for isosorbide 5-mononitrate.

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