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Original Article

Extraction of Mucilage and its Comparative Evaluation as a Binder

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ABSTRACT

A binder holds powders together to form granules and also provides the cohesiveness required for binding of the granules under compression to form a tablet. Some natural excipients are currently available for pharmaceutical formulation. Aim of this study was to isolate the mucilage from different plants and to evaluate them as a binder. Here an effort was made to investigate the efficacy of mucilage obtained from fruits of Ziziphus mauritiana and Aegle marmelos as tablet excipient. A microwave assisted extraction technique was developed to optimize the extraction of mucilage from the fruits of Ziziphus mauritiana and Aegle marmelos plants. Fruits of both plants have been extracted by conventional and microwave assisted method for isolation of mucilage. The extracted mucilage has been evaluated for various physicochemical properties. Tablets were manufactured using extracted mucilage as a binding agent and comparison was made against the tablets prepared with starch paste as standard binder on studying standard parameters like diameter, thickness, weight variation, hardness, friability, disintegration and Invitro dissolution study. Mucilage could be used as a binding agent for at low concentrations. This can be used for sustaining drug release from tablets since prepared tablets produced a sticky film of hydration on surface which ultimately reduces drug release rate and hence can be evaluated for its efficacy to sustain the drug release.

Keywords: Mucilage, Pharmaceutical excipients, Tablets, Physicochemical characterization.

INTRODUCTION

Today we have lot of pharmaceutical excipients and most of them are of plant origin. Some examples are starch, agar, alginates, Carrageenan, guar gum, xanthan gum, gelatin, pectin, chitosan, acacia, tragacanth, celluloses, sugars, etc. These natural excipients find application in pharmaceutical industry as binding agents, disintegrants, sustaining agents, protective, colloids, thickening agents, gelling agents, bases in suppositories, stabilizers and coating materials. In the design of sustained release dosage forms, they retard the drug release by forming a thick retardant gel Swellable polymers are laver. water insoluble and commonly called as hydrogels where as those which are water soluble are called as hydrophilic polymers. Also gums and mucilages obtained from different plant sources are important agrochemical used in various industries worldwide. The growing industrial utility of these plant products in the field of paper, textile, petroleum recovery and pharmaceutical industries has resulted in an impetus in India for intensified research on new sources of gums and their modified products. Mucilage is a by-product obtained as a result of metabolic mechanisms of plants.¹

The synthetic polymers used as excipients suffer from many disadvantages such as.

- 1. High cost,
- 2. Toxicity,
- 3. Non-biodegradability,

4. Environmental pollution caused during their synthesis.

Advantages of natural plant-based excipients

- 1. Low cost and natural origin.
- 2. Free from side effects.
- 3. Biocompatible and bio-acceptable.
- 4. Renewable source.
- 5. Environmental friendly processing.

6. Local availability.

7. Better patient tolerance as well as public acceptance.

8. They improve the national economy by providing inexpensive formulations to people, using locally available materials.

Natural gums and mucilages are either water soluble or absorb water to form a viscous solution. Natural gums are less economic, easily available. They have been wildly used as tablet binders, emulgents and thickeners in cosmetics and suspensions as film-forming agents and transitional colloids. Polysaccharides (gums and mucilage) are commonly used adjuvants most in pharmaceutical preparations. They find maximum use particularly in the formulation of suspensions and emulsions. The usefulness polysaccharides as emulsifying, of suspending and binding agents has been well documented.²

Some of them have also been used in tablet formulations as binding agents and also to sustain the drug release. Such matrix systems belong to the group of polymers and drug release is mainly diffusion sustained. Gums and mucilages are polysaccharide complexes formed from sugar and uronic acid units. They are insoluble in alcohol but dissolve or swell in water. They are usually formed from the cell wall (e.g. tragacanth) or deposited on it in successive layers. Gums are natural plant colloids that may be classified as anionic or non-ionic polysaccharides or salts of polysaccharides. They are translucent, amorphous substances that are frequently produced in higher plants as a protective after injury. Gums and mucilages are obtained mainly from seeds or other plant parts. Some are obtained from various marine algae, and some from selected microorganism. Also, a number of semi synthetic gums and mucilages are used for their hydrophilic properties, and they can be considered as

special hydrocolloid gums. Gums and mucilages are typically heterogeneous in composition. Upon hydrolysis, arabinose, galactose, glucose, mannose, xylose and various uronic acids are the most frequently observed components. The uronic acid may form salts with calcium, magnesium and sulfate ester substituents.

Recently for extraction of different plant products, microwave heating has been found to be a convenient source of energy in chemical laboratories. Many of the basic principles of green chemistry are best suited for microwave processes. It is one of the simple, fast, clean, eco-friendly and efficient method. It is economic in saving energy, fuel and electricity. A very short response time and better yields of the products are the main advantages of microwave heating. Commercial microwave ovens are used as a source of energy in chemical laboratories for efficient heating of water, moisture analysis, wet ash of biological and geological waste material management, materials, sterilization of pharmaceutical preparations, inactivation of enzymes in food products, etc. In the past few years, a large use of this technique is seen in acceleration of organic reaction for rapid and green synthetic procedures. Extraction is one of the most crucial point in the analytical chain in the effort of achieving a complete recovery of target compounds. Recently, microwave energy is being used for extraction of phytoconstituents from plants. Microwave extraction follows the same principle as maceration or percolation, but the speed of breaking up of plant cells and plant tissues is much higher. This reduced processing time is an economic advantage and also there is less risk of decomposition or disintegration and oxidation of the valuable plant constituents. Microwave assisted extraction methods require shorter time, less solvents, higher extraction rate and better products with lower cost. The microwave consists of a number of

radiation chambers in order to manipulate the required energy.³

MATERIALS AND METHODS

Requirements

Chemicals

Ethanol, distilled water, Paracetamol, Lactose, Sodium starch glycolate, Maize starch, Talc, Magnesium stearate, Guar gum, Gum Acacia, Gum Tragacanth, Sodium saccharin, Orange syrup, Chloroform water, Compound tatrazine solution, Phosphate buffer, Hydrochloric acid, Sulphuric acid, Ruthenium red, Molisch reagent, Iodine solution.

Equipments

Hot air oven, Bulk density apparatus, Lab Hosp Incubator, Shimadzu AUX220 Unibloc digital balance, Rotary tableting machine CIPD8 Lab press (eight station), Monsanto hardness tester, Rosch friabilator, VDA-6D USP Standards Veego tablet dissolution test apparatus, UV-vis 1800 Shimadzu UV Spectrophotometer, Catalyst scientific system Microwave oven.

Collection and authentication

The fruits of *Ziziphus mauritiana Lam.* and *Aegle marmelos Corr.* Plants were used for the isolation of mucilage. These materials were collected from the local area of Alephata, Pune Maharashtra, India and authenticated by Botanist from Botanical survey of India, Pune and voucher specimen number has submitted to Pharmacognosy department.

Isolation of mucilage

Isolation of Mucilage by Conventional Procedure

Ziziphus mauritiana and Aegle marmelos fruits (50 g) were powdered for 5

min in a mechanical blender and soaked in distilled water (500 ml) for 24 hrs in a Round bottom flask. It was boiled for 1 hr under reflux with occasional stirring and kept aside for 2 hrs for the release of mucilage into water. The material was filtered through a muslin bag and hot distilled water (100ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely. Equal volume of Ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator at 37°C, powdered and weighed. It was subjected to chemical tests to confirm its identity.^{3,4}

Isolation of Mucilage by Microwave Procedure

Ziziphus mauritiana and Aegle marmelose fruits (50 g) were powdered in a mechanical blender for 5 min and soaked in distilled water (500 ml) for 24 hrs in a 1000 ml beaker. It was kept in a microwave oven along with a glass tube inside to prevent bumping. It was subjected to microwave irradiation at 420 W intensity for7 min. The beaker was removed from the oven and kept aside for 2 hrs for the release of mucilage into water. Equal volume of Ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator at 37°c, powdered and weighed.

Yield of the mucilage obtained from microwave and conventional methods were calculated and compared to check which method serves best for extraction of the mucilage.^{3,4}

Evaluation of mucilage

Preliminary tests were performed to confirm the nature of mucilage obtained. The chemical tests such as: Ruthenium red test, Molisch test, iodine test, methylene blue, Test for uronic acid and Ninhydrin test were performed, also various properties such as color, odor, taste, nature, solubility, density, viscosity, surface tension has been determined.⁵

Physicochemical characterization of mucilage

The extracted seed mucilage was characterized for properties such as pH, specific gravity, surface tension, foreign matter, moisture content, total ash value, acid insoluble ash content, swelling index, viscosity as per WHO guidelines.^{6,7}

Preparation of granules

Preparation of binder solution

The binder solution was prepared by dissolving the mucilage of *Ziziphus mauritiana* and *Aegle marmelos* in water at 10%, 20% 30% w/v concentration. Standard binder (Maize starch) was prepared by dispersing 10 gm sample of the maize starch powder in 20ml of distilled water & adding boiled water while stirring with a glass rod to make up to 100 ml .The mucilage solution was allowed to cool & was used for binding.

Method

Wet granulation

This is the most widely & general method used in the preparation of tablets. Wet granulation involves the massing of a mixture of dry primary powder particles using a granulation fluid.

Procedure

The granules were prepared by wet granulation method. Paracetamol was used as a model drug to formulate granules. Lactose & talc were used as diluent & lubricant respectively. The binder solution was used as disintegrant, prepared by dissolving the mucilage of *Ziziphus mauritiana* & *Aegle* *marmelose* in water at 10 %, 20 % &30 % W/V concentration.

The drug, lactose & SSG mixed thoroughly & a sufficient volume of 10%, 20%, & 30% W/V of mucilage *Ziziphus mauritiana* & *Aegle marmelos* were added slowly to the powder blend & cohesive wet mass was then sieved through sieve no.16 & dried at not more than 60° C in hot air oven for 3 hrs. The dried granules were received through sieve no 20.^{8,9}

Formula for preparation of granules²

Evaluation of granules

The prepared granules were then evaluated for percentage of fine, particle size & flow properties (by measuring angle of repose). The bulk & tapped densities, carr's compressibility index were determined.^{10,11}

Bulk Density and Tapped Density

density The bulk of Ziziphus mauritiana and Aegle marmelos mucilage granules was determined by the three-tap method. Weighed quantity of granules was carefully introduced into a 100 ml graduated cylinder. The cylinder was dropped onto a hard wood surface 3 times from a height of 2.5 cm at an interval of 2 seconds. The bulk density was obtained by dividing the weight of the sample by volume of the sample contained in the cylinder. Tapped density is the ratio of weight of dry powder to its tapped volume. The weighed quantity of dry powder was taken in a graduated cylinder. The cylinder was placed on the tap density tester (Electro lab, model ETD-1020). The volume of powdered bed is measured after each increment of 250 drops until the difference of last two volume measurement is zero.

Carr's Consolidation / Compressibility $Index^{11}$

This property is also known as compressibility. It is indirectly related to the

relative flow rate, cohesiveness and particle size. It is simple, fast and popular method of predicting powder flow characteristics. It can be calculated by following formula:

Consolidation index = Tapped density- Fluff density/ Tapped density \times 100.

The weighed quantity of dry powder was taken in a graduated cylinder. The cylinder was placed on the tap density tester (Electro lab, model ETD-1020) and subjected to USP II method i.e. 250 drops per minute and drop height is 3mm±10%. The volume of powdered bed is measured after each increment of 250 drops until the difference of last two volume measurement is zero.

Hausner Ratio^{12,13}

It was determined by using the Following formula:

Hausner Ratio= Tapped bulk density/ Loose bulk density.

Angle of repose

Angle of repose was calculated by following equation,¹⁴

Tan $\Box = h/r$

Preparation of tablets

The granules made from gum were compressed into flat faced tablets of mean average weight 200 mg \pm 7.5 %, thickness 4.1 \pm 0.3 mm, and diameter 8 mm \pm 0.1 mm in Rotary tablet punching machine CIPD 8 eight station at an arbitrary pressure load unit of 6 tons. Magnesium stearate was used to lubricate the die and punch surfaces prior to tableting to prevent sticking.

Evaluation of tablets^{15,16}

The prepared tablets were evaluated for content uniformity, weight uniformity, hardness, thickness, friability, Disintegration time and dissolution property. All these tests were performed as per Indian pharmacopoeial requirements.

RESULTS AND DISCUSSION

Table 4.1 and 4.2 represent results for optimization of energy required for extraction of Ziziphus mauritiana and Aegle marmelos mucilage by microwave method. Extraction was carried out at different frequencies such as 140, 245, 420, 490 and 560 Watt for 7min. It was observed that at 140 and 245 Watt, Ziziphus mauritiana mucilage yield was low i.e. 12 % W/W at 140 W, 20 % W/W at 245 W and 33.45 % W/W at 420 W for 7 min. Also at 140, 245 Watt, Aegle marmelos mucilage yield was found to be 10 % and 18% W/W respectively and at 420 W it was 46 % W/W for 7 min. It was observed that better yields were obtained at 420 W in comparison to that at 140 and 245 Watt. Whereas, product gets degraded at higher energies such as 490 and 560 watt.

Comparative yields obtained in microwave and conventional methods are represented in table 4.3. It was observed that the yield obtained by microwave method in case of *Ziziphus mauritiana* mucilage is 33.45% W/W which is three times more as compared to that obtained in conventional method i.e 11.30 % W/W. Also yield obtained in the case of *Aegle marmelos* mucilage by microwave method is 47 % W/W which is six times greater as compared to that obtained by conventional method i.e. 8 % W/W.

Physicochemical properties of mucilage are shown in table 4.4. From evaluation of mucilage isolated from fruits of Ziziphus mauritiana and Aegle marmelos it was observed that mucilages from both the samples are found to be brownish, odorless, sticky with neutral pH and approximately equal viscosities and they produce brown and dark brown colors at 50°c and are found to be stable at temperature of 50°c. It is found to be soluble in water and insoluble in organic solvents like ethanol, methanol and chloroform. Flow properties for powdered mucilage are represented in table 4.5,

powdered mucilages from both fruits exhibit good flow properties.

Table 4.6 shows results for microbial limits in mucilage samples. It can be seen that there is complete absence of pathogenic microbes in the samples of *Ziziphus mauritiana* and *Aegle marmelos* mucilage.

Table 4.7 represents evaluation of granules prepared using Ziziphus mauritiana and Aegle marmelos mucilage. Zm1, Zm2 and Zm3 are granules prepared by using three different concentrations of Ziziphus mauritiana mucilage as a binder then Am1, Am2 and Am3 are granules prepared by using three different concentrations of Aegle marmelos as a binder and S1 represents granules prepared by using maize starch (10 %) as a binder. Table 4.8 represents evaluation of tablets prepared from drug Paracetamol using three different concentrations of binders. Tablets shown increase in hardness and disintegration time with increasing binder concentration whereas there is decrease in the friability of tablets with increase in binder concentration. In-vitro dissolution profile of tablets is performed as per Indian pharmacopoeial requirements.

Figure 4.1 and 4.2 explain results for Differential Scanning Calorimetry (DSC) for *Aegle marmelos* and *Ziziphus mauritiana* respectively, showing the heat loss or gain, resulting from physical or chemical changes within a sample as a function of temperature. A sharp symmetric melting peak can indicate relative purity of mucilage sample.

Figure 4.3 and 4.4 give peaks of IR spectroscopy for *Ziziphus mauritiana* and *Aegle marmelos* which is a useful tool in identification as well as purity of a compound. For figure 4.3 the principal absorption peaks of polysaccharide were found at 1037 cm-1 (C-O-C, ether group absorbance), 1666 cm-1 (C=O, aldehyde absorption), 2922 cm-1 (C-H stretching), 3266 cm-1 (primary OH), 3385 cm-1 (secondary OH), which indicate that isolated product was polysaccharide. For figure 4.4 the principal absorption peaks of polysaccharide were found at 1031 cm-1 (C-O-C, ether group absorbance), 1668 cm-1 (C=O, aldehyde absorption), 2922 cm-1 (C-H stretching), 3367 cm-1 (primary OH), 3385 cm-1 (secondary OH), which indicate that isolated product was polysaccharide.

Figure 4.5 represents peaks for IR spectroscopy performed on powdered Paracetamol sample. Figure 4.6 Shows peaks of IR spectroscopy performed on powdered mixture of Paracetamol and Ziziphus mauritiana mucilage sample. From comparison of peaks obtained with individual IR study of Paracetamol, i.e figure 4.5 and 4.6, it can be seen that there is no change in the peak intensities and areas for Paracetamol sample even in mixture with mucilage sample, which suggests that there is no interaction chemical between Ziziphus mauritiana mucilage and Paracetamol. Figure 4.7 represents peaks for IR spectroscopy performed on Aegle marmelos mucilage and Paracetamol powdered mixture sample. Again if we compare peaks obtained in IR study of Paracetamol alone to the peaks obtained with IR study of Aegle marmelos mucilage and Paracetamol in powdered form there is no change in the peak areas and intensities of Paracetamol even in mixture. This suggests that there is no significant chemical interaction between Paracetamol and Aegle *marmelos* mucilage sample.

Table 4.7 represents evaluation of granules prepared using Ziziphus mauritiana and Aegle marmelos mucilage as binder. Zm1, Zm2 and Zm3 are sets of granules three different prepared by using concentrations i.e 10 %, 20 %, 30 % of Ziziphus mauritiana mucilage as a binder then Am1, Am2 and Am3 are granules prepared by using three different concentrations i.e. 10 %, 20 %, 30 % of Aegle marmelos mucilage as a binder and S1 represents granules prepared by using maize starch (10%) as a

binder. It can be seen that granules have comparable properties with respect to starch granules for bulk, tap density, flow and angle of repose. Table 4.8 represents evaluation of tablets prepared from drug Paracetamol using three different concentrations of binders. Tablets shown increase in hardness and disintegration time with increasing binder concentration whereas there is decrease in the friability of tablets with increase in binder concentration. In-vitro dissolution profile of tablets is performed as per Indian pharmacopoeial requirements.

Fig 4.8 represents the release profile for Am1. Am2. and Am3 set of tablets compared with S1 sets of tablets. It can be seen that the release profile for Am1 is slightly different from that of the standard set tablets whereas release profile for set Am2 is similar to that of standard set of tablets and release profile for set Am3 is much different compared to that of standard set of tablets. The resultant difference between the dissolution profile for set Am3 and standard tablets is because of the increased concentration of binder (i.e.30 %) in case of set Am3, which retards release of drug as compared to the release of drug from tablets The higher of the standard set S1. concentration mucilage retards the drug release by producing a sticky film of hydration on the surface. Similarly, Fig 4.9 represents the release profile for Zm1, Zm2 and Zm3 set of tablets compared with S1 set of tablets. It can be seen that release profile of Zm2 matches that of S1 set of tablets, profiles of Zm1 and Zm3 do not match to that of S1 because, Zm1 has lower concentration of binder (i.e.10 %) and Zm3 has a very high concentration of binder (30 %) so to acquire steady release profile similar to S1. concentration of binder in Zm2 (i.e.20 %) seems to be appropriate. Release profile for tablets is compared with that of marketed formulation of crocin tablets and it was observed that release profile for set Zm2 and Am2 approximately matches to that of Crocin tablets.

CONCLUSION

It can be seen that the yield obtained from microwave method for extraction of mucilage is much better as compared to the conventional method because microwave method works on cellular level for extraction of the mucilage whereas conventional method is less efficient because it only uses heat externally for the extraction of mucilage. Hence we can conclude that microwave method is more suitable, fast economic and simple for extraction of mucilage as compared to the conventional method. The Aegle marmelose and Ziziphus mauritiana mucilages exhibit good binding properties for uncoated tablets. The increased concentration of mucilage showed small retardation in drug release from tablet. There was increase in the disintegration time and hardness of tablet and decrease in the friability of the tablet with increasing the concentration of mucilage. Dissolution profile shows the steady release of drug from formulation within half an hour and also with increase in binder concentration release of drug decreases slowly. So, it can be concluded that mucilage isolated from Ziziphus mauritina and Aegle marmelos, can be used as a potential natural binding agent for formulating tablets.

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Ingradients Mg/tab	Am1	Am2	Am3	Zm1	Zm2	Zm3	S1
Paracetamol	100	100	100	100	100	100	100
Lactose	64	60	56	64	60	56	64
SSG	10	10	10	10	10	10	10
Maize starch	-	-	-	-	-	-	16
Mucilage	16	20	24	16	20	24	-
Talc	9	9	9	9	9	9	9
Magnesium stearate	1	1	1	1	1	1	1
Total wt.	200	200	200	200	200	200	200

Table 1. Formula for preparation of granules

Table 4.1. Optimization of energy required for extraction of Ziziphus mauritiana mucilage by microwave method

Plant name	Energy (watt)	Time (min)	Yield (%)
Ziziphus mauritiana	140	7	12
	245	7	20
	420	7	33.45
	490	7	Product degraded
	560	7	Product degraded

Table 4.2. Optimization of energy required for extraction of *Aegle marmelos* mucilage by microwave method

Plant name	Energy (watt)	Time (min)	Yield (%)
Aegle marmelos	140	7	10
	245	7	18
	420	7	47
	490	7	Product degraded
	560	7	Product degraded

Method of extraction	Ziziphus mauritiana Yield obtained (%W/W)	Aegle marmelos Yield obtained (%W/W)
Conventional	11.30	8.6
Microwave	33.45	47

Table 4.3. Yield of mucilage obtained by conventional and microwave method

Table 4.4. Evaluation of mucilage obtained from Ziziphus mauritiana and Aegle marmelos fruits

Parameter	Ziziphus mauritiana fruit	Aegle marmelos fruit
Color	Reddish brown	Brown
Odor	Odorless	Odorless
Nature	Sticky	Sticky
Density	0.9684gm/ml	0.9657gm/ml
Thermal changes at 50 [®] c	Brown	Dark brown
pH (1% W/V solution)	7	7
LOD (%W/W)	5 %	6 %
Surface tension	59.16 d/cm	64.90 d/cm
Viscosity	2.72cp	2.13cp
Swelling index (ml)	NMT 3	NMT 3
Ash value	11%	2.5%
Foreign organic matter	0.5%	1%
Acid insoluble ash	2%	0.5%
	Form viscous solution in warm	Form viscous solution in warm
	water, Swells in cold water,	water, Swells in cold water,
Solubility	Insoluble in organic solvents like	Insoluble in organic solvents
	methanol, ethanol and	like methanol, ethanol and
	chloroform	chloroform

Table 4.5. Flow properties of powdered mucilage obtained from Ziziphus mauritiana and Aegle marmelos fruits

Parameters	Ziziphus mauritiana	Aegle marmelos
Bulk density	0.39 gm/ml	0.35 gm/ml
Tapped density	0.48 gm/ml	0.50 gm/ml
Angle of repose	30 ⁰	32 ⁰
LOD (W/W)	10%	11%

Parameter	Zm1	Zm2	Zm3	Am1	Am2	Am3	S1
Weight of granules (gm)	4.1010	4.0102	4.5012	3.0010	3.7919	3.8010	3.830
Bulk density (gm/ml)	0.2734	0.3341	0.4092	0.6012	0.4114	0.6335	0.54714
Tapped density (gm/ml)	0.3154	0.4010	0.5001	1.0020	0.5486	0.7602	0.7660
Angle of repose	30 ⁰	30.5 ⁰	32.35 ⁰	30.03 ⁰	30.28 ⁰	32.05 ⁰	30.03 ⁰
LOD (%)	0.2	0.3	0.3	0.5	0.4	0.4	0.3

Table 4.6. Evaluation of granules prepared using three different concentrations of binder

 Table 4.7. Evaluation of tablets prepared from Paracetamol

Parameter	Zm1	Zm2	Zm3	Am1	Am2	Am3	S1	Crocin
Weight variation (mg)	207	203	201	204	207	209	203	-
Hardness (kg/cm2)	4	4	5	4	4	4	4	5
Friability (%)	0.5	0.3	0.2	0.6	0.5	0.3	0.2	0.1
Disintegration test (min)	3.58	5.40	5.45	3.45	4.37	4.50	4.30	4.55
Assay (%purity)	99.5	99.8	100	99.2	99.7	99.6	100. 1	100

Table 4.8. In vitro-dissolution profile for the tablet	s prepared	using parac	etamol
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Formulation	D5	D10	D15	D20	D25	D30
Zm1	25.52	30.38	40.03	68	84	100.3
Zm2	22.06	28.24	41	64.82	87	99.36
Zm3	21.20	28.15	37.39	51.53	75	99.8
Am1	27.69	37.14	42.37	65.21	100.4	103.1
Am2	27.60	36.93	42.14	74.87	87.71	105.2
Am3	27.25	35.02	41.45	48.16	65.74	100.7
S1	27.77	37.36	42.76	80.64	100.1	103.1
Crocin	10	27.11	52.5	65	80	100

















