

Optimization of Method for Determination of Swelling Factor of Ispaghula Seeds

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Abstract

An endeavour of present study is to optimize the method for determination of swelling factor of Ispaghula seeds, so it can be determined correctly with minimum errors which will be reproducible. The polysaccharides in the mucilage of Plantago seeds constitute a diverse class of biological macromolecules with a broad range of physiochemical properties which are widely used for applications in Pharmacy and medicine. Many official and unofficial sources described different methods for determination of swelling factor with variable results. The present work is undertaken to determine the effect of parameters such as quantity of seed, volume and nature of solvent, time interval of agitation, time of measurement of result, Effect of quantity of seeds on swelling factor determination. Swelling factor of different seeds like *Lepodium sativum*, *Althia officinalis* and *Occimum sanctum* has been evaluated by IP method and optimised method for 24 h. Optimized method gives superlative results over IP method. Plantago seeds moistened with 1ml of 90% alcohol followed by addition of distilled water at room temperature along with every 3 h agitation shows exceptional swelling as compared to other methods and conditions.

Keywords: *Plantago ovata*; Laxative; Swelling; Mucilage

Introduction

Ispaghula (*Plantago ovata* Forskal) seeds and seeds of other species of *Plantago* such as *P. psyllium*, *P. arenaria*, *P. lanceolata* etc. contain mucilage in the epidermis of the seeds. The polysaccharides in the mucilage of these seeds constitute a diverse class of biological macromolecules with a broad range of physiochemical properties which are widely used for various applications in Pharmacy and medicine. All these seeds can be evaluated by measuring the volume of mucilage produced within 24 h [1]. This is termed as swelling Factor. It is defined in the B. P. as the volume in milliliter occupied by 1 g of a drug including any adhering mucilage after it has swollen in an aqueous liquid for 24 h [2]. The swelling factor reflects the mucilage content of the seeds. Skyrme and Wallis first examined the swelling factor for various seeds of *Plantago* species in 1936 [1]. The other species of *Plantago* such as *P. psyllium*, *P. arenaria*, *P. lanceolata*, *P. rhodosperma*, *P. wrightiana* can be used as adulterants/substituent in place of *P. ovata*, which can be distinguished by their swelling Factors.

Swelling factor for various species of *Plantago* is reported in Table 1 [3,4].

The seeds of *Plantago ovata* mainly contains mucilage which is a polysaccharide (20-30%) consist of highly branched, weakly acidic arabinoxylans (upto 85%) with a xylan backbone and branches of arabinose, xylose and 2-o-(galacturonic acid)-r-rhamnose residues. The seeds also contain fixed oil (~2.5%) consisting mainly linoic, oleic, palmitic acid with aliphatic hydrocarbons and oxygenated fatty acids, iridoids and protein [5]. In addition the secondary metabolites in the seeds include sterols, triterpenes and aucubin glycosides [6]. Because of its powerful ability to form a gel in water, *Psyllium* is classified as a mucilaginous fiber. This capability comes from its role as

the endosperm of the *P. ovata* seed, where it functions to retain water in order to prevent the seed from drying out.

Species	Swelling factor
<i>Plantago ovata</i>	10.25-13.50
<i>Plantago psyllium</i>	12.75
<i>Plantago arenaria</i>	14.5
<i>Plantago lanceolata</i>	4.45
<i>Plantago ovata</i> husk	20

Table 1: Swelling factor for various species of *Plantago*.

The dietary fibres from Ispaghula seeds and husk are not digested or absorbed by the body, have affinity for water [7]. When these fibers come in contact with water, gel is form that lubricates the bowel. The Ispaghula seeds are mainly used as bulk laxative [8] in habitual constipation and antidiarrhoeal in chronic amoebic as well as bacillary dysenteries and chronic diarrhoea due to irritative conditions of gastrointestinal tract. The action of drug appears to be purely mechanical being due to the mucilage which is not affected by digestive enzymes and passes through the small intestine unchanged. It lines the mucous membrane of small intestine and its demulcent properties gave it a protective and sedative action. Practically, the whole of the mucilage is passed out unchanged during 12-24 h following administration, as the intestinal bacteria have no/minimal action on it. During its passage through gut it coats the inflamed and ulcerated mucosa and protects it from being irritated by fluids and gases. This enables the lesion to heal quickly. The toxins produced by bacteria present in the gut are further absorbed by the mucilage and prevents its systemic absorption [9].

The seeds are taken in large quantities and they swell up in contact with water they increase the bulk of intestinal content and in this way relieve chronic constipation by mechanically stimulating the intestinal peristalsis. The seed act in very much the same way as liquid paraffin. It is also cheaper and is further free from injurious effect produced by habitual use of latter drug i.e., malignant disease of colon, eczema, paraffin pains, etc. [10-13].

Traditionally, Ispaghula is widely used for other medicinal purposes such as lipase inhibitor [14], hypolipidemic agent [15], arthritis, constipation [16-22], skin diseases and in cosmetic preparations [23-28]. Its activity has been found comparable to misoprostal and loperamide for its antidiarrheal activity [29-31]. Administration of Ispaghula is also beneficial in reducing gastrointestinal side effects of administrative drugs [32,33]. It has been reported that Ispaghula is capable of reducing postprandial blood glucose level in type II diabetes; non-insulin dependent diabetes with hyperlipidemia and improves insulin sensitivity [34-39]. Recently Ispaghula husk is also used in anti-obesity formulations along with other gums [40-42].

The literature survey reveals that different standard references describe different methods for determination of swelling Factor. It was practically observed that lots many variations are obtained in determination of swelling Factor of seeds. Since in all these methods the volume of water, nature of agitation and the time of agitation is not common and varies from method to method [43-48]. Also the type of water used (whether fresh, distilled, chloroform, boiled and cooled water, etc.) is not specified. Thus there are possibilities of variations in determination of swelling factor.

Determination of swelling factor of seeds is prime important to evaluate the sample on the basis of their swelling index. The reported data of these seeds show different values of swelling index from official and recognized standard books without mentioning the method used. To rectify such confusion and to avoid adulteration of inferior drug in genuine drugs as well as in formulations it is necessary to determine the swelling factor. Also swelling factor would reveal the chemical composition of different seeds which would further govern their therapeutic importance as well as their pharmaceutical applications in design of dosage forms.

Henceforth, the present work is undertaken with the aim to optimize the method, which will give reproducible results. For this the various parameters were studied such as effect of volume of solvent, nature of solvent, nature of agitation, time interval of agitation, effect of temperature, amount of seeds taken and the results were interpreted.

Material and Methods

Material

Calibrated glass stoppered measuring cylinders, (25, 50,100, 250 ml capacity), mesh (20, 40 of I.P. grade) and calibrated pipettes. Distilled water, chloroform water, hydrochloric acid (0.1N), sodium hydroxide (0.1N), alcohol (10, 20, and 90%), ethyl acetate and solvent ether were used as solvents. All the chemicals used were of Analytical grade (Loba).

Methods

Ispaghula seeds were cleaned by passing through 20, 40 mesh. Unwanted materials (grains, husk, etc.) were removed from it. 1 g of

seeds were transferred to the measuring cylinders (25, 50, 100, 250 ml) and required volume of solvent (distilled water, chloroform water, 90% alcohol, 0.1 N HCL, 0.1 N NaOH, ethyl acetate, solvent ether and alcohol moistened seeds + distilled water) was added and stoppered. The measuring cylinders were agitated after every 10 min. For first one hour and then after every 3 h. and the volume occupied by seeds along with mucilage was noted. This process was continued for 24 h. Final results were taken at the end of 24 h. The cylinders were kept for observation for next 24 h to note any change in swelling factor.

Experimental

Effect of volume of solvent

The volume of solvent specified in the already published procedures for determining the swelling factor is not same [44-48]. Hence to determine its effect 1 g of seeds were transferred to the measuring cylinders containing required volume of solvent (25, 50, 100, 250 ml). The cylinders were agitated after every 10 min for first 1 hr and then after every 3 h. Final volume occupied by seeds along with mucilage was noted at the end of 24 h.

Effect of nature of solvent

The mucilage part of the seed is mostly carbohydrate in nature (Mostly branched polysaccharides of glucouronic acid derivatives) having capacity to absorb the solvent and swells. The polysaccharides are mostly hydrolyzed either by enzymes or acid/base hydrolysis. Simpler saccharides are soluble in water while higher polymers are soluble in non-polar solvents. Hence possible acceptable solvents can be used for determination of swelling factors are distilled water, chloroform water IP, ethanol of different strength, ethyl acetate, dilute acids etc.

Based upon this, different solvents like distilled water, chloroform water, recently boiled and cooled water, hydrochloric acid (0.1 N), sodium hydroxide (0.1 N), alcohol (10, 20, 90%), ethyl acetate, and solvent ether were used. The amount of seeds (1 g) and the volume of each solvent (25 ml) were kept same and the same procedure was followed as described earlier.

Effect of agitation

The nature of agitation as well as time interval of agitation was not clearly mentioned in most of the reference book [44-49].

To observe the effect of agitation, the experiment was performed in 3 sets:

Set 1: Agitation after every 3 h.

Set 2: Agitation after every 8 h.

Set 3: Without agitation.

Again by keeping the amount of seeds (1 g) and volume of solvent (25 ml) common, the same procedure was followed and results were noted. In all 3 cases, the cylinders were agitated after every 10 min for first 1 hr.

Effect of temperature

To see whether the temperature has any effect on swelling factor or not, the cylinders containing seeds and the solvent were kept at different temperature such as room temperature, 37°C and at 50°C in

oven. Amount of seeds (1 g) and volume of solvent (25 ml) were kept common and the same procedure was followed.

Effect of quantity of seeds

By taking different quantity of seeds (1, 2, 4 g), the same procedure was followed and the effect of amount of seeds on swelling factor was observed.

Swelling factor of different seeds

Seeds of *Lepodium sativum*, *Althia officinalis* and *Occimum sanctum* were chosen to compare the swelling factor, determined using IP and optimized method.

Results and Discussion

Effect of volume of solvent

Sufficient volume of solvent is always recommended or required for swelling of mucilage. Swelling mostly depends upon character of solvent. The results showed that volume of solvent used for determination of swelling factor, affects the value of swelling factor. Irrespective of the nature of solvent in most of the solvent better results were obtained for 100 ml of solvent. Effect different solvents with different volumes are shown in Table 2.

Solvent	Volume	Swelling factor after (n=5)							
	(ml)	3 h	6 h	9 h	12 h	15 h	18 h	21 h	24 h
Distilled water	100	9 ± 0.21	9.8 ± 0.10	9.8 ± 0.31	12.5 ± 0.42	13.8 ± 0.30	14.2 ± 0.24	15 ± 0.40	15 ± 0.24
	50	10.6 ± 0.34	10.6 ± 0.26	10.9 ± 0.54	11.8 ± 0.27	11.8 ± 0.36	12.2 ± 0.05	13 ± 0.14	13 ± 0.14
	25	8.8 ± 0.25	8.8 ± 0.47	9.6 ± 0.85	10.2 ± 0.26	10.6 ± 0.38	11.2 ± 0.41	13 ± 0.49	13.1 ± 0.52
	20+5*	8.9 ± 0.66	8.9 ± 0.53	9.2 ± 0.48	9.8 ± 0.46	10.6 ± 0.33	11.2 ± 0.21	12 ± 0.09	12 ± 0.10
Chloroform water	100	9.2 ± 0.76	9.2 ± 0.63	9.5 ± 0.45	10.5 ± 0.36	10.9 ± 0.34	12.5 ± 0.46	13 ± 0.33	13 ± 0.57
	50	10.2 ± 0.47	10.5 ± 0.09	10.5 ± 0.05	11.2 ± 0.08	11.8 ± 0.2	11.8 ± 0.42	13.1 ± 0.53	13.1 ± 0.35
	25	9.1 ± 0.46	9.4 ± 0.39	9.4 ± 0.36	9.8 ± 0.23	9.8 ± 0.12	10.4 ± 0.21	12.2 ± 0.46	12.2 ± 0.54
	20+5*	9 ± 0.39	9.2 ± 0.67	9.2 ± 0.45	10.5 ± 0.46	10.8 ± 0.39	10.8 ± 0.29	12 ± 0.57	12 ± 0.44
Alcohol moist seeds + distilled water	100	10.2 ± 0.12	10.9 ± 0.36	13.9 ± 0.34	16.2 ± 0.11	16.8 ± 0.30	17.2 ± 0.30	19 ± 0.40	19.2 ± 0.31
	50	9.3 ± 0.36	9.3 ± 0.23	9.6 ± 0.25	10.2 ± 0.33	10.2 ± 0.45	12.5 ± 0.18	12.8 ± 0.05	13 ± 0.05
	25	9.2 ± 0.09	9.4 ± 0.11	9.4 ± 0.10	10.2 ± 0.18	10.2 ± 0.26	10.6 ± 0.05	12 ± 0.29	14 ± 0.68
	20+5*	9.1 ± 0.15	9.2 ± 0.36	9.2 ± 0.33	9.5 ± 0.44	9.8 ± 0.40	10 ± 0.12	12 ± 0.05	12.2 ± 0.09
Hydrochloric acid (0.1N)	100	9.4 ± 0.21	9.9 ± 0.10	9.9 ± 0.32	10.8 ± 0.41	13.2 ± 0.20	13.8 ± 0.21	16.2 ± 0.41	17 ± 0.00
	50	10 ± 0.32	11 ± 0.63	11 ± 0.18	12 ± 0.05	12.9 ± 0.36	13.2 ± 0.46	14 ± 0.34	14 ± 0.44
	25	9 ± 0.05	10.2 ± 0.32	10.5 ± 0.45	11 ± 0.32	11.8 ± 0.36	12 ± 0.37	13 ± 0.28	13.2 ± 0.22
	20+5*	9 ± 0.45	10 ± 0.12	11.5 ± 0.48	11.5 ± 0.12	11.8 ± 0.36	12 ± 0.20	12.2 ± 0.50	13 ± 0.22
Sodium hydroxide (0.1N)	100	6 ± 0.21	6.8 ± 0.33	6.8 ± 0.45	7.2 ± 0.44	8.4 ± 0.22	8.4 ± 0.20	9 ± 0.34	9.1 ± 0.34
	50	5.8 ± 0.18	6.2 ± 0.36	6.2 ± 0.29	6.3 ± 0.12	6.5 ± 0.18	6.5 ± 0.27	7.2 ± 0.05	7.2 ± 0.29
	25	6 ± 0.30	6 ± 0.18	6.5 ± 0.10	6.5 ± 0.09	6.8 ± 0.12	6.8 ± 0.31	7 ± 0.30	7.2 ± 0.44
	20+5*	5.5 ± 0.38	5.5 ± 0.32	5.9 ± 0.68	5.9 ± 0.56	5.9 ± 0.45	6.2 ± 0.50	6.2 ± 0.36	6.3 ± 0.15
Ethyl acetate	100	1.5 ± 0.15	1.5 ± 0.09	1.9 ± 0.29	1.9 ± 0.34	1.9 ± 0.36	2 ± 0.44	2 ± 0.50	2 ± 0.38
	50	2 ± 0.46	2.5 ± 0.48	2.5 ± 0.32	2.7 ± 0.18	3 ± 0.27	3.2 ± 0.33	3.2 ± 0.56	3.2 ± 0.60
	25	2.5 ± 0.42	2.6 ± 0.09	2.6 ± 0.38	2.8 ± 0.05	3.2 ± 0.12	3.2 ± 0.45	3.5 ± 0.51	3.5 ± 0.15
	20+5*	2.5 ± 0.75	2.5 ± 0.15	2.8 ± 0.05	3.2 ± 0.15	3.2 ± 0.29	3.2 ± 0.32	3.3 ± 0.29	3.3 ± 0.66
Solvent ether	100	1.5 ± 0.38	1.5 ± 0.18	1.5 ± 0.09	1.5 ± 0.66	1.5 ± 0.15	1.5 ± 0.20	1.5 ± 0.18	1.5 ± 0.22
	50	2 ± 0.66	2 ± 0.32	2 ± 0.08	2 ± 0.05	2 ± 0.36	2 ± 0.38	2 ± 0.15	2 ± 0.33

	25	2.5 ± 0.12	2.5 ± 0.29	2.5 ± 0.43	2.5 ± 0.23	2.5 ± 0.36	2.5 ± 0.09	2.5 ± 0.32	2.5 ± 0.38
	20+5*	2.5 ± 0.15	2.5 ± 0.26	2.5 ± 0.05	2.5 ± 0.12	2.5 ± 0.35	2.5 ± 0.67	2.5 ± 0.74	2.5 ± 0.25
10% alcohol	100	6.2 ± 0.08	6.5 ± 0.18	7 ± 0.48	7.5 ± 0.08	7.8 ± 0.46	7.8 ± 0.22	8 ± 0.09	8.5 ± 0.08
	50	6 ± 0.48	6.5 ± 0.08	6.5 ± 0.34	6.5 ± 0.12	6.8 ± 0.05	6.8 ± 0.18	7.2 ± 0.22	7.5 ± 0.39
	25	5.2 ± 0.65	6 ± 0.09	6.8 ± 0.32	7.4 ± 0.36	8 ± 0.88	8.4 ± 0.09	8.4 ± 0.14	9.1 ± 0.34
	20+5*	5 ± 0.36	5.5 ± 0.77	6.5 ± 0.37	6.5 ± 0.36	7.5 ± 0.45	8.2 ± 0.44	8.2 ± 0.44	9 ± 0.09
20% alcohol	100	5.8 ± 0.44	6.2 ± 0.68	6.5 ± 0.45	6.5 ± 0.56	6.8 ± 0.05	6.8 ± 0.00	7 ± 0.56	7 ± 0.68
	50	5 ± 0.57	5.2 ± 0.36	5.2 ± 0.78	5.5 ± 0.69	5.6 ± 0.36	5.8 ± 0.12	5.8 ± 0.16	6 ± 0.44
	25	5.2 ± 0.56	6.2 ± 0.37	6.8 ± 0.80	7 ± 0.05	7.2 ± 0.68	7.8 ± 0.37	7.8 ± 0.21	7.9 ± 0.36
	20+5*	4.6 ± 0.32	4.6 ± 0.25	5.2 ± 0.26	5.5 ± 0.28	5.5 ± 0.39	5.7 ± 0.25	6 ± 0.45	6.2 ± 0.68
90% alcohol	100	4.5 ± 0.12	4.5 ± 0.13	4.5 ± 0.20	5 ± 0.12	5 ± 0.50	5 ± 0.32	5 ± 0.37	5 ± 0.56
	50	2.8 ± 0.56	2.8 ± 0.42	2.8 ± 0.32	3 ± 0.78	3.2 ± 0.63	3.2 ± 0.33	3.2 ± 0.68	3.2 ± 0.45
	25	1.5 ± 0.18	1.5 ± 0.39	1.6 ± 0.50	1.8 ± 0.44	1.8 ± 0.45	1.9 ± 0.32	1.9 ± 0.36	2 ± 0.56
	20+5*	1.4 ± 0.30	1.4 ± 0.40	1.6 ± 0.42	1.6 ± 0.36	1.7 ± 0.50	1.9 ± 0.48	2 ± 0.23	2 ± 0.18
Recently boiled and cooled water	100	9 ± 0.48	9.5 ± 0.50	10.5 ± 0.48	13 ± 0.14	13.8 ± 0.36	14.5 ± 0.12	15 ± 0.20	15 ± 0.22
	50	11 ± 0.36	11.5 ± 0.12	11.5 ± 0.42	12 ± 0.05	13.5 ± 0.15	13.5 ± 0.56	13.8 ± 0.40	14 ± 0.48
	25	9 ± 0.45	9 ± 0.36	9.5 ± 0.50	10 ± 0.48	12 ± 0.09	12.5 ± 0.40	14 ± 0.12	14.2 ± 0.60
	20+5*	10 ± 0.18	10 ± 0.36	11.8 ± 0.44	12.5 ± 0.12	12.5 ± 0.64	12.8 ± 0.50	13 ± 0.05	13.1 ± 0.40

Table 2: Effect of volume of solvent and nature of solvent on 1 g of seeds (*In this case first 1 g of seeds was transferred to measuring cylinder containing 20 ml of solvent and then volume was adjusted up to 25 ml).

Effect of nature of solvents

Swelling factor in case of alcohol moistened seeds + distilled water shows better results.

It was also observed that,

- Organic solvents have a minimal/no effect on swelling of seeds.
- Acidic medium favours the swelling of seeds.
- Basic medium and alcohol (90%) has a hindering effect on the swelling of seeds (Table 2 and Figure 1).

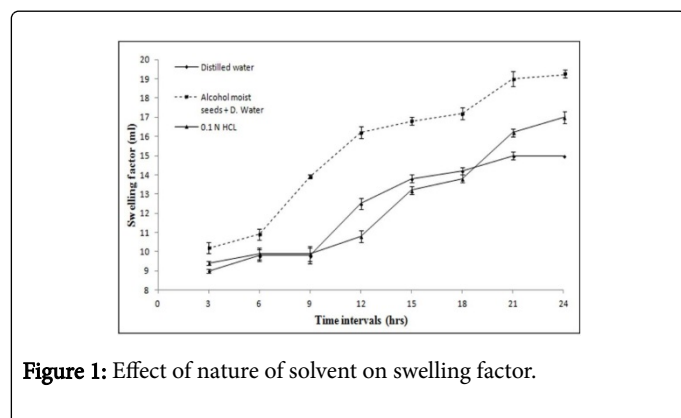


Figure 1: Effect of nature of solvent on swelling factor.

Effect of agitation

Agitation has prominent effect on swelling factor. This was based on practical outcomes as we have tried agitation at regular intervals of 2, 3, 4, 5, 6, 7 and 8 h and found maximum swelling by agitating at 3 h interval up to 24 h. The 8 h cycle was kept to check the effect of longer duration of agitation time so that we can avoid the frequency of agitations but results are not satisfactory. Noteworthy Agitation at every 3 h for 24 h has shown superior results (Table 3 and Figure 2).

Time interval of agitation	Swelling factors in solvents (n=5)			
	In distilled water	In chloroform water	In hydrochloric acid (0.1N)	In alcohol moist seeds + distilled water
Without agitation	10.2 ± 0.45	10 ± 0.54	9 ± 0.18	10.8 ± 0.22
After every 8 h	11.8 ± 0.36	10.8 ± 0.09	11 ± 0.44	11.8 ± 0.78
After every 3 h	13.2 ± 0.05	12 ± 0.36	13 ± 0.56	13.6 ± 0.40

Table 3: Effect of agitation on 1 g of seeds in 25 ml of solvents.

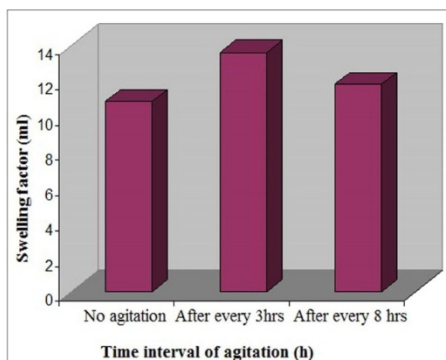


Figure 2: Effect of agitation on swelling factor in alcohol moistened seeds + distilled water.

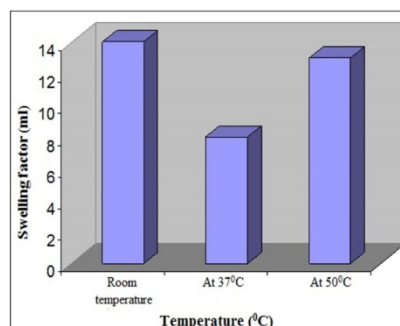


Figure 3: Effect of temperature on swelling factor in alcohol moistened seeds + distilled water.

Effect of temperature

Pre-eminent results were obtained for swelling factor determination, performed at room temperature followed by 50°C and at 37°C (Figure 3). Study at higher temperatures, like 60 and 70°C showed negative results may be due to increasing the solubility of mucilage rather than swelling and also by decomposition of mucilage due to breaking the linkages at higher temperature.

Effect of quantity of seeds

Swelling factor increases proportionally with the amount of seeds taken in case of distilled water and alcohol moistened seeds + distilled water (Table 4). But it was not the same case with hydrochloric acid (0.1 N), which shows erratic results.

Weight of seeds (g)	Volume of solvent (ml)	Swelling factors in solvents (n=5)		
		In distilled water	In hydrochloric acid (0.1N)	In alcohol moist seeds + distilled water
1	50	13	14	13
2	50	22	20	29
1	100	15	17	19
4	100	20	35	66

Table 4: Effect of quantity of seeds.

Swelling factor of different seeds

Swelling factor of different seeds like *Lepodium sativum*, *Althia officinalis* and *Occimum sanctum* has been evaluated by IP method and optimised method for 24 h. Study reveals that optimised method gives outstanding results than IP method (Table 5 and Figure 4).

Conclusion

After performing the experiment using different parameters, keeping one parameter as variable while all other parameters constant, it was observed that the swelling index of *Plantago ovata* gives better results using the solvents plain distilled water, seeds moistened with 1 ml of 90% alcohol followed by addition of distilled water and hydrochloric acid (0.1 N). Out of these three suitable solvents alcohol moistened seeds with distilled water gave significant results. The quantity of solvent used up to 100 ml gave best results for swelling index. It was also observed that agitation at every three hours till the process of 24 h is maintained would give better results for swelling factor.

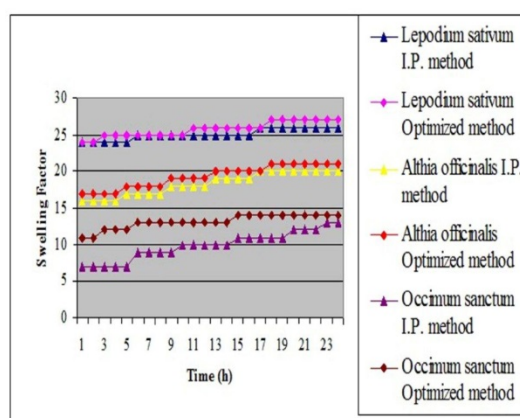


Figure 4: Swelling factor of different seeds.

Time (hr)	<i>Lepodium sativum</i>		<i>Althia officinalis</i>		<i>Occimum sanctum</i>	
	I.P. method	Optimized method	I.P. method	Optimized method	I.P. method	Optimized method
1	24	24	16	17	7	11
2	24	24	16	17	7	11
3	24	25	16	17	7	12
4	24	25	16	17	7	12
5	24	25	17	18	7	12
6	25	25	17	18	9	13
7	25	25	17	18	9	13
8	25	25	17	18	9	13
9	25	25	18	19	9	13
10	25	25	18	19	10	13
11	25	26	18	19	10	13
12	25	26	18	19	10	13
13	25	26	19	20	10	13
14	25	26	19	20	10	13
15	25	26	19	20	11	14
16	25	26	19	20	11	14
17	26	26	20	20	11	14
18	26	27	20	21	11	14
19	26	27	20	21	11	14
20	26	27	20	21	12	14
21	26	27	20	21	12	14
22	26	27	20	21	12	14
23	26	27	20	21	13	14
24	26	27	20	21	13	14

Table 5: Swelling factor of various seeds.

The effect of temperature was also observed and found that room temperature is best suitable for determination of swelling factor, since at higher temperature the results are erratic. Quantity of seeds used shows linear results with the swelling factor i.e., as the quantity of seeds increases the swelling factor also increases. The experiment was carried out for further 24 h but no change in swelling factor was observed.

Optimized method

Hence optimized method for determination of swelling factor is as follows:

Take 1 g of cleaned ripped seeds (previously passed through mesh 40 and 20) of *Plantago ovata* (or other variety) and moisten with 1 ml of 90% alcohol and then transferred in to 100 ml calibrated, well-stoppered measuring cylinder. Then add distilled water upto calibrated

mark and agitate the cylinder at every 10 min for first 1 hr and keep aside for 3 h. Repeat the agitation at regular interval of 3 h and finally measure the volume occupied by seeds along with mucilage after completion of 24 h. Simultaneously carry out 5 such determinations and calculate the mean of results.

*The alcohol used for moistening in the method also acts as a preservative. Since no growth of microorganisms was observed even after confirming the experiment for more than 36 h.

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