

Optimization Method for Determination of Swelling Factor *Linum usitatissimum* Seeds

S Dhanalakshmi^{1,*}, C N Hemalatha², Sai Ramya Bharathi³, C Dhivya⁴, S Vanishree⁵, V Rekha⁶, V Vijayalakshmi⁷, A Hari priya⁸, Sai Monisha⁹

S Dhanalakshmi^{1,*}, C N Hemalatha², Sai Ramya Bharathi³, C Dhivya⁴, S Vanishree⁵, V Rekha⁶, V Vijayalakshmi⁷, A Hari priya⁸, Sai Monisha⁹

¹Department of Pharmacognosy, Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute, Velappanchavadi, Chennai – 600 077, Tamil Nadu, INDIA.

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute, Velappanchavadi, Chennai – 600 077, Tamil Nadu, INDIA.

³Department of Pharmaceutics, Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute, Velappanchavadi, Chennai – 600 077, Tamil Nadu, INDIA.

⁴Department of Pharmacology, Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute, Velappanchavadi, Chennai – 600 077, Tamil Nadu, INDIA.

Correspondence

S Dhanalakshmi

Department of Pharmacognosy, Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute, Velappanchavadi, Chennai – 600 077, Tamil Nadu, INDIA.

E-mail: dhanadinesh2011@gmail.com

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ABSTRACT

Aim: Aspire of the investigation is to amend the method for determination of swelling factor of seeds, so it can be determined to optimize the minimum errors which will be reproducible. Swelling factor is play key role if the phytoconstituent rich in mucilage and polysaccharide which constitute a diverse class of biological macromolecules. **Background:** Sugar molecules play a broad range of physiochemical properties which are widely used for applications in Pharmacy and the present investigation work is related 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. Solvent Optimized technic gives superlative results over IP method. **Methods:** The swelling factor of *Linum usitatissimum* was evaluated by using different polar to non-polar solvents by using the standard procedure. During the determination, the role of physical parameter were calculated by comprising the swelling factor with agitation and temperature. **Results:** According to the observations, the lower concentration levels of swelling index used as an alternative binder to starch. keeping one parameter as variable while all other parameters constant, it was observed that the swelling index of gives better results using the solvents plain distilled water, seeds moistened with alcohol. It was also observed that agitation at every three hours till the process of 24 hours is maintained would give better results, keeping one parameter as variable while all other parameters constant.

Key words: Seed, Swelling index, Formulation parameter.

INTRODUCTION

During the pre-formulation studies, the swelling index needed to be considered for the preparation of muco-adhesive layer. Generally, the flax is rich in fiber and oil. Linen is the most important fiber of the flax plant. The seed contains 6.3–6.6% H₂O, 18.0–20.3% protein, 34.0–37.1% fat, 33.6–37.2 total carbohydrate, 4.8–8.8% fiber. The linseed mucilage consists of 4.57% moisture, 95.43% dry weight, 5.8% ash, and 12.3% protein. The neutral polysaccharide fraction, having a higher intrinsic viscosity ([eta] = 6.6 vs 4.6 dL/g), exhibited more pronounced shear thinning and viscoelastic responses in solution than its acidic counterpart.¹ All mucilage preparations exhibited stable viscosity in the pH range 5.0-9.0; however, large reductions in viscosity were seen with the addition of electrolytes. The water binding capacity (1600-3000 g of H₂O/100 g of solids) and theological properties of mucilage resembled those of guar gum.

MATERIALS AND METHODS

Materials

The dried seeds of *Linum usitatissimum* (Linaceae), was collected from the local market. 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 (0.1 N), sodium hydroxide

(0.1 N), alcohol (10,20 and 90%), ethyl acetate and solvent ether were used as solvents. All the chemicals used were of analytical grade (Loba) (Figures 1-9).

Methods

Seeds of *Linum usitatissimum* (Linaceae) 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% of alcohol, 0.1 NHCl, 0.1N NaOH, ethyl acetate, solvent ether and alcohol moistened seeds + distilled water) was added and stoppered.² The measuring cylinders were agitated after every 10 mins. For first one hour and then after every 3 hours and the volume occupied by seeds along with mucilage was noted. This process was continued for 24 hours. Final results were taken at the ends of 24 hours. The cylinders were kept for observation for next 24 hrs to note any change in swelling factor.

EXPERIMENTAL INVESTIGATION

Effect of volume of solvent³

The volume of solvent specified in the already published procedures for determining the swelling factor is not same. Hence to determine its effect 1 g if seeds were transferred to the measuring cylinders containing required volume of solvent (25,50,100,250

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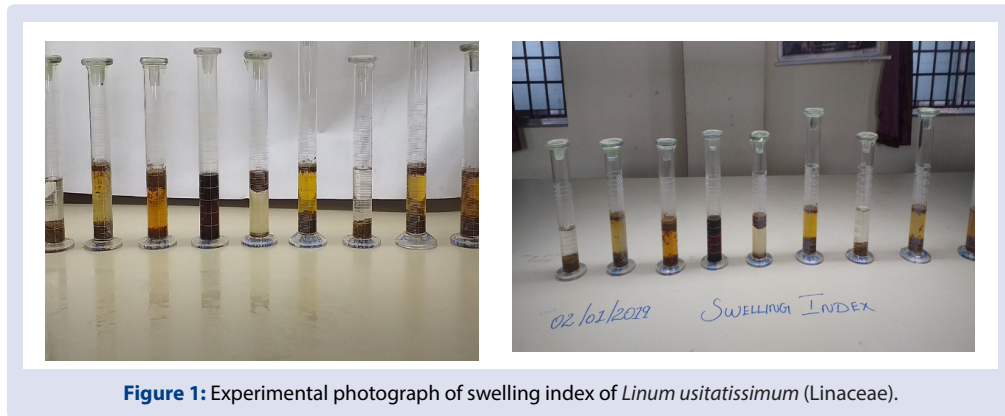


Figure 1: Experimental photograph of swelling index of *Linum usitatissimum* (Linaceae).

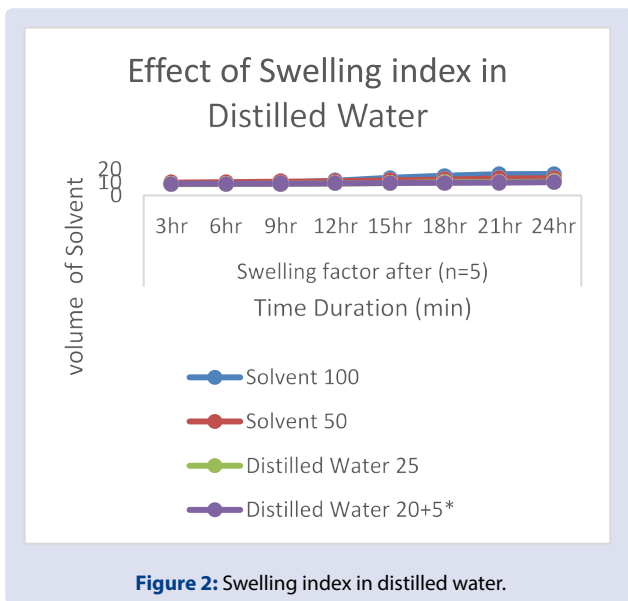


Figure 2: Swelling index in distilled water.

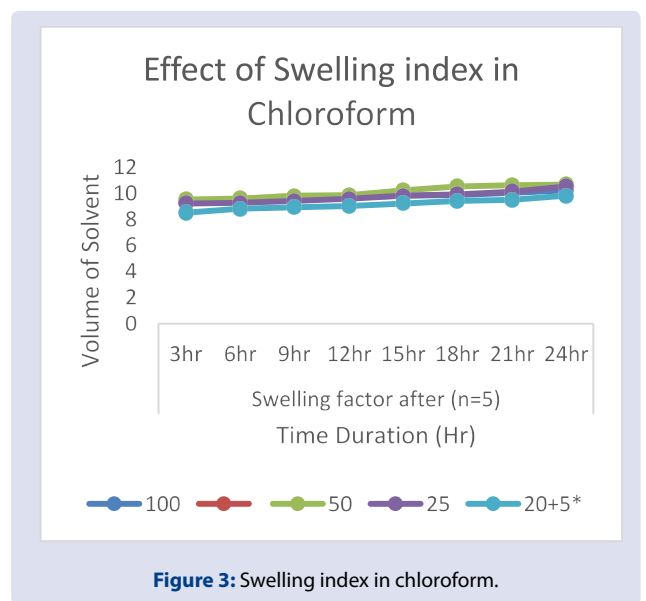


Figure 3: Swelling index in chloroform.

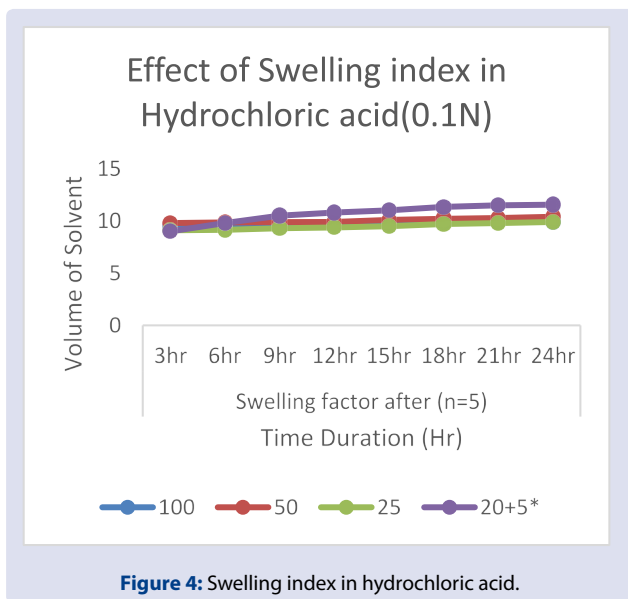


Figure 4: Swelling index in hydrochloric acid.

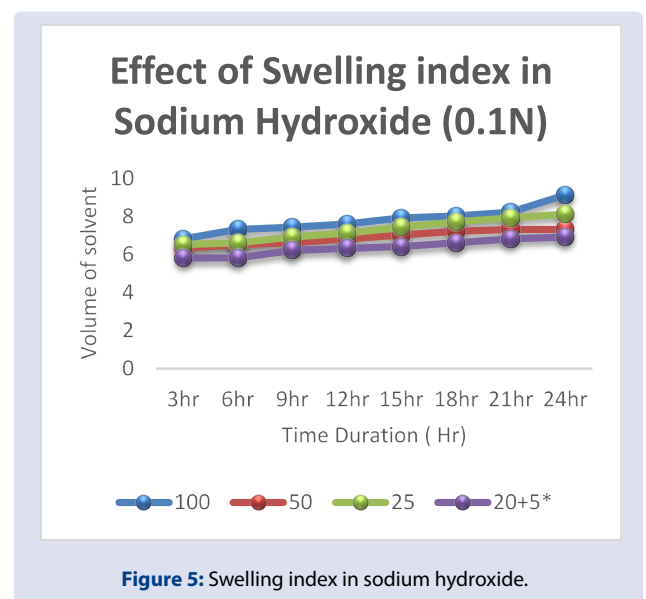


Figure 5: Swelling index in sodium hydroxide.

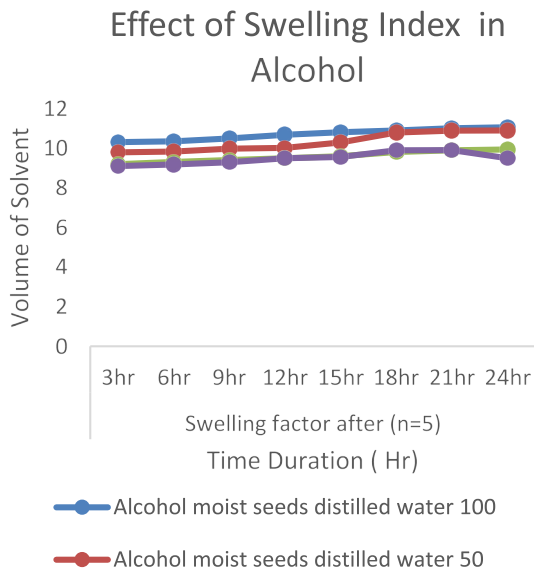


Figure 6: Swelling index in alcohol.

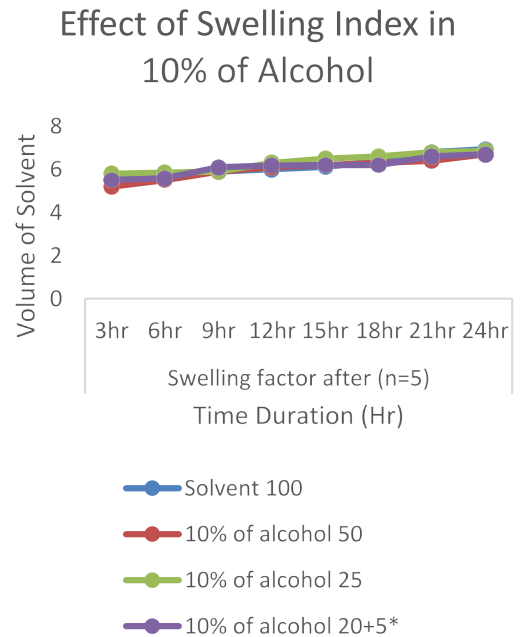


Figure 7: Swelling index in 10% alcohol.

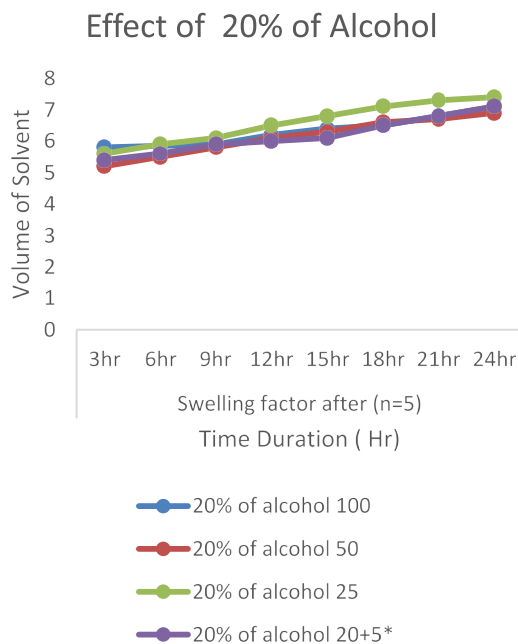


Figure 8: Swelling index in 20% alcohol.

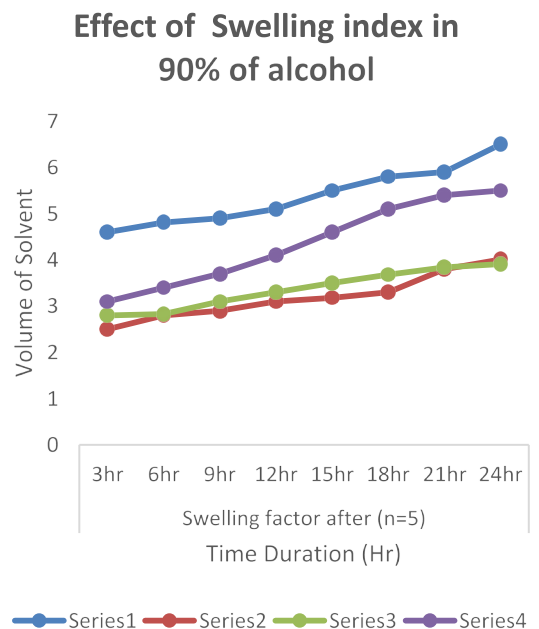


Figure 9: Swelling index in 90% alcohol.

ml). The cylinders were agitated after every 10 mins for the first 1hr and then after every 3 hr. Final volume occupied by seeds along with mucilage was noted at the end of 24 hr.

Effect of nature of solvent⁴

The mucilage part of the seeds is mostly carbohydrate in nature (Mostly branched polysaccharides of glucuronic 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 (Figures 10-12).

Effect of agitation⁵

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

Set 1: Agitation after every 3 hr.

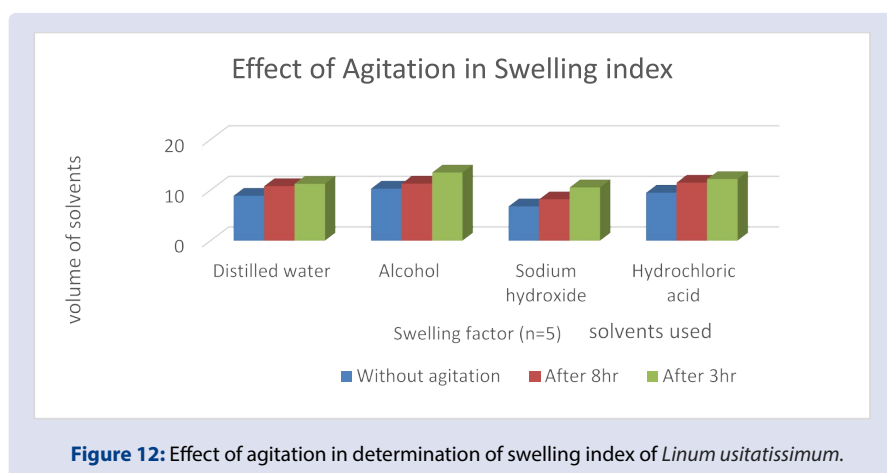
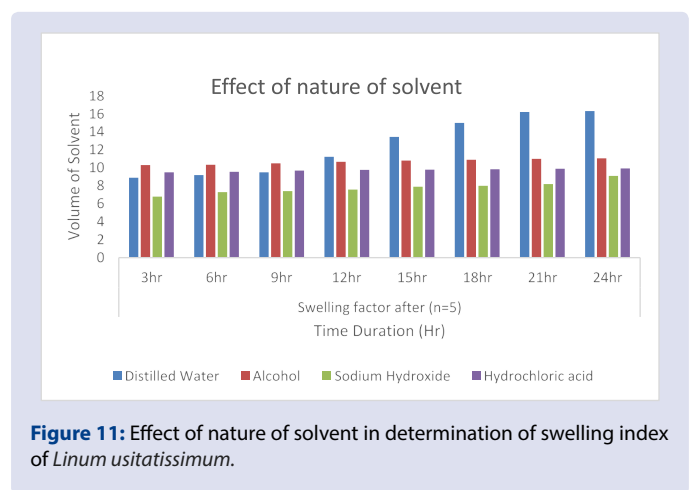
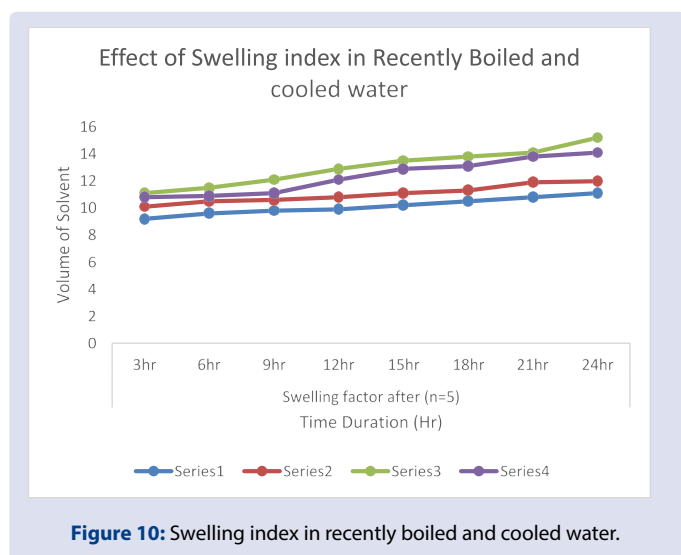
Set 2: Agitation after every 8 hr.

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 temperature⁶

To check 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 50°C in oven, Amount of seeds (1 g) and the volume of solvents (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.

RESULTS

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 favors the swelling of seeds. Basic medium and alcohol (90%) has a hindering effect on the swelling of seeds (Table 1).^{7,8}

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 8 hr and 3 hr. Noteworthy agitation at every 3 hr for 24 hrs has shown superior results compared with 8 hr (Table 2).⁹

Table 1: Effect of swelling index in *Linum usitatissimum* for optimization of solvent.

Solvent	Volume	Swelling factor after (n=5)							
	ml	3hr	6hr	9hr	12hr	15hr	18hr	21hr	24hr
Distilled Water	100	8.9 ± 0.21	9.2 ± 0.21	9.5 ± 0.33	11.23 ± 0.8	13.45 ± 0.6	15.01 ± 0.24	16.23 ± 0.40	16.33 ± 0.41
	50	9.8 ± 0.34	10.1 ± 0.61	10.5 ± 0.26	10.81 ± 0.31	11.41 ± 0.45	12.04 ± 0.15	13.02 ± 0.4	13.12 ± 0.56
	25	8.6 ± 0.11	8.7 ± 0.31	8.9 ± 0.65	9.1 ± 0.78	9.4 ± 0.58	10.1 ± 0.65	10.3 ± 0.78	10.5 ± 0.81
	20+5*	8.9 ± 0.24	8.9 ± 0.11	8.95 ± 0.8	9.2 ± 0.71	9.5 ± 0.65	9.63 ± 0.58	9.8 ± 0.56	10.1 ± 0.61
Chloroform water	100	9.3 ± 0.23	9.5 ± 0.56	9.6 ± 0.81	9.63 ± 0.21	9.8 ± 0.23	9.85 ± 0.56	10.01 ± 0.63	10.2 ± 0.85
	50	9.5 ± 0.25	9.58 ± 0.56	9.8 ± 0.78	9.84 ± 0.89	10.2 ± 0.56	10.5 ± 0.65	10.6 ± 0.56	10.65 ± 0.89
	25	9.2 ± 0.45	9.25 ± 0.33	9.4 ± 0.65	9.56 ± 0.88	9.8 ± 0.58	9.9 ± 0.89	10.1 ± 0.23	10.5 ± 0.25
	20+5*	8.5 ± 0.59	8.8 ± 0.45	8.91 ± 0.56	9.0 ± 0.54	9.2 ± 0.89	9.4 ± 0.25	9.48 ± 0.35	9.8 ± 0.89
Alcohol moist seeds distilled water	100	10.31 ± 0.56	10.35 ± 0.89	10.5 ± 0.54	10.68 ± 0.89	10.81 ± 0.87	10.9 ± 0.25	11.01 ± 0.56	11.06 ± 0.87
	50	9.8 ± 0.81	9.84 ± 0.25	9.98 ± 0.84	10.02 ± 0.58	10.3 ± 0.57	10.8 ± 0.89	10.89 ± 0.58	10.9 ± 0.12
	25	9.2 ± 0.58	9.31 ± 0.64	9.41 ± 0.58	9.5 ± 0.47	9.6 ± 0.47	9.81 ± 0.25	9.9 ± 0.89	9.94 ± 0.12
	20+5*	9.1 ± 0.25	9.18 ± 0.89	9.3 ± 0.8	9.5 ± 0.81	9.56 ± 0.58	9.9 ± 0.78	9.91 ± 0.1	9.5 ± 0.47
Hydrochloric acid (0.1N)	100	9.5 ± 0.56	9.56 ± 0.89	9.7 ± 0.52	9.78 ± 0.56	9.8 ± 0.51	9.85 ± 0.81	9.9 ± 0.41	9.94 ± 0.11
	50	9.8 ± 0.41	9.86 ± 0.2	9.88 ± 0.2	9.9 ± 0.51	10.1 ± 0.25	10.21 ± 0.12	10.28 ± 0.84	10.4 ± 0.51
	25	9.1 ± 0.51	9.15 ± 0.81	9.3 ± 0.54	9.38 ± 0.21	9.5 ± 0.54	9.7 ± 0.41	9.8 ± 0.41	9.9 ± 0.21
	20+5*	9.0 ± 0.13	9.8 ± 0.41	10.5 ± 0.85	10.8 ± 0.56	11.01 ± 0.21	11.35 ± 0.84	11.5 ± 0.58	11.56 ± 0.21
Sodium hydroxide (0.1N)	100	6.8 ± 0.21	7.3 ± 0.41	7.41 ± 0.83	7.58 ± 0.25	7.9 ± 0.45	8.0 ± 0.9	8.2 ± 0.25	9.1 ± 0.54
	50	6.3 ± 0.21	6.5 ± 0.15	6.6 ± 0.51	6.8 ± 0.51	7.0 ± 0.26	7.2 ± 0.84	7.3 ± 0.25	7.3 ± 0.21
	25	6.5 ± 0.56	6.6 ± 0.21	6.9 ± 0.54	7.1 ± 0.31	7.4 ± 0.62	7.7 ± 0.91	7.9 ± 0.21	8.1 ± 0.31
	20+5*	5.8 ± 0.32	5.81 ± 0.5	6.2 ± 0.51	6.3 ± 0.61	6.4 ± 0.61	6.6 ± 0.58	6.8 ± 0.54	6.9 ± 0.32
Ethyl acetate	100	2.1 ± 0.32	2.25 ± 0.51	2.4 ± 0.94	2.8 ± 0.54	3.2 ± 0.91	3.5 ± 0.1	3.58 ± 0.69	3.7 ± 0.51
	50	2.0 ± 0.51	2.5 ± 0.68	2.8 ± 0.21	3.1 ± 0.98	3.5 ± 0.84	3.58 ± 0.11	3.9 ± 0.36	4.1 ± 0.71
	25	2.5 ± 0.61	2.8 ± 0.62	2.9 ± 0.36	3.1 ± 0.54	3.3 ± 0.65	3.8 ± 0.32	3.9 ± 0.5	3.95 ± 0.36
	20+5*	2.6 ± 0.91	2.7 ± 0.21	2.81 ± 0.6	2.85 ± 0.35	3.1 ± 0.68	3.21 ± 0.62	3.3 ± 0.58	3.41 ± 0.1
Solvent ether	100	1.8 ± 0.31	1.83 ± 0.58	1.95 ± 0.1	2.1 ± 0.3	2.15 ± 0.61	2.21 ± 0.2	2.24 ± 0.5	2.3 ± 0.6
	50	2.1 ± 0.66	2.12 ± 0.32	2.2 ± 0.6	2.28 ± 0.61	2.9 ± 0.9	3.1 ± 0.21	3.18 ± 0.81	3.2 ± 0.62
	25	2.5 ± 0.31	2.56 ± 0.21	2.7 ± 0.36	2.71 ± 0.32	2.71 ± 0.51	2.72 ± 0.62	2.7 ± 0.8	2.8 ± 0.56
	20+5*	2.5 ± 0.2	2.5 ± 0.25	2.51 ± 0.35	2.58 ± 0.61	2.6 ± 0.6	2.62 ± 0.8	2.7 ± 0.1	2.7 ± 0.6
10% of alcohol	100	5.5 ± 0.25	5.8 ± 0.62	5.9 ± 0.8	6.01 ± 0.5	6.12 ± 0.5	6.4 ± 0.25	6.8 ± 0.35	6.94 ± 0.65
	50	5.2 ± 0.1	5.5 ± 0.81	5.9 ± 0.5	6.1 ± 0.1	6.2 ± 0.55	6.3 ± 0.31	6.4 ± 0.5	6.7 ± 0.68
	25	5.8 ± 0.56	5.85 ± 0.6	5.9 ± 0.4	6.3 ± 0.9	6.5 ± 0.8	6.6 ± 0.35	6.8 ± 0.32	6.85 ± 0.1
	20+5*	5.5 ± 0.2	5.59 ± 0.31	6.1 ± 0.3	6.18 ± 0.51	6.2 ± 0.5	6.25 ± 0.32	6.6 ± 0.9	6.7 ± 0.51
20% of alcohol	100	5.8 ± 0.44	5.85 ± 0.51	5.9 ± 0.36	6.2 ± 0.64	6.4 ± 0.47	6.5 ± 0.65	6.8 ± 0.51	7.1 ± 0.56
	50	5.2 ± 0.12	5.5 ± 0.6	5.8 ± 0.68	6.1 ± 0.8	6.3 ± 0.5	6.6 ± 0.2	6.7 ± 0.58	6.9 ± 0.57
	25	5.6 ± 0.31	5.9 ± 0.8	6.1 ± 0.2	6.5 ± 0.48	6.8 ± 0.6	7.1 ± 0.2	7.3 ± 0.51	7.4 ± 0.6
	20+5*	5.4 ± 0.5	5.6 ± 0.61	5.9 ± 0.21	6.0 ± 0.5	6.1 ± 0.87	6.5 ± 0.9	6.8 ± 0.84	7.1 ± 0.25
90% of alcohol	100	4.6 ± 0.21	4.81 ± 0.3	4.9 ± 0.65	5.1 ± 0.62	5.5 ± 0.8	5.8 ± 0.81	5.9 ± 0.2	6.5 ± 0.31
	50	2.5 ± 0.31	2.8 ± 0.51	2.9 ± 0.81	3.1 ± 0.6	3.18 ± 0.6	3.3 ± 0.2	3.8 ± 0.6	4.01 ± 0.56
	25	2.8 ± 0.6	2.83 ± 0.1	3.1 ± 0.6	3.3 ± 0.21	3.5 ± 0.25	3.68 ± 0.95	3.84 ± 0.6	3.91 ± 0.5
	20+5*	3.1 ± 0.6	3.4 ± 0.2	3.7 ± 0.26	4.1 ± 0.65	4.6 ± 0.25	5.1 ± 0.2	5.4 ± 0.3	5.5 ± 0.2
Recently boiled and cooled water	100	9.2 ± 0.3	9.6 ± 0.51	9.8 ± 0.3	9.9 ± 0.51	10.2 ± 0.3	10.5 ± 0.6	10.8 ± 0.9	11.1 ± 0.6
	50	10.1 ± 0.6	10.5 ± 0.3	10.6 ± 0.34	10.8 ± 0.65	11.1 ± 0.54	11.3 ± 0.9	11.9 ± 0.2	11.98 ± 0.1
	25	11.1 ± 0.3	11.5 ± 0.6	12.1 ± 0.3	12.9 ± 0.6	13.5 ± 0.2	13.8 ± 0.6	14.1 ± 0.2	15.2 ± 0.1
	20+5*	10.8 ± 0.5	10.9 ± 0.3	11.1 ± 0.6	12.1 ± 0.8	12.9 ± 0.2	13.1 ± 0.5	13.8 ± 0.9	14.1 ± 0.5

Table 2: Effect of agitation at regular intervals of 8hr and 3 hr.

Time interval of agitation	Swelling factor (n=5)			
	distilled water	Alcohol	Sodium hydroxide	Hydrochloric acid
Without agitation	8.9 ± 0.21	10.31 ± 0.56	6.8 ± 0.21	9.5 ± 0.56
After every 8 hr	10.8 ± 0.23	11.3 ± 0.56	8.2 ± 0.31	11.5 ± 0.98
After every 3 hr	11.25 ± 0.56	13.5 ± 0.85	10.56 ± 0.56	12.2 ± 0.41

CONCLUSION

It was observed that the swelling index seeds of *Linum usitatissimum* (Linaceae) 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. According to the observations, the lower concentration levels of swelling index used as an alternative binder to starch. Keeping one parameter as variable while all other parameters constant, it was observed that the swelling index gives better results using the solvents and plain distilled water, seeds moistened with 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. In context, present investigation showed that quantity of solvent used up to 100 ml gave best results for swelling index.¹⁰ It was also observed that agitation at every three hours till the process of 24 hrs is maintained would give better results. keeping one parameter as variable while all other parameters constant. 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. Further studies can be done to explore its role in drug delivery systems including its release retardant properties and muco-adhesive nature.

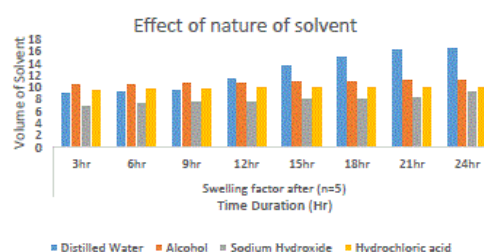
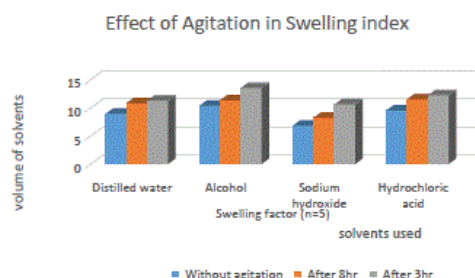
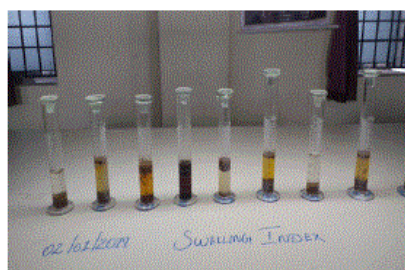
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GRAPHICAL ABSTRACT



***Linum usitatissimum* (seeds)**



ABOUT AUTHORS



Mrs. S. Dhanalakshmi M.Pharm., (Ph.D) Presently working as Asst.Professor under Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute (Deemed to be University) Velappanchavadi, Chennai – 600 077. At present her focusing research area in the mechanism of protein isolates / fraction for the Endometrial Hyperplasia with carcinoma.



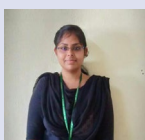
Dr. C. N. Hemalatha M.Pharm., Ph.D , Presently Working as Asst.professor under Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute (Deemed to be University). At present her focusing research area in the development of drug designing with QSAR and clinical studies.



Mrs. Sai Ramya bharathi M.Pharm (Pharmaceutics), presently working as Asst.Professor under Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute (Deemed to be University). At present here focusing research area in the for-drug development with clinical studies.



Mrs. C. Dhivya M.Pharm (Pharmacology), completed her Master degree at SRM University. At present her focusing area is to determine the mechanism for the wound healing and regenerative potential of anticarcinoma drug.



Ms. S. Vanishree, Pharma buddy (Dr.M.G.R Educational and Research Institute). Her research area is in the standardization of Herbal formulation and development of formulation.



Ms. V. Rekha, Pharma buddy (Dr.M.G.R Educational and Research Institute). Her research area is in the investigation of drug mechanism.



Ms. A. Hari Priya, Pharma buddy (Dr.M.G.R Educational and Research Institute). Her research area is in the development of nano formulation.



Ms. V. Vijayalakshmi, Pharma buddy (Dr.M.G.R Educational and Research Institute). Her research areas is designing of new drug formulation.



Mrs. V. Sai Monisha, Pharma buddy (Dr.M.G.R Educational and Research Institute). Her research interest is to design developed clinical studies.

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