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Isolation and evaluation of the emulsifying properties of tamarind seed polysaccharide on castor oil emulsion

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Abstract

Tamarind seed polysaccharide (TSP) obtained from the seed kernel of *Tamarindus indica*, possesses properties like high viscosity, broad pH tolerance, noncarcinogenicity, mucoadhesive nature, and biocompatibility. It is used as stabilizer, thickener, gelling agent, and binder in food and pharmaceutical industries. The objective of present investigation was to search for a cheap and effective natural excipient that can be used as an effective alternative for the formulation of pharmaceutical emulsions. For emulsifying activity study, castor oil was taken as a model drug and emulsified with TSP. The comparative stability studies were done with that of the emulsion prepared by taking gum acacia as standard emulsifying agent and it was found that the emulsion prepared with 2%w/v of TSP is more effective in comparison to that of the emulsion prepared by using 10%w/v of gum acacia. Thus this mucilage will be a non-toxic, bio-degradable, cheap, economic and easily available option as an emulsifier.

Key words: Emulsifier, Tamarind seed polysaccharide, Acacia gum, Pharmaceutical excipients, emulsion.

Introduction

Seed gums are important agrochemical used in various industries worldwide. The growing industrial utility of these gums 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.

Gum is a byproduct obtained as a result of metabolic mechanisms of plants. Natural gums are either water soluble or absorb water to form a viscous solution[1]. Natural gums are economic,

easily available. Gums have been wildly used as tablet binders, emulgents and thickeners in cosmetics and suspensions as film-forming agents and transitional colloids[2].

Polysaccharides are the choice of materials among the hydrophilic polymers used, because they are nontoxic and acceptable by the regulating authorities[3]. The various polysaccharides used in drug delivery application are cellulose ethers[4], xanthan gum [5], locust bean gum[6] and guar gum[7]. Another natural polysaccharide, Tamarind seed polysaccharide (TSP) obtained from the seed kernel of *Tamarindus indica*, possesses properties like high viscosity, broad pH tolerance, noncarcinogenicity, mucoadhesive nature, and biocompatibility[8]. It is used as stabilizer, thickener, gelling agent, and binder in food and pharmaceutical industries. The tamarind seed polysaccharide constitutes about 65% of the tamarind seed components[9]. It is a branched polysaccharide with a main chain of β-d-(1,4)- linked glucopyranosyl units, and that a side chain consisting of single d-xylopyranosyl unit attached to every second, third, and fourth d-glucopyrnosyl unit through an α-d-(1,6) linkage. One d-galatopyranosyl unit is attached to one of the xylopyranosyl units through a β-d-(1,2) linkage[10].

In the present study an effort was made to evaluate the efficacy of TSP as an emulsifier. The emulsifying characteristics of TSP was evaluated and compared with that of the emulsion prepared by using gum acacia as standard emulsifying agent.

Materials and Methods

Gum acacia obtained from E. Merck (India) Ltd., Mumbai, India. Tamarind kernel powder was obtained as gift sample from Sri Balaji industries, Bangalore. All the other chemicals, solvents and reagents used were of Pharmacopoeial and analytical grade were procured from Rajesh Chemicals, Mumbai.

Extraction of mucilage from tamarind seeds[9]
Tamarind seed mucilage was extracted by following the method reported earlier.200g of tamarind seeds were soaked in double distilled water and boiled for 5 hours to remove the outer dark layer. To inner white portion sufficient amount of doubled distilled water was added and boiled under stirring condition in a water bath until the slurry was prepared. The solution was cooled and kept in refrigerator overnight so that most of the undissolved portion was settled out. The upper clear solution was decanted off and centrifuged at 500 rpm for 20 minutes. The supernatant was separated and concentrated at 60ºC on a water bath until the volume reduced to one third of its original volume. Solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continuous stirring. The precipitate was washed repeatedly with acetone and dried at 50 –60ºC under vacuum. The dried material was powdered and kept in a desiccator.

Phytochemical Examination[11-12]
For the detection of the presence of carbohydrates, reducing sugars, tannins, mucilage and peroxidase enzymes the standard tests Molisch's test for carbohydrate, reduction of Fehling's solution for reducing sugars, ferric chloride test for tannins , ruthenium red test for mucilage and Benzidine test for presence of peroxidase enzymes were done.

Physicochemical characterization of mucilage[13-15]
The separated mucilage was evaluated for solubility, swelling index, loss on drying, ash value, microbial load, density, compressibility index and angle of repose.
Determination of viscosity

1 g of dried and finely powdered TSP was suspended in 75 ml of distilled water for 5 h. Distilled water added up to 100 ml to produce the concentration of 1% w/v. The mixture was homogenized by mechanical stirrer for 2 h and its viscosity determined using a Brookfield viscometer, spindle –LV2 (Brookfield LV-II, USA) at 20 rpm and 25°C.

Evaluation of Toxicity

Toxicity studies were carried out according to the method of Knudsen and Curtis[16]. The animals used in the toxicity studies were sanctioned by the Institute Animal Ethical Committee. The male albino rats of Wistar strain weighing 160-200 gm were divided into different groups comprising of six animals each. The control group received normal 0.5%CMC solution (20ml/kg i.p). The other groups received 500, 1000, 2000, 3000, 4000 and 5000 mg/kg of mucilage suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 72 hours. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 22 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 500 mg/kg was administered daily for a period of 30 d. body weights were recorded for both the groups at an interval of 10d. And at the end of 30 days, hematological and biochemical parameters were studied in both the groups and after 30 days of chronic toxicity study the animals were scarified and subjected to histopathological studies.

Characterization of TSP

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of TSP were recorded on samples prepared in potassium bromide (KBr) disks using a Shimadzu Corporation, (Tokyo, Japan) Model-1601 PC. Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 500 to 4000 cm^{-1}.

X-ray diffraction

Diffraction pattern of powdered TSP sample was recorded with an X-ray diffractometer (Panalytical spectris Pvt.Ltd., Singapore). X-ray diffraction was performed at room temperature (30^oC) with a diffractometer; target, Cu(λ=1.54Å), filter, Ni; Voltage, 40kV; current 30mA; time constant 10mm/s; scanning rate 2^o/min; measured from 10-35^o at full scale 200.

Preparation of Emulsion

To study the emulsifying characteristics, 2ml of the TSP was taken in a clean and dry mortar and pestle. The emulsification of castor oil, tried by wet gum method[17].

The oil was added to the TSP drop by drop with continuous triturating until a white primary emulsion with clicking sound results. The addition of oil continued till a white cream was obtained. From the study it was found that the primary emulsion results at the oil: gum ratio of 2:1 v/v. Now the primary emulsion diluted to 20ml with distilled water and kept in a clean and tightly closed container for stability study for 6 months. No creaming or settling of emulsion results during 6 months of storage period at room temperature, which indicate that the TSP is very effective as emulsifying agent at low concentration in comparison to other gums.

Evaluation of emulsion

Stability Studies of Emulsions

To standardize the emulsifying characteristics and stability of TSP with that of the gum acacia as standard emulsifier[18-19] an aqueous emulsifier solution was prepared by dispersing 10% w/v of gum acacia (as standard) and stirred for 6h to ensure complete dissolution. A 10% v/v of castor oil (as model drug) in water emulsion was prepared by weighing 20ml castor oil and

200ml emulsifier solution into 1000cm^3 glass beaker and homogenized with the high speed mixer (Remi Motors, Mumbai, India) for 15 min with the velocity of 5000 rpm.

Similarly an aqueous emulsifier solution of TSP (2% w/v, as test) and a 10% v/v of castor oil – in – water emulsion was prepared by weighing 20ml castor oil and 200ml emulsifier solution into 1000cm^3 glass beaker and homogenized with the high speed mixer (Remi Motors, Mumbai, India) for 15 min with the velocity of 5000 rpm.

Micromeritic measurements of emulsion stability[20]
The particle size distribution of the emulsions was measured using a laser light scattering instrument. The emulsions were diluted with distilled water prior to analysis so that the droplet concentration was less than about 0.02% w/v. The dilute emulsions were placed into the measurement cell of the instrument and each sample was analyzed 3 times and the data are presented as the average.

Zeta Potential measurements [21]
The oil-in-water emulsions (0.01%v/v) were injected directly into the measurement chamber of particle electrophoresis instrument (Zetamaster, Malvern Ltd, U.K.) capable of measuring the zeta-potential of emulsion droplets of both castor oil emulsion prepared by using gum acacia(as standard for reference) and TSP (as test) as emulsifier. The zeta-potential measurements are reported as the average of 3 separate injections with 3 readings made per injection

Centrifugal method of evaluating emulsion stability [19]
The samples of emulsion were placed in the test tube of centrifuge (Remi, C-24BL, Remi Instruments Ltd., Mumbai, India)controlled to temperature at 37^{0}C for 10 min with 3500 rpm.
The emulsion stability 'S' was determined from the formula:

$$\mathbf{S = [(V0-V)/V0] \ X \ 100\%.}$$

Where: S - emulsion stability %, V0 – volume of emulsion undergo centrifugation cm^3, V- volume of the phase given off cm^3. No phase separation was observed in both cases of formulation, indicating 100% stability after centrifugation.

Viscosity measurement of emulsion stability [22]
The emulsions were stored at 37^{0}C for 12 week and the viscosity of the emulsions were measured by using Brook field viscometer Brookfield viscometer, spindle–LV2 (Brookfield LV-II, USA) at the interval of 0, 6 and 12 weeks.

Turbidity measurement of emulsion stability [23]
Every emulsion was diluted 1 part to 1000, prior to the absorbance measurements. The absorbance was taken at 400nm and 800nm, using UV-Vis Spectrophotometer (UV-1601, Shimadzu, Japan). From the absorbance values the opacity was determined and the ratio of the absorbance at 800 to 400nm, the size index (R) and stability were predicted.

Results and Discussion

Physicochemical characterization of TSP

Polysaccharide mucilage derived from the seeds of tamarind, *Tamarindus indica* was investigated as emulsifying agent in emulsion formulation. The percentage yield of the mucilage extraction from tamarind seeds was 35% w/w. The mucilage obtained was an off white to cream color powder, and the viscosity of its 1% aqueous dispersion was 450cP indicate that the TSP is colloidal in nature following non-Newtonian bodies which do not settle down quickly. The mucilage obtained was subjected to physicochemical characteristics the results of which are summarized in table 1.

Phytochemical characterization of TSP

Phytochemical tests carried out on TSP confirmed the absence of alkaloids, glycosides and tannins. In short, in Molisch's test, the gum was treated with α-naphthol and concentrated sulphuric acid, which gave violet ring at the junction of two layers. In case of the detection of reducing sugars to the TSP, equal quantity of Fehling's solution A and B were added. After heating yellow colour precipitate was obtained.

Table 1: Physicochemical characterization of Tamarind seed polysaccharide

Parameters	Observation
Solubility	Slightly soluble in water, practically insoluble in alcohol, chloroform and acetone. Forms thick gel in water
pH (1% w/v solution)	6.0
Loss on drying	1.2%
Ash value	4.9%
Water soluble ash	3.5%
Acid insoluble ash	0.9%
Sulphated ash	2.14%
Test for foreign matter	Less than 0.1%
Test for arsenic	Less than 1ppm
Swelling ratio	
In water	12
In 0.1 N HCl	9.0
In phosphate Buffer 7.4	5.0
True density	1.9g/dl
Bulk density	0.58 g/cc
Tapped density	0.76 g/cc
Compressibity index	15.33%
Hausner ratio	0.14
Description	Powder: light brown coloured granular powder
Angle of repose	20.25

The presence of tannin was tested upon treating the gum with ferric chloride solution. There was no black precipitation for tannin with ferric chloride solution. The presence of mucilage was tested by treating the mucilage with ruthenium red solution and Benzidine solution, formation of

pink colour with Ruthenium red and blue colour with Benzidine solution indicate the presence of mucilage. To know whether the TSP contains the peroxidase enzymes, which is commonly present in some gums like gum acacia. It was treated with few drops of hydrogen peroxide, no blue colour formation; indicate the absence of enzymes in it. Thus a chance of oxidative degradation due to TSP as excipient is eliminated as compared to gum acacia. Mucilage on treating with Ninhydrin reagent does not give purple colouration indicating the absence of amino acids. The results of phytochemical screening of mucilage are summarized in table 2.

Table 2: Phytochemical screening of Tamarind seed polysaccharide

S. No.	Tests	Observation
1.	Test for Carbohydrates(Molisch's test)	+
2.	Test for Tannins(Ferric chloride test)	-
3.	Test for proteins (Ninhydrin test)	-
4.	Test for alkaloids (Wagner's test)	-
5.	Test for glycosides**(**Keller–Killaini test)	-
6.	Test for mucilage (Ruthenium red test)	+
7.	Test for flavonoids (Shinoda test)	-
8.	Test for reducing sugar (Felhing's test)	+
9.	Mounted in 95% alcohol	Transparent angular masses under microscope
10.	Mounting in the iodine	No blue colored particles (starch absent)
11.	Test with cupric –tartaric solution	Red precipitate is produced
12.	Warming with 5M sodium hydroxide	A brown color is produced
13.	Test for chlorides(silver nitrate test)	-ve
14.	Test for sulphates (barium chloride test)	-ve

Toxicity studies of TSP

To determine the safety level of the extracted TSP, acute toxicity and chronic toxicity studies were carried out. In both toxicity studies TSP revealed no behavioral changes for first four hours and no mortality, no toxic syndromes were observed even at the dose level 5000mg/kg body weight after 24 hours, indicating the safety of the TSP. To assess the suitability of TSP for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicities at regular intervals of 10 d. it was found that the body weight of both test and control and rate of increase were also comparable. Hence it is concluded that chronic administration of the TSP might not influence either the food intake or growth. Hematological and biochemical parameters that were determined at the end of 30 d of continuous administration were also found to be comparable to that of control rat. The effect of TSP on biochemical and hematological parameters is summarized in table3 and 4 respectively.

Table 3: Results of biochemical parameters in rats treated with TSP

Treatment	ALP (U/L)	ACP (U/L)	AST (U/L)	ALT (U/L)	Urea (U/L)	Creatinine (U/L)
Control*** (0.5%CMC)	69±2.45*	29±2.52	62±4.12	55±3.47	42±2.10	0.41±0.26
Treatment (TSP)**** 500 mg/kg)	71±2.63**	31±2.41	68±4.36	58±3.69	45±2.64	0.46±0.43

*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals
CMC; Carboxy methyl cellulose; TSP*; Tamarind seed polysaccharide

Table 4: Results of Hematological changes observed in rats during and after treatment of TSP for 30 days

Treatment	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (g/dl)	N	L	E
Control (0.5% CMC)	4.4±0.06*	7100±0.45	14.25±0.25	8±0.28	80± 0.13	0±0.00
Test(TSP) 500 mg/kg)	4.3±0.04**	6900±0.38	12.35± 0.32	10±0.24	84± 0.19	1± 0.42

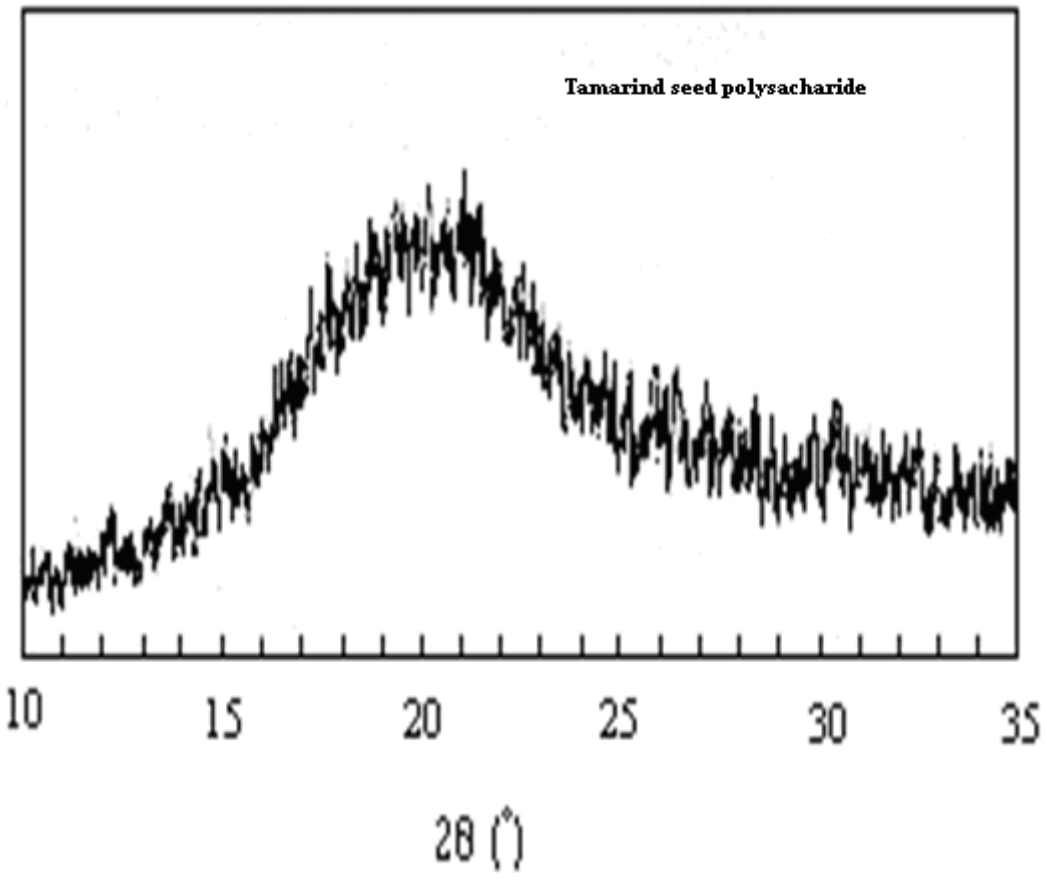
*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals

Characterization of TSP

X-ray diffraction analysis

The X-ray diffraction pattern (Figure 1) of TSP did not show any characteristic peak, which indicates that the structure is completely amorphous.

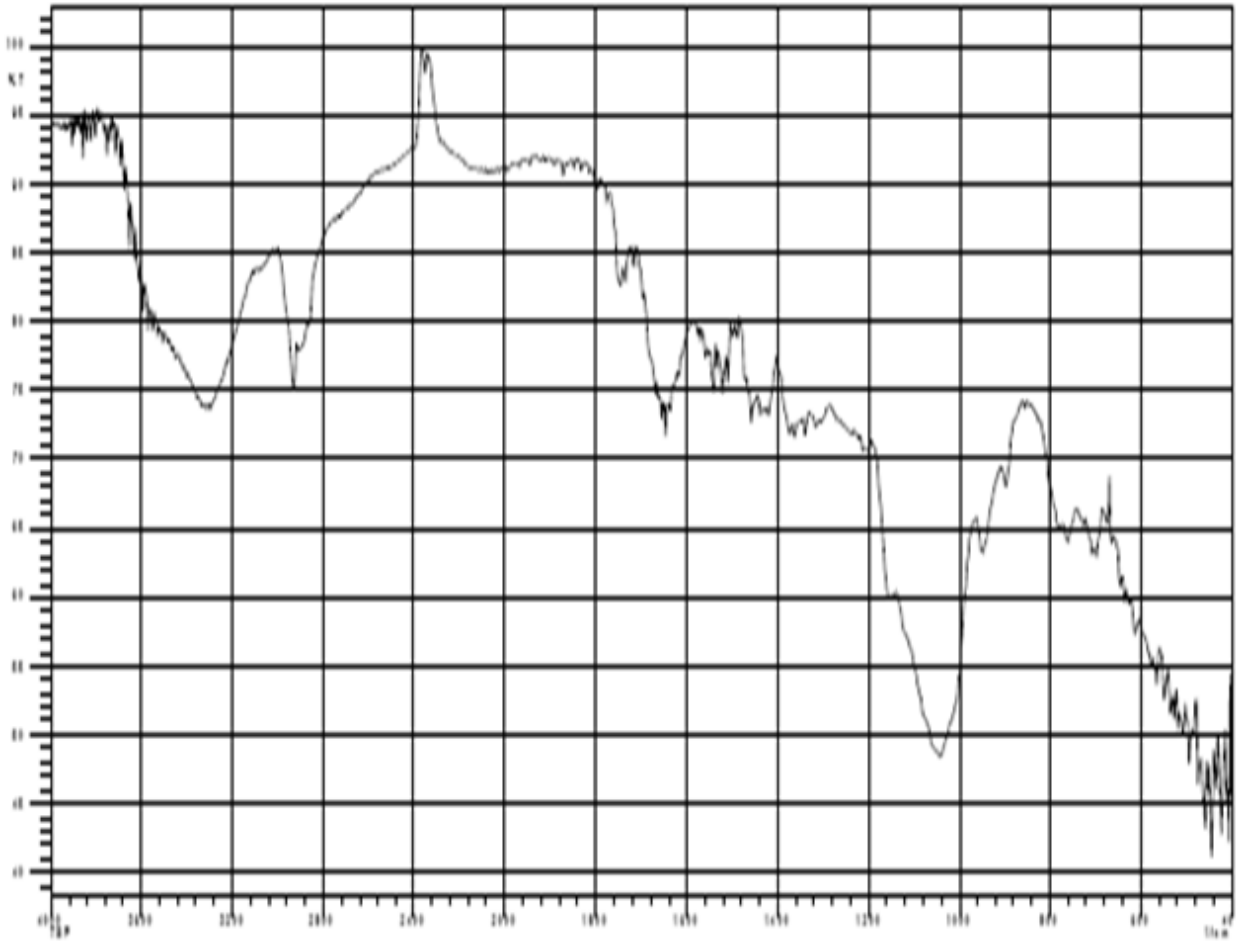
Figure 1: X-ray diffraction pattern of tamarind seed polysaccharide (TSP)



Fourier Transform Infrared (FTIR) Spectroscopy analysis

The absence of sharp peak at 1700–1800 cm^{-1} in the FTIR spectrum indicates that there is no carboxyl group in the extracted sample. On the other hand, the presence of peak at 1000–1200 cm^{-1} corresponds to the presence of alcoholic group mostly secondary alcohols. These findings proved that there were no uronic sugars or esters in the structure (Figure2).

Figure 2: FTIR Spectra of tamarind seed polysaccharide (TSP)



Evaluation of emulsion

Determination of Particle size distribution

The particle size distribution indicates that both the emulsions stabilized by using gum acacia and TSP are within 1 to 10 µm ranges. The emulsion prepared by TSP results very fine particle size distribution in comparison to gum acacia and results in stable emulsion because of fine particle size.

Determination of zeta potential

The zeta potential of the emulsion prepared by 2% w/v of TSP (test) and 10% w/v of gum acacia (standard for reference) are found to be – 30.14mv and – 26.16 mv respectively. The negative zeta potential indicates the electrostatic interactions between the droplets i.e. stearic repulsion resulting droplet dis-aggregation and formation of stable emulsion.

Determination of emulsion stability by Centrifugal method

The results of emulsion stability by centrifugal method indicate that there is no phase separation result even if with high rpm (3500) at 37^0C and the percentage stability was found to be 100% in both the cases indicating the formation of stable emulsion.

Viscosity and turbidity measurement of emulsion stability

The stability parameters such as viscosity, turbidity and size index of both the emulsion stored at 37^0C for 12 week (Table 5) results that both the emulsions are stable because there is no such

changes in their viscosity and turbidity found after 12 weeks. The emulsions prepared with 2 %w/v of TSP (test) is more stable in comparison to that of the emulsion prepared by using 10%w/v of gum acacia (standard).

Table 5: Results of stability parameters of castor oil emulsions

Parameter		Emulsion with Gum Acacia			Emulsion with TSP		
Ratio		Oil : Gum : Water 10 : 10 : 100			Oil : Gum : Water 2.0 : 10 : 100		
Particulars		Day in weeks					
		Immediate	After 6 week	After 12 week	Immediate	After 6 week	After 12 week
Viscosity in CP		12.13±0.64	12.65±0.63	13.87±0.63	7.23±0.33	8.54±0.23	8.99±0.33
Turbidity of 1 in 1000 dilution	At 400 nm	0.541±0.0011	0.587±0.0012	0.601±0.0011	0.410±0.0011	0.419±0.0012	0.425±0.0011
	At 800 nm	0.150±0.0011	0.156±0.0011	0.199±0.0011	0.147±0.0011	0.156±0.0012	0.169±0.0014
Size index		0.485	0.498	0.512	0.214	0.297	0.312

Conclusion

The result of the present study demonstrated that the TSP obtained from the from seed kernel of plant *Tamarindus indica* is having a potential emulsifying property. It is effective in a very low concentration as compared to that of the standard emulsifier (gum acacia) used. While comparing the stability characteristics of emulsions prepared by TSP and that of the gum acacia it has been found that the emulsion prepared with 2%w/v of TSP is more effective in comparison to that of the emulsion prepared by using 10%w/v of gum acacia. Moreover as this plant is widely distributed in nature, tamarind are eaten by the local tribes and used as food supplement, available chiefly in India and many other countries and easily available option without destroying the natural sources as compared to that of the other available natural option will be one of the suitable options to utilize as pharmaceutical excipient. Since the primary ingredients are in expensive, devoid of toxicity, biocompatible, biodegradable and easy to manufacture, they can be used in place of currently marketed emulsifier.

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