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FORMULATION AND EVALUATION OF ARIPIPRAZOLE LOADED LIPOSOMES FOR BRAIN DRUG DELIVERY

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ABSTRACT

Aripiprazole liposomal suspensions were formulated with a view to surpass the drug in the BBB and to increase the retention time in the brain for better therapy. The physical mixture containing Aripiprazole were mostly identical with the peaks of the pure sample of Aripiprazole indicating their compatibility. Thin film hydration technique was employed for the formulation of Aripiprazole liposomes using rotary vacuum flash evaporator with phosphatidyl choline along with and without cholesterol in molecular weight ratios. Among the various formulations, F3, F6 and F7 had shown good entrapment efficiencies. In *vitro* diffusion studies of all the formulations followed first-order kinetics & ascertained Peppas mechanism which was governed by predominant Non-fickian diffusion (slope>0.5). Based on the release profile and the entrapment efficiency, the Aripiprazole liposomal formulation 'F7' was considered as the optimized formulation. SEM analysis revealed that the particles obtained were spherical in shape. Aripiprazole liposomal formulation ranged from 60-250 nm in particle size with the average particle size of 120.9nm indicating well within the liposomal limits. The zeta potential of the optimized formulation (F7) was found to be -27.5 mV with high negative surface charge indicating its higher stability. From the above results it can be concluded that Aripiprazole liposomal suspension could act as better formulation for the treatment of psychotic disorders.

KEY WORDS: Aripiprazole, Phospholipids, Liposomes, Brain Targeting.

1. INTRODUCTION

Psychosis is a mental disorder which results due to alterations in the monoamine neurotransmitters level in the Central nervous system and variations in the brain structure including expansions of the lateral ventricle and the basal ganglion cores, changes in the limbic system, volume reduction of various brain regions. The manifestations include elusions, illusions and hallucinations. Aripiprazole is a novel dopamine D2 receptor partial agonist used in the treatment of psychotic disorders. In addition to partial agonist activity at the D2 receptor, Aripiprazole is also a partial agonist at the 5-HT1A receptor and an antagonist profile at the 5-HT2A receptor. Liposomes are the colloidal drug delivery systems having distinct advantages over conventional dosage forms. They possess a Phosphatidyl bilayer consisting of hydrophobic and hydrophilic mostly together, so can encapsulate both lipophillic and hydrophilic drug moieties. They are biocompatible, biodegradable and non-toxic. Aripiprazole entrapped liposomes can better target the brain and improves the therapeutic efficacy of the drug as the drug entrapped liposomes have more retention time in the brain.

2. MATERIALS AND METHODS

Aripiprazole was received as a gift sample from Ranbaxy, Chennai. Soyalecithin, Cholesterol, Triton-X-100 were obtained from Himedia Laboratories, Mumbai. Egg phosphatidylcholine was obtained from Sigma Aldrich, Germany. All the other chemicals and solvents were used of analytical grade.

2.1. Standard Calibration Curve of Aripipiprazole (Aviral Jain, 2006)**:** 100mg of Aripiprazole was dissolved in 100 ml of methanol. From this 10ml was taken and was made up to 100ml with methanol. From this 10ml was taken and was made up to 100 ml with pH 7.4 phosphate buffer. From this stock solution (10µg/ml) a series of concentrations 2, 4, 6 and 8µg/ml were prepared and the samples were scanned using UV-spectrophotometer. The λmax was found to be at 254nm. The absorbance was noted at 254nm using UV spectrophotometer.

2.2: Preformulation studies:

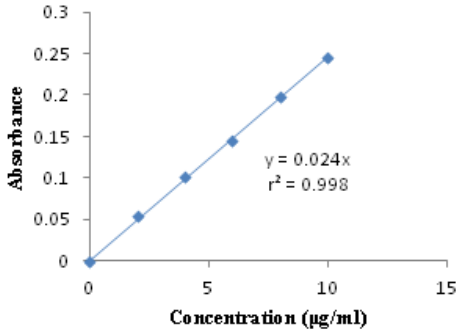
2.2.1: Characterization of Drug and Excipients: Compatibility study of Aripiprazole, soya lecithin, egg PC and cholesterol by IR spectroscopy. The physicochemical compatibility between Aripiprazole, soya lecithin, egg PC and cholesterol used in the research were carried out by IR Spectral studies using Perkin Elmer Fourier transform infrared spectrophotometer, Bruker, Germany, in the wavelength region between 4000cm^{-1} to 400cm^{-1}. The spectra obtained for Aripiprazole, soya lecithin, egg PC and cholesterol were compared.

2.2.2 Formulation of Aripiprazole Liposomes (Yasmin, 2012): Aripiprazole liposomes were formulated by employing thin film hydration technique. For this, a drug-lipid solution phase was prepared by dissolving accurately weighed quantities of drug, phosphatidyl choline of different sources(soya,egg), with and without cholesterol (Table 2,3) in 15ml of solvent mixture of chloroform and methanol (2:1 v/v).The obtained solution was placed in 800 ml evaporating flask and rotated using Rotary Vacuum Flask Evaporator at 100rpm by maintaining 60^0c temperature and 760mm Hg pressure until the solvent was evaporated and thin lipid film was deposited on the walls of the evaporating flask. Note: A chilling unit was additionally attached to the condenser for hastening the recovery of the solvent mixture. To this, 20 ml of pH 7.4 phosphate buffer was incorporated into the flask for the hydration of the thin lipid film maintained under similar conditions. The dispersion thus obtained was allowed to stand for 2-3 hrs at room temperature for complete swelling of the lipid film. Further size reduction with vibra cell ultra probe sonicator at 80% amp and pulse at 50 on and 50 off cycles for 3 minutes yielded Liposomal suspension.

Table 1: standard calibration data of Aripiprazole

Concentration(µg/ml)	Absorbance at 254nm $(\bar{X} \mp SD,\ n = 3)$
0	0
2	0.054±0.02
4	0.102±0.02
6	0.145±0.03
8	0.199±0.01
10	0.246±0.03

Figure.1.Standard calibration curve graph of Aripiprazole in pH 7.4 Phosphate buffer at 254 nm.



2.3. Evaluations of Aripiprazole Liposomes:

2.3.1. Determination of percentage entrapment efficiency by centrifugation method:

2.3.1.1. Determination of unentrapped drug (Kumar, 2010): 10 ml of liposomal suspension was placed in two centrifugal tubes separately and centrifuged at 15,000 rpm maintaining 4^0c temperature using Remi Cooling Centrifuge for 1hr.The clear supernatant was decanted and the resultant precipitate was added with 5ml of pH7.4 phosphate buffer for 30 minutes maintained under similar conditions. The supernatant was decanted and the process was repeated again by adding 5ml of pH 7.4 phosphate buffer to ensure complete removal of unentrapped drug. The amount of the drug unentrapped (i.e. supernatant aliquots) was estimated at 254nm by UV-Visible spectrophotometer under suitable dilutions.

2.3.1.2. Determination of entrapped drug (Krishna, 2012; Alina, 2013)**:** For the determination of entrapped drug, 2ml of liposomal solution was mixed with 2ml of 10% triton X-100 solution and centrifuged at 15,000rpm, maintained at 4^0c for 1hr.The contents were filtered through 0.45µ membrane filter using vacuum filter. The filtrate was analyzed by UV- Visible spectrophotometer at 254nm by suitable dilutions.

$$\% \text{ Entrapment efficiency} = \frac{W - S}{W} \times 100$$

Where, W is the amount of entrapped and unentrapped drug.
S is the amount of entrapped drug.

2.3.2. In-vitro diffusion studies (Praveen, 2012; Tanmay, 2013; Eskandar, 2012; Ganesh, 2011)**:** In-vitro drug diffusion studies were performed by dialysis technique. Liposomal suspension equivalent to 5 mg of Aripiprazole was placed in dialysis bag (12,000 Da –pore size) which was previously soaked overnight in distilled water and sealed at both the ends. The dialysis bag was immersed in beaker containing 250 ml of pH 7.4 phosphate buffer, maintaining at 37± 0.5^0c with speed of 80rpm.3ml of samples were withdrawn at regular intervals and replaced with the fresh buffer. The amount of the drug diffused was estimated from the samples at 254 nm by UV- Visible spectrophotometer.

2.3.3. Sem analysis: One drop of diluted Aripiprazole liposomal suspension was placed on a stub covered with a clean glass and subjected to SEM analysis using HITACHI S-3700 N.

2.3.4. Determination of average particle size and size distribution: The average particle size and size distribution of the Aripiprazole liposomal formulation was estimated using Horiba Nanopartica SZ-100. The number of particles present in the size range was considered and the average particle size was determined.

2.3.5. Determination of zeta potential: The zeta potential of the Aripiprazole liposomal formulation was estimated using Horbia Nanopartical SZ-100.

3. RESULTS AND DISCUSSION

Accordingly, the investigation for the compatibility of Aripiprazole, phospholipids and cholesterol were conducted by IR spectral studies**.** The results of IR spectra of Aripiprazole**,** phospholipids and cholesterol and the physical mixture of Aripiprazole, phospholipids and cholesterol suggested that the characteristic peaks observed in Aripiprazole pure samples were mostly identical with the peaks in the physical mixture of Aripiprazole**,** phospholipids and cholesterol adhering within their ranges without changes in the functionalities indicating their compatibility. The encapsulation efficiency of liposomes is the ability of formulation to retain drug molecules in the aqueous core or in the bi layer membrane of the vesicles. Here as Aripiprazole is lipophilic drug, Aripiprazole entraps the bilayer membrane of the liposomal vesicle.

The drug entrapment efficiency of all the formulated liposomes increased with the increase in the concentration of the cholesterol up to certain ratio (1:1, phospholipid:cholesterol). For F3 and F7 formulations, % drug entrapment efficiency were found to be 52.08 and 59.14.This was due to the impactness of the hydrophobicity of the bilayered membrane by cholesterol which may favor the inclusion of hydrophobic molecules. Further increment of cholesterol concentration in the formulation (F4,F8) lead to decrease in the % drug entrapment efficiency. This might be due to decreasing the micro viscosity of the bilayers by filling empty spaces among the phospholipid molecules anchoring them more strongly into the membrane thereby making membrane more rigid. *In vitro* diffusion studies of the Aripiprazole liposomes revealed that the release profile of the formulations was found to be decreased with increase in the cholesterol concentration. This may be due to the fact that cholesterol might locate itself to the glycerol back bone of the membrane pushing Aripiprazole to the inner core which may explain the decreased release rates with increasing concentrations of cholesterol. Further, increasing the cholesterol concentration resulted in more intact lipid bilayers as a barrier for drug release and decreased its leakage by improving the fluidity of the bilayer membrane and reducing its permeability, which led to lower drug elution from the liposomal vesicles.

In vitro diffusion studies of the Aripiprazole liposomes revealed that the Aripiprazole diffusion drug release from all the formulations followed first-order kinetics and ascertained Peppas mechanism. Application of Korsmeyer Peppas equation to the data of the formulations revealed that mechanism of Aripiprazole liposomes was governed by predominant Non-fickian diffusion (slope>0.5). Based on the % drug entrapment efficiency and % drug release, F3 and F7 formulations were considered to be the optimized formulations. Further, more % drug entrapment efficiency and % drug release of the egg phosphatidyl choline formulation (F7) was observed. This was due to the robustness and rigid nature of the egg phosphatidyl choline when compared with soyalecithin. When subjected to scanning electron microscopy (SEM), the particles appeared to be spherical in shape. When subjected to Horiba NanoPartica A2, the particle size distribution of the optimized liposomal formulation (F7) ranged from 60 to 250 nm and the average particle size was found to be 120.9 nm indicating well within the liposomals limits. The zeta potential is an indication of the stability of the colloidal systems and charge present on the surface of the colloidal systems.

The zeta potential value of optimized formulation (F7) was found to be -27.5 mV which indicates high negative surface charge on liposomes indicating higher stability because of the anticipated surface repulsion between similar charged particles hence inhibiting aggregation of the colloidal liposomal particles. It was observed that P value for the both the variables was less than 0.05 in case of entrapment efficiency and thus these results indicating that these selected two variables are significantly influencing the entrapment efficiency. In case of drug release (k) the p value greater than 0.05 and was found to be non significant.

Figure.2.IR Spectra of Physical Mixture Of Drug + Egg Phosphatidyl Choline + Cholesterol

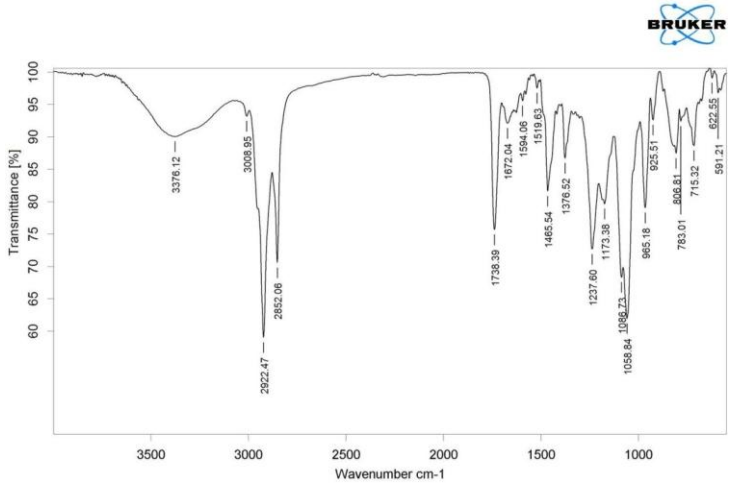


Table.2.Formulation of Aripiprazole liposomes with Soyalecithin(F1,F2,F3,F4) and Egg phosphatidylcholine (F5,F6,F7,F8,F9,F10)

Formulations PC:CH	Aripiprazole	PC (mg)	Cholesterol (mg)	VolumeofSolvent Chloroform:methanol (2:1)ml	Volume of hydration medium (ml)
F1 (1:0)	50	394.5	-	15	20
F2 (1:0.5)	50	394.5	96.75	15	20
F3 (1:1)	50	394.5	193.5	15	20
F4 (1:1.5)	50	394.5	290.25	15	20
F5 (1:0)	50	384	-	15	20
F6 (1:0.5)	50	384	96.75	15	20
F7 (1:1)	50	384	193.5	15	20
F8 (1:1.5)	50	384	290.25	15	20
F9 (0.5:0)	50	192	-	15	20
F10(0.5:1.5)	50	192	290.25	15	20

Table.3.Percentage Entrapment Efficiency of Aripiprazole Liposomal Formulations:

Formulation code	Amount of drug unentrapped(mg)	Amount of drug entrapped(mg)	% Entrapment efficiency
F1	26.01	17.94	40.81
F2	22.75	21.32	48.37
F3	20.02	21.76	52.08
F4	26.82	19.65	42.28
F5	23.01	20.72	47.38
F6	19.67	22.61	53.47
F7	18.72	27.10	59.14
F8	21.46	20.97	49.42
F9	27.98	16.53	37.13
F10	26.41	17.25	39.50

Table.3. *In vitro* Drug diffusion kinetics of Aripiprazole Liposomal Formulations

Formulation code	Correlation coefficient (r^2)				Release kinetics			Exponential coefficient (n)
	Zero order	First order	Higuchi	Peppas	K (Hr^{-1})	$T_{50\%}$ (Hr)	$T_{90\%}$ (Hr)	
F1	0.9249	0.9846	0.9822	0.9924	0.0177	39.1	130.0	0.6499
F2	0.8772	0.9757	0.9910	0.9926	0.0180	38.5	127.8	0.6257
F3	0.8875	0.9665	0.9845	0.9848	0.0149	46.7	155.1	0.7024
F4	0.9182	0.9780	0.9861	0.9889	0.0131	53.0	176.2	0.6924
F5	0.9075	0.9864	0.9846	0.9917	0.0192	36.09	119.9	0.6807
F6	0.8925	0.9763	0.9875	0.9917	0.0166	41.6	138.1	0.6225
F7	0.8692	0.9653	0.9931	0.9935	0.0157	44.3	147	0.6304
F8	0.8976	0.9699	0.9881	0.9911	0.0135	51.3	170.3	0.6630
F9	0.9256	0.9902	0.9829	0.9946	0.0223	31.1	103.2	0.6826
F10	0.9109	0.9662	0.9835	0.9836	0.0109	63.7	211.6	0.7349

Figure.3.Plot of Percentage Entrapment Efficiency Aripiprazole Liposomal Suspensions

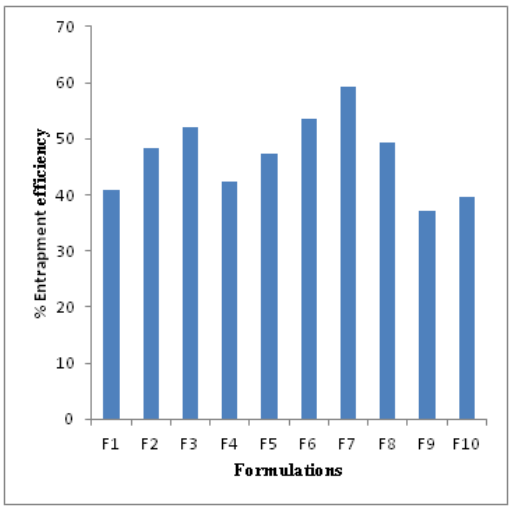


Figure.4. *In Vitro* Drug diffusion profile of Aripiprazole Liposomal Suspensions With Soyalecithin

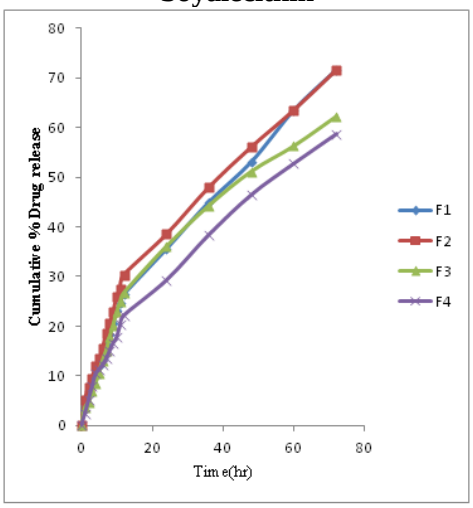


Figure.5.First Order Plot of Aripiprazole Liposomal Suspension With Soyalecithin

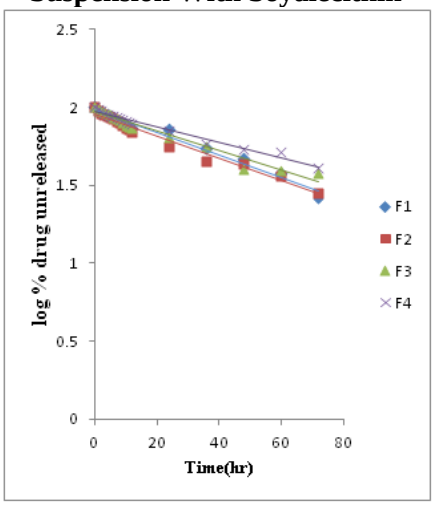


Figure.6.Peppas Plot Of Aripiprazole Liposomal Suspension With Soyalecithin

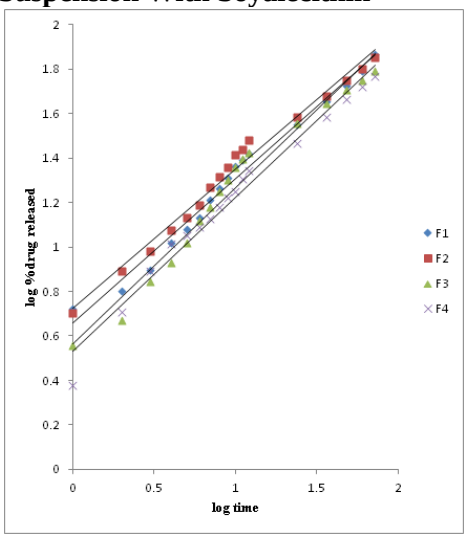


Figure.7. *In Vitro* Drug diffusion Profile Of Aripiprazole Liposomal Suspension With Egg Phosphotidylcholine

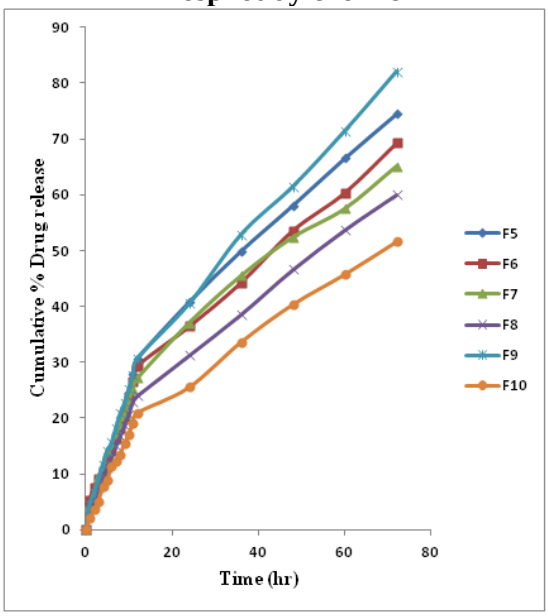


Figure.8.First Order Plot of Aripiprazole Liposomal Suspension With Egg Phosphotidylcholine

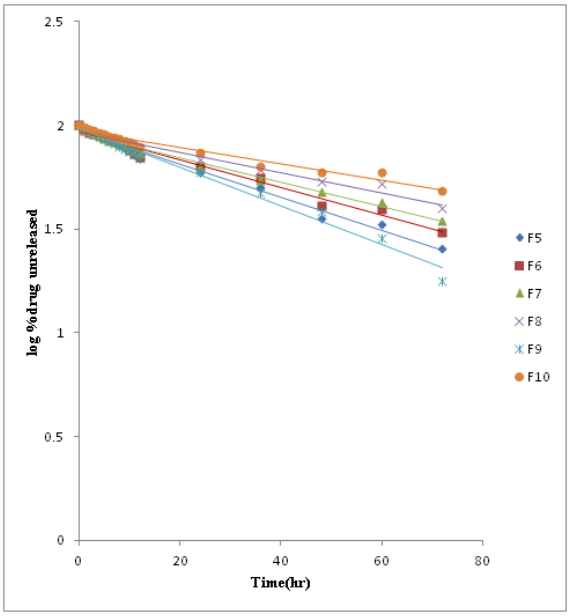


Figure.9. Peppas Plot of Aripiprazole Liposomal Suspensions With Egg Phosphotidylcholine

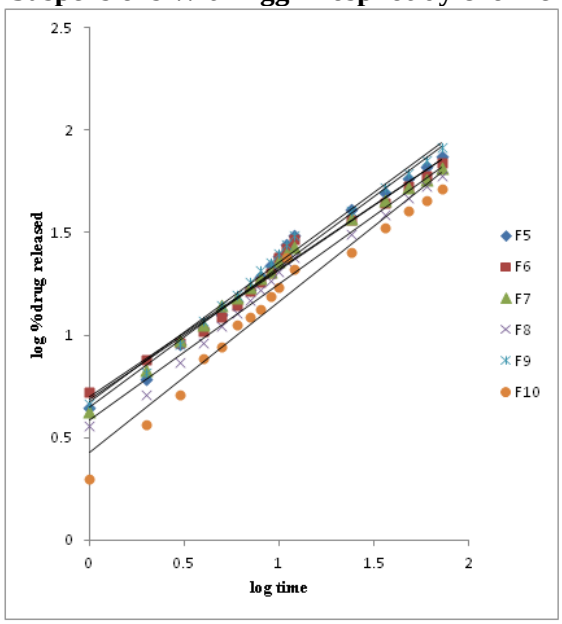
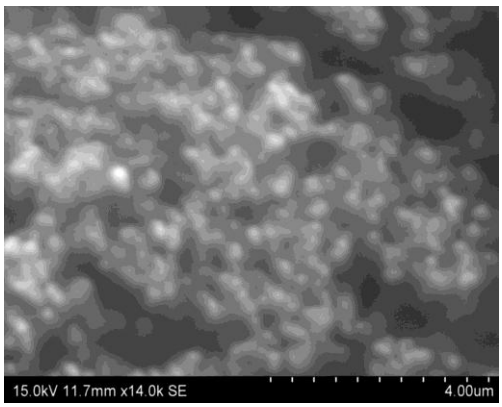


Figure 10: SEM Photograph Of Aripiprazole Liposomal Suspension: (F7)



4. CONCLUSION

Based on the release profile and the entrapment efficiency, F3 and F7 formulations were considered to be the optimized formulations. Furthermore % drug entrapment efficiency and % drug release of the egg phosphatidyl choline formulation (F7) was observed. This was due to the robustness and rigid nature of the egg phosphatidyl choline when compared with soyalecithin.

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