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Preparation and Evaluation of Microspheres of Natural Gums Containing Lamivudine

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A B S T R A C T
The current study concern with the preparation and evaluation of microspheres of naturally occurring gums in the view of effectiveness, biodegradable, easy of availability, cost effectiveness with Lamivudine as model drug. Lamivudine is an active anti-retroviral drug having biological half life of 4-6 hours and 86% bioavailability and licensed for the treatment of HIV and chronic Hepatitis B. Microspheres of Lamuvidine were prepared using xanthan gum and guar gum by solvent evaporation technique. Compatibility study was carried out by using FTIR at the range of 4000 to 400 cm-1 shows no significant change in the characteristic peaks of Lamuvidine and excipients in all the formulation, which indicates the compatibility of Lamuvidine with excipients. The prepared microspheres were analyzed for particle size, surface morphology, % yield, % drug entrapment efficiency, in-vitro drug release studies, in-vitro drug release kinetics and stability studies. Microspheres thus obtained were found to be pale yellow color and free flowing. Micromeritic studies of the prepared formulations are found within the prescribed limits and indicated good flow property. The Scanning Electron Microscopy (SEM) studies inferred the spherical shape and size range of 100µm to 200µm. In-vitro drug release shows decreases as concentration of xanthan gum increases. The release kinetics study revealed that the prepared microspheres were best fitted to the zero order and indicates that drug release from microspheres was diffusion-controlled and that the microspheres were stable. We conclude that, microspheres offer a practical and suitable approach to prepare controlled release of Lamuvidine with natural occurring xanthan gum as rate controlling agent to enhance bioavailability and reduction in dose frequency.

Keywords: Lamivudine, Xanthan gum, microspheres, Solvent evaporation, and oral controlled drug delivery.

A R T I C L E I N F O

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1. Introduction

Acquired immune deficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). As of 2009, AVERT (also known as the AIDS Education and Research Trust) estimated that there are 33.3 million people worldwide living with HIV/AIDS, with 2.6 million new HIV infections per year and 1.8 million annual deaths due to AIDS. When HIV infects a cell, a viral enzyme, reverse transcriptase copies the viral single stranded RNA genome into a double-stranded viral DNA. The viral DNA is then Integrated into the host chromosomal DNA, which then allows host cellular processes, such as transcription and translation to reproduce the virus. Reverse Transcriptase Inhibitors blocks the reverse transcriptase's enzymatic function and prevent

completion of synthesis of the double-stranded viral DNA, thus preventing HIV from multiplying.

Lamivudine comes under the class - Nucleoside Reverse Transcriptase Inhibitors (NRTIs). It is a nucleoside analogue, which was originally licensed for the treatment of HIV. It is now additionally licensed for the treatment of chronic hepatitis B with evidence of viral replication. For the treatment of AIDS, the dosage of conventional oral formulations of Lamivudine is 300mg per day (i.e. 150 mg twice daily, multiple times a day). with an absolute bioavailability of 86% ± 16%, peak serum concentration of Lamivudine (Cmax) of 1.5 ± 0.5 mcg/mL and mean elimination half-life (t½) of 4 to 6 hours, thus necessitating frequent administration for a prolonged period of time (lifelong in AIDS and for one year in hepatitis patients) to maintain constant therapeutic drug levels.[1] Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000µm.They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. Microspheres are small and have large surface-to-volume ratio. At the lower end of their size they have colloidal properties. The interfacial properties of microspheres are extremely important, oftens including their activity.[2]

The technique for the preparation of microspheres offers a variety of opportunities to control the administration of drug and also enhances the therapeutic efficacy of the given drug. There are some approaches in delivering a therapeutic substance to target site in a sustained release controlled fashion. One of the major approaches is using microspheres as carriers for drugs also known as micro particles. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug.[3]

Natural polymers are biodegradable in nature so, they are safe enough for oral consumption in the form of food additives or drug carriers. Among the advantages of natural gums over their synthetic counterparts are their biocompatibility, Low cost, Low toxicity and relative widespread availability. An additional advantage of biodegradability confers the property of complete drug release from the dosage form due to the degradation of gums by colonic bacteria and enzymes present in the distal portion of the gastro-intestinal tract.[4] Biodegradable natural polymers remain attractive primarily because they are biodegradable natural products of living organisms, and capable of a massive amount of chemical modifications. Some of the examples of natural gums like agar, Guar gum, Chitosan, Gelatin, Carboxy methyl cellulose, Xanthan gum, Sodium alginate and lotus bean gum etc., for potential pharmaceutical and biomedical applications.[5]

2. Materials and methods

Lamivudine procured from mahalakshmi chemicals, Bangalore, as model drug of choice of Antiviral agent, Xanthan gum procured from Techno Scientific products, Bangalore, as an encapsulating polymer, Gluteraldehyde as cross linking agent and petroleum ether. All chemicals and solvents used were of analytical grade.

Preparation of microspheres by solvent evaporation technique[6]

Microspheres were prepared by using different ratios of drug: natural gum (1:1.15, 1:1.20, 1:1.25). Gums were allowed to hydrate in 20 ml water for 3 hrs. Weighed quantity of drug (100mg) was dispersed in 10 ml of methylene chloride and adds the aqueous solution of gum. The above drug-gum dispersion was acidulated with 0.5 ml of concentrated sulphuric acid to give a clear viscous solution. The resultant solution was emulsified into the oily phase by poured into 200 ml of paraffin liquid containing 0.5 % w/w span 80 as an emulsifying agent. Stirred mechanically at 1800 rpm for 210 min using a stirrer and heated by a hot plate at 500C. 1.2 % w/v dichloromethane was added as encapsulating agent and 0.15 % w/v of gluteraldehyde as crosslinking agent, stirring and heating were maintained for 2.5 hrs until the aqueous phase was completely removed by evaporation. The oil was decanted and collected microspheres were washed with water to remove surfactant residue and three times with 100 ml aliquots of n-hexane, filtered through whatman filter paper, dried in an oven at 800C for 2 hr to collect discrete, solid, free flowing microspheres and stored in a desiccators at room temperature. The formulations are shown in Table no: 01.

FTIR studies

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 400 and 4000 cm-1. The spectra obtained for Lamivudine and physical mixtures of Lamivudine with polymers were compared to check compatibility of drug with polymers.

Evaluation of Lamivudine microspheres Micromeritic Studies[7,8]

The prepared microspheres are characterized by their micromeritic properties such as microsphere size, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose. The results obtained are shown in Table no: 02

Bulk Density

The bulk density is defined as the mass of powder divided by bulk volume. The bulk density was calculated by dividing the weight of the samples in grams by the final volume in cm

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres before tapping}}$$

Tapped Density

Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing Known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres before tapping}}$$

Carr's Compressibility Index

This is an important property in maintaining uniform weight. It is calculated using following equation.

$$\% \text{ Compressibility Index} = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \text{ X } 100$$

Hausner's ratio

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, where as greater than 1.25 indicates poor flow. Added glident normally improves flow of the material under study. Hausner's ratio can be calculated by formula,

$$\text{Hausner's ratio} = \frac{Tapped\ density}{Bulk\ density}$$

Angle of Repose (θ)

Good flow properties are critical for the development of any pharmaceutical tablet, capsules or powder formulation. It is essential that an accurate assessment of flow properties be made as early in the development process as possible so that an optimum formulation can be quickly identified. Interparticle forces between particles as well as flow characteristics of powders are evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane.

Particle Size Determination:

The particle size of the microspheres was determined by using optical microscopy method. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope.

Morphological Study using SEM:

The morphological study was carried out by Scanning Electron Microscope (SEM). Microspheres were scanned and examined under Electron Microscope HITACHI SU 1500, Japan connected with Fine coat, JEOL JFC-1100E Ion sputter. The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

In - vitro drug release Study[9]

The prepared microspheres were subjected to in vitro drug release sequentially in three different suitable dissolution media. USP type II dissolution apparatus was used. The dissolution medium for the first 2 hr was 900 ml of 0.1 N HCl (pH 1.2) and continued in phosphate buffer pH 6.8 for the next 7 hrs The temperature of dissolution medium was maintained at 37 ±0.5 °C and the basket was rotated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined time intervals and replaced with an equal volume of the fresh dissolution medium to maintain sink conditions. The samples were analyzed at 272 nm, for the percentage drug release using an UV Visible double beam spectrophotometer. The release study was performed in triplicates. The results obtained are shown in Table no: 04.

Details of Dissolution test Apparatus:

LABINDIA USP Type II Speed: 100 rpm

Stirrer: Paddle type Volume of medium: 900 ml Volume with drawn: 5ml

Media used: 0.1 N HCl (pH-1.2) and pH- 6.8 phosphate buffer Temperature: 37±0.5°C

λmax: 272 nm

Release Kinetics [9,10]

The matrix systems were reported to follow the Peppas release rate and the diffusion mechanism for the release of the drug. To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model. In this by comparing the r-values obtained, the best-fit model was selected.

Stability Studies[11,12]

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and enables recommended storage conditions.

ICH guidelines the length of study and storage conditions

Accelerated testing - 40°C/75% RH for six months. The accelerated stability study of the best formulations was carried out as per the ICH guidelines.

3. Results and Discussion

FTIR studies

The FT-IR spectra analysis of Lamivudine and the physical mixtures shows that there was no significant interaction between drug and polymers as shown in Fig No.1

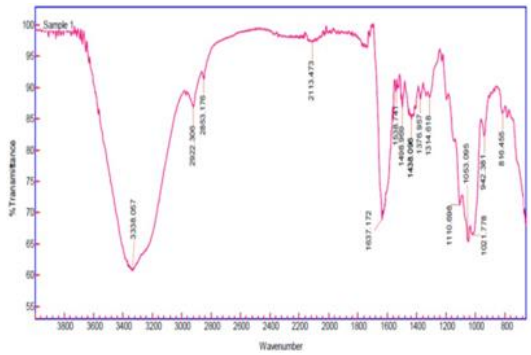


Figure 01: IR Spectrum of Lamivudine with xanthan gum + guar gum

Micromeritic properties of Lamivudine microspheres

The results of all formulations F1 to F9 of Lamivudine microsphere are shown in Table 02, which were evaluated for variable parameters such as bulk density, tapped density, % Compressibility index, Hausner's ratio and angle of repose. The % Compressibility index was in the range of 11-18 for all the formulations F1 to F9 indicating good flow property. The values of angle of repose for formulations F1,F2, F5 and F6 was found to be in the range of 25-30 which indicated the good flow potential.

Particle Size Analysis

Average particle size of microspheres as determined by optical microscopy by using stage micrometer and ocular micrometer are shown in Table 03 and in Figure 02.The mean particle size for the formulation F1 to F4 containing Xanthan gum was found to be in range from278±7.14µm to 913±6.35µm.For formulation F4 to F9 containing Guar gum the mean particle size was found to be in range from

572±12.51µm to 991±10.73 µm respectively. With increase in polymers concentration in the microspheres from F1 to F9, the particle size of microspheres increases respectively. This is because the viscosity of the polymer solution increases with increasing polymer concentration, which in turn decreases the stirring efficiency.

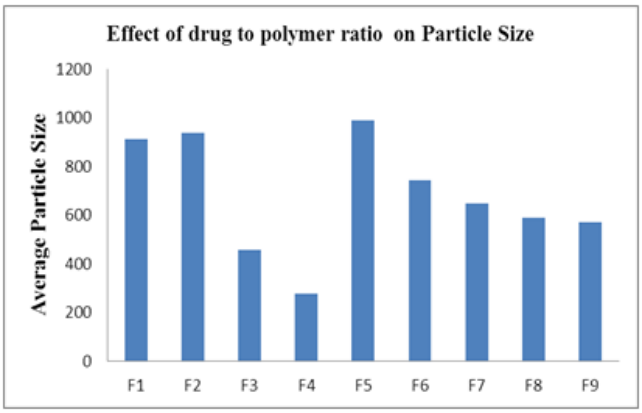
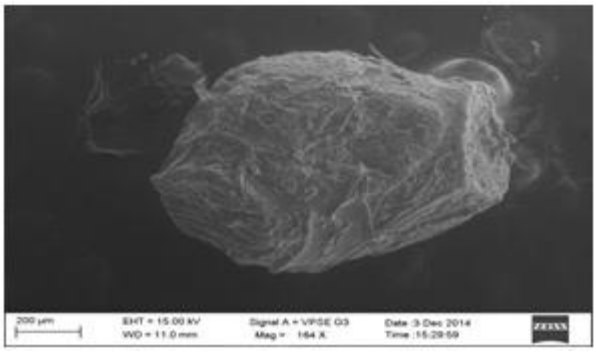
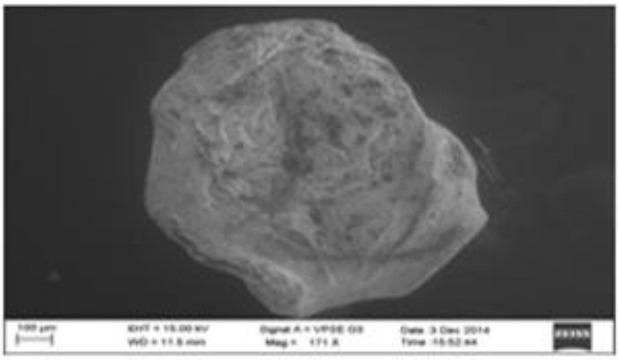


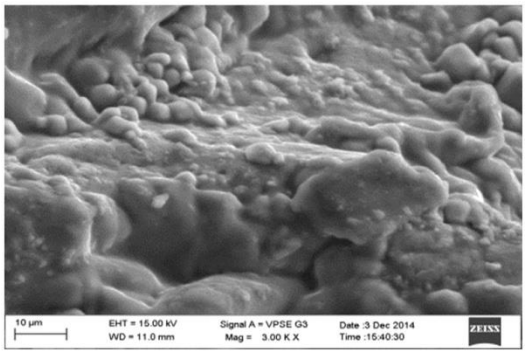
Figure no 02: Comparison of Avg. Particle Size of the Prepared Microspheres

Scanning Electron Microscopy

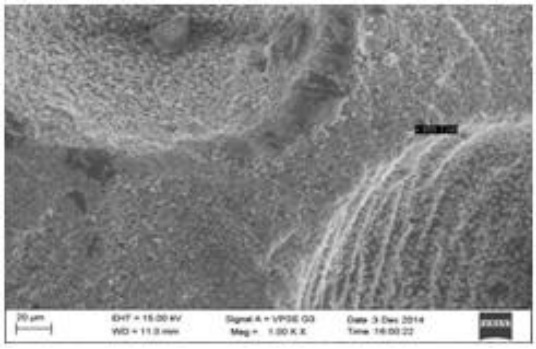
The determination of shape and surface morphology was done by scanning electron microscope HITACHI SU 1500, Japan. SEM analysis of the samples revealed that all microspheres prepared were spherical in shape. The microspheres of Lamivudine with Guar gum were smooth, spherical and slightly aggregated particles when compared with the microspheres of xanthan gum which were porous, rough, grossly, discrete spherical. Scanning electron photomicrographs of the formulations F1 and F5 are shown in Figure 03.







F1



F5

Figure no 03: SEM images of F1 and F5 formulation

In-vitro drug release studies

Dissolution studies on all the nine formulations of Lamivudine microspheres were carried out using a USP dissolution apparatus Type II. 0.1N HCl (pH 1.2) and pH 6.8 was used as the dissolution medium. The in-vitro drug release data of different formulations are shown in Table. No.04 and Figure no.04.The cumulative percent drug release after 12 hours was found to be in the range of 81.723, 79.038,76.389 and 71.558% for the formulations F1, F2, F3 and F4 respectively whereas cumulative percent drug release after 12 hours was 82.14, 80.57, 74.474, 69.093, 63.568% for formulations F5 to F9 respectively. The cumulative drug release significantly decreased with increase in polymer concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.

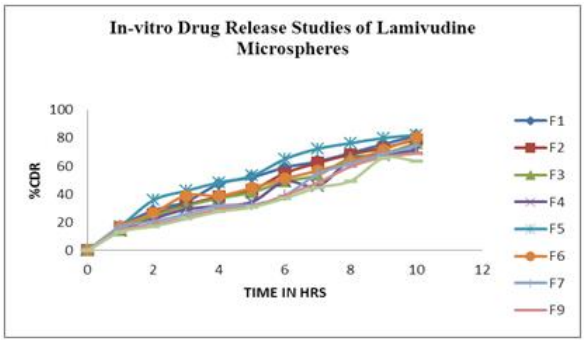


Figure 04: Comparative In-vitro Dissolution Profile of Lamivudine Microspheres

Drug release Kinetics

The rate of drug release followed by zero order kinetics and numerical data fitted into peppas model. This represents the non-fickian diffusion mechanism which indicates that the release is following zero order. The release kinetic parameters are shown in the Table no 05.

Stability studies results:

The formulations F1& F5 were selected for stability studies on the basis of their high cumulative % drug release and also results of physical appearance. The selected formulations were subjected to accelerated stability studies at 400C/75% RH for all the selected formulations observed up to till date. These formulations showed no variation in any physical parameters.

4. Conclusion

The present study reports a novel attempt to formulate microspheres of the Lamivudine by using natural gums like xanthan gum and guar gum as carrier for better treatment of HIV and chronic hepatitis B. Microspheres of Lamivudine were prepared by solvent evaporation method. Various evaluation parameters were assessed, with a view to obtain controlled release of Lamivudine. The following conclusions can be drawn from the results obtained.

FT-IR studies revealed no chemical interaction of drug with excipients. Micromeritic properties like angle of repose, bulk density, tapped density; hausner's ratio and Carr's index of all the formulations were found to be within the standard limits. The mean particle size of the prepared microspheres was within the range of 278±7.14 to 991±10.73 µm. SEM analysis of the microspheres revealed that guar gum containing microspheres were smooth, spherical and slightly aggregated particles when compared with the microspheres of xanthan gum which were porous, rough, grossly, discrete spherical. Cumulative percentage drug release significantly decreased with increase in polymer concentration. The overall curve fitting into various mathematical models was found to be on an average. The formulations F1 to F9 were best fitted to zero order kinetic model and the drug release from the formulation was by non- Fickian diffusion mechanism.

The formulations F1 and F5were selected for stability studies on the basis of their better and satisfactory evaluation studies parameter. In formulations showed there was no variation in physical parameters even after the period of 60 days. From these results it was concluded that, formulations F1 and F5 are found to be stable and retained their original properties.

Thus, the formulated microspheres seem to be a potential candidate as an oral controlled drug delivery system in prolonging the drug retention in GIT.

Table no 01: Formulation of the natural gum based Lamivudine microspheres

Formulation	Drug (mg)	Xanthan Gum (mg)	Guar Gum (mg)	Liquid Paraffin(ml)	Span 80 (v/v)
F1	100	15	-	200	0.5
F2	100	20	-	200	0.5
F3	100	25	-	200	0.5
F4	100	30	-	200	0.5
F5	100	-	15	200	0.5
F6	100	-	20	200	0.5
F7	100	-	25	200	0.5
F8	100	-	30	200	0.5
F9	100	-	35	200	0.5

Table no 02: Micromeritic properties of Lamivudine microspheres

Formulation Code	**Bulk Density(g/cm$^{3)}$**	**Tapped Density(g/cm^{3})**	**Compressibility Index (%)**	**Hausner's Ratio**	**Angle of Repose (θ)**
F1	0.4426±0.005	0.5126±0.009	13.65±1.21	1.158±0.02	26.93±0.23
F2	0.4986±0.008	0.5814±0.004	14.24±1.32	1.166±0.05	25.74±0.24
F3	0.5234±0.015	0.6243±0.008	16.16±1.27	1.193±0.011	32.94±0.17
F4	0.4813±0.009	0.5446±0.005	11.94±1.34	1.131±0.019	33.81±0.14
F5	0.5418±0.013	0.6183±0.001	12.36±1.04	1.141±0.02	28.67±0.36
F6	0.6168±0.011	0.7136±0.012	13.56±1.02	1.156±0.08	27.08±0.16
F7	0.4576±0.014	0.5228±0.008	12.47±1.21	1.142±0.03	33.61±0.64
F8	0.4754±0.013	0.5845±0.011	15.24±1.03	1.229±0.023	34.54±1.07
F9	0.5438±0.016	0.6432±0.014	15.45±0.84	1.183±0.026	37.12±1.51

Table no: 03 Average Particle Size of Lamivudine Microspheres

Formulation code	Average particle size (µm) ± SD
F1	913±6.35
F2	940±11.28

F3	456±12.42
F4	278±7.14
F5	991±10.73
F6	743±12.24
F7	650±8.69
F8	590±11.46
F9	572±12.51

Table no 04: In–vitro drug release for Lamivudine Microspheres in 0.1N HCL (pH 1.2) and (pH 6.8) phosphate buffer

Time (hrs)	**CUMULATIVE % DRUG RELEASE OF FORMULATION**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	17.215	16.557	14.472	13.959	17.215	16.959	16.553	14.141	13.093
2	28.410	25.765	24.433	22.557	35.765	26.557	20.535	18.370	17.085
3	34.714	32.406	31.723	29.146	42.406	39.146	25.844	23.465	22.431
4	47.375	38.389	37.073	31.811	48.389	38.811	31.817	29.634	27.563
5	52.038	43.050	41.369	34.477	53.050	44.477	32.497	31.050	30.774
6	59.000	54.998	49.069	49.063	64.998	51.063	39.136	39.360	37.124
7	63.306	62.318	53.716	45.712	72.318	56.712	54.469	48.156	44.744
8	69.320	68.331	65.697	64.350	76.331	63.350	61.803	59.691	49.424
9	75.633	72.994	69.020	67.669	79.994	71.669	68.447	66.313	65.768
10	81.723	79.038	76.389	71.558	82.146	80.574	74.474	69.093	63.568

Table 05 : In-vitro release kinetic parameters for Lamivudine microspheres

Formulation code	**Mathematical Models (Kinetics)**						Best FitModel
	Korsmeyer–Peppas		**Higuchi**	**Hixson–Crowell**	**First order**	**Zero order**	
	R^2	N	R^2	R^2	R^2	R^2	
F1	0.9405	0.4596	0.9633	0.9668	0.8892	0.9893	Zero order
F2	0.9352	0.4480	0.9687	0.9636	0.8979	0.9834	Zero order
F3	0.9239	0.4408	0.9499	0.9308	0.8678	0.9552	Zero order
F4	0.943	0.4374	0.9748	0.9731	0.9084	0.9914	Zero order
F5	0.9466	0.4574	0.9748	0.9686	0.9103	0.9868	Zero order
F6	0.9349	0.4231	0.9618	0.9422	0.8874	0.9636	Zero order
F7	0.967	0.4460	0.9908	0.9899	0.9304	0.9961	Zero order
F8	0.972	0.4192	0.9885	0.9868	0.9479	0.9942	Zero order
F9	0.9676	0.4358	0.9855	0.9809	0.9471	0.9896	Zero order

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