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Research

Formulation and evaluation loaded nanoparticles of dorzolamide hydrochloride

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Abstract

Objective: The study aimed to formulate and evaluate Dorzolamide Hydrochloride-loaded nanoparticles to enhance ocular bioavailability and therapeutic efficacy. The formulation utilized Eudragit RSPO and Poloxamer 188 as polymers to optimize nanoparticle characteristics and drug release profiles.

Methods: Dorzolamide Hydrochloride nanoparticles were prepared using a nanoprecipitation technique involving Eudragit RSPO and Poloxamer 188. Various formulation parameters were optimized to achieve the desired particle size, drug loading capacity. The nanoparticles were characterized for their physical properties, including size, and zeta potential. In-vitro drug release studies were conducted to evaluate the release profile of the drug from the nanoparticles.

Results: Among the different formulations tested, the formulation with Eudragit RSPO and Poloxamer 188 F2 exhibited the most favorable characteristics. The F2 formulation achieved an in-vitro drug release of 99.37%, indicating a high release efficiency and sustained release profile. This formulation was considered optimal based on its drug release performance and stability.

Conclusion: The developed Dorzolamide Hydrochloride-loaded nanoparticles using Eudragit RSPO and Poloxamer 188 demonstrated excellent drug release characteristics. The F2 formulation, with its high in-vitro release rate, holds promise for improving the ocular delivery of Dorzolamide Hydrochloride, potentially enhancing its therapeutic efficacy and reducing side effects associated with conventional dosage forms.

Keywords: Dorzolamide Hydrochloride, Nanoparticles, Eudragit RSPO, Poloxamer 188, In-vitro Drug Release.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then to maintain the desired drug concentration. That is, the drug delivery system should deliver drug at a rate dictated by the needs of the body over a specified period of treatment. This idealized objective points to the two aspects most important to drug delivery namely spatial placement and temporal

delivery of a drug. Spatial placement relates to targeting of drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. An appropriately designed controlled release drug-delivery system can be a major advance towards solving these two problems. It is for this reason that the science and technology responsible for development of controlled-release pharmaceuticals has been, and continues to be the focus of a great deal of attention in both industrial and academic laboratories.

Conventional drug therapy[1]

To gain appreciation for the value of controlled drug therapy, it is useful to review some fundamental aspects of conventional drug delivery. Consider single dosing of a hypothetical drug that follows a simple one-compartment pharmacokinetic model for disposition. Depending on the route of administration, a conventional dosage form of the drug e.g.: A solution, suspension, capsule tablet etc. can produce a drug blood level versus time profile. The term drug blood levels refer to the concentration of drug in blood or plasma, but the concentration in any tissue could be plotted on the ordinate. Administration of a drug by either intravenous injection or an extra vascular route, e.g., orally, intramuscularly or rectally does not maintain drug blood levels within the therapeutic range for extended periods of time. The short-duration of action is due to the inability of conventional dosage forms to control temporal delivery. If an attempt is made to maintain drug blood levels in the therapeutic range for longer periods by for e.g., increasing the initial dose of an intravenous injection, toxic levels can be produced at early times. This approach obviously is undesirable and unsuitable. An alternative approach is to administer the drug repetitively using a constant dosing interval, as in multiple-dose therapy. In this case the drug blood level reached and the time required to reach that level depend on the dose and the dosing interval. There are several potential problems inherent in multiple dose therapy.

1. If the dosing interval is appropriate for the biological half-life of the drug, large peaks and valleys in the drug blood level may result. For e.g., drugs with short half-lives require frequent designs to maintain constant therapeutic levels.
2. The drug blood level may not be within the therapeutic range at sufficiently early times, an important consideration for certain disease states.
3. Patient non-compliance with the multiple-dosing regimens can result in failure of this approach.

In many instances, potential problems associated with conventional drug therapy can be overcome. When this is the case, drugs given in conventional dosage forms by multiple dosing can produce the desired drug blood level for extended period of time. Frequently, however these problems are significant enough to make drug therapy with conventional dosage forms less desirable than controlled-release drug therapy. This fact, coupled with the intrinsic inability of conventional dosage forms to achieve spatial placement, is a compelling motive for investigation of controlled-release drug delivery systems.

Terminology[2,3]

Modified-release delivery systems may be divided conveniently into four categories:

1. Delayed release
2. Sustained release
3. Site-specific targeting
4. Receptor targeting.

Delayed-release systems are those that use repetitive, intermittent dosing of a drug from one or more immediate-release units incorporated into a single dosage form. Examples of delayed release systems include repeat-action tablets and capsules and enteric-coated tablets where timed release is achieved by a barrier coating. Sustained-release systems include any drug delivery system that achieves slow release of drug over an extended period of time. If the systems can provide some control, whether this is of a temporal or spatial nature, or both, of drug release in the body, or in other words, the systems is successful at maintaining constant drug levels in target tissue or cells, it is considered controlled-release systems.

Site-specific and receptor targeting refer to targeting of a drug directly to a certain biological location. In the case of site-specific release, the target is adjacent to or in the diseased organ or tissues, for receptor release, the target are the particular receptor for a drug within an organ or tissue. Both of these systems satisfy the spatial aspect of drug delivery and are also considered to be controlled drug-delivery systems.

Advantages of controlled release preparations

1. Decreased incidence and/ or intensity of adverse effects and toxicity.
2. Better drug utilization.
3. Controlled rate and site of release.
4. More uniform blood concentrations.
5. Improved patient compliance.

6. Reduced dosing frequency.
7. More consistent and prolonged therapeutic effect.
8. A greater selectivity of pharmacological activity.

Objectives[4]

Control release systems include any drug delivery system that achieves slow release of drug over an extended period of time.

The objectives of oral sustained release formulations are:

1. Frequency of drug administration is reduced.
2. Patient compliance can be improved.
3. Drug administration can be made more convenient.
4. Better control of drug absorption can be attained.

Materials

Dorzolamide Hydrochloride Procured from Aurobindo pharma Provided by Sura Labs, Dilsukhnagar, Hyderabad
Eudragit RSPO Lactel, Durect corporation Birmingham Division
Poloxomer 188 Eastman company, UK
Acetone SRL
Analytical balance Sartourius
Automatic dissolution test apparatus Electrolab TDT-60
Sartorious digital IR balance Model MA-45
Scanning electron microscope Jeol JSM-6380LV, Japan
Magnetic stirrer Electroquip, DSK instrument
Dissolution Apparatus Labindia, Mumbai, India

Methodology

Preparations of buffer

Preparation of 0.2M Potassium Dihydrogen Orthophosphate Solution: Accurately weighed 27.218 gm of monobasic potassium dihydrogen orthophosphate was dissolved in 1000 mL of distilled water and mixed.

Preparation of 0.2M sodium hydroxide solution: Accurately weighed 8 gm of sodium hydroxide pellets were dissolved in 1000 mL of distilled water and mixed

Preparation of pH 7.4 phosphate buffer: Accurately measured 250 mL of 0.2M potassium dihydrogen ortho phosphate and 195.5 mL of 0.2M NaOH was taken into the 1000 mL volumetric flask. Volume was made up to 1000 mL with distilled water.

Preparation of Standard Graph

100mg of Dorzolamide Hydrochloride pure drug was dissolved in 15ml of Methanol and volume make up to 100ml with 0.1N HCL (stock solution-1). 10ml of above solution was taken and make up with100ml by using 0.1 N HCL (stock solution-2 i.e 100µg/ml). From this take 0.5, 1, 1.5, 2 and 2.5 ml of solution and make up to 10ml with 7.4 phosphate buffer to obtain 5, 10, 15, 20 and 25 µg/ml of Dorzolamide Hydrochloride solution. The absorbance of the above dilutions was measured at 254 nm by using UV-Spectrophotometer taking 7.4 phosphate buffer as blank. Then a graph was plotted by taking Concentration on X-Axis and Absorbance on Y-Axis which gives a straight line Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis.

Method of preparation of dorzolamide hydrochloride loaded nanoparticles

Dorzolamide Hydrochloride loaded nanoparticles were prepared by using double emulsion solvent evaporation technique. Various formulations were prepared to know the effect of polymer concentration and were assigned formulation code F1 to F8 as shown in Table. Firstly polymeric solution is prepared by dissolving specific amount of Eudragit RSPO in 5 ml of Acetone dissolved. Above obtained solution is emulsified by the drop wise addition of aqueous drug solution under magnetic stirring at 1000-1200 rpm for 15 min to get primary W/O emulsion. Further this is added to 15 ml distilled water containing poloxamer188 under stirring for 10 min to achieve a stable W/O/W double emulsion. The W/O/W emulsion is separated by ultracentrifugation at 11000 rpm for 40 min. Finally the obtained product is freeze dried or lyophilized which leads to the formation of nanoparticles.

Table 1: Composition of the Nanoparticles

INGREDIENTS	FORMULATION CODES							
	F1	F2	F3	F4	F5	F6	F7	F8
Dorzolamide Hydrochloride	10	10	10	10	10	10	10	10
Eudragit RSPO (mg)	10	20	30	40	-	-	-	-
Poloxomer 188 (mg)	-	-	-	-	10	20	30	40
Acetone (ml)	5	5	5	5	5	5	5	5
Water (ml)	15	15	15	15	15	15	15	15

Evaluation of dorzolamide hydrochlorideloaded nanoparticles:

1. Mean Particle size
2. %Yield
3. Entrapment efficiency (%)
4. *In vitro* drug release
5. Application of Release Rate Kinetics to to Optimised Dissolution Data
6. FTIR studies
7. SEM for Optimised Formulation

FTIR studies

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study. A Fourier transform – infra red spectrophotometer was used to study the non-thermal analysis of drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility. The compatibility between the pure drug and excipients was detected by FTIR spectra obtained on Bruker FTIR. The spectrum of each sample was recorded over the 550-4000 cm-1. The solid powder/Liquid sample directly place on yellow crystal which was made up of ZnSe. The spectra were recorded over the wave number of 4000 to 550cm^{-1}.

Scanning Electron Microscopy Studies (SEM)

The surface morphology of the layered sample was examined by using SEM (JEOL Ltd.,Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs were coated with a thin layer (300A) of gold by employing POLARON - E 3000 sputter coater. The samples were examined by SEM with direct data capture of the images onto a computer.

RESULTS AND DISCUSSION

Preparation of Standard Graph

Table 2: Calibration curve data for Dorzolamide Hydrochloride at 254 nm

Concentrations [µg/mL]	Absorbance
0	0
5	0.125
10	0.261
15	0.388
20	0.512
25	0.633

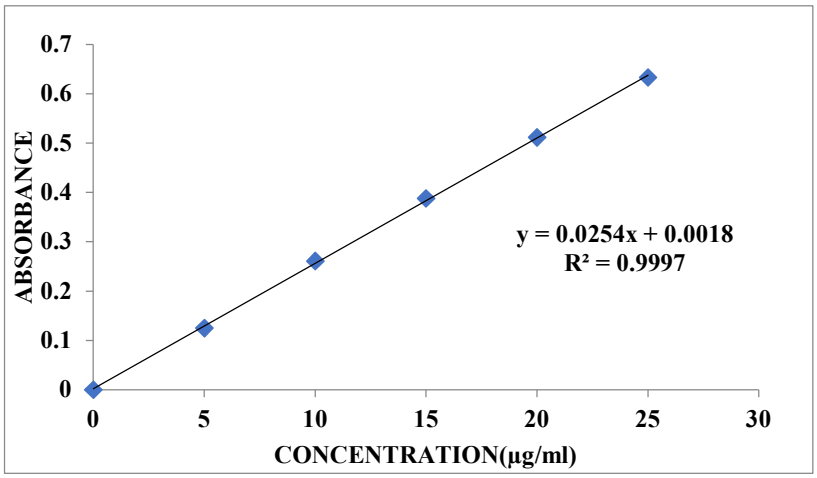

Fig 1: Standard graph of Dorzolamide Hydrochloride in 7.4 Phosphate buffer

Standard graph of Dorzolamide Hydrochloride was plotted as per the procedure in experimental method and its linearity is shown in Table 8.1 and Fig 8.1. The standard graph of Dorzolamide Hydrochloride showed good linearity with R^2 of 0.997, which indicates that it obeys "Beer- Lamberts" law.

Evaluation of dorzolamide hydrochloride loaded nanoparticles

Table 3: Evaluation of Nanoparticles

Batch No	Mean Particle size (nm)	%Yield	Drug encapsulation efficiency
F1	91.42	98.15	81.96
F2	99.14	99.48	76.33
F3	94.52	97.31	80.12
F4	95.41	96.72	93.20
F5	90.92	86.13	69.52
F6	93.86	51.25	72.60
F7	93.40	59.14	82.46
F8	91.82	63.95	70.58

***In vitro* Drug release studies**

Table 4: *In vitro* Drug release studies of Dorzolamide HydrochlorideF1, F2, F3, F4

Time (Hr)	Cumulative Percent Of Drug Released			
	F1	**F2**	**F3**	**F4**
0	0	0	0	0
1	19.93	14.59	17.41	14.17
2	24.56	21.17	21.05	22.56
3	29.02	27.35	26.81	29.53
4	37.79	35.21	31.07	36.42
5	42.38	43.79	47.19	42.01
6	56.41	58.44	54.12	59.53
7	62.57	64.63	69.52	64.12
8	78.22	81.12	73.83	69.45
10	88.86	87.59	82.51	75.09
12	92.11	99.37	89.96	86.41

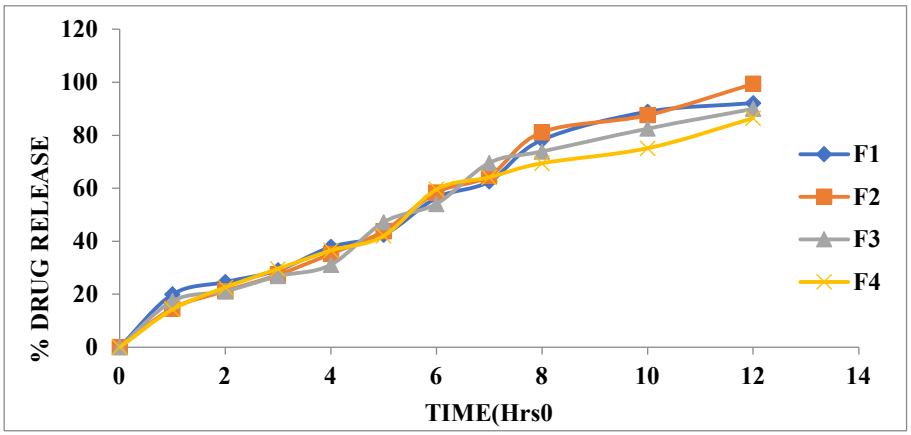

Fig 2: Dissolution study of Dorzolamide Hydrochloride Nanoparticles

Table 5: *In vitro* Drug release studies of Dorzolamide HydrochlorideF5, F6, F7, F8

TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED			
	F5	F6	F7	F8
0	0	0	0	0
1	19.68	24.47	21.94	29.63
2	26.25	29.19	27.45	36.91
3	33.89	35.58	32.28	48.85
4	48.27	43.62	36.69	56.01
5	57.53	51.46	42.58	68.19
6	61.85	59.23	59.09	74.31
7	69.72	64.91	61.51	79.79
8	76.81	71.89	74.42	82.25
10	81.09	77.65	86.21	91.01
12	92.51	91.41	96.54	97.91

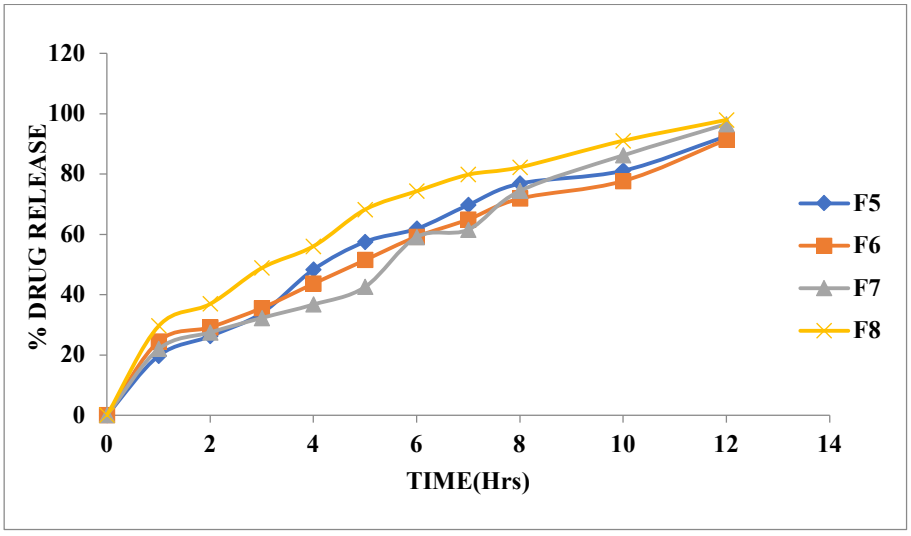

Fig 3: Dissolution study of Dorzolamide Hydrochloride Nanoparticles

Hence based on dissolution data of 8 formulations, F2 Eudragit RSPO (20mg) formulation showed better release (99.37%) up to 12 hours. So F2 formulation is optimized formulation.

Application of Release Rate Kinetics to Dissolution Data

Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of drug release from Nanoparticles. The data was fitted into various kinetic models such as zero, first order kinetics; higuchi and korsmeyer peppas mechanisms and the results were shown in below table it follows the zero order kinetics

Table 6: Release kinetics data for optimized formulation (F2)

CUMULATIVE(%) RELEASEQ	TIME(T)	ROOT (T)	LOG(%) RELEASE	LOG(T)	LOG(%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE/ t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
14.59	1	1.000	1.164	0.000	1.932	14.590	0.0685	-0.836	85.41	4.642	4.404	0.238
21.17	2	1.414	1.326	0.301	1.897	10.585	0.0472	-0.674	78.83	4.642	4.288	0.354
27.35	3	1.732	1.437	0.477	1.861	9.117	0.0366	-0.563	72.65	4.642	4.173	0.469
35.21	4	2.000	1.547	0.602	1.812	8.803	0.0284	-0.453	64.79	4.642	4.016	0.625
43.79	5	2.236	1.641	0.699	1.750	8.758	0.0228	-0.359	56.21	4.642	3.831	0.811
58.44	6	2.449	1.767	0.778	1.619	9.740	0.0171	-0.233	41.56	4.642	3.464	1.178
64.63	7	2.646	1.810	0.845	1.549	9.233	0.0155	-0.190	35.37	4.642	3.283	1.359
81.12	8	2.828	1.909	0.903	1.276	10.140	0.0123	-0.091	18.88	4.642	2.663	1.979
87.59	10	3.162	1.942	1.000	1.094	8.759	0.0114	-0.058	12.41	4.642	2.315	2.326
99.37	12	3.464	1.997	1.079	-0.201	8.281	0.0101	-0.003	0.63	4.642	0.857	3.784

Drug – Excipient compatability studies

Fourier Transform-Infrared Spectroscopy

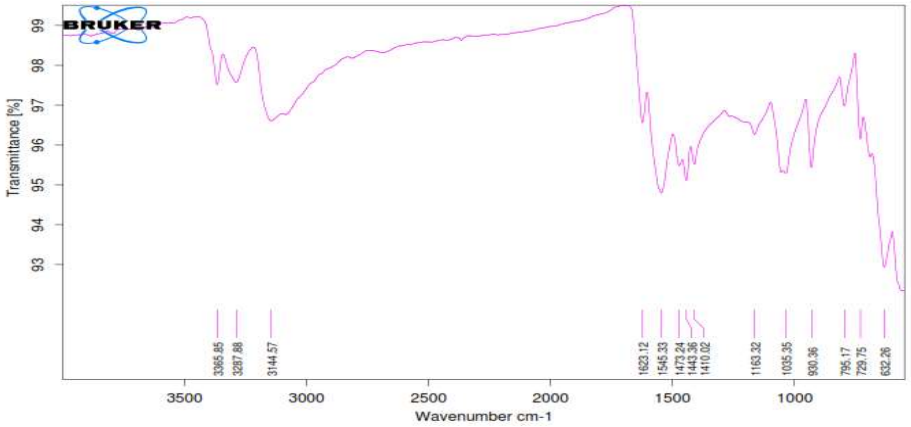

Fig 4: FT-TR Spectrum of Dorzolamide Hydrochloride pure drug

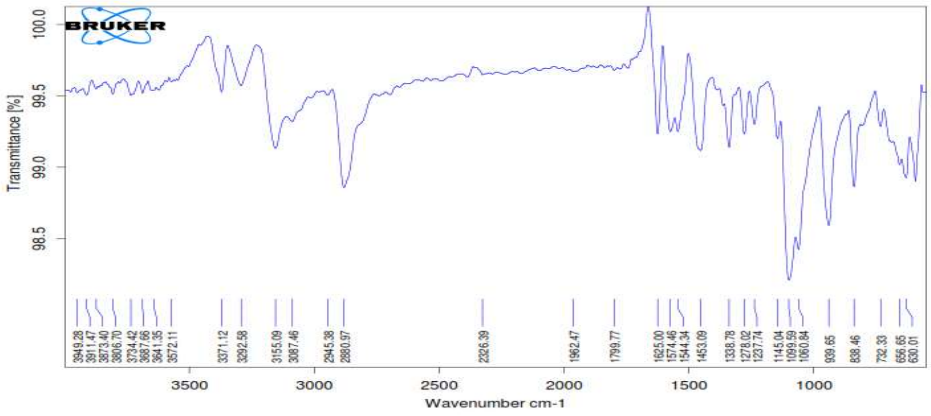

Fig 5: FT-IR Spectrum of Optimised Formulation

There is no incompatibility of pure drug and excipients. There is no disappearence of peaks of pure drug and in optimised formulation.

SEM

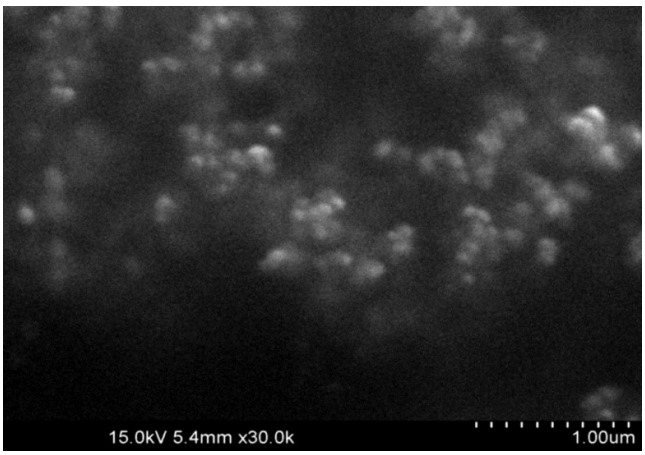

Fig 6: SEM graph of optimized formulation

CONCLUSION

The method of preparation of nanoparticles of Dorzolamide Hydrochloride was found to be simple and reproducible. Nanoparticles prepared by solvent evaporation technique. The prepared formulations were evaluated for Mean Particle size, %Yield, Drug encapsulation efficiency and *In vitro* drug release. Formulation F2 registered highest entrapment of 99.14% and practical yield of 99.48 % The incompatibility studies between the drug and polymer was evaluated using FTIR spectrophotometry. There was no significance difference in the IR spectra of pure drug & excipients. The *in-vitro* drug release of formulation F2 is found to be 99.37% over 12 h in controlled manner hence the present study was a successful attempt to formulate and extend the drug release of Dorzolamide Hydrochloride by nanoparticulate system.

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