ORIGINAL ARTICLE

CONTENTS

Formulation and characterization of lipophilic chemotherapy as ethosomal gel for cervical cancer

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ABSTRACT

Aim: methotrexate was formulated as Ethosomal gel for enhancing the therapeutic performance and stability to treat cervical cancer via targeting the drug locally through cervical mucosa to avoid the systemic adverse effect of the chemotherapy.

Materials and Methods: The formulations were produced using phospholipid and cholesterol then evaluated for particle size, polydispersity index, zeta potential, pH and drug release. The optimized ethosome formula then converted to gel. The gel evaluated for organoleptic properties, pH, viscosity and permeability.

Results: The results showed than F8 was the best ethosomal formula with 34nm in size and 92% entrapment efficiency. This formula converted into gel had 1% gelling agent that was optimized ethosomal gel formula and gave better release that ordinary methotrexate gel with same type and percent of gelling agent.

Conclusions: The drug was successfully formulated as ethosomal gel for treating cervical cancer topically in order to avoid the systemic exposure for the chemotherapy and its consequence adverse effect.

KEY WORDS: methotrexate, cervical, cancer, ethosomes, gel

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INTRODUCTION

Cervical cancer is one of the most common causes of deaths for females. The surgical excision is an effective treatment for cervical cancers in their early stages. However, the 5-year survival rate for metastatic cervical malignancies is just about 50% [1]. Cervical cancer is a malignant tumor that grows from cells that start in the cervix. The cervix connects the vagina to the top part of the uterus. While the ectocervix, that is the bottom half closest to the vagina, is fully lined with squamous cells, while the endocervix, is the top area closest to the uterus, which is lined with glandular cells. The junction of these two cervical regions is known as the transformation zone. Depending on where it appears in the cervix, cervical cancer is divided into several groups. Eighty to ninety percent of cervical cancer cases are squamous cell carcinoma, a kind of cancer that develops in the ectocervix. One type of cancer that starts in the endocervix is called adenocarcinoma. Most cervical malignancies start in the transformation zone, which is the area where glandular and squamous cells converge. The infectious human papillomavirus (HPV) is the main risk factor for cervical tumors, followed by smoking and HIV infection [2]. Cervical cancer treatment includes many strategies of surgical intervention, radiation and chemotherapy. There are many chemotherapeutic agents that can be used in the treatment of cervical cancer involving methotrexate. Methotrexate (MTX) is an antimetabolite of folate that is used to treat neoplastic conditions such as leukemia, breast, lung, bone, and cervical cancer. It has also been used to treat multiple sclerosis and psoriasis. MTX is used to treat rheumatoid arthritis since it is a member of the class of pharmaceuticals known as disease-modifying antirheumatic drugs (DMARDs). Growth and cell proliferation are inhibited by MTX because it inhibits the enzyme dihydrofolate reductase, which changes dihydrofolate into tetrahydrofolate. MTX is cytotoxic when the cell cycle is in the S phase. This process (competition with folic acid metabolism) impairs DNA synthesis and has nonspecific cytotoxic effects on healthy cells, which can result in hepatitis, nephrotoxicity, and renal insufficiency [3]. MTX is an odorless yellow to orange-brown crystalline powder with molecular weight of 454.447 g/mol, log p of -1.8, and melting point of 185–204 °C. MTX is slightly sol-

uble in diluted HCl; soluble in DMSO; insoluble in alcohol, water, ether, and chloroform. The MTX pKa is 4.7. Because of its hydrophobicity and low permeability property, it is categorized as a class IV drug by the Biopharmaceutical Classification System (BCS). Additionally, it is a chemically unstable substance that breaks down quickly in the presence of light, severe pH, or temperature changes [4-5]. The medication stability and therapeutic efficacy could be improved via formulation as nanoparticle. There are various nanoparticles types including liposome, noisome, solid lipid nanoparticles, ethosome and others. Ethosome is ethanolic vesicles because ethanol is available in the vesicular structure, 'Touitou formed a novel vesicular system which was called ethosomes [6]. The most recent researches for topical, localized or transdermal medication administration is the vesicular system. Since the drugs can reach the target and deeply penetrate the required area as well as it is considered as non-invasive delivery vehicles when applying the medication formula on the cervices to deliver the drug to the affected area avoiding the systemic exposure to the chemotherapy. However, ethosomes could be drain, hence incorporating the ethosome into gel could prolong the contact of the drug with the applied area of cancer [7-8].

AIM

Methotrexate was formulated as Ethosomal gel for enhancing the therapeutic performance and stability to treat cervical cancer via targeting the drug locally through cervical mucosa to avoid the systemic adverse effect of the chemotherapy.

HIGHLIGHTS

- This is the first time for methotrexate chemotherapy to be formulated as ethosomal gel
- Ethosomes are the nanoparticles that have advantages of Nano-sized carriers
- When methotrexate chemotherapy administered topically at the affected area (cervix in our study to treat cervical cancer, for first time), the systemic adverse effect of the drug would be avoided
- Incorporating the nanoparticle into gel will make the drug stay for longer time resisting the normal cervical clearance.

MATERIALS AND METHODS

MATERIALS

MTX was bought from YIBAI biotechnology, China. Dimethyl sulphoxide (DMSO) was purchased from Alpha Chemika, India whereas lecithin phospholipid from egg yolk and cholesterol were bought from Meyer, China. Ethanol and triethanolamine (TEA) were from Himedia, India.

METHODS

PREPARATION OF ETHOSOME

The formulas were prepared by dropping the water into the solution of phospholipid and cholesterol that dissolved in ethanol. The water was added gradually into the ethanolic phase. The mixture was stirred using magnetic stirrer (Dragon Lab- USA) at 700 rpm for a half an hour at room temperature. The drug (0.25%) was dissolved first in ethanolic phase before adding water. Table 1 shows the prepared formulas [9].

ENTRAPMENT EFFICIENCY

The entrapment efficiency (EE) of the formulas was determined by taking 2 ml of the prepared formulation, then centrifuged (Fanem Centrifuge, Brazil) at 20,000 rpm for one hour. The supernatant was got rid and one milliliter of the sediment was dissolved in ethanol to estimate the drug amount using spectrophotometric assay at the drug lambda max of 303 nm, then using the following equation [10]:

PARTICLE SIZE, PDI AND ZETA POTENTIAL

The formulations particle size, polydispersity index (PDI) and zeta potential were identified using nanotrac wave II (Microtrc, Germany). These parameters are important to identify the vesicle character and stability [11-12].

pH MEASUREMENT

The formulas pH was measured using pH meter instrument (Hanna Instruments Ltd. Singapore) in order to determine the pH of the formulas and their compatibility to the applied area.

IN VITRO PERMEABILITY STUDY

Drug penetration studies were conducted by dialysis membrane (12,000Da). The permeation study was performed using the Franz diffusion cell with an effective permeation area (1 cm^2) and receptor cell volume (50 mL). The formulas were put in the donor compartment and acetate buffer (pH 4.5) in receptor compartment which maintained at simulated body temperature of 37 ± 1°C and was constantly stirred at 50 rpm [13]. The

Table 1. Methotrexate ethosomes formulas

No.	Phospholipid	Cholesterol	MTX	Ethanol	DMSO	Water
F1	0.50%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F2	1%	0.015g	0.6 mg	20%	0.5 ml	Up to 10 ml
F3	1.50%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F4	2%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F5	2.50%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F6	1%	0.015 g	0.6 mg	30%	0.5 ml	Up to 10 ml
F7	1%	0.015 g	0.6 mg	40%	0.5 ml	Up to 10 ml
F8	1%	0.015 g	0.6 mg	50%	0.5 ml	Up to 10 ml

Source: Author's own work

Table 2. Entrapment efficiency, particle size, polydispersity index, zeta potential and pH of the ethomosal formulations (mean ± SD, n=3)

Formula Code	EE%	Size (nm)	PDI	Zeta potential (mV)	pH
F1	75.01±2.4	117.2±2.2	0.432	-19.7	5±0.4
F2	81.65±1.3	84.3±0.74	0.2376	-22.1	4.3±0.12
F3	87.68±2.2	98.25±0.39	0.3235	-25.3	4.34±0.2
F4	85.88±1.9	101±3.1	0.501	-21.2	4.66±0.1
F5	74.54±2.8	122.36±0.93	0.226	-28.76	4.34±0.2
F6	88.69±3.2	53.5±1.8	0.175	-17.85	4.17±0.3
F7	90.25±2.6	50.2±1.4	0.182	-23.35	4.38±0.2
F8	92.01±1.7	34.3±0.64	0.191	-25.0	4.51±0.1

Source: Author's own work

experiment was performed for 6 hours period. The samples from receptor compartment were withdrawn at predetermined intervals, then the same volume was replaced with fresh acetate buffer. Finally, the drawn samples were promptly examined for MTX at 303 nm using a UV (ultraviolet) spectrophotometer [14-15].

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) is a one of the popular technique for visualizing the materials' morphology and microstructure. On a small grid-coated carbon, ethosomal dispersion was applied. The specimen was viewed under the microscope after being suitably magnified. The optimized ethosomes' formula surface shape was assessed using SEM. Prior to inspection, the ethosomes samples were glued on copper stubs, platinum-coated, and put on double-sided tape. After then, the samples were seen at different magnifications [16-17].

ETHOSOMAL GEL FORMULATION

The optimized ethosome formula was added to carbopol gel (1%w/w). The liquid was agitated until thickening occurred, and then TEA was added gradually and slowly for gel formation and pH adjustment [18].

GEL CHARACTERIZATION

ORGANOLEPTIC PROPERTIES

Through visual inspection, the gel's physical appearance, clarity, occlusiveness, washability, and organoleptic properties were evaluated [19].

pH DETERMINATION

A pH meter was used to measure the ethosomal gel's pH. The average value was determined after the measurements were taken in triplicate [20].

ETHOSOMAL GEL VISCOSITY MEASUREMENT

The Atago viscometer was used to measure the prepared ethosomal gel's viscosity. Spindle A3 was used to obtain the reading at 100 rpm.

IN VITRO PERMEABILITY STUDY

Dialysis membrane was used for drug permeation studies, which were performed in Franz diffusion cell. The ethosomal gel formulations were placed in the donor compartment, which put over the receptor compartment, and the dialysis membrane in between. The experiment lasted for 24 hours, during which, the

samples were withdrawn at prearranged periods from the receptor and replenished with acetate buffer (pH 4.5) to maintain a sink condition. The samples were measured using a UV spectrophotometer for the drug content at 303 nm [21-22].

STATISTICAL ANALYSIS

One way ANOVA was used to identify significancy and the difference was considered significant when $p<0.05$.

RESULTS

ETHOSOME FORMULAS CHARACTERIZATION

ENTRAPMEMT EFFICIENCY

The range of the ethosome formulas EE% was from 74 to 92. The results shown in (Table 2).

PARTICLE SIZE, PDI AND ZETA POTENTIAL

The particle size, PDI and zeta potential, were shown in Table 2. PDI of the formulations was less than 0.501. The size of the MTX formulations was within the range of 34 to 122 nm. Vesicles benefit from having a high negative zeta potential of -17 to -28.

pH MEASUREMENT

The results of the formulas pH were shown in Table 2.

IN VITRO PERMEABILITY STUDY

Only the four optimized formulas were subjected to this study (F2, F6-F8). After six hours, the cumulative release percent of the formulas was above 77%. Formulation F8 had the highest cumulative release percent. The study results shown in Figure 1.

SCANNING ELECTRON MICROSCOPY

The SEM findings and the micro-morphology of the optimal ethosome formulation are shown in figure 2.

ETHOSOMAL GEL CHARACTERIZATION

GENERAL PROPERTIES OF THE ETHOSOMAL GEL

The gel formulation's physiochemical characteristics demonstrated uniformity and a pleasing look. There were no gritty particles in the gel, and it was readily washing [35]. The gel formulation had a pale yellow physical appearance. It was discovered that the gel formulation's pH was 6.1 within the typical skin pH range, 1% w/w carbopol concentration produced the acceptable viscosity (6200 cP).

IN VITRO PERMEABILITY STUDY

As indicated in Figure 3, the percentage permeated of the drug ordinary gel (containing the same Carbopol percentage) following a 24-hour investigation was 81%, whereas the permeability percent of ethosomal gel was 94%.

DISCUSSION

ETHOSOME FORMULAS EVALUATION

Entrapment efficiency EE% is utilized to determine the nanocarrier efficacy to retain the active ingredient and to ensure delivery of an adequate drug amount to the targeted. The range of the EE% was from 74 to 92. The results shown in Table 2. It had been determined that increasing the amount of ethanol led to improve the EE. Higher EE was for formula F8 that contained 50% ethanol. The value of log P is -1.8 for MTX, therefore the ethanol concentration had a significant influence on EE than the lipid. Evidence suggests that concentration of ethanol has a greater impact on MTX EE [23-24].

PARTICLE SIZE, PDI AND ZETA POTENTIAL

The formulas were tested via Nanotrac device to determine particle size, PDI and zeta potential, as shown in table 2. The effect of different factors on the prepared ethosomal formulations can be quite interesting. When the concentration of ethanol is increased, it has a solubilizing effect on the vesicles. Some research suggests that higher ethanol concentrations cause the ethanol hydrocarbon chains to intertwine, which ultimately reduces the thickness of the vesicular membrane and results in smaller vesicles. Ethanol, known for its ability to enhance penetration, interacts with the hydrophilic surface of the lipid bilayer on the outer layer of the skin. This interaction improves the fluidity of the lipids, thereby affecting various aspects such as zeta-potential, entrapment efficiency, stability, and the average size of the ethosomes. Increasing ethanol concentration from 20% in F2 to 30% in F6, was significantly reduced the particle size from 84 nm to 53 nm. Further increase ethanol in F7 and F8 also showed significant reduction in Particle size [25-26]. Therefore, the presence of ethanol alters the overall surface charge of

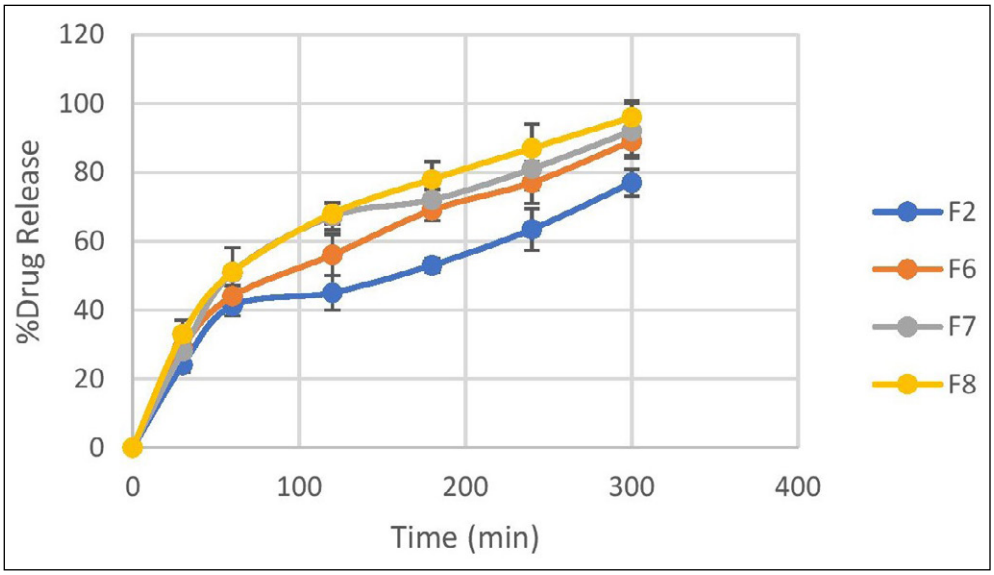


Fig. 1. MTX optimized ethosome formula permeability study results. *Source: Own materials*

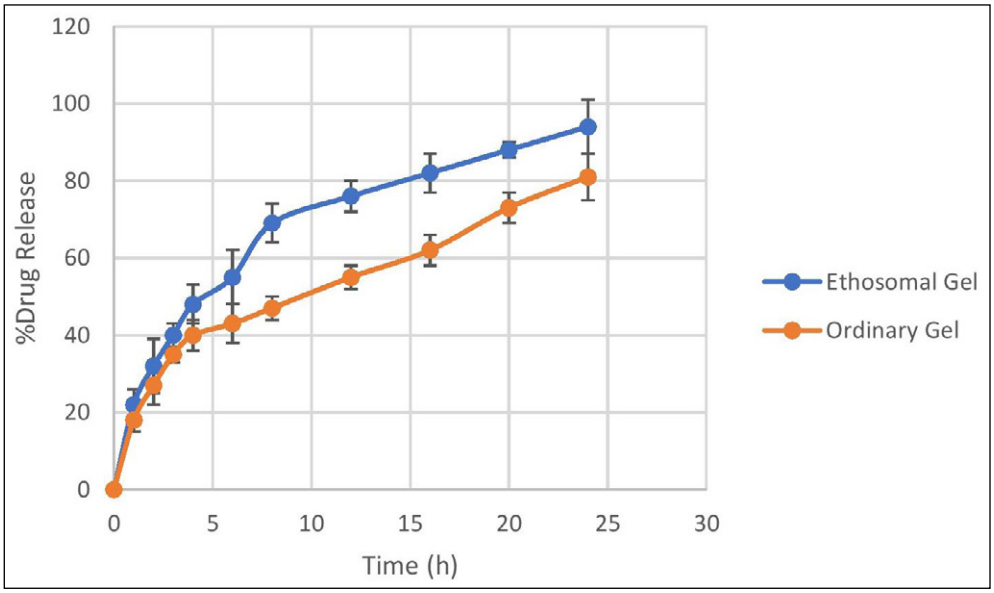


Fig. 2. SEM result of optimized formula, F8; (A) with 3 µm magnification, while (B) 500 nm. *Source: Own materials*

the ethosomes, providing some stability and increasing the repulsion between particles. This helps prevent aggregation and leads to smaller vesicle sizes. On the other hand, higher levels of phospholipids tend to result in larger vesicles. This is likely due to the thickening of the vesicle's structure caused by an increase in phospholipid concentration. Consequently, when the phospholipid levels are low while levels of ethanol are high, the smallest vesicle size is observed. This clear explained in F2-F5 as increasing the lecithin resulted in increased particle size [27-28]. PDI of the formulas was less than 0.5 and this indicating the uniformity of the particle size. The charge of the vesicle significantly reduces (increases in negative value) when the concentration of soya lecithin and ethanol rises. Vesicles benefit from having a high negative zeta potential because it stops them from aggregating due to electrostatic repulsion, increasing product stability. Potential increases as a result of (i) ethanol's electrostatic repulsion, which prevents aggregation by producing a net negative charge of the surface, and (ii) lecithin's phosphate group ionization, which produces a high negative charge [29-30].

pH MEASUREMENT

The pH of the formulas was compatible with the cervix (around 4.5) to prevent irritability [31], as shown in table 2.

IN VITRO PERMEABILITY STUDY

Selected optimized produced ethosomal formula was used in the release test (F2, F6-F8). At the end of the study, the cumulative release percent of the formulas was above 77%. Formulation F8 had the significantly highest cu-

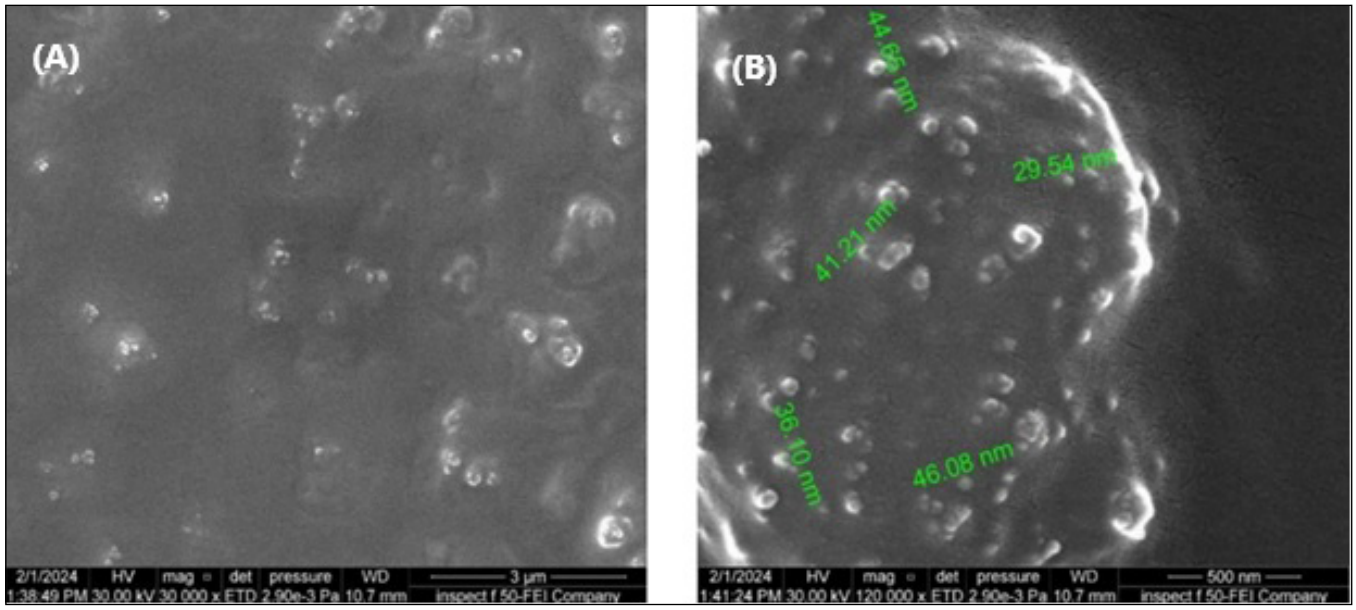


Fig. 3. The percent of MTX from the ethosomal gel and the ordinary gel
Source: Own materials

mulative release percent of 98% since the particle size is small of 34 nm. As the particle size decreased, the surface area increased and the overall drug solubility enhanced leading to better drug release then better bioavailability. Ethosomal formulation can improve drug accumulation, and increase penetration through the cervical cancer barrier [32-33], the study results shown in (Figure 1).

SCANNING ELECTRON MICROSCOPY

The SEM findings and the micro-morphology of the optimal ethosome formulation demonstrated that the ethosomes were rounded, and free of the drug crystals [34], as seen in (Figure 2).

ETHOSOMAL GEL CHARACTERIZATION

GENERAL PROPERTIES OF THE ETHOSOMAL GEL

The gel formulation's general characteristics were uniformity and a homogenous, with compatible skin pH [36]. The gel had an occlusive property, which increases the active drug's penetration and boosts effectiveness [37], 1% w/w carbopol concentration produced the appropriate viscosity [38-39].

IN VITRO PERMEABILITY STUDY

As shown in Figure 3, the percentage permeated of the drug ordinary following a 24-hour was 81%, whereas the permeability percent of ethosomal gel was 94%. It was shown that the ethosomal gel had a superior permeability profile and that could relate to the nanosized particle of the ethosome, which improves the release and penetration. Ethanol was thought to be efficient booster of permeability. As a result, ethosomal gel has a higher skin penetration rate than the ordinary drug gel [39-42].

CONCLUSIONS

The ethosomal gel of MTX was successfully prepared, evaluated and optimized in order to treat cervical cancer. The ethosomal MTX gel was better release the drug than ordinary gel of the drug with same amount and type of gelling agent for topical treatment of the cancer avoiding the peripheral adverse drug effect. This was the first time formulating MTX as ethosomal gel in order to enhance the drug targeting and permeability as well as avoiding the systemic exposure to the chemotherapy and its consequence adverse effect.

AUTHORS' CONTRIBUTIONS

M. Alaayedi and Z.M. Kadhum designed and directed the study; All author performed the experiments; each author analyzed specific part of the results. M. Alaayedi, Z.M. Kadhum, J.A. Ashoor, and A.M.H. Saeed wrote the article.

CONFLICT OF INTEREST

The authors would like to confirm that there are no conflicts of interest with this publication and that this study has not received any funding. We confirm that all writers indicated on the document have read it and given their approval. We also confirm that the authors' specified order has received our unanimous approval.

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CONFLICT OF INTEREST
The Authors declare no conflict of interes

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