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SELECTION OF CRYSTALLIZATION CONDITIONS FOR SUPRAMOLECULAR COMPLEXES OF GLYCYRRHIZIC ACID SALTS: A STUDY ON SOLVENT SYSTEM OPTIMIZATION

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Annotatsiya. Ushbu ishda glitsirrizin kislota (GK) va uning monoammoniy tuzi (GKMAT) asosida flavonoidlar (rutin, kversitin), 5-ftoruratsil va Lagochilus inebrians diterpenoidlari (lagoxilin, lagoxirzin, tetraatsetillagoxilin, izopropiliden) bilan supramolekulyar komplekslarni hosil qilish uchun erituvchi muhitlarini tanlash va kristallizatsiya sharoitlarini optimallashtirish tadqiq etilgan. Sekin bug‘latish va gradientli sovutish usullari qo‘llanilgan. EtOH:H_2O (70:30, 40–60°C) tizimi ko‘pchilik komplekslar uchun universal erituvchi sifatida aniqlangan. Lagoxilin hosilalari uchun pH nazorati (5–6) kristallizatsiyaning muhim parametri

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ekanligi ko‘rsatilgan. Rentgen difraksion tahlilga (XRD) yaroqli namunalar olish bo‘yicha tadqiqotlar davom ettirilmoqda.

Kalit so‘zlar: glitsirrizin kislota, GKMAT, kokristall, supramolekulyar kompleks, erituvchi skrining, kristallizatsiya, rutin, lagoxilin, 5-ftoruratsil, rentgen difraksion tahlil.

Аннотация. В данной работе исследован выбор растворительных систем и оптимизация условий кристаллизации для получения супрамолекулярных комплексов глицирризиновой кислоты (ГК) и её моноаммонийной соли (МАСГК) с флавоноидами (рутин, кверцетин), 5-фторурацилом и дитерпеноидами Lagochilus inebrians (лагохилин, лагохирзин, тетраацетиллагохилин, изопропилиден). Применялись методы медленного испарения и градиентного охлаждения. Система EtOH:H_2O (70:30, 40–60°C) определена как универсальная для большинства комплексов. Для систем с лагохилином контроль pH (5–6) является критическим параметром. Исследования по получению образцов для рентгеноструктурного анализа (XRD) продолжаются.

Ключевые слова: глицирризиновая кислота, МАСГК, кокристалл, супрамолекулярный комплекс, скрининг растворителей, кристаллизация, рутин, лагохилин, 5-фторурацил, рентгенодифракционный анализ.

Abstract. This study investigated the selection of solvent systems and optimization of crystallization conditions for supramolecular complexes of glycyrrhizic acid (GA) and its monoammonium salt (MASGA) with flavonoids (rutin, quercetin), 5-fluorouracil and Lagochilus inebrians diterpenoids (lagochilin, lagochirzine, tetraacetyllagochilin, isopropylidene). Slow evaporation and gradient cooling methods were applied. EtOH:H_2O (70:30, 40–60°C) was identified as a universal solvent system for most complexes. pH control (5–6) was shown to be a critical parameter for lagochilin systems. Studies aimed at obtaining samples suitable for X-ray diffraction (XRD) analysis are ongoing.

Keywords: glycyrrhizic acid, MASGA, co-crystal, supramolecular complex, solvent screening, crystallization, rutin, lagochilin, 5-fluorouracil, X-ray diffraction.

INTRODUCTION.

Multicomponent crystals (co-crystals) and supramolecular complexes have attracted significant attention in modern pharmaceutics as a promising approach to improving the solubility, bioavailability and stability of drug molecules [1, 2]. X-ray diffraction (XRD) analysis of solid-state crystals is considered the 'gold standard' for determining the molecular structure of such complexes [3].

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Glycyrrhizic acid (GA, Mr = 822 g/mol) and its monoammonium salt (MASGA, Mr = 840 g/mol), isolated from *Glycyrrhiza glabra L.*, are natural amphiphilic triterpenoid glycosides possessing broad pharmacological activity — anti-inflammatory, antiviral, hepatoprotective and immunomodulatory properties [4, 5]. The amphiphilic character of the GA molecule enables it to form supramolecular nanostructures in water, opening prospects for its use as an effective carrier for poorly soluble bioactive compounds [1, 6].

Lagochilus inebrians is an endemic plant of Uzbekistan flora whose main diterpenoid compounds include lagochilin, lagochirzine and their derivatives. These compounds possess hemostatic and anti-inflammatory properties; however, their bioavailability is limited due to poor water solubility [7]. Forming complexes with GA salts can overcome this limitation: the presence of the MASGA:lagochilin (1:2) complex in the Glilagin pharmaceutical product serves as practical evidence of this [8].

Flavonoids — rutin (Mr = 610 g/mol) and quercetin (Mr = 338 g/mol) — are compounds with broad biological activity whose bioavailability is limited by poor solubility. The literature has shown that forming supramolecular complexes with GA and MASGA can enhance their biological activity: the GA:Rutin (4:1) complex was found to increase capillary protection activity 3-fold and to show superior results with 12-fold less rutin compared to the free compound [1]. 5-Fluorouracil (5-FU, Mr = 130 g/mol) is a widely used oncological drug whose complex with MASGA was obtained in previous studies [9].

However, the solid-state crystal structure of these complexes and the conditions for obtaining single crystals have not yet been fully investigated. The objective of this study is to select solvent systems and to optimize crystallization conditions for obtaining crystal samples of multicomponent complexes of GA and MASGA with flavonoids, 5-FU and *Lagochilus inebrians* diterpenoids suitable for X-ray diffraction analysis.

LITERATURE REVIEW.

Contemporary research in the field of co-crystals demonstrates that selecting a compatible solvent system is the key factor determining crystallization success [2, 3]. Berry and Steed [2] conducted a detailed review of solvent screening methodology for pharmaceutical co-crystals and emphasized the high efficiency of EtOH:H_2O mixtures for triterpenoid-based systems.

The supramolecular properties of GA have been studied in depth by Li et al. [6], where the mechanisms of GA nanofibril and hydrogel structure formation were demonstrated. The biological activity and supramolecular structure of the GA:Rutin complex were confirmed by Matchanov et al. [1] using UV/IR

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spectroscopy and molecular modeling: the complexation energy was determined to be Eint = −159.36 kJ/mol.

Complete NMR and mass spectrometric characterization of Lagochilus inebrians diterpenoids — in particular lagochilin and lagochirzine — in various solvent media was performed by Baranovsky et al. [7]. These data serve as the theoretical basis for complex formation with GA salts and are of critical importance for interpretation of the crystal structures obtained. Dzhuraev et al. [5] reported the synthesis and spectral characterization of derivatives of the GA aglycone — glycyrrhetic acid — representing part of the group's broad research into GA chemistry.

RESEARCH METHODOLOGY.

GA (purity 94%, by HPLC [1]) and MASGA (purity 93%, by HPLC [1]) were used in the study. As biologically active guest molecules, the following were employed: rutin (Mr = 610 g/mol, purity 93%), quercetin (Mr = 338 g/mol), lagochilin (Mr = 356 g/mol), lagochirzine (Mr = 352 g/mol), tetraacetyllagochilin (Mr = 524 g/mol), isopropylidene (Mr = 394 g/mol) and 5-fluorouracil (Mr = 130 g/mol). The NMR and MS spectral characterization of GA, MASGA and Lagochilus inebrians compounds is reported in the literature [7, 8].

Slow evaporation and gradient cooling methods were used for co-crystal screening. Slow evaporation: compounds were dissolved in the designated solvent at 40–60°C and slowly evaporated over 3–7 days in an open vessel or under a semipermeable cover. Gradient cooling: the solution was prepared at 60°C and cooled in a thermostat to room temperature at 1°C/hour. Concentration range: 5–50 mg/mL; molar ratios were set at 1:1, 2:1 and 4:1.

The following solvent systems were screened: EtOH:H_2O (70:30; 50:50), MeOH:H_2O (30:70, pH 5–6; 50:50), acetone:H_2O (60:40), isopropanol:H_2O (60:40), DMSO→H_2O gradient system and CH_2Cl_2. The crystallization process was monitored and assessed by optical microscopy (×40, ×100). pH control for lagochilin systems was performed using 0.1 M hydrochloric acid.

RESULTS AND DISCUSSION.

Supramolecular complexes with flavonoids and 5-fluorouracil. In GA and MASGA systems with rutin, crystallization processes were observed in EtOH:H_2O (70:30) at molar ratios of 2:1 and 4:1 at 40–60°C. The MeOH:H_2O (50:50) system also showed partially positive results. For the MASGA:Quercetin (2:1) system, crystallization signs were detected in MeOH:H_2O (50:50) at 50°C; however, further optimization is required to obtain samples fully suitable for X-ray analysis. The supramolecular structure of these complexes has been confirmed in the literature using UV/IR spectroscopy and molecular modeling (MD, DFT

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B3LYP-D3BJ/6-31G): GA and MASGA form stable supramolecular complexes with rutin through hydrogen bonding and π→π* interactions, with a complexation energy of Eint = −159.36 kJ/mol [1].

For the MASGA:5-fluorouracil complex, slow evaporation in EtOH:H_2O (70:30) at 40–60°C was identified as the most promising crystallization condition. The DMSO→H_2O gradient system was identified as an alternative option. Taking into account that the MASGA:5-FU complex was obtained in previous studies [9], the present research is directed toward determining conditions for obtaining crystal samples.

Table 1

Crystallization conditions for flavonoid and 5-FU systems

Complex	System	Ratio	°C	Method	Result
GA:Rutin	EtOH:H_2O 70:30	2:1; 4:1	40–60	Slow evaporation	Promising ✓
MASGA:Rutin	EtOH:H_2O 70:30	2:1; 4:1	40–60	Slow evaporation	Promising ✓
MASGA:Quercetin	MeOH:H_2O 50:50	2:1	50	Slow evaporation	Partial ✓
MASGA:5-FU	EtOH:H_2O 70:30	2:1	40–60	Slow evaporation	Promising ✓
MASGA:5-FU	DMSO→H_2O	2:1	40–60	Gradient	Alternative ✓

Complexes with Lagochilus inebrians diterpenoids. The most important finding for the MASGA:Lagochilin (1:1 and 2:1) system was the strong pH dependence of crystallization. No crystallization was observed in MeOH:H_2O (30:70) under neutral conditions (pH 7); when pH was lowered to 5–6, crystallization began. This demonstrates that the protonation state of the hydroxyl groups in the lagochilin molecule directly affects supramolecular interactions. The spectral characterization and NMR data for lagochilin with GA and MASGA are reported in [7, 8] and serve as the basis for interpretation of the crystal structure.

In the GA:Lagochirzine (2:1 and 4:1) system, gradient cooling from 60°C to room temperature (1°C/hour) in EtOH:H_2O (70:30) gave promising results. Rapid cooling produced amorphous precipitate, indicating the necessity of slow crystallization. The MASGA:Tetraacetyllagochilin (2:1) system exhibited partial crystallization signs in EtOH:H_2O (70:30) at 50–60°C. For MASGA:Isopropylidene (1:1 and 2:1), slow evaporation in EtOH:H_2O (70:30) at 40–60°C was identified as the promising condition.

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Table 2

Crystallization conditions for *Lagochilus inebrians* diterpenoid systems

Complex	System	Ratio	(°C)	Method	Result
MASGA:Lagochilin	MeOH:H_2O 30:70 pH 5–6	1:1; 2:1	40–50	Slow evaporation	Promising ✓
MASGA:Lagochilin	MeOH:H_2O 30:70 pH 7	1:1	40–50	Slow evaporation	Negative ✗
GA:Lagochirzine	EtOH:H_2O 70:30	2:1; 4:1	60→RT	Gradient cooling	Promising ✓
MASGA:Tetraacetyl	EtOH:H_2O 70:30	2:1	50–60	Slow evaporation	Partial ✓
MASGA:Isopropylidene	EtOH:H_2O 70:30	1:1; 2:1	40–60	Slow evaporation	Promising ✓

Analysis of solvent systems and crystallization parameters. Analysis of the obtained results highlights the universality of the EtOH:H_2O (70:30, 40–60°C) system. In this system, the majority of GA and MASGA dissolve well, and favorable conditions are created for the formation of ordered crystal structures during slow evaporation. Increasing the EtOH:H_2O ratio (e.g., to 90:10) accelerated GA precipitation and resulted in amorphous structure formation. Increasing the H_2O fraction (30:70 and higher) did not allow complete dissolution of poorly soluble compounds such as lagochilin.

The critical significance of pH for lagochilin systems represents an interesting scientific finding. The tetrahydropyran ring and hydroxyl groups in the lagochilin molecule (at positions C-3, C-15, C-16, C-18) can alter their interaction character depending on their protonation state. At pH 5–6, a favorable electrostatic environment is created for hydrogen bond formation between the carboxyl group of GA and the hydroxyl groups of lagochilin. This finding is consistent with spectral data for lagochilin and its derivatives reported in the literature [7].

The significance of the gradient cooling method for lagochirzine systems also warrants special emphasis. Lagochirzine is a complex molecule with a spiro-fused ring system [7] whose crystallization requires a slow process. Cooling at 1°C/hour provided optimal crystal morphology.

CONCLUSIONS AND RECOMMENDATIONS.

A study on the selection and optimization of crystallization conditions for supramolecular complexes of GA and MASGA with flavonoids (rutin, quercetin), 5-fluorouracil and *Lagochilus inebrians* diterpenoids (lagochilin, lagochirzine,

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tetraacetyllagochilin, isopropylidene) was conducted. The following main conclusions were drawn:

1. EtOH:H_2O (70:30, 40–60°C, slow evaporation) was identified as a universal crystallization condition for most complexes of GA and MASGA with biologically active compounds.

2. pH control (5–6) was shown for the first time to be the decisive parameter for crystallization of lagochilin systems: no crystallization was observed under neutral conditions.

3. Gradient cooling (1°C/hour) was found to be more effective than slow evaporation for lagochirzine systems.

4. Promising crystallization conditions were established for all studied systems; optimization studies aimed at obtaining samples fully suitable for XRD analysis are ongoing.

The results obtained expand the prospects for applying GA and MASGA as carriers in novel pharmaceutical formulations of bioactive compounds and serve as the foundation for solid-state structural analysis of these complexes.

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