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Structural Characteristics of Agar-Based Emulsified Fluid Gels Designed for Enteral Nutrition

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Abstract

This study aimed to develop a novel method for preparing agar-based emulsified fluid gels as a thickened enteral feeding formula using different oil-structuring approaches: no emulsifier, the emulsifier polyglycerol polyricinoleate (PGPR), or monoglycerides/diglycerides of fatty acids (MDFA), which function as both emulsifiers and gelling agents. We examined sample stability, oil-holding capacity, particle sedimentation, size, morphology, rheological and textural properties, and the maximum injection force required to deliver the system through syringes fitted with tubes of different diameters. Adding PGPR, decreased the polydispersity index (span 2.21), apparent viscosity (588.88 mPa·s), and yield stress (0.39 Pa), reducing the force required to inject the fluid gel through the syringe. MDFA addition destabilized the system, resulting in a high polydispersity index (span 14.07) and reduced stability (68.75% under static and 79.64% under dynamic conditions). While the fluid gel with oil without emulsifier/stabilizer exhibited high stability (100%), it also showed high viscosity and yield stress values, affecting the increase in maximum injection force, which is a negative aspect. The results showed that such a different oil structuring approach affects the microstructure of the fluid gel formulations, resulting in differences in stability and rheological properties. This study provides mechanistic insights into the structural relationships between suspended agar particles and oil droplets, providing a feasible strategy for designing agar-based fluid gels with a lipophilic phase. The PGPR-containing fluid gels could serve as enteral nutrition formulations for the targeted delivery of lipophilic and hydrophilic nutrients and drugs. While lipophilic phase inclusion without an emulsifier or stabilizer also looks very promising, the high viscosity and yield stress should be reduced by adjusting the fluid gel preparation conditions.

Keywords: agar; emulsified; fluid gels; enteral nutrition; oil structuring

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1. Introduction

Enteral nutritional therapy (ENT) provides nutrients to individuals unable to meet their daily needs orally, especially in severe illness that impairs swallowing or restricts gastrointestinal access. It also supports patient recovery and reduces mortality in conditions such as traumatic brain injury, pancreatitis, malnutrition, burns, trauma, and post-surgical

recovery. In these cases, enteral feeding is an effective and safe way to deliver nutrition and oral medications [1,2].

Enteral feed (EF) formulations can be either prepackaged, ready-to-use formulas or powders that require reconstitution. The primary commercial EF categories include standard polymeric, oligomeric, and monomeric (elemental) formulations, as well as disease-specific formulations [3,4]. In most cases, enteral feeding formulations are liquid-based and flow easily through a feeding tube; however, they can cause complications that require attention. Common complications associated with EF infusion include aspiration pneumonia and vomiting, often due to gastroesophageal reflux [5]. Lower viscosity, also linked to faster infusion rates, may cause diarrhea and malabsorption [6]. Adjusting diet viscosity is a promising strategy to improve early feeding tolerance in critically ill patients after surgery. Recent studies indicate that viscous semi-solid nutrient formulations can effectively reduce gastroesophageal reflux (GER) and speed up gastric emptying in patients with percutaneous endoscopic gastrostomy [7]. Texture affects motility recovery beyond caloric content [8]. Higher-viscosity formulations may include blenderized foods made from natural whole-food ingredients. These can be prepared commercially, at home, or in a hospital [9]. Blenderized enteral nutrition is associated with positive effects, including reduced nausea, vomiting, and abdominal pain, as well as improved tolerance, compared with commercially available enteral formulas [10,11]. However, blended enteral nutrition formulations have several disadvantages, including lower nutritional value, time-consuming preparation, a higher risk of microbiological contamination, reduced physical stability, and specific physical properties that may increase the risk of feeding tube obstruction [9,11].

To address these challenges, we developed a concept that requires higher-viscosity formulations while ensuring they retain flowability and structural suitability for encapsulating and delivering micronutrients by intentionally avoiding thickeners to increase the viscosity of liquid formulations. We believe fluid gels are well suited for this purpose because they exhibit properties similar to granular materials and soft glassy substances [12]. Fluid gels consist of suspensions in which soft-gelled particles are densely embedded in a continuous, non-gelled phase [13]. Researchers have used biopolymers such as agar, low-acyl gellan gum, and egg, whey, and pea proteins to prepare these systems [12–15]. To date, these systems have been successfully applied to dysphagia foods, for confectionary applications and foam stabilisation [14,16–19]; however, they have not been applied to enteral nutrition formulations. Additionally, many of those publications addressed structure formation mechanisms, rheological behavior, and stability, with limited studies on oil incorporation [20]. The majority of these systems are also purely hydrophilic and constructed from water-soluble biopolymers. Our approach, however, involves adding an oil phase to these systems, a novel concept. Authors reported incorporating a lipophilic phase into fluid gels by producing whey protein emulsion fluid gels, focusing on a shear-gel approach to an initially prepared emulsion, whereas our approach creates the emulsified and gelled system directly under shear [20]. Developing such a structure, where the lipophilic phase appears, would align the formulation more closely with commercial enteric products, improving nutrient complexity and transforming these gels into a two-phase system that can encapsulate both hydrophilic and lipophilic ingredients/drugs, which is especially beneficial for critically ill patients. Emulsions, emulgels, and bigels have been developed as delivery systems for co-encapsulating lipophilic and hydrophilic bioactives [21,22]. However, we believe fluid gels may offer advantages over these systems by improving thermodynamic stability and providing a free-flowing consistency.

We also believe such systems could support medication delivery that would otherwise be challenging. Based on our previous study, we selected agar, a polysaccharide, for fluid-gel structuring [17]. We also noted that most studies focus on the nutritional value of enteral

formulas [23–25], whereas much less attention is paid to the physicochemical properties of enteral formulations [26] and to how these properties affect tube administration.

This work goes beyond similar studies by evaluating the effect of lipophilic phase entrapment in agar-based fluid gel formulations and exploring their potential application for enteral nutrition. This study aims to elucidate the properties of emulsified agar fluid gels produced using three oil structuring techniques: (a) without emulsifiers or stabilizers; (b) with polyglycerol polyricinoleate (PGPR), an emulsifier; (c) with monoglycerides and diglycerides of fatty acids (MDFA), which act as both an emulsifier and a gelator. PGPR is a synthetic lipophilic emulsifier that provides unique stabilization for W/O and W/O/W emulsions [27] (Su et al., 2023). This emulsifier is expected to create small, evenly distributed oil droplets by reducing interfacial tension between the phases. In addition to its emulsifying function, MDFA acts as a gelling agent. We believed this would increase the stability of emulsified agar-based fluid gel systems by gelling the dispersed oil droplets and slowing their movement. However, for the same reason, its rheological properties are difficult to predict [28].

The formulated systems were evaluated for morphology, physical stability, rheological behavior, structural changes, and their potential as enteral nutrition formulations. This work aims to offer valuable insights into the design and development of two-phase agar-based fluid gel formulations, providing essential guidance for food product developers and helping to establish more robust scientific methodologies for future research.

2. Results and Discussion

We incorporated oil with different compositions into the agar-based fluid gels during production. Because fluid gels are suspensions of jammed microgel particles that form when a gelling hydrocolloid undergoes shearing during its sol–gel transition, incorporating additional oil could affect system stability and characteristic properties. The following sections characterize agar-based fluid gels to examine both the final properties of these systems and the mechanisms underlying their development.

2.1. Emulsified Agar-Based Fluid Gels Characterization

2.1.1. Stability

All formulated fluid gels were flowable when the container was inverted, indicating a characteristic fluid gel structure with no adverse effects from the incorporated oil. All samples, except the MDFA (68.75%) sample, showed high physical stability (100%), with no visible phase separation or particle sedimentation (0% sedimentation volume) over a 30-day storage period (Table 1). Sedimentation rate is critical, as it can increase the risk of tube clogging and lead to uneven distribution of active ingredients throughout the formulation. A similar trend was observed in centrifugal stability, with the sample containing MDFA showing the lowest value (79.64%). This sample appeared non-homogeneous, with white droplets of varying sizes and shapes dispersed in the continuous phase, unlike the other samples. This structure likely forms because fatty acid monoglycerides and diglycerides cause both emulsification and gelation. Mono- and diacylglycerols form an inverse lamellar crystal structure (below the melting temperature of 60 °C), which then aggregates into oleogel droplets entrapped and partially emulsified within the fluid gel matrix [28,29].

Oil-binding capacity was very high across all tested samples, likely because the centrifugation speed was 2.5-fold lower. At this speed, agar particles formed during the sol–gel transition are assumed to maintain weak interactions within the agar-agar domains, aided by the increased viscosity of the continuous phase, which also traps free, emulsified, or gelled oil within the fluid gel matrix [18]. The following sections examine these assumptions.

Table 1. Physical and centrifugal stability, oil-binding capacity, and span index of emulsified agar fluid gels with different oil compositions.

Sample	Physical Stability, %	Oil Binding Capacity, %	Centrifugal Stability, %	Span	Particle Sedimentation Volume, %
Control	100 ^{b}	100	100 ^{b}	3.37 ± 0.16 ^{b}	0.00
Oil without additives	100 ^{b}		99.99 ± 0.01 ^{b}	5.56 ± 0.19 ^{c}	
Oil with MDFA	68.75 ± 3.43 ^{a}		79.64 ± 8.81 ^{a}	14.07 ± 4.55 ^{d}	
Oil with PGPR	100 ^{b}		90.41 ± 4.21 ^{b}	2.21 ± 0.04 ^{a}	

Values are reported as means ± standard deviation; data labeled with different lowercase letters (a–d) showed statistically significant differences ($p < 0.05$) between different formulations with different oil compositions.

2.1.2. Particle/Droplet Size

Particle size is a crucial factor in enteral nutrition formulations and should be controlled to prevent irreversible clogging, especially in fine-bore tubes [27]. The control agar fluid gel exhibited a unimodal particle size distribution ranging from 10 to 150 µm. This suggests that shear-induced breakup during network formation formed small agar domains. Interparticle interactions arise from steric repulsion or van der Waals forces, forming a multidomain fluid gel system [14]. This particle size is considered a positive outcome, as particles ≥500 µm are often difficult to pass through various enteral feeding tubes and are more likely to clog them [30,31].

Particle size is influenced by process conditions and preparation methods. Some studies reported [32] smaller particles in agar fluid gels, typically produced using a pin stirrer or a rheometer. In contrast, our larger-scale systems employing an overhead stirrer produced larger particles and yielded a higher amount of fluid gels. This benefit might be valuable when moving from laboratory prototypes to full-scale production.

As expected, adding oil produced larger oil droplets, indicated by yellow circles in Figure 1. The less stable fluid gel formulation with MDFA displayed a clear peak at ~900 µm and the highest span index (14.07), indicating significant polydispersity (Table 1). Samples with no additives and with PGPR showed much lower polydispersity and a characteristic oil droplet peak shifted toward smaller particles.

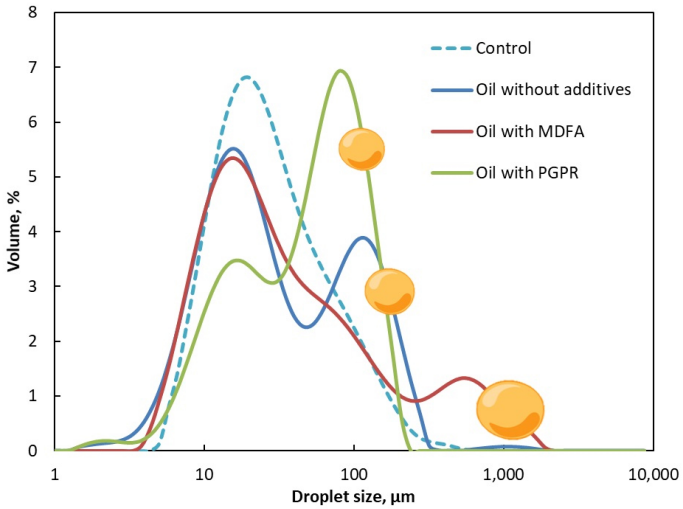


Figure 1. Droplet-size distribution in agar-based emulsified fluid gels produced using three oil-structuring techniques. Yellow circular shapes indicate areas of oil droplet formulation.

2.1.3. Microstructure

Figure 2 micrographs confirm the particle size distribution. In the control sample, agar particles exhibit an anisotropic shape, with dangling chains extending from their surfaces into the surrounding phase, forming weak, aggregate-like structures, as indicated by yellow arrows in Figure 2A. This structure is characteristic of agar-based fluid gels and has been reported in studies by other researchers [14,18].

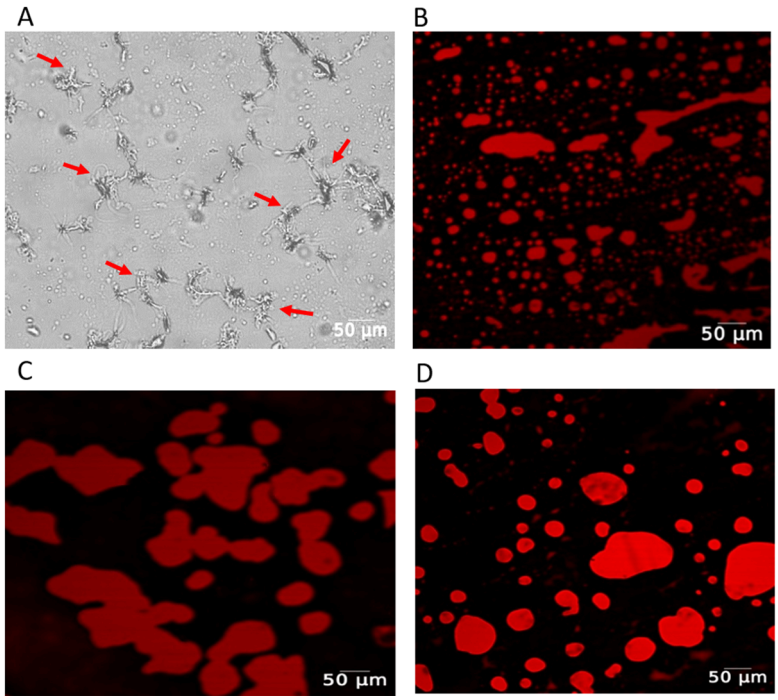


Figure 2. Phase-contrast image of the control agar fluid gel (**A**) and confocal micrographs of emulsified agar fluid gels: (**B**) without additives; (**C**) with MDFA; (**D**) with PGPR. Red arrows indicate agar particles.

Adding oil to the fluid gel system caused oil droplets to disperse throughout the structure, with the distribution pattern depending on the oil's composition. In samples without an emulsifier, irregularly sized droplets dispersed across the agar network, indicating that the oil droplets were incorporated between agar-agar domains, thereby stabilizing the system and preventing observable phase separation. When the oil contained MDFA, larger, flocculated droplets emerged and tended to cream more frequently [33], which accounts for the system's reduced stability. Meanwhile, samples containing PGPR exhibited more uniform, evenly distributed particles (Figure 2D), consistent with the particle size distribution and polydispersity index (span 2.21). This is associated with PGPR's function as an effective emulsifier, forming a protective coating around oil globules and further increasing the viscosity of the oil phase [34].

2.1.4. Proposed Mechanism of Structure Formation

Figure 3 illustrates the proposed schematic mechanism by which we predict oil is structured within the agar fluid-gel network, based on particle-size distribution and morphological image analysis. First, agar serves as the primary structural component. After gelation under a shear field, it forms small agar-particle domains, with unbound chains extending from their peripheries. As previously reported, fluid-gel structure formation begins with the formation of gel nuclei, which then grow into larger domains by linking

with additional polymer chains from the continuous phase [18]. Van der Waals interactions between the particles result in weak, aggregate-like structures [14].

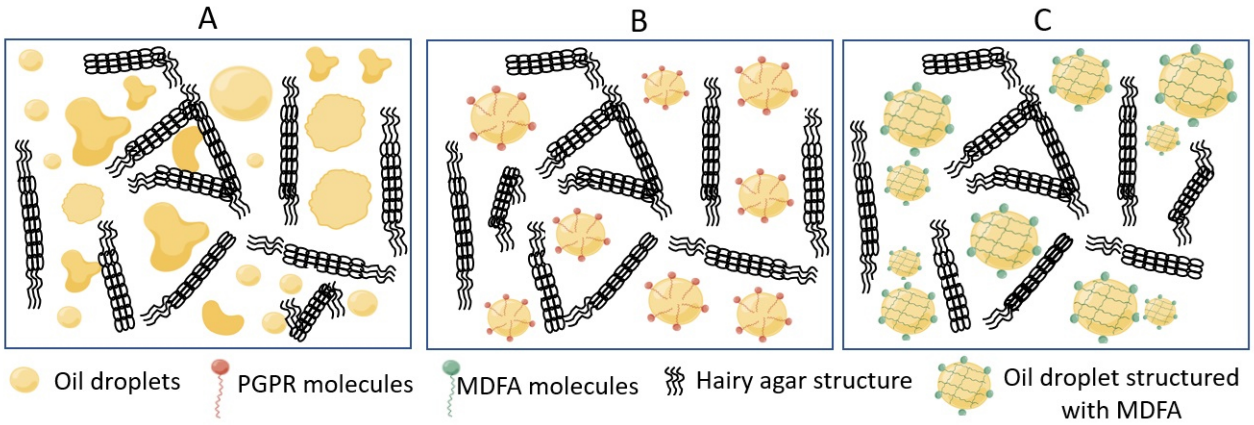


Figure 3. Schematic representation of the emulsified agar fluid gels with different oil incorporation methods: (**A**) oil without additives; (**B**) oil with PGPR; (**C**) oil with MDFA.

Oil without additives did not form well-defined droplets; instead, it comprised isolated or coalesced droplets embedded within the agar network (Figure 3A). Surprisingly, this system remained quite stable, likely due to its increased viscosity—a point to be examined in the next section. Additionally, we hypothesize that oil droplets dispersed during the formation of the agar-agar domain network became trapped between weak, aggregate-like helices that collectively form a three-dimensional network [35]. Given the different polarities of the phases, the oil dispersed in the continuous aqueous phase likely acted as an inert filler, with no potential to interact with the agar particles. Regarding PGPR, this system exhibited the most predictable structure, as PGPR functions as an emulsifier, reducing interfacial tension and dispersing oil droplets within the continuous phase of suspended agar particles (Figure 3B). We make the assumption that structural differences likely arise not only from the oil-phase composition but also from the characteristic fluid-gel preparation conditions. During preparation, the system is mixed at a constant 300 rpm with an overhead mixer, promoting turbulent flow. In this continuously mixing flow, during the cooling stage, in the sample with MDFA the monoglycerides in the oil begin to form crystalline regions, leading to gelation of the oil phase [28]. However, because mixing continues, partially or fully crystallized oil droplets are likely to be disrupted, leading to partial coalescence, as confirmed by droplet-size distribution and microstructural images. Based on this, we assume that coalescence and oil crystallization were the main destabilization mechanisms behind the weak stability of MDFA-containing fluid gels. Preventing these mechanisms may require changing the production conditions by reducing the shear speed; however, this could also lead to the formation of larger agar-agar domains.

2.1.5. IDDSI Tests

The IDDSI test offers a practical approach to assessing the flowability of formulated systems based on properties such as viscosity, cohesiveness, adhesiveness, and surface tension [36]. The gravity flow test showed that all formulations had a moderately thick consistency and were classified as IDDSI level 3 (remaining liquid <10 mL). The fork drip test further confirmed this, showing that the formulations dripped gradually in small amounts through the prongs of a fork (Figure 4). Higher viscosity is considered beneficial because semi-solid enteral nutrition formulations have been shown to prevent gastroesophageal reflux, which helps to ensure effective delivery of nutrients or medications through feeding tubes [37,38]. For this reason, researchers previously used pectin and alginate as viscosity-

regulating agents in enteral nutrition formulations [39]. Adding oil did not visibly change the flow properties of the systems (Figure 4). Oil might function as a lubricant, helping the fluid move through the tubes. The spoon tilt test indicated that emulsified fluid gel systems are flowable, leaving only a thin film on the spoon. This implies low adhesiveness and cohesiveness in the formulated systems, which likely facilitates flow and reduces the risk of tube clogging.

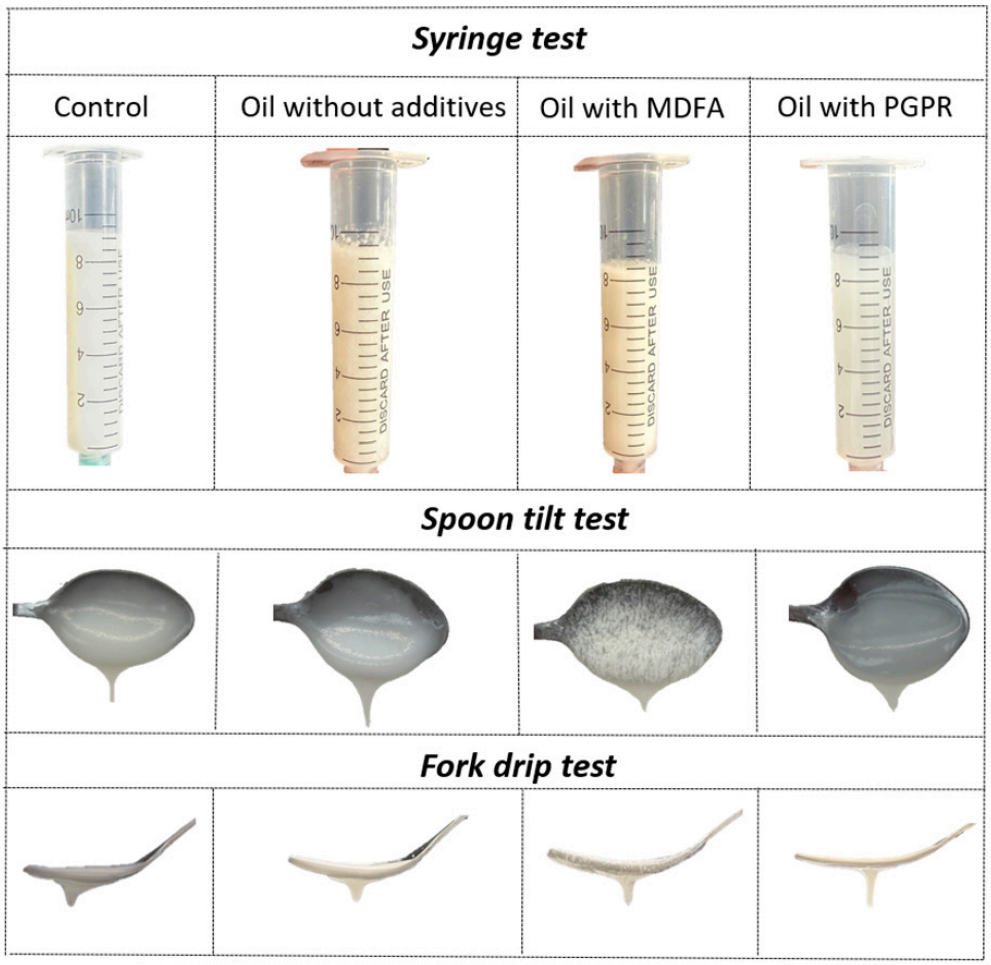


Figure 4. IDDSI tests (syringe test, spoon tilt test and fork drip test) of agar-based emulsified fluid gels produced using three oil-structuring techniques.

These IDDSI tests are appropriate for assessing system properties in hospital or home environments. However, developing enteral nutrition formulations requires more precise, detailed rheological analysis, which will be discussed in the next chapter.

2.1.6. Rheological Properties

Beyond viscosity, we examined the consistency coefficient, flow index, and yield stress to better characterize the formulations. These properties can influence the choice of administration method and tube features. The formulation's high viscosity could be a key factor, potentially causing enteral feeding tube blockage during administration. However, previous reports indicate that liquids with viscosities of 1000 mPa·s or less can flow adequately through 10Fr and 18Fr enteral feeding tubes [40]. In our case, the apparent viscosity measured at 50 s^{-1} was below 1000 mPa·s, ranging up to 760 mPa·s. Higher viscosity has an advantage over liquid formulations, as it can help prevent sedimentation, which often causes tube blockage. To safely transport nutritional components in higher-viscosity

formulations, large-diameter gastronomy tubes are commonly used to prevent tube occlusion [41]. However, higher-viscosity products can increase infection risk due to tube retention, as shown by other studies [42], which should also be considered. All formulations exhibited pronounced shear-thinning behavior, with apparent viscosity decreasing as shear rate increased and a flow index below 1 (Table 2), consistent with reports for enteral nutrition-oriented formulations [43].

Table 2. Rheological characteristics of agar-based emulsified fluid gels produced using three oil-structuring techniques.

Sample	Apparent Viscosity *, mPa·s	k	n	τ_0, Pa	Critical Stress, Pa	Structural Recovery, %
Control	749.50 ± 24.90 ^{bc}	4.89 ± 0.57 ^{ab}	0.43 ± 0.03 ^{a}	1.04 ± 0.16 ^{b}	1.07 ± 0.01 ^{b}	143.30 ± 14.72 ^{b}
Oil without additives	760.70 ± 94.29 ^{c}	4.61 ± 0.17 ^{b}	0.47 ± 0.03 ^{ab}	5.99 ± 0.32 ^{d}	1.40 ± 0.07 ^{c}	104.23 ± 8.07 ^{a}
Oil with MDFA	738.73 ± 26.00 ^{bc}	5.92 ± 0.82 ^{ab}	0.42 ± 0.03 ^{a}	3.74 ± 0.34 ^{c}	1.08 ± 0.02 ^{b}	103.49 ± 3.17 ^{a}
Oil with PGPR	588.88 ± 33.23 ^{a}	4.28 ± 0.65 ^{a}	0.52 ± 0.02 ^{b}	0.39 ± 0.07 ^{a}	0.61 ± 0.04 ^{a}	112.95 ± 5.70 ^{a}

*—designation of viscosity category based on the viscosity at 50 s^{-1}, measured at 25 °C. Values are reported as means ± standard deviation; data labeled with different lowercase letters showed statistically significant differences ($p < 0.05$) between different formulations with different oil compositions.

An evaluation of how oil addition and different incorporation methods affect rheological properties showed that these factors influence the observed variations. Oil addition increased the dispersed-phase fraction, while the presence or absence of a stabilizer/emulsifier resulted in different droplet size and distribution, as previously confirmed. Oil incorporation had no visible effect on apparent viscosity but significantly increased yield stress compared with the control sample. At higher dispersed-phase fractions, hydrodynamic interactions increase energy dissipation and restrict droplet motion, thereby increasing flow resistance [44]; this likely drove the increase in our case as well. This result agrees with a previous study linking the effective volume fraction of the dispersed phase in suspensions of starch granules and fat droplets to increased apparent viscosity and yield stress in mixed systems [45].

When developing enteral nutrition formulas, the expected yield stress value may be more important than viscosity alone. Yield stress (τ0) reflects the formulation's capacity to resist applied forces [46]. It indicates how flow is initiated and sustained, whether it flows under gravity or minimal pressure, or requires pumping. Adding PGPR effectively reduced viscosity and yield stress, with values lower than the control (588.88 Pa · s and 0.39 Pa). Previous studies have shown that PGPR, when used as an emulsifier, can decrease yield stress by up to 50% at 0.2% concentration [47]. This reduction may result from a different mechanism of oil emulsification and stabilization. PGPR molecules pack tightly at the water-oil interface and form a viscoelastic film that helps stabilize oil droplets by preventing coalescence, thereby modulating rheological properties [48]. The higher consistency index and yield stress of the MDFA-emulsified fluid gel can be explained by the formation of a three-dimensional network of aggregated crystals, as reported by other authors [27,49]. The highest yield stress was also observed in the sample with no emulsifiers. The absence of emulsifiers is widely believed to have caused the formation of small oil droplets (previously confirmed by droplet-size measurements and microscopic images), which were randomly scattered within a network of agar particles held together by weak interparticle forces. Smaller oil droplets allow stronger droplet interactions and, therefore, greater flow resistance [50].

Another important property to consider is the extent of post-shear structural recovery in emulsified fluid gel systems. After applying mechanical force to initiate flow and inject the formulation, it is important that the formulation maintains the same rheological properties once it reaches the gastrointestinal tract. As shown in Table 2, the control non-emulsified agar fluid gel sample achieved the highest structural recovery, exceeding 100% (143.30%). Other studies on agar-based fluid gels report this behavior and link it to rapid rearrangements among agar particles and new agar-agar domain interactions [17,51]. Such an increase in viscosity is undesirable because the goal is to restore the original structure after mechanical action, not to produce a formulation more viscous than the original one. Adding oil and distributing oil droplets within the agar domains hinder new interactions and reduce the rate of structural recovery, bringing it to 112.95–104.23% (closer to the expected result). This indicates that adding oil can enable complete structural recovery, with viscosity values remaining close to the original.

The structural integrity of the emulsified fluid gel samples was assessed using amplitude and frequency sweep tests. Across the entire frequency range, the elastic modulus G′ exceeded the viscous modulus G″, indicating predominantly weak-gel-like behavior ($\tan\delta > 0.5$) (Figure 5A). This aligns with the findings of Bazrafshan et al. (2023) [44] for an enteral nutrition formula made from a blend of gelatin and maltodextrin. In emulsion-type systems, G′> G″ behavior is related to percolated droplet networks with a low amount of freely moving droplets [52]. The elastic modulus G′ of the samples gradually increased with increasing frequency, which is related to rearrangement of non-covalent interconnections between agar domains and the stiffness of the formulated network [53]. It can also be seen that at the highest frequency, a sharp decrease in G″ was found in the control and the sample with PGPR.

Under large-amplitude oscillatory shear, emulsified fluid gels transitioned from the linear viscoelastic region to the nonlinear region at critical stress values that varied with emulsified fluid gel composition (Table 2). At that point, the emulsified fluid gel network disrupted, and the viscous modulus exceeded the elastic modulus (G″>G′). As expected, the oil-only sample had the highest critical stress (1.40 Pa), suggesting greater particle deformability. The PGPR-containing sample had the lowest critical stress (0.61 Pa), consistent with the yield stress values. This behavior is also likely linked to the much smaller oil droplet size, which probably not only increases the dispersed-phase fraction but also minimally affects agar-agar domain interactions, thereby strengthening the structure. Systems with oil addition exhibit dual behavior characteristic of both concentrated suspensions and emulsified systems, with a suspension-dominated structural pattern, as evidenced by an amplitude-sweep response similar to that of the control sample (without oil).

2.1.7. Maximum Injection Force

The visual assessment and rheological data indicate that the formulated fluid gels cannot flow through tubes without additional force, making gravity drip inappropriate. In this context, the administration of formulations in the gastrointestinal tract using a volume syringe or electronic infusion pumps appears promising [54]. The volume syringe is a common method for administering tube feeding at home and in long-term care facilities and should be used for gastric tube feeding only. However, to use this method effectively, determine the maximum injection force required to deliver the formulation. Lower injection force is associated with better injectability during tube administration [55].

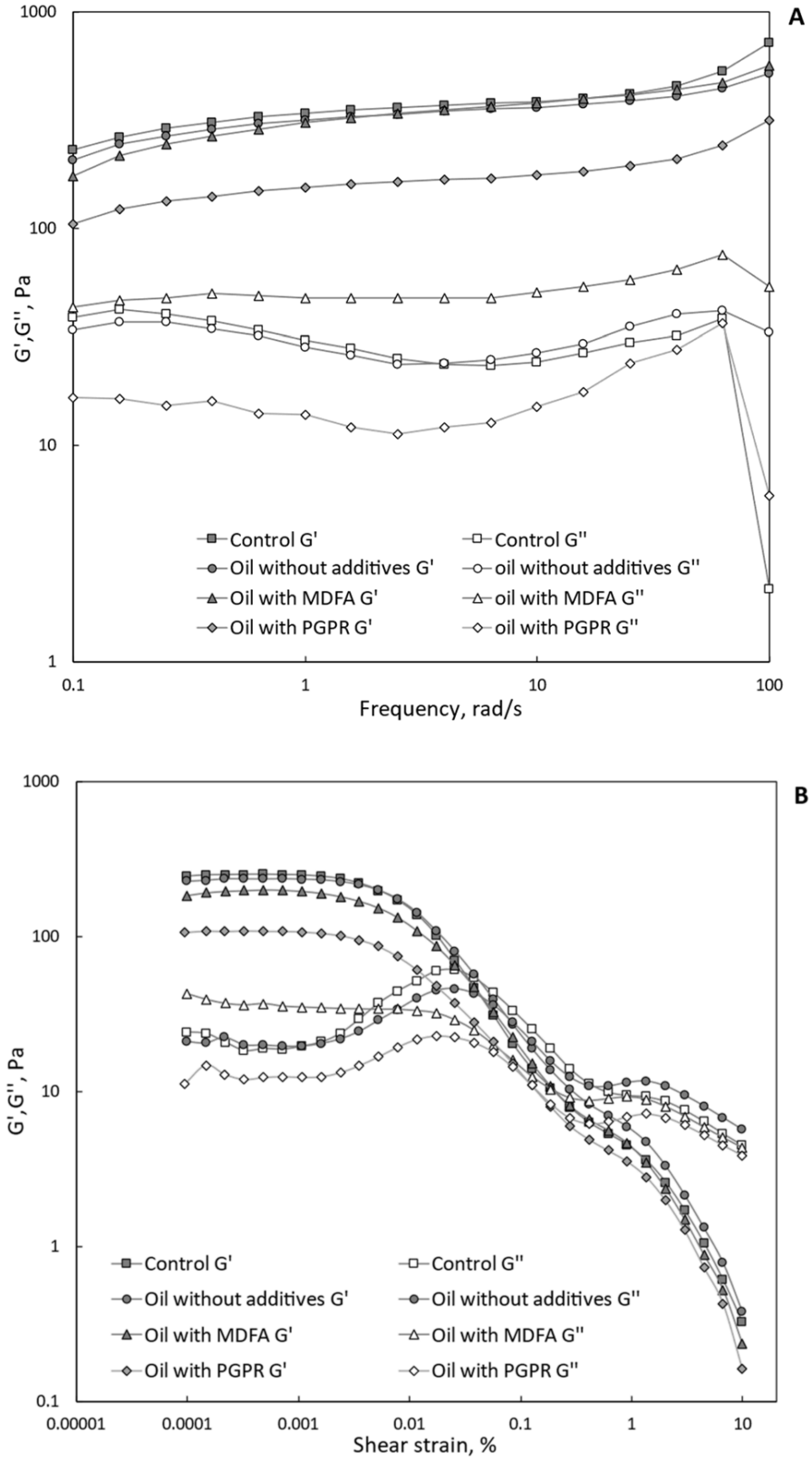


Figure 5. Frequency sweep (**A**) and amplitude sweep (**B**) in agar-based emulsified fluid gels produced using three oil-structuring techniques.

Formulation composition significantly ($p < 0.05$) affected the maximum injection force (via gastric tube). The initial assumption was that adding oil would act as a lubricant and reduce the force required to push the samples through the feeding tube. However, adding oil increased the injection force by 1.5 to 2 times relative to the control sample. Based on the

previous data, yield stress and dispersed-phase volume likely drove this behavior, with the latter having a greater influence. As previously reported, when particle/droplet concentration increases, the injection force rate appears to increase. A higher droplet volume fraction leads to a more "crowded" syringe barrel and increases the friction force [56]. We also assume that the sample with oil incorporated without emulsifiers or stabilizers exhibits much higher interfacial tension, which could also result in lower interfacial viscoelasticity under external forces. The sample containing PGPR exhibited the lowest yield stress; however, its injection force was higher than that of the control sample. This shows that the effect still depends on droplet size and distribution. These results also contradict other authors' data, indicating a higher viscosity higher injection force correlation [56]. Other studies reported a non-linear correlation between sodium carboxymethyl cellulose solution viscosity and injection force, which they attributed to shear-thinning behavior [57]. Previous studies reported a wide range of injection forces for pureed diets, from 9.62 N to 117.59 N. These values varied by energy level and were positively correlated with carbohydrate content [58]. Additionally, the study assessed the formulation's injectability through larger-diameter tubes (Duodenum tubes with outer diameters of 5.3 mm and 6.0 mm). Expectedly, the maximum injection force decreased as tube diameter increased, lowering system resistance and facilitating easier sample injection. This trend remained consistent—the control required noticeably less force (Figure 6). Nonetheless, with the largest tube (6.0 mm), differences between oil-containing samples became indistinct. This shows that injection force can be adjusted not only by formulation composition but also by tube diameter. These data are consistent with previous studies that found needle diameter substantially affects injection force [56]. This also indicates that, when selecting a syringe for enteral nutrition delivery, a wider-diameter tube type should be chosen. Additionally, injectability depends on the end user's ability to push the syringe plunger rod [57], suggesting this method may not suit users with limited hand strength. In this context, electronic pumps could be more effective.

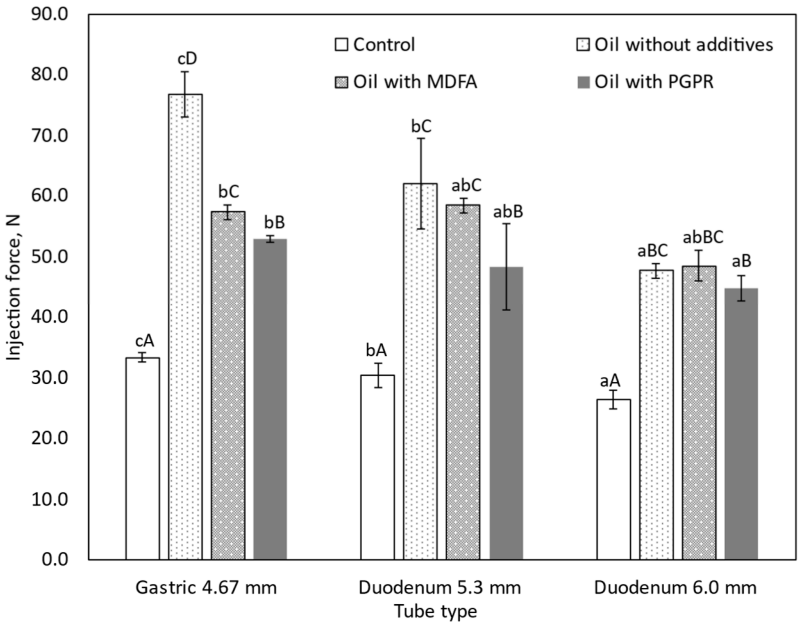


Figure 6. Maximum injection force in agar-based emulsified fluid gels produced using three oil-structuring techniques. Data labeled with different lowercase letters showed statistically significant differences ($p < 0.05$) between different formulations with different oil compositions; data labeled with different uppercase letters showed statistically significant differences ($p < 0.05$) between different tube sizes.

However, these highly stable, dual-phase formulations with higher viscosity and no observable sedimentation may serve not only as a nutrient source but also as a drug-delivery platform for critically ill patients, particularly those who frequently experience complications associated with enteral nutrition infusion. As previously reported, higher-viscosity enteral nutrition formulations may prevent leakage around the tube insertion site and slow intestinal glucose absorption [58,59]. In addition, these systems offer an advantage over formulations thickened with commercial thickeners before feeding, as they help prevent bacterial contamination during the mixing of the liquid formula with the thickener.

3. Conclusions

This study introduced an innovative method for creating agar-based emulsified fluid gels, designed as a base for enteral nutrition and with potential to serve as a carrier for lipophilic and hydrophilic bioactive and functional ingredients. Modifying oil structuring within the continuous fluid gel phase enabled control over stability, droplet size and distribution, oil retention, rheological properties, and flow behavior during tube administration.

Results showed that oil emulsification followed by gelation using MDFA was the least suitable approach for developing these systems, yielding nonhomogeneous, highly polydisperse systems with low physical stability (68.75%) and low stability under dynamic conditions (79.64%). Whether with or without an emulsifier, droplet size distribution was comparable, with much lower system polydispersity in the PGPR sample. Additionally, this lipophilic-phase compositional difference affected viscosity, yield stress, droplet-phase volume fraction, and maximum injection force, with lower values in the PGPR-containing formulations. These results confirmed the hypothesis that different oil-structuring approaches strongly affect agar-based fluid gels' stability, structure-formation mechanism, and rheological properties. These findings are promising for the development of fluid gel formulations containing a lipophilic phase. However, existing research has not extensively examined the capacity of newly developed emulsified fluid gel systems to co-encapsulate hydrophilic and lipophilic compounds or to serve as drug carriers. A limitation of the study is the lack of gastrointestinal digestion studies and long-term storage evaluation. Future studies should investigate this capacity to better understand encapsulation efficiency, encapsulated-compound stability, and site-specific release during gastrointestinal digestion.

4. Materials and Methods

4.1. Materials

Agar powder (0.3% fat, 3% carbohydrates, 0.4% protein, and 0.1% sodium chloride) was purchased from a local supermarket (Alvo, Kaunas, Lithuania). Refined rapeseed oil (Obelių aliejus, Lomista, Kaišiadorys, Lithuania) contained 7 g/100 g of saturated fats, 66 g/100 g of monounsaturated fats, and 27 g/100 g of polyunsaturated fats. Monoglycerides and diglycerides of fatty acids with a melting point of 63–72 °C (E471, Puratos, Bijgaarde, Belgium), along with polyglycerol polyricinoleate (PGPR) (Danisco, Copenhagen, Denmark), were used as emulsifiers and stabilizers for the oil phase. Fluorescein-5-isothiocyanate (FITC, CAS No. 3326-32-7) and Nile Red (CAS No.7385-67-3) were obtained from Fluorochem (Derbyshire, UK).

4.2. Methods

4.2.1. Agar-Based Emulsified Fluid Gels Preparation

During preparation of the emulsified fluid gel, 10% rapeseed oil was incorporated into the agar- based fluid gel using three structuring approaches: (a) the oil was incorporated

without additional stabilizers; (b) the emulsifier PGPR was used; (c) the emulsifier/stabilizer monoglycerides and diglycerides of fatty acids (MDFA) was used. Before incorporating the oil into the fluid gel formulation, the oil phase was prepared by dispersing PGPR (2 g/100 g) or MDFA (4 g/100 g) in the oil. The concentrations of PGPR and MDFA were determined based on the results of preliminary experiments.

Conditions for the agar-fluid gel preparation were selected based on our previous study [17] with some minor changes at the oil incorporation step. 1.5 w/w% agar powder was dissolved in distilled water using a magnetic stirrer (IKA C-Mag HS 7, Staufen, German) until fully hydrated. The agar solution was then heated in a water bath at 95 °C for 20 min. Simultaneously, an oil phase (10 w/w%) prepared separately was placed in a glass container in a water bath under the same conditions. After heating, both phases were combined and homogenized with an Ultra-Turrax (IKAR T-18 Basic, Staufen, Germany) for 2 min at 15,000 rpm. The mixture was then stirred for 60 min at room temperature (25 °C) using an overhead stirrer (HT-DX, Witeg, Wertheim, Germany) at 300 rpm. Finally, it was stirred for another 60 min in an ice-water bath, maintaining 0–2 °C. A control sample was prepared at a 1.5% agar concentration under the same conditions, without adding an oil phase.

4.2.2. Agar-Based Emulsified Fluid Gels Characterisation

Physical stability

The physical stability of emulsified fluid gels was evaluated using a phase-separation test. Immediately after preparation, samples were transferred to 50 mL tubes and stored at approximately 20 °C. The total height of the samples and the height of the phase separation were recorded over 30 days of storage.

Dynamic stability

To evaluate centrifugal stability, freshly prepared samples were centrifuged (MPW-260RH, MPW Med Instruments, Warsaw, Poland) at 2500× g for 15 min at 4 °C [60]. Stability is expressed as a percentage, defined as the ratio of the test tube's total volume to the volume of the phase that separated during centrifugation.

Oil binding capacity

The oil-binding capacity was evaluated using the method reported by Martins et al. (2017) [61]. The freshly prepared sample was centrifuged (MPW-260RH, MPW Med Instruments, Warsaw, Poland) at 1000× g for 10 min at room temperature (20 ± 1 °C). The precipitated oil was then gently removed. The oil-binding capacity was determined using the following formula:

$$OBC\ (\%) = \left(1 - \frac{W_i - W_f}{W_i}\right) \times 100 \quad (1)$$

where Wi is the initial weight of the sample before centrifugation; Wf is the weight after the oil has been removed.

Sedimentation rate

For the sedimentation rate, fluid gel samples were placed in 30 mL glass tubes and kept at 25 °C. Visual changes and the height of the clarified phase were monitored for 48 h at room temperature.

Particle size

Particle size distribution was measured with a Mastersizer 2000 laser diffraction analyzer (Malvern, Worcestershire, UK). The parameters included a measurement speed of 1400 rpm, with the refractive indices of the dispersed phase and the solvent set at 1.42 and 1.33, respectively. The droplet absorption was 0.01, and the obscuration range was 10–15%. Results were presented as particle size distribution and polydispersity index (span).

IDDSI tests

The flow properties of fluid gels were evaluated in accordance with the IDDSI (2019) guidelines using a syringe-based method. A reference syringe (Amedi+plus Luer slip 21 G × 1½″; Jiangyin Nanquan Macromolecule Product Co., Ltd., Jiangyin, China) equipped with a Luer-Lok tip was used. The length for the 0–10 mL volume range was measured at 61.5 mm. Procedure: After covering the syringe nozzle with a finger, the syringe was filled with 10 mL of the tested sample. The needle tip was opened, then covered again after 10 s, and the volume of sample remaining in the needle was recorded. Based on these results, samples were categorized as thin (less than 1 mL remaining) to extremely thick (10 mL remaining) [62]. IDDSI, 2019

Rheological analysis

The rheological properties of the samples were measured using an MCR 92 controlled-stress rheometer (Anton Paar, Graz, Austria) with a 25-mm parallel-plate geometry and a 1-mm gap at 25 °C.

Viscosity was measured at a constant shear rate of 50 s^{-1}, after which samples were classified into four consistency categories following the National Dysphagia Diet Task Force (NDD) guidelines (American Dietetic Association, 2002) [63].

To assess shear recovery behavior, samples were subjected to three shear-rate cycles: starting with a low shear rate of 0.1 s^{-1} for 3 min, then a high shear rate of 50 s^{-1} for 1 min, and ending with another 0.1 s^{-1} low shear rate for an extra 3 min to observe structural recovery.

Samples were tested to characterize viscoelastic behavior using oscillatory frequency and amplitude sweeps. A stress sweep increased the stress from 0.01% to 1000% at 1 Hz, and the linear viscoelastic region was identified. The critical stress was defined as the stress at which a 10% decrease in the samples' elastic modulus (G′, Pa) was observed [64]. During the frequency sweep, the strain amplitude was held constant at 0.05%, while the oscillation frequency varied from 1 to 100 rad/s.

Microstructure

The microstructure of the emulsified fluid gels was analyzed using a confocal laser scanning microscope (Olympus FLUOVIEW FV1000, Olympus, Tokyo, Japan) with a 20× oil-immersion objective. Nile red (0.75 mg/mL) was added to the oil phase before the final gel structure formed. The sample was then mounted on a glass slide. Protein-containing samples were tagged with fluorescein 5-isothiocyanate (FITC) (10 µL at 1 mg/mL in acetone). After labeling, the sample was covered with a cover glass and examined under CLSM. FITC and Nile Red were excited at 488 nm and 543 nm, respectively. CLSM images were analyzed with ImageJ software.

Microstructure of the control agar fluid gel sample was evaluated with a phase-contrast inverted microscope Olympus IX73 (Olympus Europe Holding GmbH, Hamburg, Germany). Selected areas of the coverslips were randomly imaged with a 20x lens.

All images of the samples were analyzed with ImageJ version 1.53 t (National Institutes of Health, Bethesda, MD, USA).

Maximum injection force

The maximum injection force was determined following the method of Chen et al. (2025) [65]. A TA-XT Plus texture analyzer (Stable Micro Systems, Godalming, UK) was employed. Samples were injected via a 60 mL manually operated syringe linked to different tubes 14 Fr (Stomach Tube, outer diameter 4.67 mm, length 120 cm, Greetmed, Ningbo, China), 16 Ch (Duodenal Tube, outer diameter 5.3 mm, length 125 cm, Galmed, Leczycka, Poland) and 18 Ch (Duodenal Tube, outer diameter 6.0 mm, length 125 cm, Galmed, Leczycka, Poland) (Figure 7) at a speed 1 mm/s.

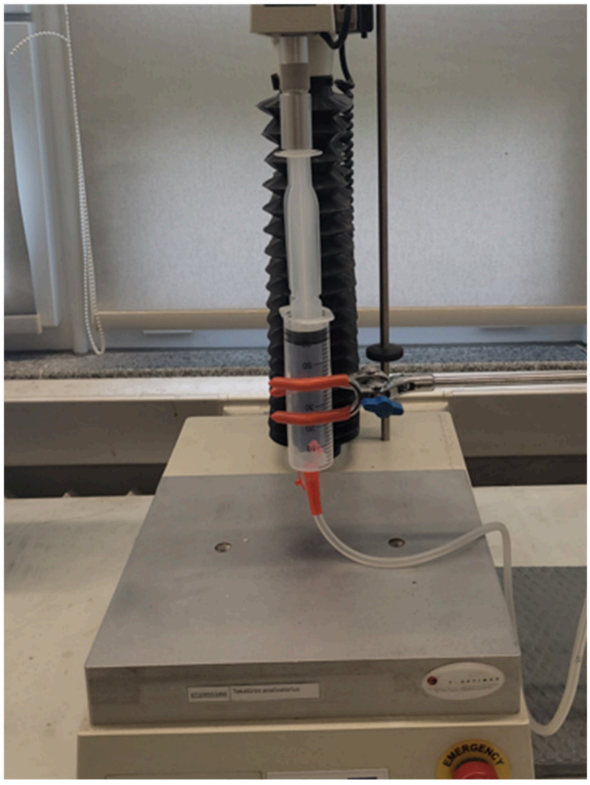


Figure 7. Setup of the maximum injection force measurement.

The peak injection force was recorded to reflect the samples' resistance as they passed through the tubes. In addition, we measured the friction force and calculated the maximum injection force using the provided formula:

$$F_{injection} = F_{sample} - F_{friction} \quad (2)$$

where F_{sample}—injection force in the syringe containing the sample, N; $F_{friction}$—average glide force of the empty syringe, N.

4.3. Statistical Analysis

All experiments were performed 3–5 times, and results are presented as mean ± standard deviation. Significant differences in the means were identified using analysis of variance (ANOVA) with a p-value < 0.05 in Statistica 12.0 (StatSoft Inc., Oklahoma, AK, USA, 2013).

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