# Preparation, in vitro evaluation, and stability studies of sodium risedronate microemulsions

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## Abstract

**Introduction:** Sodium Risedronate (SR) is a member of bisphosphonate drugs used for osteoporosis treatment. Albeit its well-known efficacy, SR suffers from low oral absorption (only 0.63%) and many adverse effects.

**The aim of the study:** formulate and characterize oil-in-water microemulsion systems for the oral delivery of SR.

**Methods:** Sunflower oil was used for the oil phase. Next, the solubility of SR in different surfactants and co-surfactants was detected. Three different ratios of (Tween 80/glycerin) microemulsions were studied: (2/1), (1/1), and (1/2). Regarding SR microemulsions, three different formulations: (2/1), (1/1), and (1/2) (Tween 80/glycerin) were characterized for their: transparency, conductivity, light transmission, refractive index, droplet size, dissolution tests, and intensive stability studies.

**Results:** As Tween 80 and glycerin were the best surfactant/co-surfactant mixture, the higher surfactant ratio (Tween 80) had the largest microemulsion region in pseudo-ternary diagrams and a new detected phase (gels). SR microemulsions were transparent liquids of the o/w type. (2/1) formulation had the smallest droplet size (8nm) with good stability results across three diverse protocols. Followed by the (1/1) formulation (10.89 nm), though this formulation showed an overloading problem (0.4% SR) in stability tests. (1/2) formulation (19.21 nm) had the highest loading capacity (0.6% SR).

**Conclusion**: The outstanding stability of the (2/1) (0.1% SR) formulation as a highly stable finished drug form or a potential step for further formulation development, along with the notable drug loading capacity of the (1/2) (0.6% SR) formulation, emphasize the significance of further studies to validate their efficacy and potentials.

**Keywords**: Sodium risedronate, Microemulsion, Tween 80, Glycerin, Stability studies

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## Introduction

Despite the recently devoted attention to the bisphosphonates family as the first-line therapy for most patients with osteoporosis: such as postmenopausal osteoporosis, glucocorticoid-induced osteoporosis, and many other bone resorption conditions, the initial industrial use of this group was as anti-scaling and anticorrosive agents in washing powders and water brines. Fleisch, Russell, and Francis introduced the clinical utility of these drugs in the 1960s (1). As class III drugs, all bisphosphonates have good water solubility with a low absorption percent, such that only 0.6%–3% of these drugs can reach the bloodstream (2). SR is a widely marketed drug of this group with the lowest bioavailability. Less than 1% of the oral dose can enter the bloodstream and go straight to bone surfaces (3). Thus, researchers made huge efforts regarding this problem, taking advantage of new systems and technologies. For instance, nanoparticles (4, 5, 6, 7), enteric-coated tablets (8), buccal mucoadhesive film (9), and bilosomes (10).

Notwithstanding the recent intensive searches on microemulsions, their discovery dates back to 1943 by Hoar and Schulman (11). They first described the microemulsion as a dispersed system consisting of

water and oil with a transparent look, unlike all previous binary colloidal systems (11). Microemulsions are thermodynamically stable, translucent systems with a nano-sized dispersed inner phase between 10-100 nm, which gives microemulsions high stability and long shelf life (12). As an emulsion, microemulsions consist of water, oil, and surfactants. However, because of the nano-sized internal phase and low surface tension in microemulsions, surfactants (single hydrophobic tail surfactants in particular) cannot ensure microemulsions' stability on their own (13). Therefore, a fourth ingredient called co-surfactants is often a must (14). Examples include medium-chain alcohols and polyols such as glycerol and propylene glycols. Some researchers classify co-surfactants as second surfactants or low molecular weight amphiphiles that cannot make a stable microemulsion individually because of their small polar heads (15). Co-surfactants play influential roles in microemulsion formation and long-term stability as they penetrate surfactant molecules on the oil-water interface and increase the film fluidity, thereby increasing the system entropy (16). Moreover, co-surfactants absorb some water molecules, which decreases the surfactants' water solubility and increases their hydrophobic tail motility, allowing greater penetration for surfactants in oil and good stability for the system (17).

Despite their easy preparation -spontaneously by ordinary mixing, microemulsions' characterization is challenging. Initial evaluation of having a microemulsion formulation or not can be done simply by two tests: A simple visual examination and polarized light microscopy detection. Then, the determination of microemulsion’s type by Electrical conductivity and Refractive Index RI. Afterwards, detect the inner phase droplet size using light transmission %T simply and zetasizer for accurate results. Finally, formulation tests: dissolution and stability tests.

The long-term goal of this work is to emphasize the advantages of microemulsions as promising dosage forms for class III drugs that have low permeability, and high solubility, unlike the intensive work on microemulsions as solubility enhancers for class II drugs. The present study used SR as a model drug and searched for the possible effects of the Smixture's components, surfactant, and co-surfactant, on the properties of resulting microemulsions.

## Materials and methods

### 2.1. Materials

SR was received as a gift from JPN Pharma (Mumbai, India), sunflower oil was purchased from Croda (Mumbai, India), Tween 20 (polysorbate 20), and Tween 80 (polysorbate 80) was purchased from Riedel-de Haën (Seelze, Germany), Glycerin and Polyethylene glycol 400 (PEG-400) were purchased from Panreac Quimica (Barcelona, Spain). Fresh, deionized water was distilled by Water Still DS 8000 (Drag lab, Germany).

### 2.2 Screening SR solubility in surfactants and co-surfactants

Different types of nonionic surfactants: (Tween 20, Tween 80) were selected due to their safety, biocompatibility, and cheap prices (18). Likewise, two types of water-soluble co-surfactants: glycerin and PEG-400, were selected as they are labeled the best in producing microemulsions (19). Note: These excipients were carefully selected after a pre-formulation study, ensuring no unwanted effects of excipients on the active Substance SR. The solubility of SR was determined in surfactants and co-surfactants by adding an excessive amount of SR to 5g of each solution (20). These mixtures were stirred for 24 hours at room temperature. The equilibrated samples were centrifuged at 6000 rpm for 30 minutes to remove the undissolved drug. The solubility of SR was calculated by analyzing the filtrate spectrophotometrically after dilution with distilled water when needed. The SR measurements were according to our previously published study at 262nm (21).

### 2.3. Selecting the best surfactant-co-surfactant (Smix) for microemulsion formulation

Four different Smix (Tween20, Glycerin), (Tween20, PEG-400), (Tween 80, Glycerin), and (Tween 80, PEG-400) were tested. For this step, a microemulsion formulation was made from each of these four mixtures with (1/9) (oil/ Smix) – to detect the highest capacity of the Smix with the lowest oil percentage and a constant ratio of surfactant/co-surfactant (1/1). Microemulsions were made by adding an adequate amount of oil, surfactant, and co-surfactant to a glass beaker, mixing by sonication (Cleaner Ultrasonic Digital PHYLLO with heating USH-10D, Italy) for 5 minutes. Then, on a magnetic stirrer, accurate water volumes were added at room temperature dropwise, varying from 90 to 10% of the microemulsion at a moderate speed (22). Finally, samples were sonicated for 5-10 minutes to accelerate equilibration (23) and then kept for detection the next day in tightly closed glass containers. Samples were classified as a microemulsion according to two criteria: visually: as stable, clear, and transparent liquids -semisolid or milky formulations are excluded- and microscopically under polarized light microscopy as an isotropic liquid with a dark background (24).

### 2.4. Construction of pseudo-ternary phase diagrams

After selecting Tween 80 and glycerin as the best active mixture, detecting each component's effect on making stable microemulsions was the second step. Therefore, three pseudo ternary phase diagrams (2/1), (1/1), and (1/2) Tween 80/glycerin were constructed. Microemulsions were made according to the previously described method; however, the water titration strategy was not helpful for this step because samples did not reach equilibrium quickly. This led us to divide the water dilution line into 10 points (99% -10%) with varying (oil/Smix) ratios (15):

1:9,2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 for each single Smix ratio. This step needed (9*10) *3= 270 samples in total. All described ratios were treated as weight ratios by the gram. Samples were evaluated visually and using the polarised microscope, as mentioned before. Diagrams were created using Chemix School v.10.0 software.

### 2.5. Preparing SR-loaded microemulsion

Table (1) represents the microemulsion's formulations chosen from the pseudo ternary phase diagrams for detecting SR loading capacity.

Table (1)

**Table (1). Composition of the microemulsion formulation and tested SR loading percent**

| Ingredient* | Tween 80/ Glycerin | | |
|---|---|---|---|
| | (2/1) | (1/1) | (1/2) |
| Oil | 9% | | |
| Tween 80 | 54% | 40.5% | 27% |
| Glycerin | 27% | 40.5% | 54% |
| Water | 10% | | |
| SR | 0.5, 0,4, 0.3, 0.2, and 0.1 % | 0.5 and 0.4 % | 0.6, 0.7, 0.8, 0.9, 1% |

* Ingredients are expressed by the weight percent (g/100g microemulsion).

This microemulsion's formula was selected because of ease of preparation. 10g SR microemulsions samples were prepared as described in 2.3: after weighing: oil, Tween, glycerin, and mixing by sonication for 5 minutes, SR powder was added gradually to the mix with sonication after each addition, and then after finishing SR's amount, water calculated amount (1ml) was added dropwise with moderate agitation on magnetic stirrers. Formulas were kept in firmly closed glass containers at room temperature for the subsequent day evaluation.

The assessment of drug-loaded microemulsions involved a two-step process. Firstly, the samples were visually examined for their clear and transparent appearance, which was done by comparing them with a blank formula prepared in the same way. Secondly, polarized light microscopy was used to validate the visual test results by observing black backgrounds that were free of undissolved SR crystals.

## 2.6. Characterization of SR microemulsions

### 2.6.1. Optical properties

- The physical appearance of microemulsion formulations is stable, transparent, and clear liquids (25).
- Polarized light microscopy is a valuable test for distinguishing between isotropic microemulsion samples, which do not react with polarized light, and other non-isotropic samples: nanoemulsions and liquid crystals (26,27).

### 2.6.2. Light transmission measurements (%T)

Microemulsions' turbidity can be easily measured using ultraviolet-visible (UV-vis) spectrophotometers (15). 1 ml microemulsion sample was diluted with deionized water to 100ml, and light transmission was measured at 650 nm using deionized water as a blank (28).

### 2.6.3. Electrical conductivity and refractive index (RI) measurements

Some physical properties of microemulsion help determine the external phase nature, such as electrical conductivity and RI (20, 29). Electrical conductivity was measured using (inoLab Cond 731, Germany) and RI (CARL ZEISS 202557, JENA, Germany).

### 2.6.4. Droplet size measurement

The droplet size of each formulation was measured after 100-fold dilution with double distilled water using the Dynamic Light scattering DLS technique by Zetasizer (Malvern Instruments, Malvern, UK).

### 2.6.5. In vitro dissolution test

The dissolution test for SR microemulsion formulations was carried out following USP-38 for a 5 mg SR dose(30). Therefore, an accurate weight of each sample containing 5 mg of SR was determined. Hence, samples of (1/1) and (1/2) Tween 80/glycerin were filled into hard gelatin capsules and tested using the USP I apparatus basket method, whereas (2/1) sample was tested using the USP II apparatus-paddle method. These three samples were introduced into 5oo ml distilled water (pH=6.8) at 37 ±0.5°C and stirred for 30 minutes at 50rpm. An aliquot of 5 ml was withdrawn periodically and filtered through 0.22-micrometer membrane filters. The concentration of SR was determined using HPLC ( JASCO PU-980 HPLC Pump, JASCO UV 970 Intelligent UV-VIS HPLC Detector, Japan) according to Moustapha et al., (31). The experiment for each formulation was done in triplicate.

### 2.6.6. Microemulsion stability studies

Centrifugation test: The formulations were centrifuged (HERMLE Z 200A, Germany) at 6000 rpm for 30 min and observed for phase separation, creaming, cracking, or SR sedimentation.

Heating-cooling cycles: As microemulsions are considered thermally sensitive pharmaceutical forms, an intensive thermal stability study was done by various protocols:

Freeze-thaw cycles (FTC): According to ICH Q1 A(R2) guidelines, 5g of each microemulsion was frozen at -20°C for 24 hours, then placed at room temperature at 25°C for another 24 hours, and this cycle was repeated three times(32). Stability was assessed based on the SR microemulsion appearance: clarity, transparency, and absence of SR precipitate under the microscope inspection. *Thermal cycles:* consisted of three consecutive cycles, each with freezing at -5°C for 24 h, room temp at 25°C for 24h, heating at 40°C for 24h, and lastly, room temperature at 25°C for 24h(33).

Constant temperature for 30 days: SR microemulsions were kept at three different temperatures: 4 °C, 25 °C, and 40 °C for one entire month, and were observed daily during the first week, then weekly till the end(34, 18).

The stability assessment for the previous two thermal stability tests was based on many properties: physical appearance, Refractive Index, light transmission (%T), and droplet size determination.

## 3. Results

### 3.1. SR solubility in surfactants and co-surfactants

As shown in Table (2), SR solubility results in both Tweens were close, with the superiority of Tween20. However, there was a remarkable difference between glycerin and PEG-400 of more than 35 folds.

Table (2).

**Table (2). SR solubility in different excipients: (Mean ± SD, n = 3)**

| Excipient | Solubility (mg/ml)± SD |
|---|---|
| Surfactants | |
| Tween 80 | 1.203 ± 0.25 |
| Tween 20 | 1.58 ± 0.11 |
| Co-surfactants | |
| Glycerin | 2.35 ± 0.27 |
| PEG-400 | 0.063 ± 0.008 |

### 3.2 Selecting the best surfactant-co-surfactant mixture ($S_{mix}$) in microemulsion construction

Table (3) displays that the combination of Tween 80 and glycerin exhibited the highest potential for producing a sunflower oil microemulsion across all five concentrations tested. However, the combination of Tween 20 and PEG-400 failed to produce a microemulsion at any of the studied concentrations.Table (3)

**Table (3). Results of the different mixtures' ability to make sunflower oil microemulsions** *

| | Water percent | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 99 % | 90 % | 80 % | 70 % | 60 % | 50 % | 40 % | 30 % | 20 % | 10 % |
| Tween 80/Glycerin | | | | | | | | | | |
| Tween 80/PEG-400 | | | | | | | | | | |
| Tween 20/Glycerin | | | | | | | | | | |
| Tween 20/PEG-400 | | | | | | | | | | |

* The shaded square represents a microemulsion sample.

### 3.3. Construction of pseudo-ternary phase diagrams

Figure (1) and table (4) clarify all the three phase diagrams results:

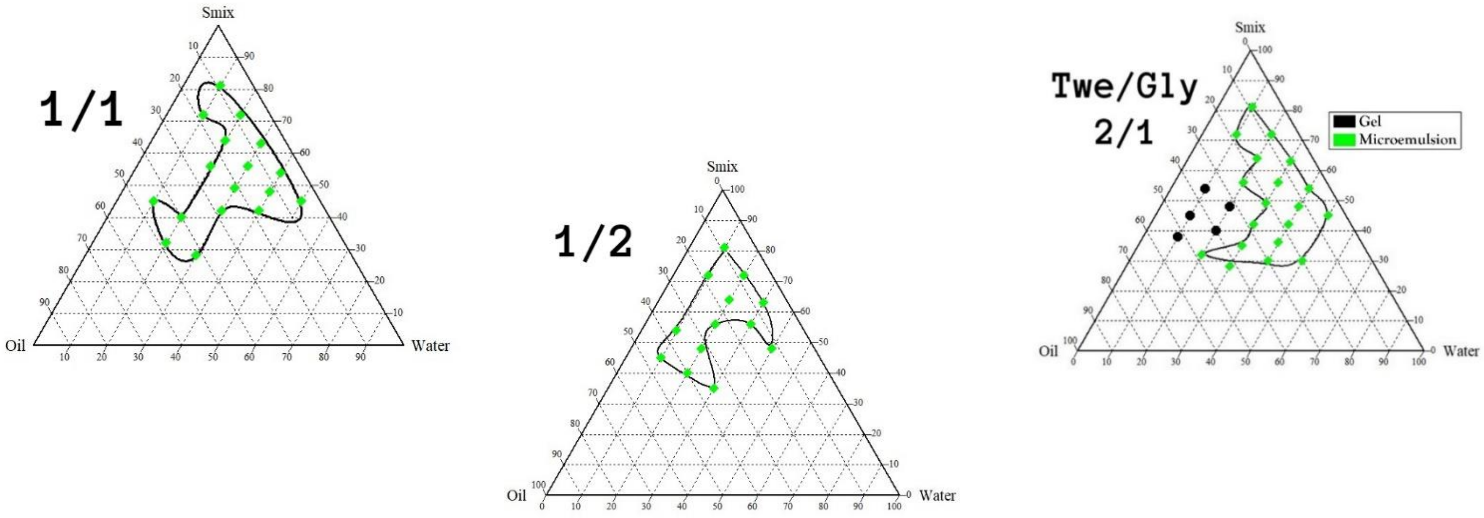


**Figure 1:** Pseudo ternary phase diagrams for oil, water, and different ratios of Smix

**Table (4). Comparison of pseudo ternary phase diagrams results for different Smix ratios**

| Tween 80/Glycerin | | | |
|---|---|---|---|
| | **(1/2)** | **(1/1)** | **(2/1)** |
| **Microemulsion region** | 11.4 % | 14.8 % | 16 % |
| **Phases formed** | Microemulsion<br>other emulsions | Microemulsion<br>other emulsions | Microemulsion<br>other emulsions<br>Gel |
| **Highest oil capacity** | 45% | 48% | 48% |
| **Highest water capacity** | 45% | 50% | 50% |

The surfactant's highest ratio recorded the highest microemulsion region, while the lowest was for the co-surfactants highest ratio. Besides the different types of emulsions seen in the diagrams, a new phase was recognized in the higher Tween ratio, which is gel. This gel form was noticed in five points: (4/6) (oil/Smix) with 10% and 20% water, (5/5) (oil/Smix) with 10% and 20% water, and (6/4) (oil/Smix) with 10% water.

### 3.4. SR microemulsion preparation

A clear difference in SR loading capacity appeared as a result of the different compositions: The highest loading was for (1/2) Tween 80/glycerin microemulsion with (0.6%) SR followed by (1/1) and (2/1) with (0.4%) and (0.1%) SR, respectively. That can be detected from SR's high solubility in glycerin compared with Tween 80.

Figures (2,3) represent some of the samples' results: Figure (2): The clear transparent appearance of 0.4% SR (1/1) Tween 80/glycerin sample vs. the turbid look for a higher SR load (0.5%). Figure (3): Detecting SR overloading in (1/2) Tween 80/glycerin microemulsion samples by screening SR crystals under the polarised light microscope.

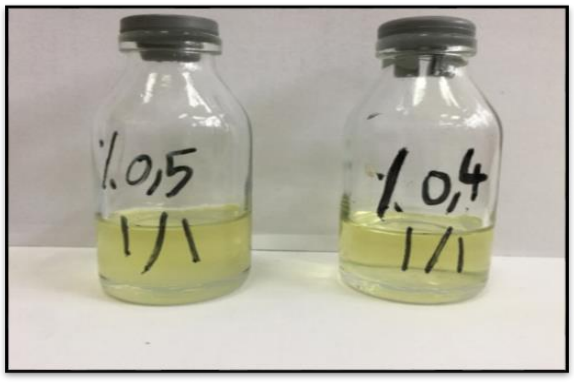


**Figure 2: The optical difference between different loading (1/1) Tween 80/glycerin samples a week after preparation.**

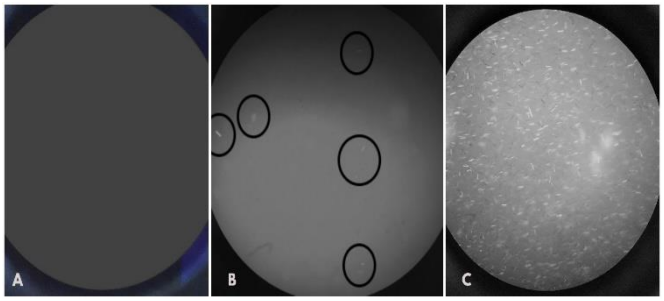


**Figure 3**: (1/2) Tween 80/glycerin samples with different SR loading under the polarized light microscope: A: 0.6% SR, B:0.7% SR, and C: 0.9% SR.

### 3.5. Characterizations of SR microemulsions

Tables (5,6) represent some of the features of SR microemulsions.

Table (5)

**Table (5). Characterizations of SR Microemulsions**

| Formula Tween 80/Glycerin | Optical test | Polarized light microscopy | Electrical conductivity (µS/cm) | Light transmission |
|---|---|---|---|---|
| (2/1): 0.1% SR | Clear transparent | Black background | 19.41 | 99.7% |
| (1/1): 0.4% SR | Clear transparent | Black background | 8.84 | 99.5% |
| (1/2): 0.6% SR | Clear transparent | Black background | 5.87 | 99.2% |

Electrical conductivity results for all three formulations show that they are o/w type (35). Microemulsions with water on the outside are possible. All SR microemulsions let more than 99% of the light through, which goes to the small internal phases as nano-sized drops.

Table (6)

**Table 6. Refractive index results for microemulsion samples with and without SR (mean standard deviation, n=3)**

| Formula(Tween 80/Glycerin) | Without SR | With SR |
|---|---|---|
| (2/1) | 1.469± 0.0025 | 1.469± 0.006 |
| (1/1) | 1.465± 0.001 | 1.466± 0.0015 |
| (1/2) | 1.462± 0.0004 | 1.463± 0 |

Previous results indicate that SR loading in microemulsions did not change the RI. Therefore, we can say that microemulsions reacted with visible light and changed their speed with the same behavior, whether with or without API.

### 3.5.1. Droplet size measurements

All three samples fulfilled the microemulsion formulation with an internal phase lower than100nm. Like many other previous studies(14,36): the smallest size was for the highest surfactant ratio (2/1) Tween 80/glycerin then (1/1), and lastly (1/2) with: 8.08 nm, 10.89 nm, and 19.21 nm respectively.

Figures (4,5,6) describe these results with PdI values.

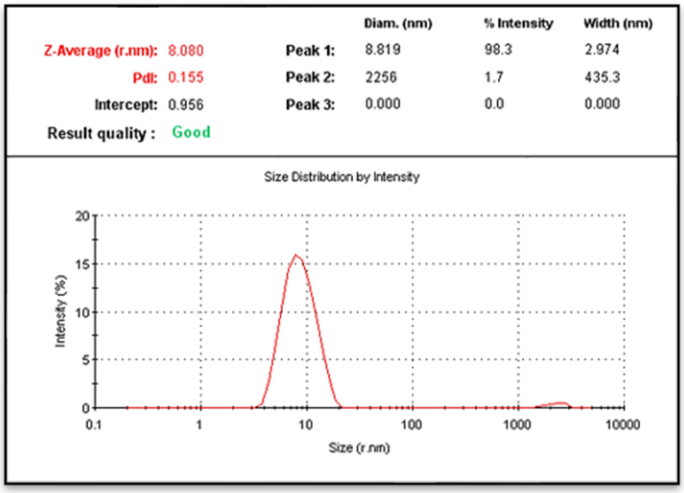


**Figure 4: (2/1) (Tween 80/glycerin) Droplet size Distribution**

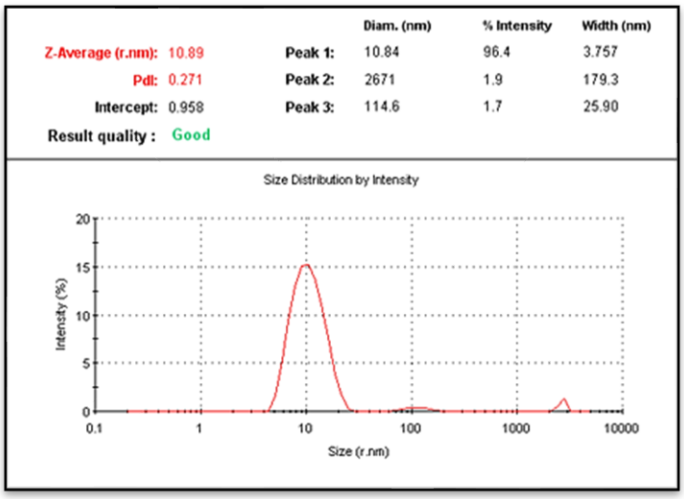


**Figure 5: (1/1) (Tween 80/glycerin) Droplet size distribution**

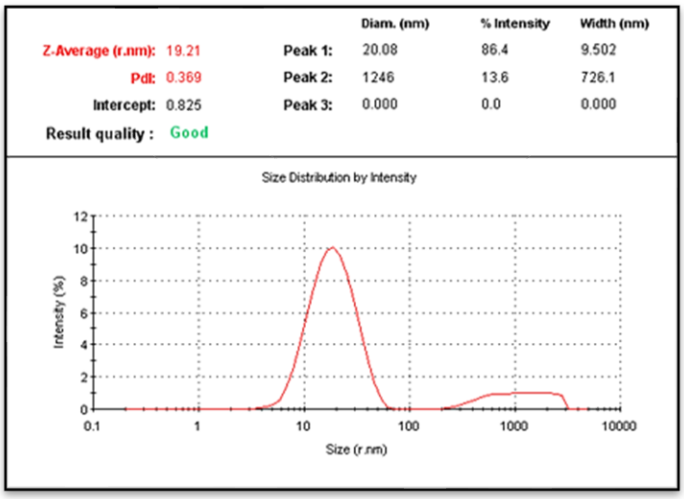


**Figure 6: (1/2) (Tween 80/glycerin) Droplet size distribution**

### 3.5.2. Dissolution test results

All three formulations achieved not less than 80% SR release in 30 minutes, but this was performed differently: (2/1) and (1/1) Tween 80/glycerin had faster dissolution rates than the higher glycerin formulation (1/2).

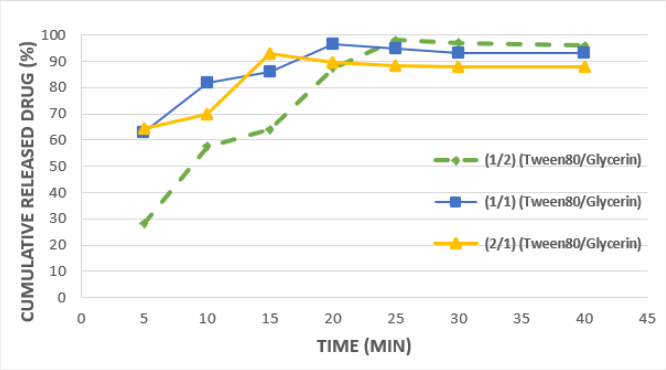


**Figure 7: In vitro release profiles of SR microemulsions**

### 3.5.4. Stability tests

Note: A Part of the Stability study results was addressed in our paper: "Stability Study of Sodium Risedronate Microemulsion in Different Physical and Thermal ways" in Journal: Arab Journal of Pharmaceutical Sciences. (37)

**a) Physical stability test:** All three SR microemulsion formulations were stable after centrifugation at 5000 rpm for 30 minutes. No separation or API sedimentation was recorded.

**b) Thermal stability tests:**

FTC cycles: After three FTCs, all three SR microemulsion formulations with different Smix compositions were stable as clear transparent liquids. However, regarding SR loading: (2/1) and (1/2) Tween 80/glycerin were stable, unlike the (1/1) dosage form where a residual powder was observed in the bottom. This might indicate SR's higher loading capacity than the excipients' ability.

Thermal cycles: Table (7) reports the various characterizations of SR microemulsions after completing three thermal cycles.

Table (7)

**Table (7). Results of thermal cycles stability test**

| Characterization | | First formula (2/1)* | Second formula (1/1) | Third formula (2/1) |
|---|---|---|---|---|
| Physical appearance | Clarity/ Turbidity | Clear | | |
| | Transparency | Transparent | | |
| | Phase separation | No separation | | |
| | SR residual | None | | |
| RI | | 1.469 | 1.466 | 1.463 |
| Light transmission (T%) | | 99.6% | 99.8% | 99.5% |
| Droplet size (nm) | | 7.138 | 10.44 | 12.45 |

*: (2/1) represents (Tween 80/glycerin).

Constant temperature for 30 days:

Table (8) clarifies the results of this test for each formulation in detail, with Zetasizer results for each formulation shown in Figure (8).

**Table (8).** Constant thermal stability for one month's results

| Formulation | Characterization | | 4°C | 25°C | 40°C |
|---|---|---|---|---|---|
| **(2/1) (Tween 80/Glycerin) 0.1% SR** | Physical appearance | Clarity/ Turbidity | Clear | | |
| | | Transparency | Transparent | | |
| | | Phase separation | No separation | | |
| | | SR residual | None | | |
| | RI | | 1.469 | 1.468 | 1.469 |
| | Light transmission (T%) | | 98.6% | 99.4% | 99.36% |
| | Droplet size (nm) | | 9.1 | 9.19 | 8.94 |
| **(1/1) (Tween 80/Glycerin) 0.4% SR** | Physical appearance | Clarity/ Turbidity | Clear | | |
| | | Transparency | Transparent | | |
| | | Phase separation | No separation | | |
| | | SR residual | noticed | None | noticed |
| | RI | | ___ | 1.464 | ___ |
| | Light transmission (T%) | | ___ | 98% | ___ |
| | Droplet size (nm) | | ___ | 12.31 | ___ |
| **(1/2) (Tween 80/Glycerin) 0.6% SR** | Physical appearance | Clarity/ Turbidity | Clear | | |
| | | Transparency | Transparent | | |
| | | Phase separation | No separation | | |
| | | SR residual | None | | |
| | RI | | 1.467 | 1.463 | 1.465 |
| | Light transmission (T%) | | 97.3% | 97.4% | 96.2% |
| | Droplet size (nm) | | 13.56 | 11.8 | 13.22 |

According to previous results: all three formulations were stable in all thermal conditions as homogenous transparent systems. Higher Tween formulations (2/1) confirmed good stability in all three temperatures for one month with a clear appearance and minor droplet size changes (one nm or less). At the same time, the (1/2) formulation also kept a stable microemulsion formulation after the test ended, and droplet size reduction was recorded in all conditions. Moreover, the (1/1) SR microemulsion formulation assured SR high loading amount as SR powder was noticed at the end of severe conditions stability tests (4°c and 40°c). What should be noted is that all three (1/1) microemulsions succeeded as one clear, stable liquid at all tested temperatures

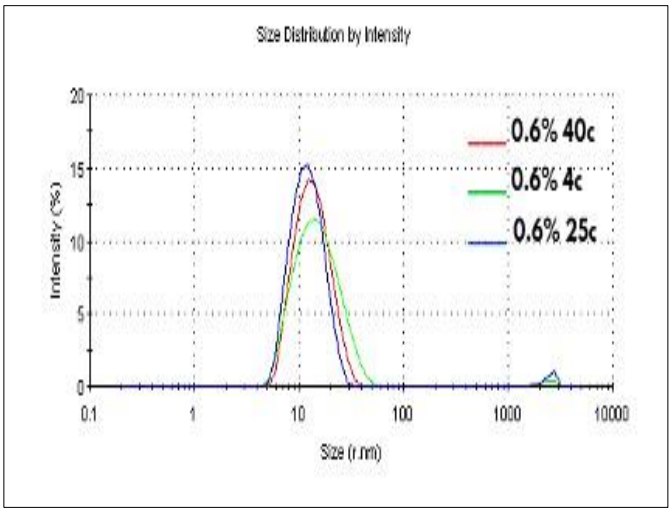


**Figure 8: Size distribution for SR microemulsions after 30 days in constant temps.**

## 4. Discussion

As the first step in preparing medical microemulsions from SR, we detected the solubility of SR in different excipients. The observed differences in the solubility capabilities between glycerin and PEG-400, may be explained by their unlike structures and SR's hydrophilic properties. As glycerin has three carbon atoms with one hydroxyl group on each, PEG-400 consists of 18 carbon atoms with only two hydroxyl groups (38,39).

Oleic acid (C18H34O2), the fatty acid in Tween 80, has a structure that makes it more likely to penetrate the separation surface with this long tail and give a stable emulsion with nano-sized drops compared with lauric acid (C12H24O2) in Tween 20. Tween 80 had a greater ability to make sunflower oil microemulsions than Tween 20. This could be explained by their unlike molecular structures, more precisely by different fatty acids in these Tweens and their effects on lipophilicity. Above all is the considerable similarity between oleic acid in Tween 80 and the principal fatty acids in sunflower oil (Linoleic acid: C18H32O2 66% and oleic acid 21.3%). That plays an essential role in making stable microemulsions (28, 29). Considering co-surfactants: glycerin was more capable of giving stable microemulsions than PEG-400. That would be explained by the superiority of glycerin in playing co-surfactant roles as a more hydrophilic substance than PEG-400.

Pseudo ternary phase diagrams showed the biggest microemulsin's region for the highest tween 80 ratio (2/1). These results seem rational and agree with many previous findings(40, 41, 36) as the surfactant is emulsification's principal factor. However, the Gel structure may be attributed to the hydration of polyoxyethylene (POE) chains in Tween 80, resulting in increased viscosity and smaller internal phases characterized by nano-sized droplets.

A first look at the comparison between the RI results for the microemulsion formulations with components' RI: water: 1.337, oil: 1.478, Tween 80: 1.478 and glycerin: 1.475 may refer to an oily external phase. However, this disagrees with the electrical conductivity results. This issue can be solved by considering the Tween 80 and glycerin's hydrophilic nature. They would be solved in water's external phase, which differs in its characterizations as a liquid, such as density viscosity and RI (42).

The delayed release of SR from formulations with higher glycerin content can be attributed to its higher affinity for glycerin, which retards SR release into the aqueous medium. These data point out the ability of microemulsion formulations to change and control the release of SR- as an excellent soluble drug supposed to move immediately to the proffered aqueous release medium.

SR microemulsions passed FTC and three thermal cycles with clear transparent systems and no sedimentation in all formulations (including (1/1)). We think the difference between (1/1) thermal cycles and FTC test results is a consequence of unlike protocols and severe conditions for FTCs -FTCs include freezing to -20°C, and thermal cycles have a heating period of 40°C- that may help in keeping SR powder dissolved. RI results after finishing the test did not change, which may indicate keeping the same microemulsion types after thermal cycles.

Regarding droplet size, all three SR microemulsion formulations had a decrease in droplet size at the end of stability tests. This could be explained for thermal cycles as follows: the solubility's behavior of tween 80 changes with heating, as it would be more lipophilic and its molecules would get closer, as well as tighter on the surface (36). Glycerin can preserve water molecules abandoned from tween 80 and therefore holds Glycerin the smaller internal phase drops after finishing the test at room temperature.

On the other hand, we think that the reduction in droplet size after one Month of stability study could be explained as follows: as glycerin is more hydrophilic than Tween 80 when adding water dropwise during the preparation, glycerin will have the superiority to mix with water molecules compared with a tween. However, during this month system's components had the chance to rearrange and take the most comfortable status. Hence tween 80 molecules could rearrange and get tighter and closer in the interfacial film.

In summary, the findings underscore the intricate interplay between molecular structures, hydrophilic properties, and environmental factors in shaping the emulsification behavior and stability of microemulsion formulations, offering insights into their potential applications in controlled drug delivery systems.

## Conclusions

In the current study, different microemulsion formulations of SR based on sunflower oil were developed and intensively studied. Starting with locating the superiority of Tween 80 and glycerin on other studied active mixtures, we explored the effect of increasing each component of this active mixture: higher Tween 80 microemulsion formulation (2/1) compared with other two studied ratios (1/1) and (1/2) had the largest emulsification region with gel phase, as well as smallest droplet size (8.08 nm), and remarkable stability results in various conditions. While a higher co-solvent ratio –glycerin- caused a decrease in the microemulsion region compared with the (1/1) ratio, this formula (1/2) was the best in SR loading capacity, with remarkable API releasing behavior and good stability results. These findings suggest that microemulsions hold promise in providing a superior drug form compared to traditional solid dosage forms (Tablets) for Bisphosphonate currently available in the market, after conducting the needed clinical studies.

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  All data generated or analyzed during this study are included in this published article.

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## التحضير والتقييم المختبري ودراسات الاستقرار لمستحلبات دقيقة من ريسدرونات الصوديوم

**الملخص:**

**المقدمة:** تعتبر ريسيدرونات الصوديوم (SR) احد افراد أدوية البيسفوسفونات المستخدمة في علاج هشاشة العظام. على الرغم من فعاليتها المعروفة جيدًا، تعاني SR من امتصاص فموي منخفض (فقط ٠,٦٣٪) والعديد من الآثار الجانبية.

الهدف: تهدف الدراسة الحالية إلى تكوين وتوصيف أنظمة مستحلبات ميكروية من نمط ز/م لايتاء SR عن طريق الفم.

**الطرائق:** تم استخدام زيت عباد الشمس كطور زيتي. ثم تم تحري ذوبانية SR في مختلف العوامل الفعالة على السطح والعوامل المساعدة على الاستحلاب. تم دراسة ثلاث نسب مختلفة من المستحلبات المايكروية (توين ٨٠/جلسرين): (١/٢)، (١/١)، و (٢/١). فيما يتعلق بالمستحلبات المايكروية الحاوية على المادة الفعالة، تم توصيف ثلاث صيغ مختلفة: (١/٢)، (١/١)، و (٢/١) (توين ٨٠/جلسرين) من حيث: الشفافية، الناقلية الكهربائية، نقل الضوء، معامل الانكسار، حجم القطرة، اختبارات الذوبان، والدراسات المكثفة للثبات.

**النتائج:** نظرًا لأن توين ٨٠ والجلسرين كانتا أفضل خليط عامل فعال على السطح/عامل مساعد، فإن نسبة العامل الاستحلابي الأعلى (توين ٨٠) كانت لها أكبر منطقة استحلاب مايكروي في المخططات الثلاثية الكاذبة وظهور شكل جديد (هلام _جل_). كانت المستحلبات المايكروية ل SR سوائل شفافة من النوع زيت/ماء. الصيغة (١/٢) أظهرت أصغر حجم للقطرات (٨ نانومتر) مع نتائج ثبات جيدة عبر ثلاث بروتوكولات متنوعة. تليها الصيغة (١/١) (١٠,٨٩ نانومتر)، على الرغم من أن هذه الصيغة أظهرت مشكلة في التحميل الزائد SR) (0.4%في اختبارات الثبات. امتلكت الصيغة (٢/١) (١٩,٢١ نانومتر) أعلى قدرة تحميل(SR 0.6%) .

**الاستنتاجات:** إن الثبات المميز لصيغة (١/٢) (SR 0.1%)كصيغة دوائية ذات ثبات عالي أو كمرحلة متوسطة في تطوير أشكال صيدلانية لاحقة، إضافةً إلى القدرة العالية على تحميل الدواء في الصيغة (2/1) , (SR ٠,٦٪) تؤكدان أهمية إجراء المزيد من الدراسات للتحقق من فعاليتها وإمكانيات استخدامها في الصناعة الدوائية.

**الكلمات المفتاحية:** ريزدرونات الصوديوم، مستحلبات مايكروية، توين ٨٠، غليسرين، دراسات الثبات.