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# Aqueous Media for Effective Delivery of Tretinoin

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**Abstract**

Tretinoin, also known as vitamin A acid or *all-trans* retinoic acid (t-RA), is a widely used dermatological and pharmaceutical agent. However, t-RA has very low water solubility ($< 0.2$ $\mu$g/ml), which restricts its use. Previous efforts have focused on using concentrated solutions of $\beta$-cyclodextrin (BCD) derivatives to increase the aqueous solubility of t-RA. The present study demonstrates that substantial enhancement to the t-RA solubility (e.g., $>$ 2000 fold) can be achieved by using low concentration of BCD (1.5%) plus a small amount of additives (0.1%–1%), such as carboxymethyl cellulose, sodium acetate and potassium phosphate. This simple yet effective approach can also improve the loading of t-RA up to 10 times over the previously published results using CD derivatives including hydroxypropyl BCD (HPBCD). The findings may lead to development of an oral t-RA formulation in an aqueous medium.

## Introduction

Tretinoin, also known as vitamin A acid or *all-trans* retinoic acid (t-RA), is widely used in the treatment of dermatological ailments. It is also a potent anti-tumor agent. However, effective use of t-RA is limited by its very low water solubility, which prevents it from forming an oral or parenteral formulation, as well as its instability toward light and air. Many efforts have been devoted to enhancing the aqueous solubility and stability of t-RA by using BCD and its derivatives such as methyl BCD (RAMEB) and HPBCD [1–6]. In our previous work, we discovered the outstanding performance of a tertiary amine substituted BCD (TABCD) in solubilizing both t-RA and its therapeutically active isomer, *13-cis* retinoic acid [7]. One of the problems associated with such use of CD derivatives is their required high usage level (typically 40% w/v). This translates not only to a very low drug loading but also potential adverse effects caused by the massive use of a solubilizer in a pharmaceutical formulation. On the other hand, although BCD remains the most effective CD at low concentrations in solubilizing and stabilizing t-RA, the relatively poor aqueous solubility of BCD restricts its use as a powerful solubilizer.

It has first been shown by Loftsson *et al.* that addition of small amount of certain water-soluble polymers to an aqueous CD solution can synergistically increase the complexation efficiency of the CD with a guest compound by forming three-way, or ternary, complexes. The pharmacologically inactive polymers, such as water-soluble cellulose derivatives, polysaccharides, and synthetic polymers, are widely used as rheological agents or excipients in various pharmaceutical applications. In aqueous solutions, they effectively increase the solubilizing efficacy of CDs on many hydrophobic drugs [8]. The purpose of the current study was to investigate various means, including use of water-soluble polymers and other additives, in order to enhance the solubility of t-RA while minimizing the usage of solubilizing agents.

## Experimental

### *Materials*

BCD was a product of Cargill, Inc. All other materials and chemicals were purchased from Aldrich Chemical Company (St. Louis, USA). Abbreviations used in the text are HPMC (hydroxypropyl methyl cellulose), PEG (polyethylene glycol, avg. MW ~3400), CMC (carboxymethyl cellulose sodium salt), PVP (polyvinylpyrrolidone), and NaAc (sodium acetate).

### *Methods*

Solubility studies were carried out following similar procedures as described in the previous publications. An excess amount of t-RA was added to water, a buffer solution, or a BCD solution with or without small amount (0.1% to 1.0%) of a water-soluble polymer. While no further treatment was given to the BCD solutions used for obtaining the phase solubility diagram of t-RA, all other samples were subjected to autoclave (121 °C for 20 or 30 min) before equilibration at room temperature for 3–5 days.

Each equilibrated sample was filtered through a 0.45 $\mu$ membrane filter and diluted with 50% aqueous 2-methoxyethanol. Quantitative determination of t-RA was carried out either by the UV-Vis method or by the HPLC method. Data acquired by both methods were in good

agreement when there was no appreciable light-induced isomerization (from t-RA to *13-cis* retinoic acid) occurring.

For the UV-Vis analysis, each sample was scanned from 250 nm to 430 nm on a Beckman DU-65 spectrophotometer. Absorption at 343 nm was used to calculate the t-RA concentration against its standard calibration linear plot.

The HPLC system (HP 1100 series) was composed of a quaternary pump, a vacuum degarsser, a thermostatted column compartment, an autosampler and a diode array detector (343 nm, Ref = 430 nm). Assays were performed using a Lichrospher RP 18 E (124 × 4.6 mm, 5 $\mu$) column and a Lichrospher RP 18 e (30 × 4.6 mm, 5 $\mu$) guard column, both manufactured by Phenomenex (California, USA). The mobile phase was methanol-acetonitrile (95:5, v/v) and the flow rate was 0.5 ml/min. Under these conditions, t-RA eluted at 5.2 min while *13-cis* retinoic acid eluted at 4.7 min. All detection was made at ambient temperature.

## Results and discussion

### *Effect of BCD and polymer additives*

As shown previously [6], BCD was able to solubilize t-RA appreciably at the usage level below 2% (w/v) and it gave an interesting phase solubility diagram. As concentration of BCD rises, the solubility of t-RA increases initially until leveling off (Type A), increases again, then decreases (Type Bs) when BCD solution approaches saturation. A plausible explanation may be a two-stage complexation process, that is, 1:1 (t-RA: BCD) complex formation followed by the 1:2 complexation.

Among the water-soluble polymers under investigation (each at 0.5%, w/v), PEG and PVP did not aid BCD in solubilizing t-RA while HPMC produced about 30% increase in the solubility of t-RA versus using BCD alone (Table 1). Addition of CMC, however, generated nearly 7-fold solubility enhancement to t-RA over BCD. Separate experimental results showed that 0.5% CMC alone did not have much impact on the aqueous solubility of t-RA. Therefore, the combined use of 1.5% BCD with CMC achieved true synergy in solubilizing t-RA. Furthermore, this synergistic effect was found to be a function of the CMC concentration (Figure 1). About 5-fold solubility increase was attained by 0.1% or 0.25% CMC as an additive to 1.5% BCD. Addition of 0.5% CMC furthered the upward trend. However, there was a steep drop in the solubility of t-RA at 0.75% CMC, followed by a substantial jump at 1.0% CMC. This rather unusual pattern was confirmed by our repeated experiments.

*Table 1.* Effect of polymer addition

| Solubilizing agent | [t-RA] $\mu$g/ml | Ratio$_{(H_2O)}$ | Ratio$_{(BCD)}$ |
|---|---|---|---|
| None (water only) | 0.19 | 1.0 | 0.01 |
| Saturated BCD | 21.2 | 111.6 | 1.0 |
| Sat. BCD + 0.5% HPMC | 27.8 | 146.3 | 1.3 |
| Sat. BCD + 0.5% PEG | 19.8 | 104.2 | 0.9 |
| Sat. BCD + 0.5% CMC | 147.3 | 775.3 | 6.9 |
| Sat. BCD + 0.5% PVP | 20.4 | 107.4 | 1.0 |

*Table 2.* Synergy of BCD with additives

| Solubilizing agent | [t-RA] $\mu$g/ml | Ratio$_{(H_2O)}$ | Ratio$_{(BCD)}$ |
|---|---|---|---|
| None (water only) | 0.19 | 1.0 | 0.01 |
| 0.5% NaAc (pH 7.65) | 0.68 | 3.6 | 0.04 |
| 0.5% pot. phosphate (pH 7.65) | 0.34 | 1.8 | 0.02 |
| 1.5% BCD | 15.7 | 82.6 | 1.0 |
| BCD + 0.5% CMC | 91.7 | 482.6 | 5.8 |
| BCD + 0.5% NaAc (pH 7.65) | 267.9 | 1410.0 | 17.0 |
| BCD + 0.5% pot. phosphate (pH 7.65) | 407.7 | 2145.8 | 25.9 |

### *Synergy of BCD and salts*

Since t-RA bears a carboxylic acid and the CMC in use is a sodium salt, interactions between the ionic groups were thought to be mainly responsible for the observed synergistic effect. If so, other forms of similar functional groups might work as well. NaAc was hence chosen to test the hypothesis because it has a sodium carboxylate group just as CMC has. The data in Figure 2 were consistent with the hypothesis. As the concentration of NaAc increased, so did the aqueous solubility of t-RA. Moreover, the magnitude of the enhancement by NaAc far surpassed what CMC brought about at the same concentration levels. Thus, 17-fold increase over 1.5% BCD was obtained with addition of 0.5% NaAc and 30-fold solubility enhancement was achieved with 1.0% NaAc.

Additional testing results showed that potassium phosphate was even more potent in improving the solubilizing efficacy of BCD (Table 2). It might be postulated that the pH of the solution had a great influence on the carboxylic acid group, thus the solubility, of t-RA. That is why salts of retinoic acid are more soluble than the acid form. It is also known that RA salts have surface-active properties and this might lead to micelle formation. However, our control experiments using each salt alone negated a pH effect occurring under the experimental conditions. Furthermore, the data confirmed presence of the BCD cavity as a necessary prerequisite for any significant improvement of the t-RA solubility. A charged additive provided extra interactions with the guest. As the charge density increased, the solubilizing effect magnified. Hence, solubility enhancement of more than 2000-fold over water and about 26-fold over 1.5% BCD to t-RA was achieved merely with addition of 0.5% phosphate.

The results obtained by our simple, inexpensive, yet effective approach are more significant when the practicality of t-RA formulations is considered. For instance, the t-RA loading when using HPBCD [2] was about 0.2% (0.8 mg/ml in 40% HPBCD) while it was 2% when using 1.5% BCD in 0.5% phosphate (0.41 mg/ml, 2% solubilizers). Moreover, by minimizing the usage of solubilizing agents, poten-

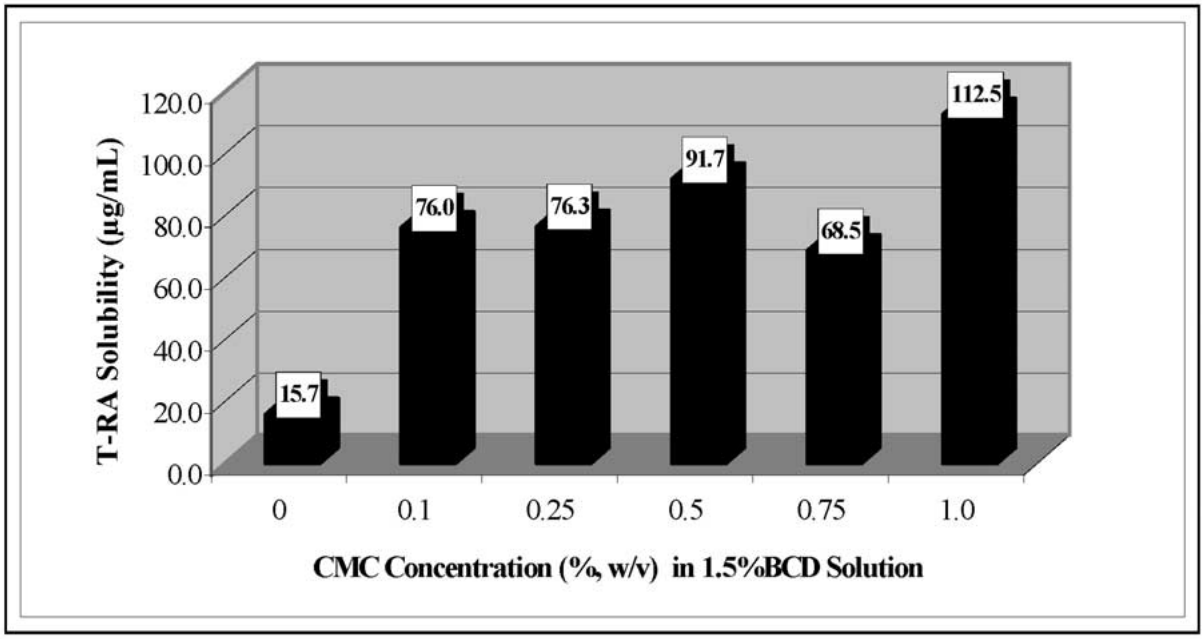


*Figure 1.* Effect of CMC concentration on aqueous solubility of t-RA.

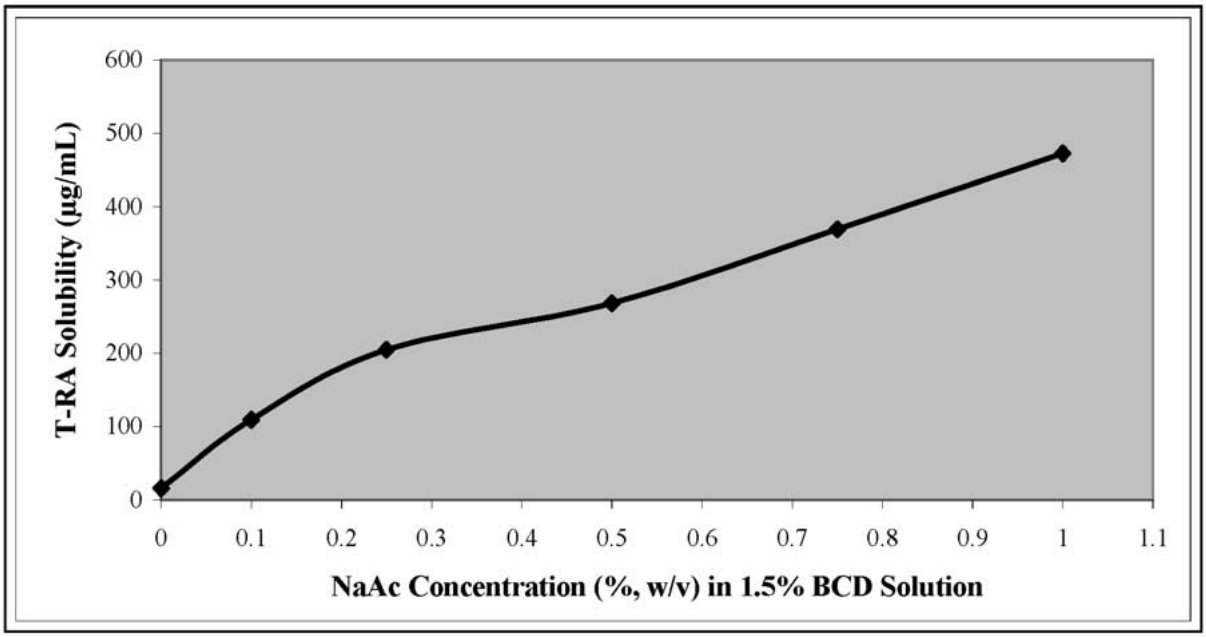


*Figure 2.* Effect of NaAc concentration on aqueous solubility of t-RA.

tial adverse side effects caused by these agents are also minimized.

## Conclusion

In comparison with previously published results using high concentration of modified cyclodextrins including hydroxypropylated or methylated CDs, our findings can lead to an approach that has two distinctive advantages. First, only unmodified BCD is needed along with other readily available and inexpensive additives. Second, the required total amount of solubilizing agents is much less while substantial solubility enhancement to t-RA can be attained. This in turn means fewer potential problems with solubilizers and a higher payload for t-RA, making its delivery in an aqueous medium more practical and economical.

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