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# Efficient mechanochemical complexation of various steroid compounds with $\alpha$ -, $\beta$ - and $\gamma$ -cyclodextrin

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## Abstract

Mechanochemical technology enables solvent-free micronized solid dispersions and efficient molecular host-guest inclusion complexes to be formed in matrices which contain cyclodextrins (CDs). This type of complexation has been studied using  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin with the dual aims of improving overall solubility and enhancing the bioavailability of common steroid compounds, such as cholic acids and  $\beta$ -sitosterols or lowering cholesterol content in products of animal origin. Several parameters have been studied and optimized: CD/compound molar ratio (1:1, 1:2, 2:1 and 3:1) in function of the cavity sizes of the three different CDs, milling time (from 5 to 40 min) and rotation speed (from 100 to 300 rpm). DSC (differential scanning calorimetry) analyses have revealed that inclusion complexes were efficiently formed after 40 min milling (200 rpm) for  $\beta$ -CD/cholesterol and  $\beta$ -CD/ursodeoxycholic acid (encapsulation efficiency 96% and 77% respectively). Besides steroid encapsulation/vehiculation, the mechanochemical technique may pave the way for new ideas in solventless steroid extraction from vegetal matrices with CDs.

## Keywords

Cyclodextrins, inclusion complexes, planetary ball mill, cholesterol,  $\beta$ -sitosterol, cholic acid, ursodeoxycholic acid.

## Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides which arise from the degradation of starch and are formed of ( $\alpha$ →1,4)- linked D-anhydroglucopyranose units. Due to their specific conical toroid shape,  $\alpha$ -  $\beta$ - and  $\gamma$ -CDs (made up of 6, 7 and 8 glucose units respectively), are able to form host-guest inclusion complexes with various structures thus improving the solubility, chemical stability,

bioavailability and dissolution rate of hydrophobic compounds. The food, cosmetic and pharmaceutical industries have found several applications for CD inclusion complexes and their ability to entrap molecules by forming reversible non-covalent interactions [1-3].

Cholesterol is the principal sterol synthesized by animals and an abnormal seric content increase represents a risk factor in cardiovascular disease. Many methods have been developed to reduce cholesterol content in foods, from extraction using organic solvents or supercritical carbon dioxide to blending in vegetable oils or via degradation using cholesterol oxidases [4].  $\beta$ -CD's sequestering action is widely applied as a selective method for lowering cholesterol levels in many dairy foods such as cream, milk, butter, cheese and eggs, while maintaining quality [5]. Several studies have also reported the optimal stoichiometric ratio between CD/guest molecules and the stability of inclusion complexes [6-8].

Bile acids are polar derivatives of cholesterol, are synthesized in the liver and are made up of a hydrophobic steroid skeleton to which various hydrophilic groups are attached. They are surfactant-like molecules that assist fat digestion, forming micelles and micellar aggregates. They also regulate cholesterol homeostasis. From a pharmacological point of view, bile acids are substances that stimulate the physiological function of the liver to secrete bile while also favouring the contractility of intestinal muscles. These substances can be administered in therapy for the treatment of cholesterol gallstone diseases and to decrease the cytotoxicity of retained bile acids in cholestatic liver disease [9, 10]. The poor aqueous solubility of bile acids at physiological pH makes it necessary to enhance their dissolution rate to increase their bioavailability. It is well known that CDs are able to form inclusion complexes and exhibit size specificity in the binding of bile acids [11-15]. These peculiar properties of CDs have also been proposed as a means to mask the bitter taste of solid pharmaceutical formulations and to improve their dissolution properties [16,17].

Phytosterols, which share the same basic cyclopentanoperhydrophenanthrene structure, are the counterparts of cholesterol in animal products and have been employed in the development of functional foods or bioactive products. It is, in fact, well known that these kind of substances can positively influence cardiovascular risk factors as well as promoting general health and well-being as they reduce intestinal cholesterol absorption [18]. Moreover, it has been reported that  $\beta$ -sitosterol may inhibit the development of most common cancer (i.e. colon and breast tumour) and prostate disorders [19]. The importance of supplementing the nutritional value of some food preparations through the addition of sitosterols makes the study of processes which are able to enhance their water solubility and bioavailability vital. Some authors have reported that the preparation of inclusion complexes between phytosterols and CDs in solution may be useful for this purpose and for evaluating inclusion mechanisms and efficiency [20]. Meng *et al.* [21] have shown that phytosterol aqueous solubility can be significantly increased thanks to an inclusion complex between  $\beta$ -CD and phytosterol's hydroxypropyl derivative. In a previous work we exploited  $\beta$ -CD water solution for the selective extraction of resveratrol and polydatin from the roots of *Poligonum cuspidatum* [22].

In the last decade the development of alternative sustainable chemical processes exploited suitable enabling technologies [23], noteworthy is the chemical modification of bile acids under high-intensity ultrasound and microwave irradiation [24].

Mechanochemistry is a dynamic interdisciplinary research field in material science and solid state chemistry that brings advantages in term of time, simplicity, cost and waste reduction [25]. Solventless grinding is a technique which is able to provide products or material forms that cannot be obtained, or which are difficult to obtain, using classical solution-based methods. Among its many advantages are easy scaling as well as quantitative and *in situ* reaction monitoring.

Mechanochemical activation has been used for CDs complexation of drugs since 1986 [26]; steroid, such as methylhydroxyprogesterone complexes have been prepared [27]. Grinding treatments may dramatically enhance solubility and bioavailability of poorly water-soluble drugs [28] due to particle size reduction, higher surface area and porosity [29]. Ultrafine milling is a straightforward technique based on impact forces and friction effects which result in large amounts of mechanical energy that usually cause structural changes and even chemical reactions including the enhancement of molecular interaction and complexes formation [30,31].

A Planetary Ball Mill (PBM) is known to install jars on a disk, and both jars and disk are simultaneously and separately rotated at a high speed. The high speed of rotation of the jars and revolution of the disk make the balls move strongly and violently, leading to fine grinding of a product due to generation of large ball impact energy [32]. The ball mill jar is partially filled with the material to be ground together with grinding media, typically stainless steel balls. The high-energy of balls during the milling is attributed to an extremely high centrifugal force acting on the balls by the two rotations at high speed. Several investigations have provided information on the effect of operational conditions on the mechanical milling product by the experimental and mathematical analyses [33,34]. Processes inside PBM are complex and strongly depend on the processed material, synthesis or complexation type, thus, the optimum milling conditions have to be assessed for each individual system. This green technology allows reactions and complexations to be carried out in the solid state and can also reduce solvent use (when required), which makes it an attractive option for several application fields [35].

A number of studies on the complexation of bioactive molecules and drugs with CDs via the milling method have been published [36-41].

The aim of this study is to exploit the ball mill ability to form CD-complexes in solventless condition. We analyzed the milling effect on the physical dispersion of steroid structures such as cholesterol,  $\beta$ -sitosterol and bile acids (cholic acid and ursodeoxycholic acid) in  $\alpha$ -,  $\beta$ - and  $\gamma$ -CDs while optimizing complex stoichiometry in relation to CD cavity size. Furthermore, we investigate whether the co-grinding of steroid structures with CDs in the solid state can enhance the interaction between oligosaccharides and guest molecules. The intermolecular interactions have been characterized using differential scanning calorimetry (DSC) [42].

## Materials and Method

### Materials and reagents

All chemicals were purchased from Sigma-Aldrich and used without further purification. CDs were kindly provided by Wacker Chemie (Munich, Germany). The estimated water content was 10.6%, 14.4% and 9% for  $\alpha$ - (MW = 972.84),  $\beta$ - (MW = 1134.98) and  $\gamma$ -CD (MW = 1297.12) respectively. The  $\beta$ -Sitosterol ( $\geq 70\%$  purity) and cholesterol ( $\geq 92.5\%$  purity) were purchased from Sigma-Aldrich. Cholic acid and ursodeoxycholic acid were kindly provided by PCA - Prodotti Chimici e Alimentari S.p.A. (Basaluzzo, AI - Italy).

### Preparation of the solid inclusion complex and the physical mixture

Steroid-CD inclusion complexes were prepared in a PBM (PM100 Retsch GmbH) using 50 mL grinding stainless steel jars and milling balls (mixture of  $48 \times 5$  mm and  $1500 \times 2$  mm; stainless steel). The grinding jar is arranged eccentrically on the sun wheel of the PBM. The direction of movement of the sun wheel is opposite to that of the grinding jar in the ratio 1:2. The grinding balls are subjected to superimposed rotational movements, the so-called Coriolis forces. The difference in speed between balls and jar produces an interaction between frictional and impact forces, which releases high dynamic energies. The interplay between these forces produces the high and very effective degree of size reduction of the PBM.

The physical mixture was prepared by mixing the sterol molecules and CDs using a spatula and a mortar. 1 gram of total powder (steroid + CD) was introduced respectively in the PBM's jar or in a mortar to be grinded. The energy involved in the mortar grinding is clearly much lower and experiments reproducibility is a critical issue.

Various CD/compound ratios (1:1, 1:2, 2:1 and 3:1), milling times (from 5 to 40 min) and revolutions per minute (from 100 to 300 rpm) values were evaluated in order to find the optimal conditions for the complexation. All the experiments were carried out using 1 mmol of CD.

### DSC- Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analyses were performed using a DSC 7 Perkin Elmer differential calorimeter (Norwalk, CT, USA). The samples were placed in conventional aluminum hermetic pans and heated from 50 to 300°C at 10 K/min under a 10 mL/min nitrogen purge and all the tests were carried out in duplicate.

The DSC was used to determine the melting point ( $T_m$ ) and the Enthalpy of fusion ( $\Delta H$ ) of the pure compounds, the physical mixtures and the complexes. Encapsulation efficiency (EE) was calculated according to the following equation:

$$\%EE = 100 \cdot \left( 1 - \frac{\Delta H_C}{\Delta H_F} \right)$$

where  $\Delta H_C$  is the enthalpy of the complex and  $\Delta H_F$  is the enthalpy of the respectively pure sterol molecule.

Duplicate experiments were carried out for each sample and average values are reported. The calculated confidence interval for 95% certainty was between 3 and 5% of the absolute values.

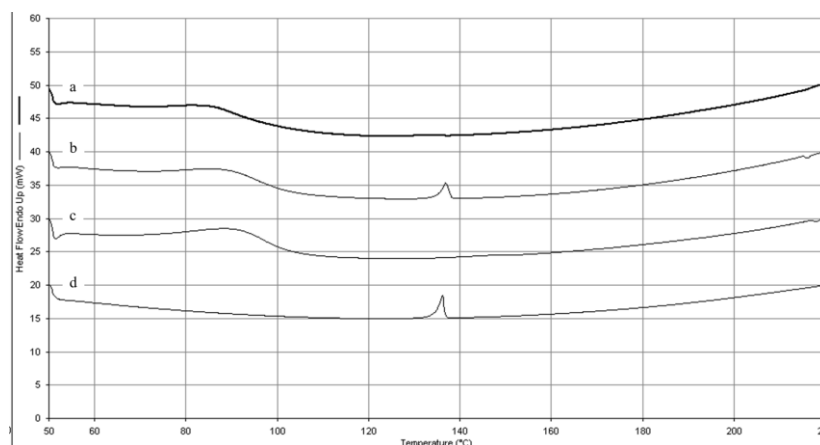
## Results and discussion

A PBM was used as it provided an easy and efficient method for the formation of sterol structure-CD complexes without the addition of a solvent [32].

The coordination of sterol molecules and CDs is a complex reaction system which is influenced by many factors. The effect of reaction parameters (reaction time, molar ratio, ball-mill speed) on the inclusion ratio was studied.  $\beta$ -sitosterol was chosen as the target structure in order to optimize the PBM method.

DSC was used to confirm the formation of the complexes. The proof of complexation is generally considered to be the total or partial disappearance of the melting point peak of the free guest molecules [41]. It indicated the formation of an amorphous solid dispersion and existence of the molecules' encapsulation inside the CD cavity via intermolecular hydrogen bonding or van der Waals interactions which occurred thanks to mechanochemical processing [42].

The DSC results presented in Fig. 1d demonstrate an endothermic peak for  $\beta$ -sitosterol at 137.6°C, which corresponds to the melting point. The melting point reported for  $\beta$ -sitosterol is within the 136 to 140°C range [43]. Complex formation was proven by a lack of a sharp endothermic peak in the temperature range investigated (Fig. 1a), which however, is present with a slight shift in the physical mixture Fig. 1b.



**Figure 1.** Differential scanning calorimetry (DSC) thermograms of: a)  $\beta$ -CD/  $\beta$ -sitosterol inclusion complex 2:1; b) physical mixture of  $\beta$ -CD/  $\beta$ -sitosterol 2:1; c)  $\beta$ -CD; d) free  $\beta$ -sitosterol.

In the PBM method optimization, the first parameter taken into account was the molar ratio between host (H;  $\beta$ -CD) and guest (G;  $\beta$ -sitosterol) molecules: 1:1, 1:2, 2:1 and 3:1 molar ratios were used.

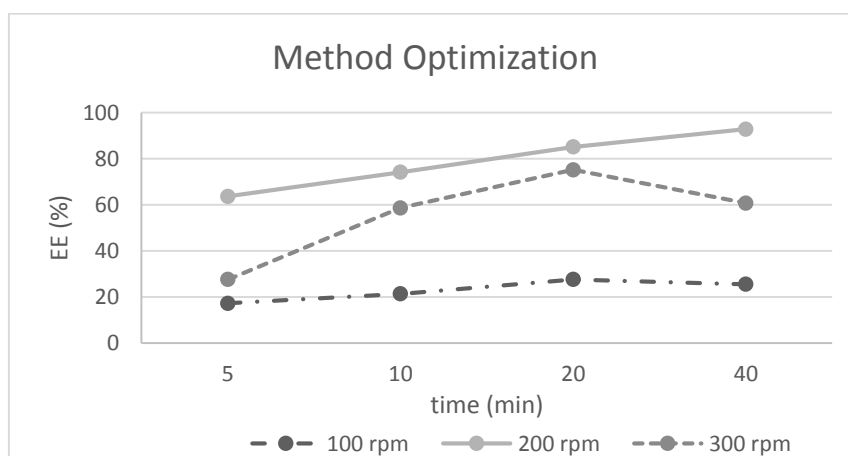
As shown in Table 1, the best results were obtained with a molar ratio of 2:1 (H:G) leading to an EE of 79%.

**Table 1.** EE of different type of steroids with CDs.

Entry	CD (H)	Sterol type molecule (G)	ratio (H:G)	EE (%) <sup>a</sup>
1 <sup>b</sup>	β	β-Sitosterol	1:1	32
2 <sup>b</sup>	β	β-Sitosterol	1:2	52
3 <sup>b</sup>	β	β-Sitosterol	2:1	85
4 <sup>b</sup>	β	β-Sitosterol	3:1	77
5 <sup>c</sup>	β	β-Sitosterol	2:1	93
6 <sup>c</sup>	α	β-Sitosterol	2:1	87
7 <sup>c</sup>	γ	β-Sitosterol	2:1	83
8 <sup>c</sup>	β	Cholic acid	2:1	89
9 <sup>c</sup>	β	Cholesterol	2:1	96
10 <sup>c</sup>	β	Ursodeoxycholic acid	2:1	77

<sup>a</sup> Calculated by DSC analysis; <sup>b</sup> Reaction conditions: 200 rpm for 20 min; <sup>c</sup> Reaction conditions: 200 rpm for 40 min.

Other parameters were changed to further improve the efficiency of the technique and the H:G molar ratio was set at 2:1. Method optimization was performed and complexation process time and revolutions per minutes (rpm) were varied (Fig. 2).



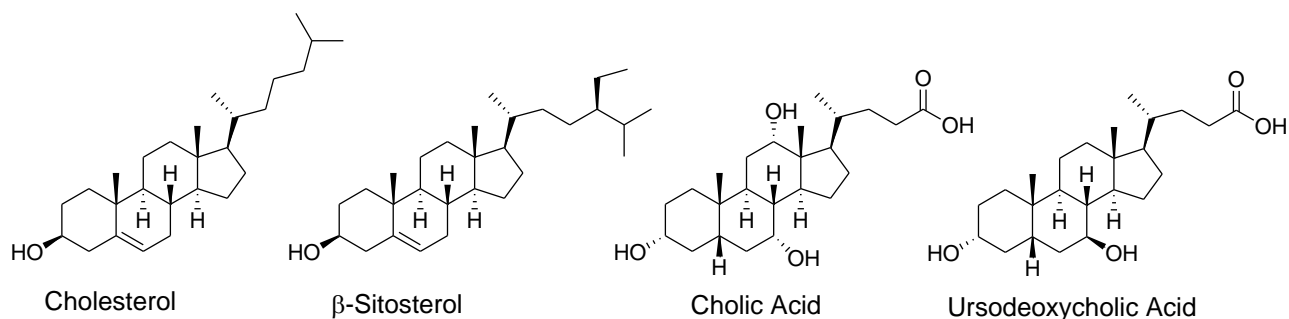
**Figure 2.** EE monitoring.

The best EE was achieved after 40 minutes at 200 rpm. The EE was not satisfactory at higher or lower rpm values because too much or too little energy, respectively, was provided to the system. In the former case, probably the energy did not allow for adequate complex formation since the complexation degree decreases with increasing temperature, as demonstrated in solution complexation experiments. In the latter case, we hypothesized that not enough energy was provided to displace water molecules inside the cavity. In fact, the apolar CD cavity is assumed to be occupied by water molecules, whose presence is unfavourable from an energy point of view (polar-apolar

interaction) and therefore can be substituted by suitable, less polar guest molecules. 200 rpm is a good compromise for complex formation and a suitable EE. 93% EE was achieved at this rotation speed in 40 minutes in solvent free conditions. No further improvements in EE were noticed when time was increased.

Upon observing these promising results, we decided to extend our investigation to the other most common CDs;  $\alpha$ - and  $\gamma$ -CD, and then to other sterol structures to prove the general application of this procedure to analogue molecules.

The most suitable CD cavity for the guests, as considered in this study, was demonstrated to be the  $\beta$ -CD cavity: EE values of 87% and 83% were obtained with  $\alpha$ - and  $\gamma$ -CD respectively.  $\beta$ -CD was therefore used with the following structures: cholesterol (Cho), cholic acid (CA) and ursodeoxycholic acid (UA) (Fig. 3).



**Figure 3.** Sterol structures employed in the mechanochemical complexation with CDs.

As has already proven in the literature [42], Cho has an incredibly high affinity for  $\beta$ -CD. In fact, many studies and patents have presented the use of CDs in the removal of cholesterol from dairy foods [3]. This property was also herein confirmed using the PBM complexation technique and DSC analysis gave a high EE of 96%. Other molecules also provided worthy CD cavity encapsulation, as shown in Table 1.

Conventional extraction techniques are usually time consuming and require large amounts of organic solvents which causes environmental pollution and entails toxicological and safety issues. For these reasons, “green”, efficient and sustainable extraction methods like solid phase extraction and enrichment via the ball milling technique and using CDs may solidify its place as a promising investigation trend in the extraction field as some matrix molecules may become entrapped in cyclic oligosaccharides causing a higher dissolution rate and improved hydrophobic substance bioavailability on the one hand and the removal of undesirable substances on the other.

## Conclusions

In conclusion, fast solvent-free mechanochemical treatments in PBM enable an efficient preparation of steroids-CDs inclusion complexes. In addition, steroid interaction with CDs has been strengthened



and the amorphous solid inclusion complexes obtained can be directly used in pharmaceutical, dietary and cosmetic preparations.

## Reference

- [1] Cravotto G, Binello A, Baranelli E, Carraro P, Trotta F. Cyclodextrins as Food Additives and in Food Processing. *Curr. Nutr. Food Sci.* 2006;2:343-50.
- [2] Pinho E, Grootveld M, Soares G, Henriques M. Cyclodextrins as encapsulation agents for plant bioactive compounds. *Carbohydr. Polym.* 2014;101:121-35.
- [3] Astray G, Gonzalez-Barreiro C, Mejuto JC, Rial-Otero R, Simal-Gandara J. A review on the use of cyclodextrins in foods. *Food Hydrocolloids* 2008;23:1631-40.
- [4] Jiang HM, Zhang S, Sui Q. Removal of Cholesterol by beta-Cyclodextrin. *Asian J. Chem.* 2011;23:3783-6.
- [5] Martina K, Binello A, Lawson D, Jicsinszky L, Cravotto G. Recent applications of cyclodextrins as food additives and in food processing. *Curr. Nutr. Food Sci.* 2013;9:167-9.
- [6] Dos Santos C, Buera MP, Mazzobre MF. Phase solubility studies and stability of cholesterol/beta-cyclodextrin inclusion complexes. *J. Sci. Food Agric.* 2011;91:2551-7.
- [7] Nishijo J, Moriyama S, Shiota S. Interactions of cholesterol with cyclodextrins in aqueous solution. *Chem. Pharm. Bull.* 2003;51:1253-7.
- [8] Yamamoto S, Kurihara H, Mutoh T, Xing X, Unno H. Cholesterol recovery from inclusion complex of beta-cyclodextrin and cholesterol by aeration at elevated temperatures. *Biochem. Eng. J.* 2005;22:197-205.
- [9] Nakagava S, Makino I, Ishizaki T, Dohi I. Dissolution of cholesterol gallstones by ursodeoxycholic acid. *Lancet* 1977;2:367-9.
- [10] Hofmann AF, Hagey LR, Bile Acids: Chemistry, Pathochemistry, Biology, Pathobiology and Therapeutics. *Cellular Molec. Life Sci.* 2008;65: 2461-83.
- [11] Yang ZW, Breslow R. Very strong binding of lithocholic acid to beta-cyclodextrin. *Tetrahedron Lett.* 1997;38:6171-2.
- [12] Tan ZG, Zhu XX, Brown GR. Formation of inclusion complexes of cyclodextrins with bile-salt anions as determined by nmr titration studies. *Langmuir* 1994;10:1034-9.
- [13] Yao L, Mori Y, Takano K. Theoretical Study on Intermolecular Interactions in Complexes of Cyclodextrins with Bile Acids: DFT and Ab Initio Fragment Molecular Orbital Calculations. *Bull. Chem. Soc. Jpn.* 2014;87:258-66.
- [14] Holm R, Schonbeck C, Askjaer S, Westh P. Thermodynamics of the interaction of gamma-cyclodextrin and tauro- and glyco-conjugated bile salts. *J. Inclusion Phenom. Macrocyclic. Chem.* 2013;75:223-33.
- [15] Vandelli MA, Sergi S, Ruozi B, Forni F. Preformulative and formulative studies on inclusion complexes between cyclodextrins and ursodeoxycholic acid. *STP Pharma Sci.* 2001;11:157-65.

- [16] Vandelli MA, Salvioli G, Mucci A, Panini R, Malmusi L, Forni F. 2-hydroxypropyl-beta-cyclodextrin complexation with ursodeoxycholic acid. *Int. J. Pharm.* 1995;118:77-83.
- [17] Ventura CA, Tirendi S, Puglisi G, Bousquet E, Panza L. Improvement of water solubility and dissolution rate of ursodeoxycholic acid and chenodeoxycholic acid by complexation with natural and modified beta-cyclodextrins. *Int. J. Pharm.* 1997;149:1-13.
- [18] Dhankhar J. Cardioprotective effects of phytosterols. *IJPSR* 2013;4:590-6.
- [19] Awad AB, Fink CS. Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J. Nutr.* 2000;9:2127-30.
- [20] Castelli F, Sarpietro MG, Micieli D, Trombetta D, Saija A. Differential scanning calorimetry evidence of the enhancement of beta-sitosterol absorption across biological membranes mediated by beta-cyclodextrins. *J. Agric. Food Chem.* 2005;54:10228-33.
- [21] Meng XH, Pan QY, Liu Y. Preparation and properties of phytosterols with hydroxypropyl beta-cyclodextrin inclusion complexes. *Eur. Food Res. Technol.* 2012;235:1039-47.
- [22] Mantegna S, Binello A, Boffa L, Giorgis M, Cena C, Cravotto G. A One-Pot Ultrasound-assisted water Extraction/Cyclodextrin encapsulation of Resveratrol from *Polygonum cuspidatum*. *Food Chem.* 2012;130:746-50.
- [23] Tabasso S, Carnaroglio D, Calcio Gaudino E, Cravotto G. Enabling technologies for bio-waste valorization. *Green Chem.* 2015, DOI:10.1039/C4GC01545B.
- [24] Cravotto G, Boffa L, Turello M, Parenti M, Barge A. Chemical Modifications of Bile Acids under High-Intensity Ultrasound or Microwave Irradiation. *Steroids* 2005;70:77-83.
- [25] Ball Milling Towards Green Synthesis: Applications, Projects, Challenges. 2014, Ed. Ranu BC, Stolle A. RSC. ISBN: 978-1-84973-945-0.
- [26] Nakai Y. Molecular behavior of medicinals in ground mixtures with microcrystalline cellulose and cyclodextrins. *Drug Dev. Ind. Pharm.* 1986;12:1017-39.
- [27] Carli F., Colombo I., Torricelli C. Drug beta-cyclodextrin systems by mechanochemical activation. *Chim. Oggi* 1987;5:61-64.
- [28] Xie J, Yang F, Shi XJ, Zhu XY, Su WK, Wang P. Improvement in solubility and bioavailability of puerarin by mechanochemical preparation. *Drug Dev. Ind. Pharm.* 2013;39:826-35.
- [29] Dantu AS, Durai R, Vedha BNV, Effect of impact and attrition milling on Nimesulide for solubility enhancement. *IJAP* 2013;5:1-7.
- [30] Bragagni M, Maestrelli F, Mura P. Physical chemical characterization of binary systems of prilocaine hydrochloride with triacetyl- $\beta$ -cyclodextrin. *J. Inclusion Phenom. Macrocyclic Chem.* 2010;68:437-45.
- [31] Dushkin AV, Rykova ZU, Shakhtshneider TP, Boldyrev VV. Aggregation processes in the reactivity of mechanically activated organic solids. *Int. J. Mechanochem. Mech. Alloying.* 1994;1:48-55.

- [32] Mio H, Kano J, Saito F, Kaneko K. Effects of rotational direction and rotation-to-revolution speed ratio in planetary ball milling. *Mater. Sci. Eng.* 2002;A332:75-80.
- [33] Schilz T, Riffel M, Pixius K, Meyer H-J. Synthesis of thermoelectric materials by mechanical alloying in planetary ball mills. *Powder Technol.* 1999;105:149-54.
- [34] Kudaka K, Iizumi K, Izumi H, Sasaki T. Effect of milling media on the reaction kinetics of the mechanochemical synthesis of pentatitanium trisilicide. *J. Am. Ceram. Soc.* 2000;83:2887-9.
- [35] Cravotto G, Garella D, Carnaroglio D, Calcio Gaudino E, Rosati O. Solvent-free chemoselective oxidation of thioethers and thiophenes by mechanical milling. *Chem. Commun.* 2012;48:11632-4.
- [36] Bandarkar FS, Vavia PR. An optimized commercially feasible milling technique for molecular encapsulation of meloxicam in beta-cyclodextrin. *Drug Dev. Ind. Pharm.* 2011;37:1318-28.
- [37] Iwata M, Fukami T, Kawashima D, Sakai M, Furuishi T, Suzuki T, Tomono, K, Ueda H. Effectiveness of mechanochemical treatment with cyclodextrins on increasing solubility of glimepiride. *Pharmazie* 2009;64:390-4.
- [38] Adhage NA, Vavia PR.  $\beta$ -cyclodextrin inclusion complexation by milling. *Pharm. Pharmacol. Commun.* 2000;6:13-17.
- [39] Wongmekiat A, Yoshimatsu S, Tozuka Y, Moribe K, Yamamoto K. Investigation of drug nanoparticle formation by co-grinding with cyclodextrins: Studies for indomethacin, furosemide and naproxen. *J. Inclusion Phenom. Macrocyclic Chem.* 2006;56:29-32.
- [40] Rajendrakumar K, Pralhad T, Madhusudan S. Comparative Study on Co-Ground Products of Rofecoxib with  $\beta$ -Cyclodextrin and Its Sulfobutyl Ether-7 Derivative in Solution and in the Solid State. *J. Inclusion Phenom. Macrocyclic Chem.* 2004;49:259–266.
- [41] Wang X, Luo Z, Xiao Z. Preparation, characterization, and thermal stability of  $\beta$ -cyclodextrin/soybean lecithin inclusion complex. *Carbohydr. Polym.* 2014;101:1027–1032.
- [42] Giordano F, Novak C, Moyano JR. Thermal analysis of cyclodextrins and their inclusion compounds. *Thermochim. Acta* 2001;380:123-51.
- [43] Oja V, Chen X, Hajaligol MR, Chan WG, Sublimation Thermodynamic Parameters for Cholesterol, Ergosterol, beta-Sitosterol, and Stigmasterol. *J. Chem. Eng. Data* 2009;54:730-4.

## Highlights

- Cyclodextrins/steroids inclusion complexes are easily formed in ball mills
- Solid state grinding enhances the interaction between cyclodextrins and steroids
- DSC was used to confirm the formation of inclusion complexes
- Cyclodextrins/steroids 2:1 ratio gave high encapsulation efficiency (77-96%)