

Full Length Review Paper

Co-crystals of agrochemical actives

Bhupinder Singh Sekhon

PCTE Institute of Pharmaceutical Sciences, near Baddowal Cantt, Ludhiana-142 021, India.
Email: sekhon224@yahoo.com

Accepted 12 January, 2014

Design of agrochemical active ingredients is a multidimensional task. Co-crystals are crystalline compounds that contain two different molecules that pack together in a fixed way. Co-crystallization can be used as a reliable technique to form alternative solid forms of agrochemical actives, and co-crystals do indeed have potential to increase the apparent solubility of compounds with poor dissolution properties. No marketed agrochemicals have been actively developed as co-crystals so far, but several companies are actively investigating them. Use of the Cambridge Structural Database which screens potential co-formers for a given active compound to find suitable, strongly interacting co-crystal partners is desirable.

Key words: Co-crystals, agrochemical actives, pesticides, solubility, co-formers.

INTRODUCTION

Bulk solids physical properties are typically governed by the molecular arrangement of individual building blocks with respect to each other in the crystal lattice. Co-crystallisation is a fast growing field in the area of crystal design and co-crystals are of utmost importance for development of new types of agrochemicals. In this context, identification of a suitable set of co-formers is an important part of design and preparation of co-crystals.

Until, recently co-crystallization process has got tremendous success in developing the co-crystals of active pharmaceutical ingredients in order to alter their physicochemical properties such as solubility, dissolution rate, stability and bioavailability, however, its implementation in agrochemical industry research is scarce. In agrochemical research, the broad diversity of the target organisms presents a specific and complex challenge which must be carefully considered and addressed for each screening program.

The term "agrochemical" refers to a collection of chemicals (pesticides) such as fungicides, herbicides and insecticides or acaricides. Agrochemicals are used to protect crops from pests and thus help improve the quality and quantity of crop production. Design of agrochemical active ingredients is a multidimensional task (Schleifer, 2013). Agrochemicals usually marketed as liquid or solid formulations comprise one or more agrochemically active organic compounds and suitable formulation additives. For several reasons, solid formulations types such as dusts, powders or granules

and liquid formulations such as suspension concentrates are preferred (Krapp et al., 2011; Encyclopedia of Agrochemicals, 2003). Physical properties such as solubility, hygroscopicity, thermal stability, filterability and flow ability are influenced by the solid forms of materials. These properties affect the overall usability and efficacy of a given agrochemical.

Designing different solid forms can affect various physical properties and make them more suitable for a particular form of application. Issues such as amorphous nature of solid forms, Ostwald ripening in a liquid formulation, and polymorphic transformation of most of the agrochemical actives suggest the need for modification of the physico-chemical properties of the actives that influence the morphology and behaviour of the actives, resulting in better end use properties.

Co-crystals are molecular crystals composed of two or more compounds, and refer mainly to crystals which contain compounds that are solids at standard conditions. Co-crystallization is an established technique that is generating renewed interest in the agrochemical industry. Co-crystals incorporate guest molecules into a crystal lattice along with the active ingredient, changing the physical properties of the solid. Recently, co-crystals have been tested as an efficient alternative form for solid-state modification of the active ingredient (George et al., 2011; Mereiter, 2011; Nauha et al., 2011; Nauha and Nissinen, 2011). Co-crystallization, a tool for probing intermolecular interactions between molecules (Aakeröy

and Salmon, 2005) is a promising strategy for improving the critical characteristics of agrochemicals.

Co-crystallization utilizes non-covalent interactions to form a regular solid-state arrangement from two or more neutral/charged molecular components within the same crystal lattice. Co-crystals are new supramolecules comprising at least one active ingredient and a partner molecule linked to it by non-covalent bonds. Co-crystals are formed from heteromeric species while homomeric species formation is due to recrystallization.

The formation of co-crystals needs to be controlled in any industrial crystallization processes. In the field of pharmaceuticals, co-crystallization has been shown to be an effective means of altering a drug's physical properties, such as stability, solubility, bioactivity, moisture uptake, melting point etc (Remenar et al., 2003; Walsh et al., 2003).

Co-crystallization can be used as a reliable technique to form alternative solid forms of agrochemical actives which display improved physical properties: i) improved melting points, ii) decreased solubility iii) improved storage and formulation stability, and iv) modified crystal morphology and so on. In this context, researchers have synthesized co-crystals of two agrochemical pesticides, namely, Cyprodinil and Terbutylazine with co-formers (even chain dicarboxylic acids (C₄ to C₁₀) such as Succinic, Adipic, Suberic, Sebacic and Dodecandioic acid). Both cyprodinil and terbuthylazine showed the potential to form co-crystals with a series of diacids with 100% supra molecular yield. The co-crystals displayed melting points different from the parent active. In cyprodinil co-crystals, the solubility of the active could be altered using co-crystallization and a very good correlation between the co-crystal solubility and co-former solubility was reported. No appreciable change in hygroscopicity was observed at 84% and 43% relative humidity conditions for cyprodinil co-crystals. The PXRD patterns collected at the end of humidity experiments indicated no degradation or change in form of the solid samples (Nauha, 2012).

Bringing active agrochemical ingredients in solution often is the most demanding step in agrochemical development (Klamt, 2012). Melting point is an important consideration for agrochemical development. A higher melting point is important in formulation processing and stability of co-crystals. High melting points are usually desirable, but may contribute to poor solubility and are as troublesome as low melting points, which can hinder processing, drying, and stability. The melting point can typically be tuned according to which coformer is chosen; for example, if a higher melting cocrystal is desired, then a higher melting co-former should be selected and vice versa. For example, the agrochemical pyrimethanil has a melting point of 96°C. Its 1:1 co-crystal with benzoic acid

can raise melting point by 32°. Pyrimethanil-dithianon co-crystals have a melting point in the range of 165 to 175°C compared to dithianon (230°C) and pyrimethanil 96°C. Processes for preparing the co-crystals of pyrimethanil-dithianon and their use for controlling phytopathogenic fungi have been reported (Sowa et al., 2009; Sowa et al., 2012; Sowa et al., 2013).

Co-crystals of cyprodinil with benzoic acid, succinic acid, fumaric acid, maleic acid, oxalic acid, pyrazine carboxylic acid, glycolic acid, levulinic acid, (2-methylphenoxy)acetic acid, hexanedioic acid, 4-(methylamino) benzoic acid, trimethyl acetic acid, pyruvic acid or 4-hydroxy-4'-biphenyl carboxylic acid have been reported (George et al., 2008).

4-[[6-chloropyrid-3-yl)methyl](2,2'-difluoroethyl)amino}furan-2-(5H)-one is an active ingredient (AI) with insecticidal properties. Co-crystals of the AI with salicylic acid (1:1) has been synthesized which displays a higher melting point, 82°C when compared to that of the active by itself (72-74°C). Due to the increased thermal stability, the AI can be treated and handled at higher temperatures. The co-crystal also displays improved storage stability and solubility than the pure substance (Weiss et al., 2012). The elevated melting point of the inventive cocrystal is particularly advantageous because the cocrystal, in contrast with the pure substance 4-[[6-chloropyrid-3-yl)methyl] (2,2-difluoroethyl)amino}furan-2(5H)-one, can be treated at higher temperatures without melting. Co-crystal of 4-[[6-chloropyrid-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one with oxalic acid was reported for use as pesticide (Weiss et al., 2011).

The crystal form screening of two analogous agrochemical actives, thiophanate-methyl and thiophanate-ethyl, as well as the discovery of seven 4-hydroxybenzoic acid co-crystals of selected agrochemical actives have been reported. Three co-crystals of thiophanate-methyl and eight co-crystals of thiophanate-ethyl were reported. The seven co-crystal forms of agrochemical actives with 4-hydroxybenzoic acid showed better properties in comparison to the pure forms, and highlight the importance of co-crystallization in industrial applications (Nauha, 2012).

Researchers have developed the most reliable technology (Virtual Co-crystal Screening) to predict which co-formers will co-crystallize with higher probability with a specific molecule. In this context, calculated gas phase molecular electrostatic potential surfaces have been used to identify sets of H-bond donor and H-bond acceptor sites that describe the possible intermolecular interaction sites on the surface of a molecule. The approach assumes that all of the interactions that can be made in the solid are made and that the details of three-dimensional structure and crystal packing are of secondary importance. Comparisons of the energy of two pure solids with co-crystals of various stoichiometries pro-

vide an energy difference, ΔE , which is a measure of the probability of forming a co-crystal. The results provided a calibration between the value of ΔE and the probability of co-crystal formation: when the co-crystal is favoured by more than 11 kJ mol^{-1} over the two pure solids, the probability of obtaining a co-crystal is better than 50% (Musumeci, 2011). CIRCE has acquired the intellectual rights for the use of this software, with the objective of offering it to the agrochemical and pharmaceutical companies.

Recently, a virtual co-crystal screening method based on calculated gas phase molecular electrostatic potential surfaces (MEPS) of the individual components has been validated using experimental co-crystal screens reported in the literature. The non covalent interactions of a molecule with its environment were described by a discrete set of independent surface site interaction points (SSIPs), whose properties can be calculated from the *ab initio* MEPS. The stability of a crystal was estimated based on pairing SSIPs such that the sum of the pair wise interaction energies was optimized. This provided a means of calculating the relative stability of a co-crystal compared with the pure components without knowing anything about the three-dimensional structures of the crystalline states. For a set of potential crystal co-formers (CCF), the difference between interaction site pairing energies of different solid forms (ΔE) provided a method for ranking CCFs based on the calculated probability of co-crystal formation. The method was applied to co-crystal screens of 18 compounds that reported both hits and misses, and in most cases, the virtual co-crystal screen reproduces experimental results well. In lists of CCFs ranked by ΔE , the experimentally observed hits were significantly enriched at the top, and this indicated that virtual screening is a promising tool for focusing experimental efforts on the most promising CCF candidates (Greco et al., 2013).

The co-crystallization of agrochemical actives (fungicides) thiophanate-methyl (TM) and thiophanate-ethyl (TE) with 2,2'-bipyridine, 4,4'-bipyridine and 1,2-bis(4-pyridyl)ethane was investigated with conventional crystallization, the slurry method and liquid-assisted grinding. Both thiophanates formed co-crystals with all bipyridines and the structures solved with single crystal X-ray diffraction. The thiophanate to co-former stoichiometry of the hydrogen bonded co-crystals, however, was found variable with three 2:1 co-crystals (TM-4,4'-bipyridine, TM-1,2-bis(4-pyridyl)ethane and TE-1,2-bis(4-pyridyl)ethane), three 1:1 co-crystals (TE-4,4'-bipyridine, TE-di(2-pyridyl)ketone and TE-2-benzoylpyridine) and one 1:2 co-crystal (TE-3-benzoylpyridine). Whereas the 2,2'-bipyridine co-crystals seem to form because of a combination of weak interactions, and in the case of the thiophanate-methyl, partly because of close packing incentives, the 4,4'-bipyridine and 1,2-bis(4-

pyridyl)ethane co-crystals formed mainly because of a favourable N-H/N-pyridine hydrogen bonding synthon. The co-crystal forms of TM and TE with 4-hydroxybenzoic acid showed better properties in comparison to the pure forms, thereby, highlighting the importance of co-crystallization in industrial applications (Nauha et al., 2011).

Co-crystal screening for a number of active agrochemical ingredients (pyraclostrobin, epoxiconazole, tebuconazole, boscalid, imazethapyr, imazamox and acetamiprid containing a heterocyclic nitrogen) with 4-hydroxybenzoic acid were reported using a supra molecular synthon approach. The co-crystals showed improved properties in comparison to the pure compounds. Co-crystals of pyraclostrobin, epoxiconazole, tebuconazole, boscalid and acetamiprid showed an increased melting point compared to pure agrochemical actives. For the pyraclostrobin and tebuconazole cocrystals, especially, the melting point increase was found around 50°C (Nauha, 2012).

Five novel co-crystals of TE with di(2-pyridyl)ketone (1), 2-benzoylpyridine (2), 3-benzoylpyridine (3), 4-phenylpyridine (4) and biphenyl (5) were reported and crystal structures of four of them (TE1-TE3, TE5) solved by single crystal X-ray diffraction. Three of the co-crystals (TE1-TE3) formed by way of a reliable pyridine-amine hydrogen bond synthon and one (TE5) because of close packing effects. The fifth co-crystal was identified by X-ray powder diffraction (Nauha and Nissinen, 2011).

Co-crystals comprising i) a herbicide compound A (3,6-dichloro-2-methoxybenzoic acid, dicamba), and ii) a co-crystal former B (caffeine, theophylline, 2-aminopyrimidine, 4-aminopyrimidine, 2-aminothiazole, 3-hydroxypyridine, isocytosine, 4,4'-pyridine) use in agrochemical compositions were reported (Chiodo et al., 2013).

The co-crystals of itraconazole, a potent antifungal drug, with C_2 - C_7 dicarboxylic acids have been successfully synthesized and characterized by powder X-ray diffraction, solid state nuclear magnetic resonance, Raman spectroscopy, and thermal analysis. The characterized multi component compounds include anhydrous co-crystals (malonic, succinic, glutaric, and pimelic acids), a co-crystal hydrate (adipic acid), and co-crystal solvates with acetone and tetrahydrofuran (oxalic acid) (Shevchenko et al., 2013).

Both commercially available cyprodinil and pyrimethanil are anilinopyrimidine fungicides. Cyprodinil is used as a foliar fungicide on cereals, grapes, pome fruit, stone fruit, strawberries, vegetables, field crops and ornamentals and as a seed dressing on barley to control a wide range of pathogens such as *Tapesia yallundae* and *T. acuformis*, *Erysiphe* spp., *Pyrenophora teres*, *Rhynchosporium secalis*, *Botrytis* spp., *Alternaria* spp., *Venturia* spp. and *Monilinia* spp. Pyrimethanil is used to control grey mould (*Botrytis cinerea*) on vines, fruit, vege-

tables and ornamentals and in the control of leaf scab (*Venturia inaequalis* or *V. pirina*) on pome fruit. Both are described in The Pesticide Manual (The Pesticide Manual). Co-crystals of cyprodinil or pyrimethanil and a cocrystal forming compound which has at least one imide and/or oxime functional group were reported (George et al., 2011).

Co-crystal of propiconazole with a co-crystal forming compound which has at least one functional group selected from hydroxyl (including alcohol and phenol), ketone, carboxylic acid, amide, primary amine, secondary amine, tertiary amine, sp^2 amine, diazo, N-heterocyclic ring, pyrimidine or pyridine or with a biphenyl derivative wherein at least one of the ortho, meta or para positions of one or both phenyl rings is independently substituted with a suitable hydrogen bonding functional group selected from $-OH$, $-ROH$, $-C(O)H$, $-C(O)R'$, $-COON$, $-RCOOH$, $-NH_2$, $-RNH_2$, $-NHR'$, $-RNHR'$, $-NR'_2$, $-RNR'_2$, $-NHOR'$, $-RNHOR'$ wherein R is an alkylene group or an acyl group ($-C(O)R''-$), R' is an alkyl group and R'' is an alkylene group. Cocrystalline forms of propiconazole with a higher melting point than the commercially available versions of propiconazole were reported (George et al., 2012).

Co-crystals of cyprodinil with benzoic acid, succinic acid, fumaric acid, maleic acid, oxalic acid, pyrazine carboxylic acid, glycolic acid, levulinic acid, (2-methylphenoxy)acetic acid, hexanedioic acid, 4-(methylamino) benzoic acid, trimethyl acetic acid, pyruvic acid or 4-hydroxy-4'-biphenyl carboxylic acid was reported. Hydrogen-bonding occurs between the co-crystal forming compound and the cyprodinil (George et al., 2013). Co-crystals of metalaxyl and prothioconazole were reported to treat crops and plants (David, 2013). Co-crystallization used for tuning the physical properties of two agrochemicals, cyprodinil and terbuthylazine improved the solubility and melting point of cyprodinil, while ensuring that the hygroscopicity of the active was unaltered (Panikkattu, 2013).

A kinetic barrier hindered co-crystal formation in some cases. In one such case, the addition of a molecular species (fluorobenzoic acid derivative) similar to that found in the nucleus of the caffeine-benzoic acid co-crystal as a seed facilitated crystallization, because fluorine and hydrogen are so similar in size (Bucar et al., 2013). A 1:1 co-crystal of the herbicide triflurosulfuron-methyl (systematic name: methyl 2-[[4-dimethylamino-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoylsulfamoyl]-3-methylbenzoate) and its degradation product triazine amine [systematic name: 2-amino-4-dimethylamino-6-(2,2,2-trifluoroethoxy)-1,3,5-triazine] was reported. Two components were found connected *via* a pair of complementary $N-H\cdots N$ hydrogen bonds, similar to the monoclinic crystal structure of the parent compound triflurosulfuron-methyl (Mereiter, 2011) in which a pair of molecules related by a

twofold axis are linked by two $N-H\cdots N$ bonds. The triflurosulfuron-methyl molecules of both crystal structures were found similar in geometric parameters and conformation. Intermolecular $N-H\cdots O$ hydrogen bonds and slipped $\pi-\pi$ stacking interactions between the diaminotriazine moieties linked the two constituents of the co-crystal into columns parallel to the *a* axis. An intramolecular $C-H\cdots O$ hydrogen bond occurs in the triflurosulfuron-methyl molecule and intermolecular $C-H\cdots O$ interactions between triflurosulfuron-methyl molecules occur in the crystal structure (Mereiter, 2011). 1-Acetyl-3-phenyl-5-(1-pyrenyl)-pyrazoline (APPP) was synthesized and formed APPP-phenol co-crystal (III) (Feng et al., 2013).

Photosensitive Co-crystals for Controlled Release of Agrochemicals

A key problem in the agrochemical industry is the lack of reliable methods for releasing the active ingredient (AI) (agrochemical, pesticide) in a slow, controlled manner. Cocrystals with a combination of the active ingredient [agrochemical, pesticide] and a photoactive compound were reported. Upon UV irradiation, the photoactive molecule undergoes a structural change which in effect would result in breakdown of the crystal thus releasing the AI. In a particular case, azobenzene and stilbene based compounds were used as photoactive backbone, which undergo a cis-trans isomerization upon UV irradiation (DeHaven et al., 2011; Panikkattu et al., 2011).

CONCLUSION

Bringing active agrochemical ingredients in solution often is the most challenging step in agrochemical development. Co-crystals are a viable alternative solid form that can improve the dissolution rate and bioavailability of poorly soluble agrochemical actives. Co-crystals optimize agrochemical active product stability and enhance solubility. Scientists are definitely opting for co-crystals to improve the solubility of agrochemical actives. There is really a need to screen for different crystal forms very thoroughly as co-crystals themselves can suffer from is polymorphism where the active ingredient can exist in several different crystal forms, each with their own set of physicochemical properties and solubility.

REFERENCES

Aakeröy CB, Salmon DJ (2005). Building co-crystals with molecular sense and supra molecular sensibility. *Cryst.*

- Eng. Comm. 7: 439-448.
- Walsh RDB, Bradner MW, Fleischman S, Morales LA, Moulton B, Hornedo NR, Zaworotko MJ (2003). Chem. Comm. 186-187.
- Bucar DK, Day GM, Halasz I, Zhang GGZ, Sander JRG, Reid DG, MacGillivray LRG, Duer MJ, Jones W (2013). The curious case of (caffeine)·(benzoic acid): How heteronuclear seeding allowed the formation of an elusive co-crystal. Chem. Sci. 4: 4417-4425.
- Chiodo T, Klimov E, Schafer A, Wolfgang H, Hellmann R, Kabat A, Israels R, Schnabel G, Bratz M, Kibat C, Houy W (2013). Co-crystals of dicamba and a co-crystal former B. Patent WO2013143927 A1, Oct 3, 2013.
- David F (2013). Metalaxyl and prothioconazole cocrystals and method of making and using. WIPO WO/2013/162725A1, Oct 31.
- DeHaven B, Aakeroy CB, Panikkattu S, Desper J (2011). Co-crystals of photochromic compounds. Department of Chemistry, Kansas State University, Manhattan, KS. MOSSCS XXI, Eastern Illinois University, June 10-11th, p. 9.
- Encyclopedia of Agrochemicals (2003). John Wiley and Sons, Inc. DOI: 10.1002/047126363X.
- Feng Q, Wang M, Dong B, He J, Xu C (2013). Regulation of arrangements of pyrene fluorophores via solvates and cocrystals for fluorescence modulation. Cryst. Growth Des. 13(10): 4418- 4427.
- George N, Forrest J, Bonnett PE, Gavin PT (2012). Co-crystals of propiconazole. Patent No: US 8318789 B2, Nov 27.
- George N, Forrest J, Gavan PT, Burton RC, Gregory L (2008). Co-crystals of cyprodinil and pyrimethanil. Patent No:WO/2008/117060, Oct 2.
- George N, Forrest JO, Burton RC, Aakeroy CB (2011). Co-crystals of pyrimethanil or cyprodinil. Patent WO 2011128618A1, Oct 20.
- George N, Forrest JO, Gavin PT, Burton RC, Gregory L (2013). Co-crystals. US Patent 8470832, June 25.
- Greco T, Hunter CA, Gardiner EJ, McCabe JF (2014). Validation of a computational co-crystal prediction tool: Comparison of virtual and experimental co-crystal screening results. Cryst. Growth Des. 14(1): 165-171.
- Klamt A (2012). Solvent-screening and co-crystal screening for drug development with COSMO-RS. J. Chem. Inform. 4 (1): O14.
- Krapp M, Gregori W, Saxell HE (2011). Crystalline complexes of pendimethalin and metazachlor. Patent US 8212054 B2, 3 Jul. 2012.
- Mereiter K (2011). 1:1 Cocrystal of herbicide triflusalflufuron-methyl and its degradation product triazine amine. Acta. Crystallogr. Sect. E.67: 2321–2322.
- Musumeci D, Hunter CA, Prohens R, Scuderi S, McCabe JF (2011). Virtual co-crystal screening. Chem. Sci. 2: 883-890.
- Nauha E (2012). “Crystalline forms of selected agrochemical actives: design and synthesis of cocrystals”: Ph D Thesis Department of Chemistry, University of Jyväskylä, p.132.
- Nauha E, Kolehmainen E, Nissinen M (2011). Packing incentives and a reliable N-H...N-pyridine synthon in co-crystallization of bipyridines with two agrochemical actives. Cryst. Eng. Comm. 13: 6531-6537.
- Nauha E, Nissinen M (2011). Co-crystal design using a pyridine-amine synthon for an agrochemical active containing a thioamide group, J. Mol. Struct. 1006: 566-569.
- Panikkattu S (2013). Designing molecular solids with structural control and tunable physical properties using co-crystallization techniques. Ph D Thesis, Department of Chemistry. College of Arts and Sciences, Kansas State University, Manhattan, Kansas, pp. 1-144.
- Panikkattu S, Christer B, Aakeroy B, Desper J (2011). “A new approach to controlled release of agrochemicals”: MOSSCS XXI, Eastern Illinois University, June 10-11th, p. 6.
- Remenar JF, Morissette SL, Peterson ML, Moulton B, MacPhee JM, Guzmán HR, Almarsson O (2003). J. Am. Chem. Soc. 125: 8456- 7.
- Schleifer KJ (2013). Challenges in agrochemicals design. J. Chem. Inform. 5(1): O17.
- Shevchenko A, Miroshnyk I, Pietilä LO, Haarala J, Salmia J, Sinervo K, Mirza S, Van Veen B, Kolehmainen E, Nonappa, Yliruusi J (2013). Diversity in itraconazole cocrystals with aliphatic dicarboxylic acids of varying chain length. Cryst. Growth Des. 13(11): 4877- 4884.
- Sowa C, Saxell HE, Vogel R (2009). Co-crystals of pyrimethanil and dithianon. BASF SE, WO/2009/047043.
- Sowa C, Saxell HE, Vogel R (2012). Co-crystals of pyrimethanil and dithianon. BASF SE, US 8324233.
- Sowa C, Saxell HE, Vogel R (2013). Co-crystals of pyrimethanil and dithianon. Patent application number: EP 2197278 B1.
- Weiss M, Storch D, Wirth W, Olenik B, Weiss HC, Schwiedop U (2012). Co-crystal of 4-[[6-chloropyrid-3-yl)methyl]](2,2'-difluoroethyl)amino}furan-2-(5H)-one with salicylic acid and use thereof as pesticide. USPA No 20120252766 A1.
- Weiss W, Storch D, Wirth W, Olenik B, Schweid U, Weiss HC (2011). Cocrystal of 4-[[6-chloropyrid-3-yl)methyl]](2,2-difluoroethyl)amino}furan-2(5H)-one with oxalic acid and use thereof as pesticide. WO/2011/051241.